

# Kinetics of Retting of Fresh Tubers of Five Cassava Accessions in Collection at SAMBA in Ombella M'poko in the Central African Republic

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**Abstract** Cassava (*Manihot esculenta*) is a perennial shrub of the Euphorbiaceae family. Thus, the objectives assigned to this study consist of studying the kinetics of retting of fresh tubers of 5 accessions of cassava in collection at Samba at concentration respectively: 100.66 mg/kg (ICRA14), 103.33 mg/kg (ICRA51), 134.66 mg/kg (MAMBERE), 135.1 mg/kg (ICRA21) and 144 mg/kg (DOGBO) considered higher than the FAO standard (10 mg/kg), specifically it involves evaluating the concentration of HCN in fresh tubers of five cassava accessions grown in CAR, every 7 hours for 70 hours and observing the variations in HCN levels during the different stages of processing (soaking, fermentation, drying, cooking); Determine the pH every 7 hours for 70 hours in the retting water from this transformation process ;Determine the temperature every 7 hours up to 70 hours in the retting water of this transformation process; Analyze the effectiveness of various traditional transformation methods to reduce the HCN level, Identify if possible the accessions presenting a significant risk due to their residual HCN content after transformation. And provide practical recommendations to improve traditional methods and reduce the exposure of populations to cyanide. 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 to determine the level of hydrocyanic acid contained in fresh cassava tubers. The results obtained showed respectively the open system (before and after retting): 102.8-142 mg/kg and 19.3-23 mg/kg depending on the HCN content in the tubers of these accessions; the closed system (before and after retting): 103-142 mg/kg and 13.3-18.1 mg/kg. Thus in the cossettes of the open-closed system: 8-9.23mg/kg and 7.7-8.3mg/kg and in the couscous of the open-closed system: 0.25-0.60mg/kg; 0.23-0.48mg/kg. The pH values varied from 3.5-6.01 from retting to the cossettes. The temperature varied from 22°C -26°C for all systems along this retting process. These HCN results obtained in the cossettes as well as the cassava couscous were considered acceptable according to the FAO standard (10mg/kg of HCN in the cossettes) and CODEX (2mg /Kg of HCN) in the cassava couscous. This demonstrates the effectiveness of the improved cassava production diagram implemented in the Central African Republic through this study.

**Keywords:** Cassava, Hydrocyanic acid, Titrimetry, Detoxification, Kinetics, Retting

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## 1. Introduction

Cassava (*Manihot esculenta* Crantz) is a plant native to Brazil (South America), cultivated throughout the tropics for its starchy roots [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 eaten 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 advantages, cassava is one of the 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 in themselves are not toxic 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 due to the consumption of cassava containing a high level of HCN [6]. KONZO is an irreversible neurological disorder affecting the upper body that is prevalent in rural areas of sub-Saharan Africa, where the main crop is bitter varieties of cassava. Due to the dangers of chronic toxicity of this plant, there is talk of more intensive efforts to achieve low and acceptable levels of cyanide. The first possibility of reducing the cyanide level in cassava lies in the selection of cultivars that are free or low in HCN [7]. It is difficult to find cyanide-free varieties, but studies will always continue. Cassava varieties that produce less than 50mg/kg of HCN from peeled and fresh roots are considered harmless [8,9]. CAR is one of the countries where cassava is the staple food and its cultivation is widespread throughout the country. The leaves are eaten as a vegetable and the roots as an energy source. In the Central African Republic, numerous studies have been carried out on its improvement [10,11]. An epidemiological survey confirmed the presence of KONZO disease (epidermal paralysis induced by chronic poisoning by cyanide of food origin) in health district No. 2 in 2009 [12]. 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 safety, it is a question of launching research on the toxicological aspect of this plant. This is why, through this research project, we have already evaluated the HCN content in the leaves of 58 cassava accessions in circulation in the Central African Republic, whose determined HCN concentrations vary from 13 mg to 144 mg, exceeding the FAO standard of 10 mg [13]. This is a worrying situation, because recent work has shown that the frequency of consumption of cassava

leaves is 1 to 4 times per week in households [14]. This would expose consumers to a health risk due to the cyanide effect. What required the verification of the effects of the toxicity of the leaves of these accessions on mice helped in the search for solutions in humans. The results obtained from this work showed a very significant elevation of liver and kidney markers, accompanied by weight loss and visible changes in the organs examined, highlighting the presence of Atrophy, Dystrophy, Ischemia and Necrosis in the livers, kidneys and hearts of the mice tested except for the control group. These results also showed that the accessions studied DOGBO, ICRA21, MAMBERE, ICRA51 and ICRA14 have considerable toxic potential. It is therefore crucial to carry out a thorough assessment before any consumption of leaves and derived products, particularly by-products in the Central African Republic. This is how this study aims to evaluate the Kinetics of retting of fresh cassava tubers of these toxic accessions in collection at Samba in Embella M'poko in the Central African Republic.

### Research questions

What is the risk of consuming poorly processed cassava?

### General objective

To analyze how hydrocyanic acid (HCN) is released during the various processing phases of cassava (*Manihot esculenta* Crantz) in the Central African Republic, with the aim of better understanding the toxic risk and offering practical advice to ensure food safety.

### Specific objectives

- To assess the HCN concentration in fresh tubers of five cassava accessions grown in CAR each 7h for 70h and to observe the variations in HCN levels during the different stages of processing (soaking, fermentation, drying, cooking);
- Determine the pH every 7 hours for 70 hours in the retting water from this transformation process
- Determine the temperature every 7 hours up to 70 hours in the retting water of this transformation process;
- Analyze the effectiveness of various traditional processing methods to reduce HCN levels, Identify, if possible, local accessions presenting a significant risk due to their residual HCN content after processing. Provide practical recommendations to improve traditional methods and reduce population exposure to cyanide.

## 2. Materials and Methods

### 2.1. Plant Material

#### 2.1.1. Materials and Methods

#### 2.1.2. Sample Collection

The samples were collected at the LASBAD experimental station located 20 km south of Bangui and transported to the laboratory for analysis. These roots were harvested in the Embella M'poko region (Central African Republic) 24 months after planting.

### 2.2. Measurement of Hydrocyanic acid (HCN)

The measurement was carried out by the titration method (argentometric procedure).

Process :

- 20 g of tubers chopped mixed with 200 mL of distilled water, left to macerate for 20 hours at room temperature;
- Steam distillation, with recovery of 130 mL in 20 mL of NaOH at a concentration of 0.5 g/20 mL;
- Redistillation, followed by titration with 0.02 N  $\text{AgNO}_3$  in the presence of  $\text{NH}_4\text{OH}$  and KI;
- The appearance of turbidity indicates the end of equivalence [15.a] [15.b].

**Calculation formula:**

- Hydrogen cyanide (mg/100g) =  $1.08 \text{ Veq} \cdot 2.5 \cdot 100 / \text{Me}$
- where Veq is the volume of  $\text{AgNO}_3$  poured at equilibrium and Me is the mass of the sample. Results converted to mg/kg for comparison with CODEX and WHO standards.

### 2.3. pH Measurement

The measurement was carried out using a pH measuring device (Seven Compact S220, Mettler Toledo).

Samples (20 g in 200 mL of distilled water) were evaluated before and after the maceration period.

### 2.4. Temperature Assessment During Retting

The roots were previously:

- Roots cleaned, peeled and cut into 5 cm segments (10 kg).
- Immersed in mains water, volume increased to 200 ml (open and closed systems).
- Temperature recorded every 7 hours over a period of 70 hours.

*Fresh tuber → maceration → fermentation → distillation → HCN measurement, pH, T°C)*



Figure 1. Retting process

## 3. Results

**Basic notation:** SO = Open System; SF = Closed System; Lx = Batch x; x = 1, 2, 3, 4 and 5Q = Quantity of HCN; Time = Temperature; pH.

**3.1.** Results of retting work on HCN values in open system tubers over 70 hours.

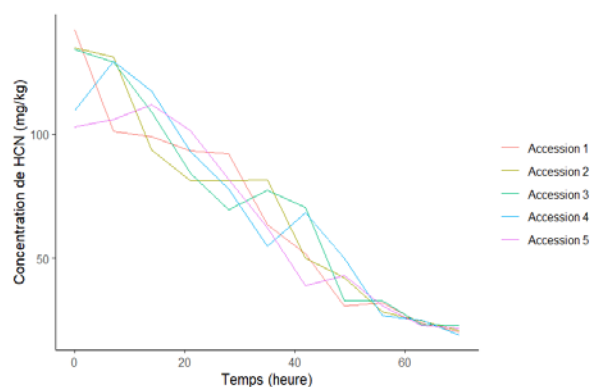


Figure 2. HCN decrease curves of different accessions during open-system retting of cassava tubers



From the above, the results of open system retting showed that the x-axis exclusively comprises time in h/s and the y-axis exclusively comprises hydrocyanic acid (HCN) concentration in mg/kg. Thus the slope of different HCN curves is negative resulting in the decrease of HCN concentration in cassava tubers as a function of time. The different specific points of interest are as follows:

- The beginning of these different curves indicates the initial behavior before the reaction
- The final state: the final values on the x-axis after reaching a plateau are stable.

The results also show the variation of different curves depending on the HCN concentration in the tubers of these accessions which differs from one accession to another.... And the time factor in the transformation process. This being: accession 1 (before-after retting) = 142mg/Kg to 20.3mg/kg; accession 2 (before-after retting) = 134.8mg/Kg to 21.1mg/Kg; accession 3 (before-after retting) = 134.1mg/Kg to 23mg/Kg; accession 4 (before-after retting) = 109.5mg/Kg to 19.3mg/Kg; accession 5 (before-after retting) = 102.8mg/Kg to 21.9mg/Kg.

#### The Open System Hydrocyanic Acid Value Comparison Test of Five Accessions

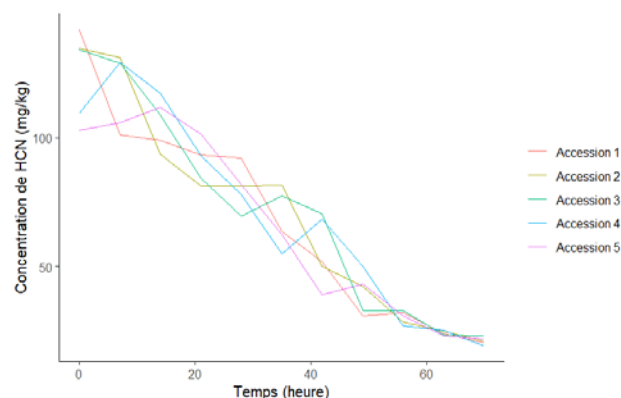
> t.test (Q.L1.SO, Q.L2.SO, conf.level = 0.95) .Welch Two Sample t-test .Data: Q.L1.SO and Q.L2.SO

t = -0.39291, df = 19.933, p-value = 0.6986. Alternative hypothesis: true difference in means is not equal to 0 95 percent confidence interval: -45.31842 30.95479. Sample estimates: mean of x mean of y 70.38182 77.56364.> t.test (Q.L1.SO, Q.L3.SO, conf.level = 0.95).Welch Two Sample t-test .Data: Q.L1.SO and Q.L3.SO ,t = -0.22497, df = 19.846, p-value = 0.8243. Alternative hypothesis: true difference in means is not equal to 095 percent confidence interval: -41.66789 33.55880. Sample estimates: mean of x mean of y 70.38182 74.43636 .> t.test (Q.L1.SO, Q.L4.SO, conf.level = 0.95).Welch Two Sample t-test

Data: Q.L1.SO and Q.L4.SO.t = 0.60563, df = 18.916, p-value = 0.552 .Alternative hypothesis: true difference in means is not equal to 0 95 percent confidence interval: -25.12829 45.58283. Sample estimates: mean of x mean of y 70.38182 60.15455 > t.test (Q.L1.SO, Q.L5.SO, conf.level = 0.95).Welch Two Sample t-test.Data: Q.L1.SO and Q.L5.SO.t = 0.43356, df = 17.752, p-value = 0.6698. Alternative hypothesis: true difference in means is not equal to 0 95 percent confidence interval: -26.95424 40.95424. Sample estimates: mean of x mean of y 70.38182 63.38182.> t.test (Q.L2.SO, Q.L3.SO, conf.level = 0.95). 19.982, p-value = 0.8598. Alternative hypothesis: true difference in means is not equal to 095 percent confidence interval: -33.33390 39.58844. Sample estimates: mean of x mean of y 77.56364 74.43636 .> t.test (Q.L2.SO, Q.L4.SO, conf.level = 0.95). Q.L4.SO.t = 1.0677, df = 19.345, p-value = 0.2988. Alternative hypothesis: true difference in means is not equal to 0 95 percent confidence interval: -16.67603 51.49422. Sample estimates: mean of x mean of y 77.56364 60.15455 .> t.test (Q.L2.SO, Q.L5.SO, conf.level = 0.95).Welch Two Sample t-test.Data: Q.L2.SO and Q.L5.SO.t = 0.91287, df = 18.305, p-value = 0.3732. Alternative hypothesis: true difference in means is not equal to 095 percent confidence interval:-18.41777 46.78141. Sample estimates: mean of x mean of y 77.56364 63.38182.> t.test (Q.L3.SO, Q.L4.SO, conf.level = 0.95). p-value = 0.3834. Alternative

hypothesis: true difference in means is not equal to 0 95 percent confidence interval: -19.18099 47.74463. Sample estimates: mean of x mean of y 74.43636 60.15455 .> t.test (Q.L3.SO, Q.L5.SO, conf.level = 0.95). Q.L5.SO.t = 0.72568, df = 18.58, p-value = 0.4771. Alternative hypothesis: true difference in means is not equal to 095 percent confidence interval: -20.87823 42.98732. Sample estimates: mean of x mean of y 74.43636 63.38182 > t.test (Q.L4.SO, Q.L5.SO, conf.level = 0.95).Welch Two Sample t-test.Data: Q.L4.SO and Q.L5.SO.t = -0.23266, df = 19.681, p-value = 0.8184. Alternative hypothesis: true difference in means is not equal to 0 95 percent confidence interval: -32.19241 25.73786

Sample estimates: mean of x mean of y 60.15455 63.38182



**Figure 3.** HCN decrease curves of different accessions during closed-system retting of cassava tubers

The results of closed system retting showed that the x-axis exclusively contains time in h/s and the y-axis contains hydrocyanic acid (HCN) concentration in mg/kg. Thus, the slope of different HCN curves is negative, resulting in a decrease in HCN concentration in cassava tubers as a function of time. The different specific points of interest are as follows:

- The beginning of these different curves indicates the initial behavior before the reaction
- The final state: the final values on the x-axis after reaching a plateau are stable.

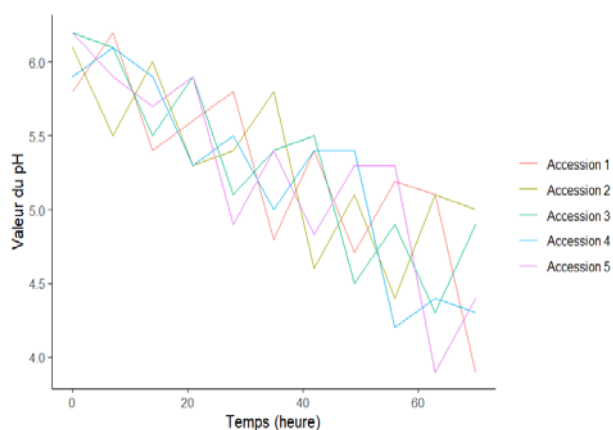
These results also show the variation of different curves depending on the HCN concentration in the tubers of these accessions which differs from one accession to another.... And the time factor in the transformation process. This being: accession 1 (before-after retting) = 142mg/kg to 18.1mg/kg; accession 2 (before-after retting) = 135mg/Kg to 17.5mg/Kg; accession 3 (before-after retting) = 134.1mg/Kg to 18.2mg/Kg; accession 4 (before-after retting) = 109.5mg/Kg to 17.1mg/Kg; accession 5 (before-after retting) = 103mg/Kg to 13.3mg/Kg.

#### The Closed System Hydrocyanic Acid Value Comparison Test of Five Accessions

> t.test (Q.L1.SF, Q.L2.SF, conf.level = 0.95).Welch Two Sample t-test.Data: Q.L1.SF and Q.L2.SF

t = 0.47547, df = 19.993, p-value = 0.6396. Alternative hypothesis: true difference in means is not equal to 0 95 percent confidence interval:-29.25339 46.52611. Sample estimates: mean of x mean of y 62.89091 54.25455 > t.test (Q.L1.SF, Q.L3.SF, conf.level = 0.95).Welch Two Sample t-test. Data: Q.L1.SF and Q.L3.SF.t = -0.059447, df =

19.983, p-value = 0.9532. Alternative hypothesis: true difference in means is not equal to 0 95 percent confidence interval: -38.05982 35.95073. Sample estimates: mean of x mean of y 62.89091 63.94545 .> t.test (Q.L1.SF, Q.L4.SF, conf.level = 0.95).Welch Two Sample t-test.Data: Q.L1.SF and Q.L4.SF.t = 0.95873, df = 18.767, p-value = 0.3499.Alternative hypothesis: true difference in means is not equal to 0 95 percent confidence interval: -18.23762 49.01943.Sample estimates: mean of x mean of y 62.89091 47.50000 .> t.test (Q.L1.SF, Q.L5.SF, conf.level = 0.95). 18.353, p-value = 0.4112. Alternative hypothesis: true difference in means is not equal to 095 percent confidence interval: -19.84282 46.38827. Sample estimates: mean of x mean of y 62.89091 49.61818 .> t.test (Q.L2.SF, Q.L3.SF, conf.level = 0.95).Welch Two Sample t-test.Data: Q.L2.SF and Q.L3.SF.t = -0.54104, df = 19.955, p-value = 0.5945. Alternative hypothesis: true difference in means is not equal to 095 percent confidence interval: -47.05914 27.67732. > t.test (Q.L2.SF, Q.L4.SF, conf.level = 0.95).Welch Two Sample t-test.Data: Q.L2.SF and Q.L4.SF.t = 0.41583, df = 18.607, p-value = 0.6823.Alternative hypothesis: true difference in means is not equal to 095 percent confidence interval: -27.29263 40.80172.Sample estimates: mean of p-value = 0.775. Alternative hypothesis: true difference in means is not equal to 095 percent confidence interval: -28.90752 38.18025. Sample estimates: mean of x mean of y 54.25455 49.61818 .> t.test (Q.L3.SF, Q.L4.SF, conf.level = 0.95).Welch Two Sample t-test .Data: Q.L3.SF and Q.L4.SF.t = 1.043, df = 19.002, p-value = 0.31.Alternative hypothesis: true difference in means is not equal to 095 percent confidence interval: -16.55608 49.44698.Sample estimates: mean of x mean of y 63.94545 47.50000 .> t.test (Q.L3.SF, Q.L5.SF, conf.level = 0.95).Welch Two Sample t-test.Data: Q.L3.SF and Q.L5.SF.t = 0.92474, df = 18.612, p-value = 0.3669.Alternative hypothesis: true difference in means is not equal to 0 95 percent confidence interval: -18.14617 46.80072.Sample estimates: mean of x mean of y 63.94545 49.61818 .> t.test (Q.L4.SF, Q.L5.SF, conf.level = 0.95).Welch Two Sample t-test.Data: Q.L4.SF and Q.L5.SF.t = -0.15655, df = 19.956, p-value = 0.8772.Alternative hypothesis:true difference in means is not equal to 095 percent confidence interval: -30.34626 26.10989.Sample estimates: mean of x mean of y 47.50000 49.61818



**Figure 4.** pH decrease curves of different accessions during open-system retting of cassava tubers

The results from open system retting showed that the x-axis exclusively comprises time in h/s and the y-axis the variation of pH. Thus the slope of different pH curves is negative leading to the decrease of pH values in cassava tubers as a function of time. The different specific points of interest are as follows:

- The beginning of these different curves indicates the initial behavior before the reaction
- The final state: the final values on the x-axis after reaching a plateau are stable.

These results also show the variation of different curves depending on the HCN concentration in the tubers of these accessions which differs from one accession to another. And the time factor in the transformation process. This being:accession 1 (before-after retting) = 5.8-3.9; accession 2 (before-after retting) = 6.1-5; accession 3 (before-after retting) = 6.2-4.9; accession 4 (before-after retting) = 5.9-4.3 ; accession 5 (before-after retting) =6.2-4.4

#### The Open System pH Value Comparison Test of Five Accessions

> t.test (pH.L1.SO, pH.L2.SO, conf.level = 0.95).Welch Two Sample t-test.Data: pH.L1.SO and pH.L2.SO

t = -3.5476, df = 16.171, p-value = 0.002643. Alternative hypothesis: true difference in means is not equal to 095 percent confidence interval: -1.3066763 - 0.3296873. Sample estimates: mean of x mean of y 4.809091 5.627273 .> t.test (pH.L1.SO, pH.L3.SO, conf.level = 0.95).Welch Two Sample t-test.Data: pH.L1.SO and pH.L3.SO.t = -2.8932, df = 16.425, p-value = 0.01038.Alternative hypothesis: true difference in means is not equal to 095 percent confidence interval: -1.1630401 -0.1805962.Sample estimates: mean of x mean of y 4.809091 5.480909 .> t.test (pH.L1.SO, pH.L4.SO, conf.level = 0.95)

Welch Two Sample t-test.Data: pH.L1.SO and pH.L4.SO.t = -0.77066, df = 19.655, p-value = 0.4501

Alternative hypothesis: true difference in means is not equal to 095 percent confidence interval: -0.7554456 0.3481729.Sample estimates: mean of x mean of y 4.809091 5.012727 .> t.test (pH.L1.SO, pH.L5.SO, conf.level = 0.95).Welch Two Sample t-test.Data: pH.L1.SO and pH.L5.SO

t = -2.1147, df = 19.997, p-value = 0.04721. Alternative hypothesis: true difference in means is not equal to 0 95 percent confidence interval: -1.173801557 -0.008016624. Sample estimates: mean of x mean of y 4.809091 5.400000. > t.test (pH.L2.SO, pH.L3.SO, conf.level = 0.95).Welch Two Sample t-test.Data: pH.L2.SO and pH.L3.SO.t = 0.87414, df = 19.986, p-value = 0.3924.Alternative hypothesis: true difference in means is not equal to 0 95 percent confidence interval:-0.2029214 0.4956487.Sample estimates: mean of x mean of y 5.627273 5.480909.> t.test (pH.L2.SO, pH.L4.SO, conf.level = 0.95). 17.494, p-value = 0.009107

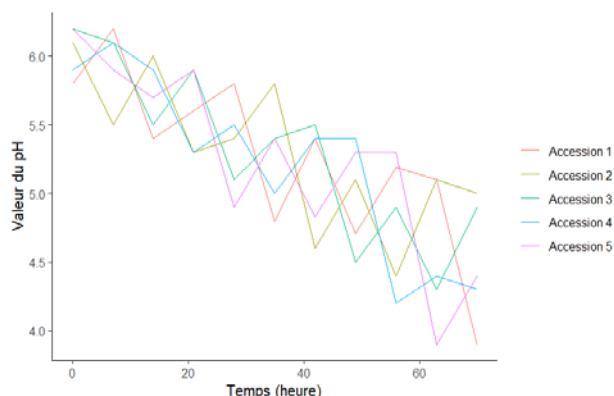
Alternative hypothesis: true difference in means is not equal to 0 95 percent confidence interval: 0.1732539 1.0558370.Sample estimates: mean of x mean of y 5.627273 5.012727 .> t.test (pH.L2.SO, pH.L5.SO, conf.level = 0.95).Welch Two Sample t-test.Data: pH.L2.SO and pH.L5.SOt = 0.99476, df = 16.294, p-value = 0.3344. Alternative hypothesis: true difference in means is not equal to 0 95 percent confidence interval: -0.2563510 0.7108965. Sample estimates: mean of x mean

of y 5.627273 5.400000

> t.test (pH.L3.SO, pH.L4.SO, conf.level = 0.95). Welch Two Sample t-test. Data: pH.L3.SO and pH.L4.SO

t = 2.2153, df = 17.75, p-value = 0.04007. Alternative hypothesis: true difference in means is not equal to 0.95 percent confidence interval: 0.02371581 0.91264782. Sample estimates: mean of x mean of y 5.480909 5.012727. > t.test (pH.L3.SO, pH.L5.SO, conf.level = 0.95). Welch Two Sample t-test. Data: pH.L3.SO and pH.L5.SO. t = 0.35169, df = 16.551, p-value = 0.7295

Alternative hypothesis: true difference in means is not equal to 0.95 percent confidence interval: -0.4054832 0.5673013. Sample estimates: mean of x mean of y 5.480909 5.400000. > t.test (pH.L4.SO, pH.L5.SO, conf.level = 0.95). Welch Two Sample t-test. Data: pH.L4.SO and pH.L5.SO. t = -1.4761, df = 19.716, p-value = 0.1557. Alternative hypothesis: true difference in means is not equal to 0.95 percent confidence interval: -0.9350380 0.1604926. 5.400000



**Figure 5.** pH decrease curves of different accessions during closed-system retting of cassava tubers

The results of closed-system retting showed that the x-axis exclusively contains time in h/s and the y-axis the variation of pH. Thus, the slope of different pH curves is negative, resulting in the decrease of pH values in cassava tubers as a function of time. The different-specific points of interest are as follows:

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- The final state: the final values on the x-axis after reaching a plateau are stable.

These results also show the variation of different curves depending on the HCN concentration in the tubers of these accessions which differs from one accession to another. And the time factor in the transformation process. This being: accession 1 (before-after retting) = 5.8-3.5; accession 2 (before-after retting) = 6.1-3.5; accession 3 (before-after retting) = 6.2-4.1; accession 4 (before-after retting) = 6.3-3.9; accession 5 (before-after retting) = 5.9-4.

#### The Closed System pH Value Comparison Test of Five Accessions

> t.test (pH.L1.SF, pH.L2.SF, conf.level = 0.95). Welch Two Sample t-test. Data: pH.L1.SF and pH.L2.SF

t = -0.24219, df = 18.259, p-value = 0.8113. Alternative hypothesis: true difference in means is not equal to 0.95 percent confidence interval: -0.8787025 0.6968844. (pH.L1.SF, pH.L3.SF, conf.level = 0.95). Welch Two Sample t-test. Data: pH.L1.SF and pH.L3.SF. t = -3.2353,

df = 19.881, p-value = 0.004171. Alternative hypothesis: true difference in means is not equal to 0.95 percent confidence interval: -1.600135 -0.345320. Sample estimates: mean of x mean of y 4.281818 5.254545. > t.test (pH.L1.SF, pH.L4.SF, conf.level = 0.95). 0.2876. Alternative hypothesis: true difference in means is not equal to 0.95 percent confidence interval: -1.0316497 0.3225588. Sample estimates: mean of t-test. Data: pH.L1.SF and pH.L5.SF. t = -1.5537, df = 19.946, p-value = 0.136. Alternative hypothesis: true difference in means is not equal to 0.95 percent confidence interval: -1.1075273 0.1620728. (pH.L2.SF, pH.L3.SF, conf.level = 0.95)

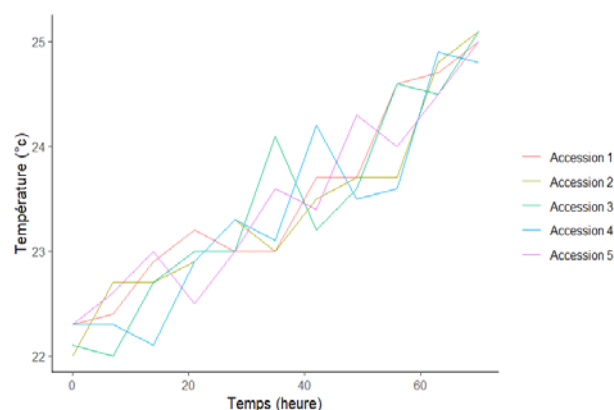
Welch Two Sample t-test. Data: pH.L2.SF and pH.L3.SF. t = -2.4098, df = 17.51, p-value = 0.02721

Alternative hypothesis: true difference in means is not equal to 0.95 percent confidence interval:

-1.6521428 -0.1114936. Sample estimates: mean of x mean of y 4.372727 5.254545. > t.test (pH.L2.SF, pH.L4.SF, conf.level = 0.95). Welch Two Sample t-test. Data: pH.L2.SF and pH.L4.SF. t = -0.68346, df = 18.917, p-value = 0.5026. Alternative hypothesis: true difference in means is not equal to 0.95 percent confidence interval: -1.0712342 0.5439615. Sample estimates: mean of x mean of y 4.372727 4.636364

> t.test (pH.L2.SF, pH.L5.SF, conf.level = 0.95) Welch Two Sample t-test. Data: pH.L2.SF and pH.L5.SF

t = -1.035, df = 17.761, p-value = 0.3145. Alternative hypothesis: true difference in means is not equal to 0.95 percent confidence interval: -1.1575829 0.3939465. Sample estimates: mean of x mean of y 4.372727 4.754545. > t.test (pH.L3.SF, pH.L4.SF, conf.level = 0.95). Welch Two Sample t-test. Data: pH.L3.SF and pH.L4.SF. t = 1.9717, df = 19.551, p-value = 0.06296. Alternative hypothesis: true difference in means is not equal to 0.95 percent confidence interval: -0.03678222 1.27314586. Sample estimates: mean of x mean of y 5.254545 4.636364. > t.test (pH.L3.SF, pH.L5.SF, conf.level = 0.95). 0.1029. Alternative hypothesis: true difference in means is not equal to 0.95 percent confidence interval: -0.1102786 1.1102786. Sample estimates: mean of x mean of y 5.254545 4.754545. > t.test (pH.L4.SF, pH.L5.SF, conf.level = 0.95). Two Sample t-test. Data: pH.L4.SF and pH.L5.SF. t = -0.37283, df = 19.685, p-value = 0.7133. Alternative hypothesis: true difference in means is not equal to 0.95 percent confidence interval: -0.7800794 0.5437158.



**Figure 6.** Temperature evolution curves of different accessions during retting of cassava tubers in an open system

The results of open system retting showed that the x-axis exclusively comprises time in h/s and the y-axis the temperature variation. Thus the slope of different temperature curves is positive leading to the increase of temperature values in the cassava tuber solution as a function of time. The different specific points of interest are as follows:

- The beginning of these different curves indicates the initial behavior before the reaction
- The final state: the final values on the x-axis after reaching a plateau are stable.

These results also show the variation of different curves depending on the HCN concentration in the tubers of these accessions which differs from one accession to another.... And the time factor in the transformation process. This being: accession 1 (before-after retting) = 22.3-25; accession 2 (before-after retting) = 22-25.1; accession 3 (before-after retting) = 22.1-25.1; accession 4 (before-after retting) = 22.3-24.8; accession 5 (before-after retting) = 22.3-25.

#### The Open System Temperature Value Comparison Test of Five Accessions

> t.test (Temp.L1.SO, Temp.L2.SO, conf.level = 0.95) Welch Two Sample t-test Data: Temp.L1.SO and Temp.L2.SO  $t = 0.3838$ ,  $df = 18.721$ ,  $p\text{-value} = 0.7055$  Alternative hypothesis: true difference in means is not equal to 0 95 percent confidence interval: -0.6891177 0.9982086 Sample estimates: mean of x mean of y 23.34545 23.19091 > t.test (Temp.L1.SO, Temp.L3.SO, conf.level = 0.95) Welch Two Sample t-test

Data: Temp.L1.SO and Temp.L3.SO  $t = -0.97849$ ,  $df = 19.129$ ,  $p\text{-value} = 0.34$  Alternative hypothesis: true difference in means is not equal to 0 95 percent confidence interval: -1.1981669 0.4345305 Sample estimates: mean of x mean of y 23.34545 23.72727 > t.test (Temp.L1.SO, Temp.L4.SO, conf.level = 0.95)

Welch Two Sample t-test Data: Temp.L1.SO and Temp.L4.SO  $t = -0.026952$ ,  $df = 19.945$ ,  $p\text{-value} = 0.9788$

Alternative hypothesis: true difference in means is not equal to 0 95 percent confidence interval:

-0.7128035 0.6946217 Sample estimates: mean of x mean of y 23.34545 23.35455

> t.test (Temp.L1.SO, Temp.L5.SO, conf.level = 0.95) Welch Two Sample t-test data: Temp.L1.SO and Temp.L5.SO  $t = -0.57876$ ,  $df = 19.517$ ,  $p\text{-value} = 0.5694$  Alternative hypothesis: true difference in means is not equal to 0 95 percent confidence interval: -1.005802 0.569438 Sample estimates: mean of x mean of y 23.34545 23.56364 > t.test (Temp.L2.SO, Temp.L3.SO, conf.level = 0.95) Welch Two Sample t-test

Data: Temp.L2.SO and Temp.L3.SO  $t = -1.2157$ ,  $df = 19.949$ ,  $p\text{-value} = 0.2383$  Alternative hypothesis: true difference in means is not equal to 0 95 percent confidence interval: -1.4568126 0.3840853

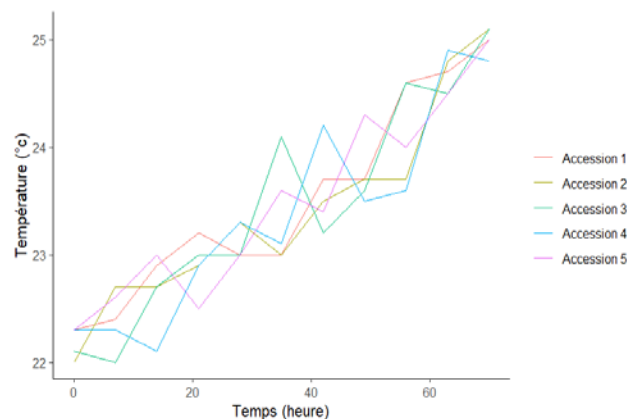
Sample estimates: mean of true difference in means is not equal to 0 95 percent confidence interval:

-0.9930209 0.6657482 Sample estimates: mean of  $t = -0.86776$ ,  $df = 19.767$ ,  $p\text{-value} = 0.3959$  Alternative hypothesis: true difference in means is not equal to 0 95 percent confidence interval: -1.2693885 0.5239339 Sample estimates: mean of x mean of y 23.19091 23.56364 > t.test (Temp.L3.SO, Temp.L4.SO, conf.level = 0.95) Welch Two Sample t-test

Data: Temp.L3.SO and Temp.L4.SO  $t = 0.97459$ ,  $df = 18.704$ ,  $p\text{-value} = 0.3422$  Alternative hypothesis: true difference in means is not equal to 0 95 percent confidence interval: -0.4285974 1.1740519

Sample estimates: mean of difference in means is not equal to 0 95 percent confidence interval:

-0.7082046 1.0354773 Sample estimates: mean of  $t = -0.56674$ ,  $df = 19.169$ ,  $p\text{-value} = 0.5775$  Alternative hypothesis: true difference in means is not equal to 0 95 percent confidence interval: -0.9808288 0.5626470 Sample estimates: mean of x mean of y 23.35455 23.56364



**Figure 7.** Temperature evolution curves of different accessions during retting of cassava tubers in a closed system

The results of closed system retting showed that the x-axis exclusively contains time in h/s and the y-axis the temperature variation. Thus the slope of different temperature curves is positive leading to the increase of temperature values in the cassava tuber solution as a function of time. The different specific points of interest are as follows:

- The beginning of these different curves indicates the initial behavior before the reaction
- The final state: the final values on the x-axis after reaching a plateau are stable.

The results also show the variation of different curves depending on the HCN concentration in the tubers of these accessions which differs from one accession to another.... And the time factor in the transformation process. This being: accession 1 (before-after retting) = 23-26.2; accession 2 (before-after retting) = 23-26.2; accession 3 (before-after retting) = 23.4-26; accession 4 (before-after retting) = 22.3; accession 5 (before-after retting) = 22-26.1.

#### The Closed System Temperature Value Comparison Test of Five Accessions

> t.test (Temp.L1.SF, Temp.L2.SF, conf.level = 0.95) Welch Two Sample t-test

Data: Temp.L1.SF and Temp.L2.SF  $t = -0.88807$ ,  $df = 19.384$ ,  $p\text{-value} = 0.3854$  Alternative hypothesis: true difference in means is not equal to 0 95 percent confidence interval: -1.4024412 0.5660776

Sample estimates: mean of true difference in means is not equal to 0 95 percent confidence interval: -0.9676627 0.6585718 Sample estimates: mean of

$t = -1.1693$ ,  $df = 18.267$ ,  $p\text{-value} = 0.2573$  Alternative hypothesis: true difference in means is not equal to 0 95 percent confidence interval: -1.6769231 0.4769231 Sample

estimates: mean of x mean of y 24.47273 25.07273 > t.test (Temp.L1.SF, Temp.L5.SF, conf.level = 0.95) Welch Two Sample t-test Data: Temp.L1.SF and Temp.L5.SF  $t = -0.70624$ ,  $df = 19.551$ ,  $p\text{-value} = 0.4884$  Alternative hypothesis: true difference in means is not equal to 0.95 percent confidence interval: -1.2953416 0.6407962 Sample estimates: mean of x mean of y 24.47273 24.80000 > t.test (Temp.L2.SF, Temp.L3.SF, conf.level = 0.95) Welch Two Sample t-test Data: Temp.L2.SF and Temp.L3.SF  $t = 0.6038$ ,  $df = 17.587$ ,  $p\text{-value} = 0.5537$  Alternative hypothesis: true difference in means is not equal to 0.95 percent confidence interval: -0.6552319 1.1825046 Sample estimates: mean of x mean of y 24.89091 24.62727 > t.test (Temp.L2.SF, Temp.L4.SF, conf.level = 0.95)

Welch Two Sample t-test Data: Temp.L2.SF and Temp.L4.SF  $t = -0.3304$ ,  $df = 19.63$ ,  $p\text{-value} = 0.7446$

Alternative hypothesis: true difference in means is not equal to 0.95 percent confidence interval:

-1.3311053 0.9674689 Sample estimates: mean of x mean of y 24.89091 25.07273

> t.test (Temp.L2.SF, Temp.L5.SF, conf.level = 0.95) Welch Two Sample t-test Data: Temp.L2.SF and Temp.L5.SF  $t = 0.18029$ ,  $df = 19.985$ ,  $p\text{-value} = 0.8587$  Alternative hypothesis: true difference in means is not equal to 0.95 percent confidence interval: -0.9609815 1.1427997

Sample estimates: mean of difference in means is not equal to 0.95 percent confidence interval: -1.4659062 0.5749971 Sample estimates: mean of x mean of y 24.62727 25.07273

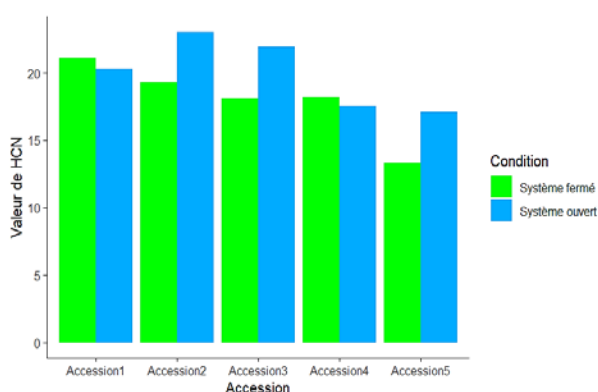
> t.test (Temp.L3.SF, Temp.L5.SF, conf.level = 0.95) Welch Two Sample t-test Data: Temp.L3.SF and Temp.L5.SF  $t = -0.40305$ ,  $df = 17.857$ ,  $p\text{-value} = 0.6917$  Alternative hypothesis: true difference in means is not equal to 0.95 percent confidence interval: -1.0735887 0.7281342 Sample estimates: mean of x mean of y 24.62727 24.80000 > t.test (Temp.L4.SF, Temp.L5.SF, conf.level = 0.95) Welch Two Sample t-test

Data: Temp.L4.SF and Temp.L5.SF  $t = 0.50142$ ,  $df = 19.475$ ,  $p\text{-value} = 0.6217$

Alternative hypothesis: true difference in means is not equal to 0.95 percent confidence interval:

-0.8638057 1.4092603 Sample estimates: mean of x mean of y 25.07273 24.80000.

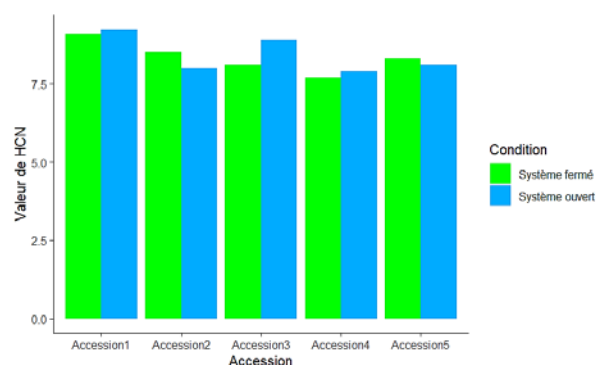
Results of the work after retting in tubers with open and closed systems over 70 hours, as well as in cossettes and couscous.



**Figure 8.** Variation diagram of HCN values of different accessions after retting of cassava tubers in open and closed systems

The results of open-closed system retting showed that the x-axis exclusively comprises cassava accessions and the y-axis the concentration of hydrocyanic acid (HCN) in mg/kg. Thus the slope of different HCN diagrams is negative resulting in the variation of HCN concentration in cassava tubers depending on the cassava accessions.

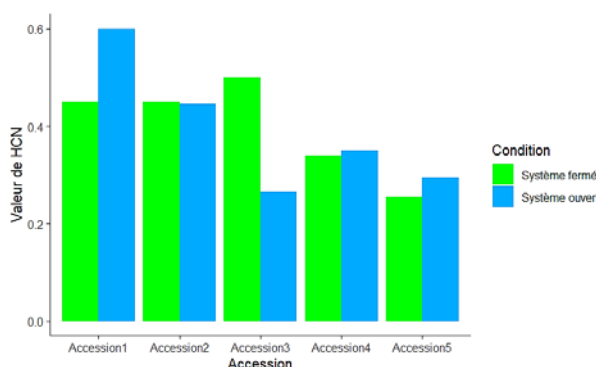
The results also show the variation of different diagrams depending on the HCN concentration in the tubers of these accessions which differs from one accession to another. And the time factor in the transformation process. This being: accession 1(open-closed)=20.3 mg/Kg -18.1 mg/kg; accession 2(open-closed)=21.1mg/kg -17.5mg/kg; accession 3(open-closed)=23mg/Kg -18.2 mg/kg; accession 4(open-closed)=19.3mg/Kg -17.1 mg/kg; accession 5 (before-after retting) =21.9mg/Kg -13.3 mg/kg.



**Figure 9.** Variation diagram of HCN values of different cassava accessions in open and closed system cossettes

The results of open-closed system retting showed that the x-axis exclusively comprises cassava accessions and the y-axis the concentration of hydrocyanic acid (HCN) in mg/kg. Thus the slope of different HCN diagrams is negative resulting in the variation of HCN concentration in cassava tubers depending on the cassava accessions.

These results also show the variation of different diagrams depending on the HCN concentration in the tubers of these accessions which differs from one accession to another. And the time factor in the transformation process. This being: accession 1(open-closed)=9.23 mg/Kg -8.1 mg/kg; accession 2(open-closed)=9.1mg/Kg -7.9 mg/kg; accession 3(open-closed)=8 mg/Kg -7.7 mg/kg; accession 4(open-closed)=8.5mg/Kg -8.1mg/Kg; accession 5 (before-after retting) = 8.9mg/Kg -8.3 mg/kg .



**Figure 10.** Variation diagram of HCN values of different cassava accessions in open and closed system couscous

The results of open-closed system retting showed that the x-axis exclusively comprises cassava accessions and the y-axis the concentration of hydrocyanic acid (HCN) in mg/kg. Thus the slope of different HCN diagrams is negative resulting in the variation of HCN concentration in cassava tubers depending on the cassava accessions.

The results also show the variation of different diagrams depending on the HCN concentration in the tubers of these accessions which differs from one accession to another. And the time factor in the transformation process. This being: accession 1(open-closed)=0.6mg/Kg -0.5mg/Kg; accession 2(open-closed)=0.45mg/Kg-0.35 mg/Kg; accession 3(open-closed)=0.44mg/Kg-0.34mg/Kg; accession 4(open-closed)=0.45mg/Kg-0.29mg/Kg; accession 5 (before-after retting) =0.26 mg/Kg-0.25mg/Kg .

## 4. Synthesis of Physicochemical Parameters

### 4.1. Determining the Optimal Temperature

Previous experiments have confirmed that temperature is the most influential factor in retting. We therefore undertook a more detailed study of the influence of this factor on the duration (70 hours) of retting in both systems: open and closed, and on the quality of the cossettes and couscous obtained.

The results confirm the close correlation between retting time and temperature. This time is minimum between 22 and 26°C with an optimum at 26°C, where retting takes place in less than 70 hours. The quality of the couscous produced by the 2 retting temperatures is similar (good to very good).

The hydrocyanic acid concentration after retting is below 10 mg/kg for all the cossettes tested. This demonstrates that under the experimental conditions described, detoxification is not a limiting factor for the optimization of retting conditions.

### 4.2. Determination of pH

The pH value measured overall for all accessions decreases, having the Minimum value of 3.5 and the Maximum value of 6.1 during the retting process of both open and closed systems.

### 4.3. Determination of Hydrocyanic Acid

According to the progressive decrease in the hydrocyanic acid level during the transformation process evaluated in this work, the different HCN contents observed in the fresh tubers of these cassava accessions from the experimental field clearly showed the difference between open and closed system retting over a period of 70 hours.

## 5. Discussions

The results obtained showed respectively the open system (before and after retting): 102.8-142 mg/kg and

19.3-23 mg/kg depending on the HCN content in the tubers of these accessions; the closed system (before and after retting): 103-142 mg/kg and 13.3-18.1 mg/kg. Thus in the cossettes of the open-closed system: 8-9.23 mg/kg and 7.7-8.3 mg/kg and in the couscous of the open-closed system: 0.25-0.60 mg/kg; 0.23-0.48 mg/kg. The pH values varied from 3.5-6.01 from retting to the cossettes. The temperature ranged from 22°C to 26°C for all systems along this retting process. These HCN results obtained in cassava chips and couscous were considered acceptable according to the FAO standard (10mg/kg of HCN in chips) and CODEX (2mg/kg of HCN) in cassava couscous. The various statistical tests clearly showed that there are differences between the accessions compared and this is due to the diversification of concentrations as well as accession-specific ones. Moreover, retting allows the elimination of more than 90% of the endogenous cyanide compounds from the root. This disappearance is essentially due to the action of the endogenous linamarase of cassava as many authors have already shown [16], but the bacteria which, thanks to their pectinase activities, can lyse plant cells, promote this process [17]. However, the retting root undergoes a set of subsequent transformations which further decreases the concentration of free cyanides in chikwangue and fufu (average = 6 to 8 ppm [18], The main interest of retting lies in the softening of the root which allows its further preparation. This Softening is due to the lysis of certain plant cell walls of the roots composed mainly of pectin and cellulose. The presence of significant pectinase activities and the absence of cellulase activity indicate that the former are at the origin of this softening. The pectinases detected are composed of three enzymes (pectinesterase, pectate lyase and polygalacturonase) whose plant and bacterial origin has since been clarified [17]. The presence of pectinesterase in retting has already been mentioned in the case of traditional retting [19].

Drying at temperatures of 40°C, boiling, fermentation, roasting [20] and storage [21] lead to a significant decrease in the initial content of cyanogenic glycosides in cassava. Furthermore, retting is an important unit operation for the detoxification of cassava. It consists of immersing fresh cassava roots in water for 3 to 6 days. Fermentation occurs during which microbial activities combine with the action of enzymes to soften the roots, degrade the cyanogenic compounds and give the finished products their characteristic tastes [22]. Cyanogenic glycosides, as such, are not toxic, but once ingested by humans, they undergo decomposition into hydrocyanic acid by enzymes of the intestinal flora [23]. The dose of 1 mg HCN.kg-1 of body weight is considered likely to cause acute poisoning in humans [24]. Other authors such as [25] 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. However, polygastric animals are capable of ingesting doses 3 to 4 times higher without danger [24]. The hydrocyanic acid content set by the FAO must be less than or at most equal to 10 mg HCN.kg-1 in product chips [26]. [27] concludes that toxicity due to cassava is especially dangerous when there is a natural context of protein deficiency, which was confirmed by Jones [28]. Indeed, sulfur, which is involved in protein synthesis reactions, is present in amino acids

such as methionine and cysteine. It plays an important role in redox reactions during cellular respiration. Sulfur binds to cyanide ions to give thiocyanate, a form of detoxification following the reaction:  $8\text{CN}^- + \text{S} \rightarrow 8\text{SCN}^-$ . Cyanide poisoning is treated by administering amyl nitrite [29] which facilitates cellular respiration. Studies by [30] show that the use of intravenous sodium nitrite, intramuscular dimethylaminophenol and hydroxylamine hydrochloride, and inhalation of amyl nitrite are effective in overcoming the lethal dose of cyanide. Cyanide detoxification is also facilitated by the administration of thiosulfate, which is necessary for the formation of thiocyanates:  $\text{CN}^- + \text{S}_2\text{O}_3^{2-} \rightarrow \text{SCN}^- + \text{SO}_3^{2-}$  [31]. Regular consumption of cassava is the cause of several pathological disorders due to the presence of degradation products of cyanogenic compounds [32]. These include: thyroid goiter, dwarfism and tropical ataxic neuropathy [33]. Individuals with neuropathy have low blood sulfur and high thiocyanate concentrations [34] showed that cassava ingestion with iodine deficiency is an etiological factor in endemic goiter. In addition, high consumption of cyanide from cassava flour causes a disease called Konzo. This consists of irreversible paralysis of the legs in children and women of childbearing age, which occurs in many countries of southern, eastern and central Africa [35]. Discussion of HCN contents in Cassava (*M. esculenta*):

Comparing the HCN levels in cassava with other cyanogenic food samples studied, it is noted that the levels in cassava vary from 91ppm to more than 1000ppm and according to the FDA the HCN levels in cassava can reach 1500ppm in poorly processed bitter varieties, which explains the harmful effects of daily consumption of cassava, such as neurological disorders, diabetes, congenital malformations and goiter. Based on these disorders, several national and international health authorities have assessed the danger of this food.

In 2000, the Australian Expert Committee on Flavors (ACEF) based its assessment on a toxicological study of a population in the Democratic Republic of Congo affected by Konzo, whose daily HCN intake is 0.19 to 0.37 mg CN-/kg body weight / day, to set a tolerable daily intake of 20 µg HCN/kg body weight per day. Furthermore, the consumption of 200 g of cassava flour by a 60 kg adult leads to an estimated intake level of 30 µg HCN / kg body weight, such an intake is not considered by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) of the World Health Organization (WHO) and the Food and Agriculture Organization (FAO) as associated with acute toxicity. In 2008, analyses carried out on different batches of food products manufactured in Australia from cassava pellets imported from Indonesia revealed a total cyanide content of up to 145 mg/kg. This led Food Standards Australia New Zealand to impose a recall of these products, and to develop a set of recommendations to limit the consumption of cassava-based products (biscuits and chips), particularly among children. The safety threshold of 10 ppm of total cyanide in cassava flour, established by the WHO, appears to be acceptable for protecting people sensitive to the effects of HCN. Levels above 100 ppm are considered by the WHO to be dangerous to health. [35]

## 6. Conclusion

The various dosages of hydrocyanic acid (HCN), and the measurement of temperature (T°C), pH during this retting process allowed us to obtain the different HCN concentrations which are considered acceptable in view of the WHO and Codex standards in the by-products and finished products of detoxified cassava accessions. The comparative study of the HCN concentrations in these fresh tubers of the accessions along this transformation process until obtaining the couscous shows that the technological treatments (crushing, fermentation, drying and cooking) reduce the HCN content and this demonstrates the effectiveness of the improved cassava production diagram implemented in the Central African Republic through this study carried out. The HCN content observed in the different couscous tested is safe for the body as highlighted above by the WHO and Codex. The absence of specialized processing units can have a negative impact on public health in terms of the lack of knowledge of hydrocyanic acid in cassava by-products or derivatives which could be the cause of several pathologies. This is why it is very crucial to evaluate the HCN content in the cossettes from different prefectures of the Central African Republic including in the districts of the capital Bangui in order to ensure the quality of these products before any consumption. As a solution, actions must be undertaken, namely awareness raising and training of processors, increasing the capacity of production units through the availability of adequate equipment, and the selection of improved cultivars both genetically and chemically.

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## References

- [1] Ufuan Achidi, A., Ajayi, O.A., Bokanga, M. and Maziya-Dixon, B. (2005) The Use of Cassava Leaves as Food in Africa. *Ecology of Food and Nutrition*, 44, 423-435.
- [2] Bellotti, A. and Kawano, K. (1980) Breeding Approaches in Cassava.
- [3] McMahon, J.M., White, W.L.B. and Sayre, R.T. (1995) Review Article: Cyanogenesis in Cassava (*Manihot esculenta* Crantz). *Journal of Experimental Botany*, 46, 731-741.
- [4] Kerharo, J. and Bouquet, A. (1950) Medicinal and Toxic Plants of Ivory Coast and Upper Volta. Vigot Brothers.

- [5] Keita, Å.V., Lindqvist, CM, Öst, Å., et al. (2018) Gut Barrier Dysfunction—A Primary Defect in Twins with Crohn's Disease Predominantly Caused by Genetic Predisposition. *Journal of Crohn's and Colitis*, 12, 1200-1209.
- [6] Cliff, J., Mårtensson, J., Lundqvist, P., Rosling, H. and Sörbo, B. (1985) Association of High Cyanide and Low Sulfur Intake in Cassava-Induced Spastic Paraparesis. *The Lancet*, 326, 1211-1213.
- [7] Tylleskär, T. et al. (1992) Cassava Cyanogens and Konzo, an Upper Motor-Neuron Disease Found in Africa. *Lancet*, 339, 208-211.
- [8] FAOSTAT (2016) 2017 Food and Agriculture Organization of the United Nations. [https://fr.wikipedia.org/wiki/Organisation\\_des\\_Nations\\_unies\\_pour\\_l'alimentation\\_et\\_l'agriculture](https://fr.wikipedia.org/wiki/Organisation_des_Nations_unies_pour_l'alimentation_et_l'agriculture).
- [9] Tylleskär, T., Banea, M., Bikangi, N., Fresco, L., Persson, LA and Rosling, H. (1991) Epidemiological Evidence from Zaire for a Dietary Etiology of Konzo, an Upper Motor Neuron Disease. *Bull World Health Organ*, 69, 581-589.
- [10] Koch, L. (1933) Cassavesselectie (Cassava breeding) Landbouwhogeschool, Genin.
- [11] Bolhuis, GG (1954) The Toxicity of Cassava Roots. *Netherlands Journal of Agricultural Science*, 2, 176-185.
- [12] Tisserant, C. (1953) Agriculture in the Savannas of Oubangui. *Bulletin of the Institute of Central African Studies (Brazzaville)*, New Series, 6, 27.
- [13] Ignaleamoko, EF, Zinga, I., Semballa, S., Longue, DRS, Sako, EP, Fulgence, IJ, Sania, M. Eni, A., Tiendrebio, F. and Pita, J. (2025) 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. *American Journal of Plant Sciences*, 16, 525-536.
- [14] Fokunang, C.N., et al. (2001) Cyanogenic Potential in Food Crops and Its Implication in Cassava (*Manihot Esculenta* Crantz) Production. *Pakistan Journal of Biological Sciences*, 4, 926-930.
- [15] Vetter, J. (2000) Plant Cyanogenic Glycosides. *Toxicon*, 38, 11-36.; [15.a].National Institut of Agronomic Research-INRA-Agronomy Stations of Arras,Avignon. ; [15.b].National Higher School of Agronomy(Biochemistry and Plant Physiology Laboratory)-ENSAM, Montpellier Overseas Office of Scientific and Technical Research(Foliar Diagnostic Laboratory)-ORSTOM,Bondy
- [16] MADUAGIVU (EN), 1983.- Differential effects on the cyanogenic glycoside content of fermenting cassava root pulp by 6-glucosidase and microbial activities. *Toxicology Letters*, 15:335-339.
- [17] AMPE (F.), BRAUW (A), TRECHEAGOSSO OS SOU (A.), 1994- The fermentation of cassava: optimization by the experimental research methodology.J, of the Science of Food and A/\$CtdtU?e, 65:355-361.
- [18] TRECHE (S.) 1995- Importance of cassava in human nutrition in different regions of the world. In: Agbor (E)l Brauman (A), Griffon (DJ, Trtkhe (S.), ed.: *TrnnsformntiotlAlimentaire du Ahnioc*, ORSTOM, Paris.
- [19] OYEWOLE (OB), ODUNFA (SA), 1990 - Optimization of cassava fermentation for fufu production: effects of single starter cultures. [*Appl. Bactekd.*, 68: 49-54.
- [20] Padmaja G., 1995. Cyanide detoxification in cassava for food and feed uses. *Crit. Rev. Food Sci. Nutr.*, 35, 299-339.
- [21] Mahungu NM, Yamaguchi Y., Alamazan AM & Hahn SK, 1987. Production of cyanide during processing of cassava into some traditional African foods. *J. Food Agric.*, 1, 11-15.
- [22] Ampe F. & Brauman A., 1992. Cassava retting: a traditional fermentation revealed. *Biofutur*, 114, 36-37.
- [23] Ouegnin EA, 1988. Contribution to the study of hydrocyanic acid in a cassava processing product: "Attiéké". Doctoral thesis in pharmacy: Cheikh Anta Diop University of Dakar (Senegal).
- [24] Silvestre P. & Arraudeau M., 1983. Cassava. Paris: Maisonneuve & Larose; Paris: ACCT.
- [25] Shibamoto T. & Bjeldanes LF, 1993. Introduction to food toxicology. New York, London, Toronto: Academic Press Inc.
- [26] Yéo G., 2007. Potential for cassava processing in West Africa. In: Amani G. et al., eds. *Proceedings of the 1st International Workshop on the Potential for Cassava Processing in West Africa*, June 4-7, 2007, Abidjan, Ivory Coast, 48-79.
- [27] Wilson J., 1973. Cyanide and human disease. In: *Chronic cassava toxicity. Proceedings of an interdisciplinary workshop*, 29-30 January 1973, London, England. Ottawa: IDRC, 121-125.
- [28] Jones DA, 1998. Why are so many food plants cyanogenic? *Phytochemistry*, 47(2), 155-162.
- [29] Akanji AO, Adeyefa I., Charles-Davies M. & Osotimehin BO, 1990. Plasma glucose and thiocyanate responses to different mixed cassava meals in nondiabetic Nigerians. *Eur. J.Clinic. Nutr.*, 44, 71-77.
- [30] Vick JA & Froehlich H., 1991. Treatment of cyanide poisoning. *Mil Med.*, 156(7), 330-339.
- [31] Fokunang CN et al., 2001. Cyanogenic potential in food crops and its implication in cassava (*Manihot esculenta* Crantz) production. *Pak. J. Biol. Sci.*, 4(7), 926-930.
- [32] Tylleskär T. et al., 1992. Cassava cyanogens and konzo, an upper motor neuron disease found in Africa. *Lancet*, 339, 208-211.
- [33] Kobawila SC et al., 2005. Reduction of the cyanide content during fermentation of cassava roots and leaves to produce bikedi and ntoba mbodi, two food products from Congo. *Afr. J. Biotechnol.*, 4(7), 689-696.
- [34] BennettF.,Morgan O.,Golden MH&Stennett-Dawkins M., 1987. Plasma thiocyanate and free radicals in Jamaican neuropathy. *West Indian Med. J.*, 36, 163-165.
- [35] Essers AJ, Alsen P. & Rosling H., 1992. Insufficient processing of cassava induced acute intoxications and the paralytic disease Konzo in a rural area of Mozambique. *Ecol. Food Nutr.*, 27, 17-27.

