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Investigation of Levels of Cyanide in Cassava Tubers Marketed in Kampala

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Abstract: Cassava is one of the cyanogenic plants poisonous to humans once consumed in unprocessed state. Cassava is characterized by a cyanogenic glycoside, linamarin and hydrolyzed to cyanohydrin by an enzyme called linamarase, cyanohydrin is hydrolyzed to a toxic hydrogen cyanide (HCN). This study purposed investigation of the levels of cyanide in the sweet cassava subspecies marketed in various markets within Kampala City. The mean concentration levels of cyanide in cassava samples from markets within Nakawa Division were 50.83±2.93, 53.54±2.46, 52.25±2.64, 52.20±2.45 and 47.25±3.12 ppm for Nakawa Market, Kisasi Market, Banda Market, Kirombe Market and Bugolobi Local Market respectively. The mean concentration levels of cyanide in cassava samples from markets within Kawempe Division were 56.25± 3.43, 54.52± 2.84 and 61.08± 3.32 ppm for Kalerwe Market, Mperewe Market and Kawempe Market respectively. The mean concentration levels of cyanide in cassava samples from markets within Rubaga Division were 59.69±2.41, 57.83±2.96 and 61.55±2.52 ppm for Busega Market, Kawala Market and Nakulabye Market respectively. The mean concentration levels of cyanide in cassava samples from markets within Makindye Division were 58.41±2.51, 52.58±2.43, 54.53±2.76 and 54.41±2.93 ppm for Gaaba Market, Kabalagala Market, Kansanga Vendor Market and Bunga Market respectively. All samples analyzed for cyanide in cassava gave values higher than 10ppm which is WHO's recommended level of cyanide in cassava. This article gives vital information on the levels of cyanide in cassava from the various markets in Kampala City.

Keywords: Cyanide, Cassava, Picrate Paper Kit, Kampala City, Toxicity

1. Introduction

Cassava (*Manihot esculenta* Cranzt) is a crop having a life cycle of more than two years autochthonous to tropical America [1, 2]. Cassava originated from Brazil and was introduced to Africa by Portuguese traders in the 16th century. The plant parts used are the storage root (tuber) and leaves [3]. It belongs to the dicotyledonous family, Euphorbiaceae. The *Manihot* genus is reported to have about 100 species, among which the only commercially cultivated one is *Manihot esculenta* Crantz. There is high level of morphological variability among cassava varieties hence, it is difficult to reliably distinguish individual varieties using only morphological characteristics but there are basically two distinct types of cassava plant that is erect, with or without branching at the top type and the spreading type [4].

Cassava root is an energy-dense food giving very efficient carbohydrate production per hectare of about 250000 calories/hectare/d, which places it above maize, rice, sorghum, and wheat [5]. Cassava root is a physiological energy store with high carbohydrate content, which ranges from 32% to 35% on a fresh weight (FW) basis, and from 80% to 90% on a dry matter (DM) basis and eighty percent of its carbohydrates produced being starch [6]; 83% is in the form of amylopectin and 17% is amylose [6]. Roots also contain small amounts of sucrose, glucose, fructose, and maltose [7]. Cassava has basically bitter and sweet varieties. In sweet cassava varieties, up to 17% of the root is sucrose with small quantities of dextrose and fructose [5, 8]. Raw cassava root has more carbohydrate than potatoes and less carbohydrate than wheat, rice, yellow corn, and sorghum on a

100-g basis. The fiber content in cassava roots depends on both the variety and the age of the root which usually does not exceed 1.5% in fresh root and 4% in root flour [6].

Cassava is primarily grown for its enlarged storage roots, which are used for human consumption after a number of traditional processing methods like boiling, roasting, processing into flour, and fermentation [4, 9]. Cassava plays an important dietary role in the diets of almost 1 billion people worldwide although it has the lowest protein-to-carbohydrate ratio among major crops [10, 11]. In some regions, particularly in Africa and Brazil, the foliage is harvested for human consumption and animal feed, providing supplemental dietary protein [4]. Cassava is also grown for industrial purposes, such as the production of starch and for fermentation into ethanol [12, 13].

Cyanides are found in a number of plants constrained to sugar molecules as cyanogenic glycosides and are usually invoked by some algae, bacteria and fungi. Cyanides play plant's defensive role against herbivores [14, 15]. Cassava's linamarin is majorly synthesized in the leaves and taken down to the root tuber and therefore, the leaves have higher concentration of linamarin (900-2000 ppm) [16]. An enzyme HNL found in the leaves hydrolyses acetone cyanohydrin to hydrogen cyanide and acetone [17]. Cassava tuber is nearly 20-times lower in linamarin than the leaves and hence HNL is in relatively lower amount in the root tubers (less than 6%) of HNL in leaves. Cyanogen levels varies from cultivar to cultivar with its total in tubers ranging between 100 and 500 ppm for low- cyanogenic and high-cyanogenic cultivars, respectively. Cyanogenic glycosides are present in all cassava cultivars with no exception [18]. Boiling and other processing methods greatly reduce Cyanide levels to within acceptable level of 10 ppm by WHO [19]. However, when animals and humans consume inadequately processed bitter root tuber with varying levels of linamarin, the result is dietary cyanide exposure that has been linked to acute poisoning and some other toxicological-nutritional malfunction [20]. Human body has the capacity to detoxify the biggest portion of cyanide by enzymatic conversion to the less toxic compound called thiocyanate which is an excrete in the urine [21]. Hydrogen cyanide released to the body through ingested or cyanogens is quickly absorbed by bonding to iron in hemoglobin into the blood and quickly distributed to key organs like liver, kidney, brain and the blood tissue [22, 23]. Acute intoxication leads to death, exacerbate goitre and cretinism in iodine-deficient regions leads to konzo and are linked to Tropical Ataxic Neuropathy (TAN) and stunting in children [24, 25].

Previous studies have described a number of analytical methods for cyanide in cassava, all of them having the method of extraction, hydrolysis of cyanogenic glycosides and experimental determination of the released free cyanide. [26]. Cyanogenic compounds in plants are extracted using dilute acidic solution because linamarase is inactive at low pH [27]. Linamarin extracted is removed and subjected to a series of hydrolyses to obtain free cyanide [28]. Concentration of free cyanide in solution is determined either by titration of cyanide with $AgNO_3$, reaction with alkaline picrate [29] using color indicator based on the König reaction where free cyanide (CN^-) oxidizes into cyanogen halide by chloramines T or N-chloro succinimide [30] or use of specific electrode for cyanide and voltmeter to measure the potential difference [27, 31]. In this study colorimetric technique was used in determination of cyanide concentration in cassava samples.

2. Materials and Methods

2.1. Study Area

Kampala is the capital and largest city of Uganda. It occupies a series of hills at an elevation of about 3,900 feet (1,190 metres) and is situated in the southern part of the country, just north of Lake Victoria. It was selected in 1890 by Capt. Frederick Lugard as the headquarters of the Imperial British East Africa Company. Lugard's fort on Old Kampala Hill remained the Ugandan colonial administrative headquarters until 1905, when it was moved to Entebbe. In 1962 Kampala a municipality since 1949 became the capital of independent Uganda. Situated in the country's most prosperous agricultural section, Kampala exports coffee, cotton, tea, tobacco, and sugar. Although second industrially to Jinja, the city has numerous food, metal-products, and furniture enterprises and a tractor-assembly plant. It is the headquarters for most of Uganda's large firms and the chief market for the Lake Victoria region. Kampala has parliament, universities, technical and business institutes. Kampala also has the Uganda Museum. The city is home to several mosques, Hindu temples, and Christian churches. Kampala is the hub of the nation's road network and lies on the railway from Kasese to Mombasa, Kenya. It is also served by Port Bell on Lake Victoria and by Uganda's international airport at Entebbe 21 miles southwest.

2.2. Apparatus

The following apparatus were used. Analytical balance, 100 ml round bottomed flasks, Borosilicate volumetric flasks (25, 50 ml, 100 ml & 1000 ml), Measuring cylinders, Pipettes, UV- Vis Spectrophotometer.

2.3. Chemicals

All chemicals of high purity analytical grade reagents were used; 0.1 M phosphate buffer, picric acid, sodium carbonate solution, Potassium cyanide, 0.01 M NaOH, Deionized water.

2.4. Sample Collection

Fresh raw cassava root tuber samples were collected from selected markets during morning hours when they are just brought from different farmers.

2.5. Sample Preparation

The labeled Cassavas samples obtained from the different farmers were cleaned using tap water and refrigerated at -4°C until time for laboratory analysis.

2.6. Determination of Cyanide Levels

To obtain the actual concentration of HCN equivalent in the sample, a linear calibration curve was drawn using standard solutions. Picrate solutions were made by placing the picrate papers in 5 ml of distilled water. The absorbance of the picrate solution was then obtained at 510 nm using UV-visible spectrophotometer Cecil CE 2041 2000 series. The linear calibration curve for cyanide was used to determine HCN content in cassava samples.

A sample of cassava root (parenchyma) was obtained by cutting a 3 mm thick section across the root about halfway along its length. After removal of the peel, 100 mg sector was cut from this disc. A round paper disc containing buffer at pH 6 was placed in flat bottomed plastic bottle and the weighed sector of cassava root placed on top of it. 0.5 ml of distilled water was added using the plastic pipette provided in the kit. Immediately the picrate paper attached to a plastic strip was placed on the plastic bottle and the bottle closed with a screw capped lid. It was allowed to stand for 24 hours at room temperature. The bottles were then opened and the color of the picrate papers matched against the shades of color in the color chart provided in Bradbury picrate paper kit. The total cyanide content in mg/kg in the cassava root was determined using UV-visible spectrophotometer.

3. Results and Discussion

The samples were analyzed using picrate paper kit for the determination of cyanide in cassava tuber. The concentrations of cyanide in samples collected from different markets in Nakawa Division were obtained in mg/kg as recorded in Table 1 below.

Table 1. *Concentration of cyanide (ppm) in cassava samples collected from different markets in Nakawa Division.*

Market	N	Concentration of cyanide (ppm)		
		Mean	Minimum	Maximum
Nakawa Market	40	50.83±2.93	40.34	58.08
Kisasi Market	32	53.54±2.46	44.62	67.65
Banda Market	32	52.25±2.64	36.75	64.34
Kirombe Market	25	52.20±2.45	43.28	66.45
Bugolobi Local Market	30	47.25±3.12	31.47	61.68

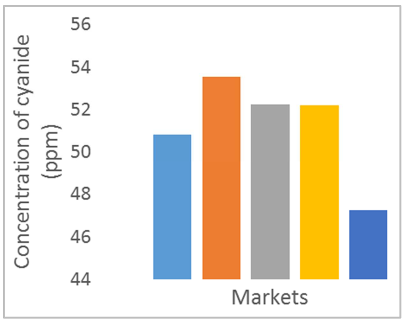

Figure 1. *Concentration of cyanide (ppm) in cassava samples collected from different markets in Nakawa Division.*

The concentrations of cyanide in samples collected from different markets in Kawempe Division were obtained in mg/kg as recorded in Table 2 below.

Table 2. *Concentration of cyanide (ppm) in cassava samples collected from different markets in Kawempe Division.*

Markets	N	Concentration of cyanide (ppm)		
		Mean	Minimum	Maximum
Kalerwe Market	45	56.25± 3.43	40.73	74.55
Mperewe Market	30	54.52± 2.84	45.05	70.82
Kawempe Market	40	61.08± 3.32	46.36	84.35

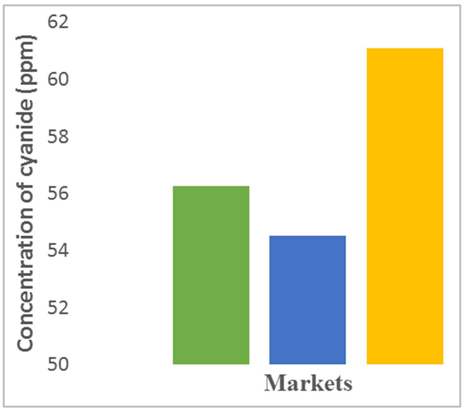

Figure 2. *Concentration of cyanide (ppm) in cassava samples collected from different markets in Kawempe Division.*

The concentrations of cyanide in samples collected from different markets in Rubaga Division were obtained in mg/kg as recorded in Table 3 below.

Table 3. *Concentration of cyanide (ppm) in cassava samples collected from different markets in Rubaga Division.*

Markets	N	Concentration of cyanide (ppm)		
		Mean	Minimum	Maximum
Busega Market	30	59.69±2.41	44.53	73.27
Kawala Market	24	57.83±2.96	40.32	80.50
Nakulabye Market	20	61.55±2.52	43.06	77.85

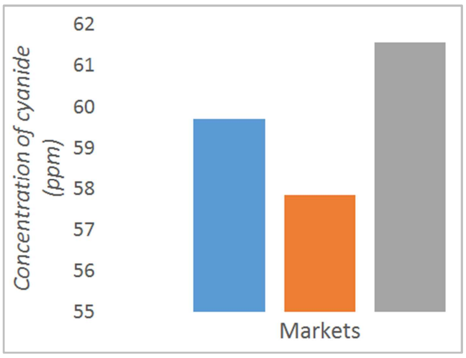

Figure 3. *Concentration of cyanide (ppm) in cassava samples collected from different markets in Rubaga Division.*

The concentrations of cyanide in samples collected from different markets in Makindye Division were obtained in mg/kg as recorded in Table 4 below.

Table 4. *Concentration of cyanide (ppm) in cassava samples collected from different markets in Makindye Division.*

Markets	N	Concentration of cyanide (ppm)		
		Mean	Minimum	Maximum
Gaaba Market	20	58.41±2.51	46.44	71.68
Kabalagala Market	25	52.58±2.43	40.22	66.62
Kansanga Vendor Market	20	54.53±2.76	38.36	69.44
Bunga Market	20	54.41±2.93	37.18	75.31

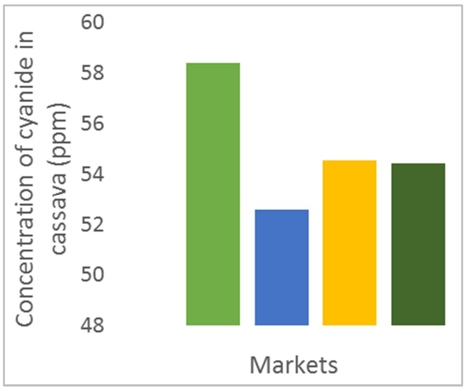

Figure 4. *Concentration of cyanide (ppm) in cassava samples collected from different markets in Makindye Division.*

Results for the amount of cyanide in cassava are presented in Table 1, Table 2, Table 3 and Table 4. cyanide was detectable in all samples taken. The mean concentration levels of cyanide in cassava samples from markets within Nakawa Division were 50.83±2.93, 53.54±2.46, 52.25±2.64, 52.20±2.45 and 47.25±3.12 ppm for Nakawa Market, Kisasi Market, Banda Market, Kirombe Market and Bugolobi Local Market respectively. The mean concentration levels of cyanide in cassava samples from markets within Kawempe Division were 56.25± 3.43, 54.52± 2.84 and 61.08± 3.32 ppm for Kalerwe Market, Mperewe Market and Kawempe Market respectively. The mean concentration levels of cyanide in cassava samples from markets within Rubaga Division were 59.69±2.41, 57.83±2.96 and 61.55±2.52 ppm for Busega Market, Kawala Market and Nakulabye Market respectively. The mean concentration levels of cyanide in cassava samples from markets within Makindye Division were 58.41±2.51, 52.58±2.43, 54.53±2.76 and 54.41±2.93 ppm for Gaaba Market, Kabalagala Market, Kansanga Vendor Market and Bunga Market respectively.

All the markets sampled showed different levels of cyanide in cassava, these variations can be attributed to the fact that chemical composition of cassava display differences in accordance to variety, site of production, age, analytical method, and environmental conditions [32]. Cassava cyanogenic potential varies with variations in agro-ecological zone [32] as is revealed in the previous studies where concentration of cyanide was dependent on environmental conditions in which the cassava is cultivated [33]. Besides the regions where cassava supplied to Kampala being ecologically different, the differences in levels of cyanide can be attributed to the different soil chemistry of the regions [33].

The reported levels of cyanide in cassava gave values higher than 10ppm which is WHO's recommended level of cyanide in cassava [34] and therefore they can be toxic to humans and animals if consumed unprocessed.

The ability of cyanogenic plant to be toxic to humans depends mainly on hydrogen cyanide (HCN) production. The residual cyanogens, linamarin and acetone cyanohydrin in cassava plant, are the obvious source of cyanide toxicity to human when converted to hydrogen cyanide inside the body [35]. Usually, minute quantities of cyanide are detoxified by cellular enzymes and thiosulfates in numerous tissues to form relatively harmless thiocyanate, which is excreted in the urine [36].

Larger quantities of cyanide cannot be detoxified by cellular enzymes and thiosulfates, consumption of 50 to 100 mg of hydrogen cyanide by an adult within 24 hours is capable of blocking celluar respiration hence causing death [35]. Consumption of raw and unprocessed cassava releases β-glycosidase during digestion, this enzyme catalyzes the hydrolysis of cyanogenic glycoside to harmful hydrogen cyanide and it remains active until it is deactivated by the low pH of the stomach [37]. Massive consumption of cassava or continuous ingestion of improperly processed cassava is linked to chronic cyanide toxicity in many parts of Africa where cassava is staple [20, 21] and is the cause of food poisoning and fatal cases relating to Cassava contains cyanogenic glycosides which slowly releases a toxic hydrogen cyanide [38].

Cyanide is a chemical which causes death immediately after exposure due to the fact that cyanide causes acute lack of oxygen [39]. Cyanide inhibits cytochrome oxidase hence stopping oxygen utilization causing cytotoxic anoxia. Acute manifestation of this occurrences depends on the extent of histotoxic hypoxia and CNS depression results to death [40]. Signs of acute intoxication by cyanide include rapid breathing, gasping, headache, salivation, nausea, anxiety, vertigo, cardiac arrhythmias, tremors, hypotension, respiratory failure, convulsions and death. Venous blood remains oxygenated and victim may appear pink. Average dosage by mouth of cyanide in adult human believed to be lethal is in the range of 50 to 200 mg and death is rarely delayed more than one hour [41].

4. Conclusion

In this study the levels of cyanide in raw cassava tuber marketed within Kampala City were determined by picrate paper kit. The objective of the study was to determine the levels of cyanide in raw cassava tuber marketed within Kampala City. Cyanide concentration in cassava root indicates a significant concentration of cyanide in cassava from different markets within Kampala City and the reported levels of cyanide are comparatively higher than the WHO recommended values of 10 mg of HCN/kg body weight. Consumers should be sensitized on effective methods of cassava processing and should be encouraged to use them before consumption of cassava. Studies on cyanide content in cassava based products like flours, cassava crisps should be undertaken.

Conflicts of Interest

Neither of the authors have any conflict of interest relating to this study.

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