Journal of Food and Nutrition Research, 2017, Vol. 5, No. 11, 817-823
Available online at http://pubs.sciepub.com/jfnr/5/11/4
©Science and Education Publishing
DOI:10.12691/jfnr-5-11-4

Evaluation of Cyanide Levels in Two Cassava Varieties (*Mariwa* and *Nyakatanegi*) Grown in Bar-agulu, Siaya County, Kenya

Moriasi Gervason A[1,*], Olela Ben O.[2], Waiganjo Bibianne W.[3], Wakori Edith W. T[2], Onyancha Jared M.[2]

[1]Department of Medical Biochemistry, Medical school, Mount Kenya University, Thika, Kenya
[2]Department of Pharmacognosy and Pharmaceutics, School of Pharmacy, Mount Kenya University, Thika, Kenya
[3]Department of Research Grants and Endowments, Directorate of Research, Grants and Endowments, Mount Kenya University, Thika, Kenya
*Corresponding author: gmoriasi@outlook.com

Abstract *Mannihot esculenta* Crantz (Cassava) is the second highest producer of carbohydrates after sugarcane. However, some varieties have high levels of cyanogenic glycosides and therefore are potentially toxic for human consumption. *Nyakatanegi* is one of the local varieties cultivated in Bar-agulu village in Siaya County (Kenya) and has been severally in the recent past reported to cause fatal cyanide toxicity. The other variety which is rarely associated with cassava poisoning is *Mariwa*. The objective of the current study was to determine cyanide levels in the two cassava varieties (*Mariwa* and *Nyakatanegi*) which are locally cultivated in Siaya County. The study was based on three laboratory assays: the picrate paper, picrate in solution and titration. Whole root cassava tubers were collected from a farmer in Bar-agulu village, Siaya County, packaged separately and transported to Mount Kenya University Pharmaceutical Chemistry Laboratory for analysis. Voucher specimens were authenticated in the cassava laboratories at Jomo Kenyatta University of Agriculture and Technology. It was observed that all the cortex samples of *Nyakatanegi* variety had the highest concentrations of cyanide giving 400 ppm for the picrate paper assay, 14.10 mg/Kg for the picrate in solution and 63 mg/Kg for the titration assays. On the other hand, the cortex samples of *Mariwa* reported cyanide concentrations of 30 ppm, 11.20 mg/Kg and 27.20 mg/Kg for the picrate paper, picrate in solution and titration assays respectively. The results showed higher amounts of cyanide in the tested cassava varieties than the WHO recommended levels (10 mg/Kg) especially the *Nyakatanegi* variety. Based on these results, sensitization campaigns are necessary to minimize cases of cyanide poisoning following cassava consumption.

Keywords: *cyanogenic glycoside, cyanide toxicity, picrate, titration and variety*

Cite This Article: Moriasi Gervason A, Olela Ben O., Waiganjo Bibianne W., Wakori Edith W. T, and Onyancha Jared M., "Evaluation of Cyanide Levels in Two Cassava Varieties (*Mariwa* and *Nyakatanegi*) Grown in Bar-agulu, Siaya County, Kenya." *Journal of Food and Nutrition Research*, vol. 5, no. 11 (2017): 817-823. doi: 10.12691/jfnr-5-11-4.

1. Introduction

Manihot esculenta Crantz (cassava) is the second highest producer of carbohydrates among crops after sugarcane [1]. It is majorly used as a staple food in the cuisine of Latin America and Africa [2]. Primarily, cassava is cultivated for human culinary consumption after boiling with water and in the form of flour. It is also a source of starch and starch derivatives which have significantly contributed to the wellbeing of humanity and livestock [1].

Cassava root contains natural toxic cyanogenic glycoside compounds; linamarin and methyl-linamarin. Injury to root tuber of cassava releases linamarase enzyme from the ruptured cells, which then converts linamarin to poisonous hydrocyanic acid (HCN) [3]. Consumption of raw cassava root results to potential toxicity of cyanoglycosides which arises from enzymatic degradation to produce hydrogen cyanide thus causing acute cyanide poisoning to the subjects [4]. The acute dose of cyanide responsible to elicit toxicity is in the region of 1mg/kg body weight. Cases of acute poisoning have been associated with the substantial higher cyanide content in the outer part or cassava peel [5].

Prolonged consumption of a monotonous cassava diet especially the bitter cultivar results in chronic illnesses like tropical ataxia neuropathy (TAN), goiter and cretinism due to iodine deficiency among other conditions. Sudden deaths of whole families due to consumption of poorly processed cassava have been reported [6,7,8]. In Kenya, deaths of four children of the same family caused by cassava intake were recently reported among other previous reports [9]. The current study aimed at determining cyanide levels in the cassava varieties cultivated in Bar-agulu village in Siaya County (Kenya).

2. Materials and Methods

2.1. Study Area and Sample Collection

Fresh cassava root tubers were obtained from Bar-agulu village (Siaya county) located in the Southwest region of Kenya. The site is defined by the following GPS cordinates: 0°03'40.18''N, 34°17.63''E. It is bordered by Busia county to the north, Kakamega and Vihiga counties to the North East and Kisumu county to the South East (Figure 1). It has a population of over 885, 762 according to the 2012 census, [10]. The residents practice subsistence farming and fishing as their main economic activities with sorghum and cassava being their staple foods [11]. The samples were separately packaged and transported to Mount Kenya University laboratory and stored in a refrigerator at 4°C awaiting analysis.

2.2. Laboratory Methods for Cyanide Determination

2.2.1. Preparation of the Samples

The labeled Cassava samples were cleaned using tap water to remove soil and sectioned with a sharp blade into peel (cortex), parenchyma and pith. The respective sections were placed into separately labeled clean dishes for analysis.

2.2.2. Preparation of Picrate Papers

Picrate solution was prepared by dissolving 1.4 g of moist picric acid in 2.5 w/v of sodium carbonate. Whatman filter paper No. 1 sheet measuring 10 cm x 10 cm was cut and placed in the yellow picrate solution in a dish for about 20 seconds after which it was removed and left to air-dry. Unevenly colored sections of the paper were cut off and the paper cut into 30 mm x 10 mm rectangular pieces. Each piece was attached to plastic strip (10 mm x 50 mm) using vinyl acetate glue. The picrate papers were stored in a deep freezer at -4°C to ensure stability.

2.2.3. Preparation of Phosphate Buffer (1.0 M)

One molar solutions of sodium dihydrogen phosphate and disodium hydrogen phosphate were separately prepared. Acidic sodium dihydrogen phosphate (39 parts) solution was carefully added to disodium hydrogen phosphate (11 parts) to give a solution of pH 7.5 that was adjusted to 6.0 with one normal hydrochloric acid (1N HCl). To make 0.1 M phosphate buffer, 100 ml of 1.0 M phosphate buffer was mixed with 900 ml distilled water.

2.2.4. Preparation of Alkaline Picrate Solution

Equal volumes of 2.56 w/v picric acid and 5 w/v sodium carbonate solutions were prepared in 100 ml of distilled water and mixed to have alkaline picrate for analysis.

2.2.5. Preparation of Standard Potassium Cyanide

Potassium cyanide (6.5 g) was dissolved in 900 ml of distilled water and made up to 1000 ml with 0.01 M H_2SO_4. Stock solutions of various volumes were diluted with 0.01 M H_2SO_4 in 100 ml flask as shown in Table 1 below [12].

Figure 1. A map of Alego Usonga constituency, Siaya county showing sample collection area (Bar-agulu)

Table 1. Potassium cyanide (KCN) dilutions

	Stock solution	H_2SO_4
Flasks	ml	ml
Control	0	100
Flask A	1	99
Flask B	2	98
Flask C	5	95
Flask D	10	90
Flask E	15	85
Flask F	25	75
Flask G	50	50

Various dilutions of the Stock Solutions (Table 1) (0.04 ml) were added to 1.96 ml of picrate solution, the mixture was incubated for 15 minutes at 37 ^{0}C. After incubation, the absorbance of various solutions was measured. The standard calibration curve of potassium cyanide was obtained by plotting absorbance values of various HCN equivalents against HCN concentrations and values for standard curve obtained as shown in Table 2 and Figure 2.

Table 2. Values for standard calibration curve preparation

Test tube	HCN concentration (µg of HCN ml^{-1})	Absorbance (Mean±SEM)
Control	0.000	0.00±0.00
Test tube A	0.026	0.09±0.00
Test tube B	0.052	0.06±0.00
Test tube C	0.135	0.05±0.01
Test tube D	0.270	0.21±0.01
Test tube E	0.405	0.34±0.06
Test tube F	0.675	0.38±0.00
Test tube G	1.350	0.34±0.02

Key: The control contained alkaline picrate (1.96 ml) and 0.01 M Sulphuric acid (0.04 ml).

2.3. Qualitative Determination of Cyanide Using Picrate Paper

Root tuber parts (parenchyma, peel and pith) were obtained from the test samples and 100 mg sections were cut from each sample. Round paper discs containing phosphate buffer of pH 6 were put in labeled flat bottomed plastic bottles. Weighed sections of each cassava samples were placed on top of the buffered paper discs. Distilled (0.5 ml) water was carefully added on top of the sections using a micropipette and immediately the picrate paper was attached to a bottle top via a plastic strip and the bottle was stoppered with a screw capped lid. The setup was left to stand for 24 hours at room temperature and the bottles were then opened and the colour of the picrate papers matched against the color chart shown in Figure 3 for qualitative determination of cyanide in ppm. This procedure was repeated for blank (same procedure used but no cassava/KCN put in the bottle) and the standard KCN solution.

2.4. Semi-quantitative Determination of Cyanide Using Picrate Paper

Following the qualitative assay, the picrate papers were soaked in 5 ml of distilled water for 3 minutes to prepare picrate solution. Absorbance of the picrate solution was read at 510 nm using UV-visible spectrophotometer (Shimadzu-1601, Tokyo Japan) [13]. The linear calibration curve for cyanide used to determine HCN content in cassava samples was as drawn in Figure 2. Experiments were carried out in triplicate analyses [12].

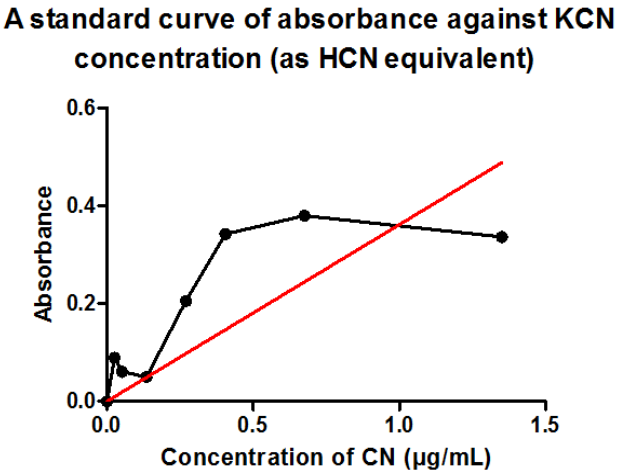

Figure 2. Standard calibration curve for HCN determination

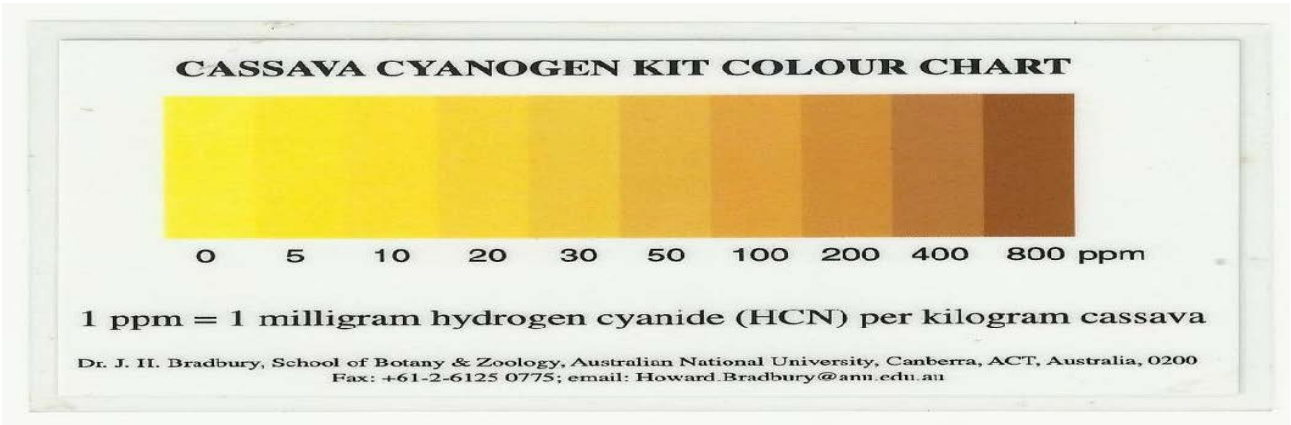

Figure 3. Cassava Cyanogen Kit color chart for cyanide determination (adopted from Bradbury, 1999)

2.5. Quantitative Determination of Cyanide Using Picrate in Solution Method

Three parts of the cassava root tuber (parenchyma, pith and cortex) were weighed (30 g of each) into a mortar, the weighed samples were mixed with 50 ml of distilled water for one minute and mashed using a pestle. Each of the extracts were filtered using Whatman filter paper No. 1. A volume of 0.04 ml of each extract was mixed with 2 ml of alkaline picrate solution and 1.96 ml of distilled water. The mixture was then incubated for 15 minutes in a water bath at 37°C after which the reaction was terminated by addition of 15 µl of concentrated sulfuric acid (H_2SO_4) so as to increase the stability of reading. Absorbances of the solutions were read in triplicate at 535 nm.

For both the picric paper and picrate in solution methods, the cyanide content of test samples was determined from the standard calibration curve shown in Figure 2 above. Amount of cyanide in the tested samples was calculated using the formula:

$$\text{mg / kg cyanide} = \frac{\mu g / mL \text{ of cyanide x final volume(L)}}{\text{Sample weight}} \text{ [12]}$$

Where: µg/mL CN is the HCN equivalent obtained from the KCN calibration curve; Final volume - Represents the volume of sample measured from filtered extract; Sample weight represents the weight of sample extracted in Kilograms.

2.6. Determination of Cyanide Content by Titration Method

Twenty grams of cassava root tissues (parenchyma, pith and peel) were ground using a pestle and mortar and 250 ml of distilled water added. The homogenates were transferred into distillation flasks and left to stand for 3 hrs. Distillation was performed until 150 ml of the distillate was collected. To the distillate, 20 ml of 0.02 M sodium hydroxide was added and thereafter the volume of the mixture was topped up to 250 ml in a volumetric flask using distilled water. The mixture was divided into three portions, two of 100 ml each and one of 50 ml for *Mariwa* and *Nyakatanegi* varieties. 8 ml of 6 M ammonium solution and 2 ml of 5 % potassium iodide were added to 100 ml aliquots, 4 ml and 1 ml of ammonium solution and potassium iodide were respectively added to the 50 ml portions. They were titrated using 0.02 M silver nitrate, with the 50 ml portions as trials. The amount of silver nitrate consumed to the endpoint of the titration was recorded. The equation below was used to determine HCN in the various parts in mg.

1 ml of 0.02 M silver nitrate = 1.08 mg HCN

according to AOAC [12].

2.7. Data Analysis

The obtained data was tabulated and expressed as standard error of the mean. The student t-test was employed to determine statistical significance of the obtained results.

2.8. Ethical Considerations

All reagents and chemicals were handled and disposed of according to the set laboratory regulations. Good laboratory practice was upheld to ensure safety.

3. Results and Discussion

3.1. Picrate Paper Assay

3.1.1. Estimated Cyanide Content in Parts Per Million (ppm) from Color Chart

The poisonous variety showed the highest amounts of CN with its cortex having 400 ppm as compared with the edible variety whose cortex showed 30 ppm. The piths of both the edible and the poisonous variety had the lowest amounts of 10 and 75 ppm respectively. Table 3 shows the results.

Table 3. Table showing average cyanide concentration in ppm from color chart

Part of cassava root tuber	Local variety	Average cyanide content
Cortex	*Nyakatanegi*	400.0±0.00
	Mariwa	30.0±0.00
Parenchyma	*Nyakatanegi*	150.0±50.00
	Mariwa	20.0±0.00
Pith	*Nyakatanegi*	75.0±25.00
	Mariwa	10.0±0.00

3.1.2. Determined Cyanide Content from the Picrate Paper

Spectrophotometric determination of the picrate papers demonstrated that the pith and the cortex of the *Nyakatanegi* variety had cyanide concentrations of 11.10 and 14.10 mg/Kg respectively. The parenchyma and the pith of the *Mariwa* variety gave a CN content of 11.60 mg/Kg and 14.20 mg/Kg respectively. The results are presented in Table 4 below.

Table 4. Table showing the determined cyanide content from the picrate paper

Root parts	*Mariwa*			*Nyakatanegi*			$t_{calculated}$
	Measured Absorbance	HCN equivalent (µg/ml)	Concentration of HCN (mg/Kg)	Measured Absorbance	HCN equivalent (µg/ml)	Concentration of HCN (mg/Kg)	
Cortex	0.08±0.00	0.22	11.20	0.1±0.00	0.28	14.10	0.17
Parenchyma	0.08±0.00	0.23	11.60	0.08±0.01	0.22	11.10	
Pith	0.1±0.00	0.28	14.20	0.08±0.01	0.22	11.10	

The differences in cyanide concentrations between the standard and the edible and poisonous cultivar parts at $p<0.05$ are not significant.
WHO recommended HCN level (10 mg/Kg body weight).

Table 5. A table showing the concentrations of CN following Picrate paper assay

Cassava root	*Mariwa*			*Nyakatanegi*			$t_{calculated}$
	Measured Absorbance	HCN equivalent (µg/ml)	Concentration of HCN (mg/Kg)	Measured Absorbance	HCN equivalent	Concentration of HCN (mg/Kg)	
Cortex	0.08±0.01	0.23	3.80	0.78±0.01	2.17	36.10	$t_{calculated}$=3.47
Parenchyma	0.09±0.02	0.23	3.90	0.33±0.19	0.90	15.00	
Pith	0.07±0.01	0.20	3.40	0.52±0.31	1.43	23.80	

The differences in cyanide concentration between the edible and the poisonous variety were significant at $p<0.05$.
WHO recommended HCN level (10 mg/Kg body weight).

Table 6. Table presenting the CN amounts following titration

Cassava parts	*Mariwa*		*Nyakatanegi*		t calculated
	Amount of Silver Nitrate (L)	Amount of HCN (mg)	Amount of Silver Nitrate (L)	Amount of HCN (mg)	
Cortex	25.17±0.44	27.18	58.50±1.61	63.18	t = 3.4
Parenchyma	20.70±0.15	22.35	38.30±0.42	41.36	
Pith	23.70±0.15	25.60	40.03±0.32	43.23	

The differences in cyanide concentration between the edible and the poisonous variety were significant at $p<0.05$. WHO recommended HCN (mg/Kg body weight=10).

3.2. Picrate in Solution Assay

The spectrophotometric assay revealed that the cortex of the poisonous variety had the highest amount of HCN (36.10 mg/Kg) followed by the pith (23.80 mg/Kg) while the parenchyma had the lowest amount of 15.00 mg/Kg. On the other hand, the edible cultivar had less than 10 mg/Kg of HCN content in the three parts tested with the parenchyma having 3.90 mg/Kg followed by cortex and pith with 3.80 and 3.40 mg/Kg respectively as shown in Table 5.

3.3. Titration Method

3.3.1. Amount of Cyanide in the Two Local Cassava Cultivars

Following titration method for cyanide determination in the cassava cultivars, the study showed that the cortex of the *Nyakatanegi* cultivar had the highest concentration of cyanide of 63.20 mg followed by the pith with 43.2 mg, while parenchyma had the lowest concentration of 41.40 mg. In contrast, the cortex of the *Mariwa* variety had a concentration of 27.18 mg followed by the pith and parenchyma with concentrations of 27.60 and 22.40 mg respectively. The results are shown in Table 6.

4. Discussion

Based on the obtained results from both picrate in solution and titration methods, the determined HCN equivalents (mg/L) of the *Mariwa* and *Nyakatanegi* cultivars differ significantly ($t_{calculated} > t_{critical}$ at 95% confidence level). (Table 3 – Table 6). Picrate and titration methods showed higher HCN values for the *Nyakatanegi* variety than the *Mariwa*. The cyanide concentration values obtained in the titration method can be attributed to interferences from other background substances such as nitrates and nitrites that could be present in the assay solution [14]. In addition, these high amounts can be attributed to inconsistency that usually occur in collection of titer volumes during titration. The application of turbidity as reaction endpoint is subject to being arbitrary depending on the experimenter and the precise endpoint is difficult to determine.

The picrate paper method yielded high consistency and reproducibility where uniform concentrations of HCN in all replicate analyses were reported. This means that using the picrate paper offers a possibility of reducing the interferences as opposed to the titration and the picrate in solution methods. In the picrate paper, there is minimal interference with anions that could be potential sources of exaggerated results [14].

According to Bradbury *et al*., (1999), the picrate paper method is able to detect cyanide concentration over the range of 0-800 mg HCN equivalent per kilogram of cassava (ppm). This means that despite the fact that this technique is reproducible, it may not detect low concentrations of cyanide. Therefore, the replacement or improvement of this method with modern electrochemical and potentiometric methods in which the reaction end point determination is accurate is warranted [15].

In spite of the fact the two cassava varieties are grown in the same ecological niche, it is worth noting that their concentrations of cyanide are significantly different with the *Nyakatanegi* variety possessing higher levels than the *Mariwa variety*. Generally, the reported levels of cyanide of the *Nyakatanegi* variety in this study are comparatively higher than the WHO recommended values of 10 mg of HCN/kg body weight [13,21]. This means that this variety is potentially toxic to the consumer, if consumed either raw or unprocessed. It is thus for primary processing methods like drying for at least a week, fermenting, boiling, soaking overnight the cassava tubers before consumption as a a strategy to minimize intoxication [19].

Cyanide concentration in cassava varieties has been linked to the activity of various genes involved in cyanogenesis pathway in which linamarin and lotaustralin are produced when glycosyl transfarase catalyses the production of L-valine and L- isoleucine [16,17]. Two scaffold genes; scaffold 09743 and scaffold 01206 code for the synthesis of linamarase enzyme and hydroxyl nitrile lyase which play a critical role in cyanogenesis pathway [18]. Research has demonstrated that interferences

in these two scaffold genes in the course of cassava development lead to variations in cyanide content of different varieties [18].

The close relationship between genes and cyanide concentration in cassava varieties has led to the deployment of molecular biology and genetic engineering techniques as a strategic intervention geared towards at reduction of Post-Harvest Processing Deterioration (PHPD) of cassava [19]. Expression of the cyanide-insensitive mitochondrial enzyme alternative oxidase (AOX) from *Arabidopsis spp* in cassava roots has facilitated the production of transgenic cassava varieties with delayed PHPD for approximately three weeks after harvesting [20].

The current study showed that the cortex of the bitter (*Nyakatanegi*) variety had the highest concentration of cyanide followed by the pith, while parenchyma had the lowest concentration (Table 3 - Table 6). Owing to the individual differences among the three tissues, each has distinct biochemical characteristics which are attributable to the distribution of vital enzymes associated with cyanide metabolism. For instance, Linamarase enzyme has been previously described to reduce from the cortex towards the pith. This feature confers the varied accumulation of cyanogenic glycosides in the three different parts of the cassava cultivars [19]. This is consistent with the trend of cyanide levels in the three parts of cassava varieties reported in this study (Table 3 - Table 6). The results revealed a gradual decrease in cyanide concentration from the cortex/peel through to the pith.

The strong relationship existing between gene expression patterns and the age of the cassava plant explains the noted variations of cyanide composition in the different parts of cassava samples investigated in this study. It is well established that different tissue parts mature at different times, therefore, different genes and degrees of gene activity occur at different ages of the cassava plant [19].

5. Conclusion and Recommendations

Based on the experimental results obtained from the three methods of cyanide determination in cassava, picrate paper method was most effective and reliable method for determining cyanide concentration due to its reproducibility although it may not be sensitive to small amounts. Although all the samples were obtained from the same ecological niche, geographical area in the same climatic conditions significant differences cyanide concentration were noted especially between the sweet and the edible varieties. The study also revealed that the three parts the cassava root examined (pith, parenchyma and cortex) reported significant differences in cyanide concentration with the cortex possessing high amounts as compared to the other parts. Due to the increasing use of cassava as a staple food and source of carbohydrates in many households especially those of low income, the cyanide concentrations reported herein prompt urgent measures to curb potential toxicity and fatality. Farmers and consumers of cassava should be educated on effective methods of cassava processing and encouraged to use them before consumption of cassava as some may not be able to distinguish between the edible and the poisonous varieties. The administrative arms are urged to facilitate public awareness campaigns to sensitize the public on high concentrations of HCN equivalent per kilogram of cassava compared to the recommended standard WHO reference values to avoid chances of toxicity and potential death. It is also important to investigate cyanide concentration in cassava-based products like flour, cassava crisps among others so as to determine their safety profiles. Furthermore, it is recommended that further studies be carried out on the leaves that are consumed as vegetables to ascertain their HCN equivalent/kg and determine their safety.

Acknowledgements

Authors owe gratitude to a number of people for this piece of work, first and fore most the local farmers at Baragulu who assisted in identification and collection of the cassava varieties. Secondly, the laboratory technologists at Jomo Kenyatta University of Agriculture and Technology for authentication of the cassava varieties of this study. The authors thank Mount Kenya University for provisions of pharmacognosy, pharmaceutical chemistry, Medical biochemistry and the research center laboratories where this study was carried.

Conflict of Interest

The authors declare that there are no conflicts of interests in regard to this work

References

[1] New World Encyclopedia; Cassava. http://www.newworldencyclopedia.org/entry/Cassava; 2008. (Accessed on 19, February, 2017).

[2] Cereda, M.P.; Processing of cassava as a mechanism of detoxification. In: Cereda, M.P.; Vilpoux, O.F. Technology, use and potentialities of Latin American starchy tubers. Cargill Foundation 03: 2003, 47-80.

[3] Greenwood N., Earnshaw A.; Chemistry of the Elements (2nd Edition.), Oxford Butterworth-Heinemann, 1997.

[4] Gosselin R., Smith R., Hodge H.; Clinical toxicology of commercial products. 5th edition. Williams and Wilkins, Baltimore, M.D, 1984.

[5] Agency for Toxic Substances and Disease Registry (ATSDR).; Toxicological Profile of Cyanide, Prepared by Research Triangle Institute for the US Department of Health and Human Services, Agency for Toxic Substances and Disease Registry (ATDSR), Atlanta, Georgia, August 1995.

[6] Adindu MN, Aprioku ABI.; Cyanogenic content of garri from some processing centers in River State, Nigeria. Nig. Food J. 24(1):135-138, 2006.

[7] Nhassico D, Muquingue H, Cliff J, Cumbana A, Bradbury HJ.; Review: Rising African cassava production, diseases, 2008.

[8] Dufour DL.; Assessing diets in population at risk for konzo and neurolathyrism. Food Chem. Toxicol. 49:655-661, 2006.

[9] Daily Nation.; News bulletin: children succumb to cassava consumption in Siaya county, 18th September, 2016

[10] Kenya National Bureau of Statistics (KNBS); Population and housing census highlights, Kenya, 2012.

[11] County Integrated Development plan; Siaya county integrated development plan for 2013-2017, 2013.

[12] Association of Official Analytical Chemist AOAC.; Official Method of Analysis. 15 Edn. Washington D.C., 1990.

[13] Bradbury, M., Egan, S., Bradbury, J.; Determination of all forms of cyanogens in cassava roots and cassava products using picrate paper kits. *Journal of the Science of Food and Agriculture*, 79: 593-601, 1999.

[14] US EPA.; Method for determining total and amenable cyanide: Distillation. Method 9010C US Environmental Protection Agency. November 2004.

[15] Courtney A.; Hydrometallurgy: Proceedings of sixth International Symposium, 2008.

[16] Food and Agricultural Organization.; June 2003 cassava market assessment due to high cyanide intake and control measure. J. Sci. Food Agric. 88:2043-2049; 2007.

[17] Conn E.; Cyanogenesis - a personal perspective. Acta Horticulturae. 375: 31-43, 1994.

[18] Sukhuman W., Supannee P., Supanath K., Sithichoke T., Opas B., David A., Kanokporn T.; A genome scan for quantitative trait loci affecting cyanogenic potential of cassava root in an outbred population. BMC Genomics, 12: 226, 2011.

[19] Westby A.; Cassava utilization, storage and small-scale processing. In: Hillocks RJ, Thresh JM, Belloti AC (eds.) Cassava: biology, production and utilization. CAB International Publishing, Wallingford.pp. 281-300, 2002.

[20] Sayre R., Beeching J., Cahoon E., Egesi C., Fauquet C., Fellman J., Fregene M.; The BioCassava plus program: biofortification of cassava for sub-Saharan Africa. *Annual Review of Plant Biology*, 62: 251-72, 2011.

[21] WHO; Toxicological evaluation of certain food additives and naturally occurring toxicants. WHO Food Additive Series: 30. World Health Organization, Geneva, 2010.