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Tracking Cyanogenic Potentials of Cassava Tuber Processing into "GARI" from Farm to Table and Assessing Toxicity Levels

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Abstract

The most important requirement in the processing of cassava tubers into "gari", an important African food, is its detoxification by the reduction of the total cyanide content (bound or free) to an acceptable level. Cyanogenic glucoside is referred to as bound cyanide while hydrocyanic acid (HCN) is referred to as free cyanide. The study was conducted to examine the cyanide level at every step and stage in "gari" processing chain. The study was conducted in Onipepeye, Oremeji, Agugu cassava processing industry in Ibadan, Nigeria. A multistage random sampling technique was used to select one processor out of 20 processors screened. Sixteen samples from the processing steps of raw, fermented and finished (ready to eat) cassava food product were analyzed for cyanide (toxicity) level using Good Manufacturing Practices (GMP) concept. Analysis of Variance (ANOVA) and T-test were used to determine significant differences in "gari" processing steps and stages for cyanide level changes. Post HOC Test was used to identify processing step(s) that contributed to the significant level differences in the cyanide level or toxicity in the "gari" processing steps. Changes in cyanide levels associated with "gari" processing steps were computed at 5% confidence level. The mean cyanide level (mg HCN eq. Average/100 gm) in "gari", unpeeled cassava 3.622, peeled cassava 9.35, peeled and washed cassava 10.16, pressed mash 7.48, "gari" hold and sell 2.09, showed significant changes in cyanide level ($p < 0.0001$). Post HOC Test showed that many step(s) contributed to the significant level differences in the cyanide level or toxicity. At the step of unpeeled cassava, peeled cassava, peeled and washed cassava, grated mash (fermented), sieved mash, fried "gari" from pan to ready-to-eat "gari" and held at 0.05% level, some mean differences in the processing step(s) contributed to the statistically significant difference in the cyanide level or toxicity in "gari" processing stages. This study shows that there is statistically significant difference in cyanide level (toxicity) among "gari" processing steps.

Keywords

Cassava, Cyanide level changes, Toxicity, "Gari" processing chain

Introduction

Cassava, one of the over 3,000 types of plants that produces cyan genic compounds, [1-4] releases hydrogen cyanide (HCN) upon hydrolysis [3-5]. This process of HCN Production is known as cyan genesis and makes cassava potentially toxic food to humans [1, 3]. In cassava roots and leaves, two cyan genic glucosides, linamarin and lotaustralin are known to occur in a ratio of about 93.7 [6]. Linamarase, an enzyme which hydrolyses the Cyanogenic glucoside to glucose and cyanohydrins is also present in cassava tissues, but in a separate

compartment from the glucosides. Linamarase is located in the cell wall, while the glucosides are located inside vacuoles in the cytoplasm, [1, 4, 7].

When cassava tissues are disrupted as occurs with grating or crushing of cassava roots during processing, linamarin and lotaustralin come into contact with linamarase and are hydrolysed to produce acetone cyanohydrin and 2-butanone cynohydrins respectively, [8-12]. These cyanohydrins being relatively unstable decompose spontaneously to the corresponding ketones and hydrogen cyanide (HCN) at pH above 5 and at temperature above 35°C. Once HCN is produce, it dissipates in air because of its low boiling point at 25.7°C [13] The decomposition of cyanohydrins may also be catalyzed by a-hydroxynitrile lyase, aytaplasmic enzyme that is present in cassava leaves [11] but is not expressed in tissues of cassava roots [14]. Poor manufacturing practices and handling in cassava tuber processing into "gari" chain from farm-to-table can increase Cyanogenic potential [CNP] in "cassava processed products and finished product ("gari") to unsafe level [15].

Alphonse Laya et al. 2018 [16], cassava roots yield more carbohydrates per hectare than cereal crops and can be grown at a considerably lower cost, [17]. Cassava roots are a staple food that provides carbohydrates for more than 2 billion people in the tropics. However, cassava roots spoil quickly after harvest. In order to avoid this loss, they must be sold or processed into by-products after harvest. Generally, cassava and its products are poor in proteins. The deficiency in certain essential amino acids depends mostly on the varieties and geographical conditions. In order to enhance the nutritional quality of cassava, it is processed into fermented products such as "gari". "Gari" is one of the most popular cassava products consumed in Africa, Southeast Asia, and Brazil [18]. In Africa, fermented foods and beverages are produced using fermentation.

Processed cassava food products are the major staple food in the tropics, where an estimated half a billion people depend on cassava as a staple [19, 20]. Utilization of cassava as staple [21] is limited by various factors, but prominently the potential toxicity [22, 23]. In communities where cassava is used for food, it is usually processed after harvesting. The presence of HCN in the root limits cassava utilization [24] but with proper processing [25] this problem can be eliminated [26-29]. Numerous methods of processing have been developed for cassava in different parts of the world resulting in the production of a wide variety of food [25]. The effectiveness and extent of the processing chain can determine the quality and nutritional status of cassava food products.

The extent of removal of cyanogens from cassava roots by the different methods of processing varies [25, 30-32]. All methods of processing cassava involve different combination and sequence of two or more of the following steps: peeling, chipping, slicing, boiling, grating, fermentation, dewatering, steaming, roasting and sun or smoke drying. Different combination of these steps results in the production of different food products, which are either intermediate products that need further cooking before eating, or products that can be eaten without further processing [33, 34]. Understanding Good Manufacturing Practices (GMP) in each processing step and stage affecting toxic level trend is important so that the implication of modification in processing technique (to scale up manufacturing practices in the processing chain) can be understood. Processing of raw cassava tubers into "gari" is proceeded in many steps and stages; unpeeled cassava, peeled cassava, peeled and washed cassava, grated mash (raw stage); pressed mash before sieving (dewatering), sieved mash (fermentation stage); fried "gari" from frypan, cool "gari" and hold (finished product stage). The toxicity which is due to the Cyanogenic glucoside, linamarin, and to a lesser extent, lotaustralin [35] is reduced with varying degree of effectiveness during processing into different foods. The manufacturing practices affecting toxic trends in each processing step and stage are not yet understood.

In practice, most traditional and non-informal sector cassava processing industry technique appear to be designed to bring together enzyme (linamarase) acid substrate (Cyanogenic glucoside) by cell rupture, followed by elimination of the liberated hydrocyanic acid (HCN). "Gari" is the most widely consumed and traded of all cassava foods in Nigeria [36-39] and many countries of West Africa [40]. "Gari" is also becoming a major export food to immigrant from West Africa living in Europe [40]. "Gari" is processed from harvested cassava tubers; hence there is usually effect of pre-harvest and post-harvest handling of cyanogenic potentials of cassava tubers. The term cyanogenic potential (or CNP) is proposed to mean the sum of cyanogenic glucoside, cyanohydrins and hydrocyanic acid present in a plant or food sample. It should be evaluated following the method of [21, 41]. Prior to processing of cassava tubers into "gari" are usually transported from the farm to the home, processing area or industry. Most cassava tubers are not always brought to the industry market on the day of harvesting, while some are not sold to the processing industry the same day they are transported to the industry market. Hence, most cassava tubers spend 2 to 3 days after harvesting before being processed into cassava food in the industry.

Harvesting and storage should be done in a way that minimizes bruising as these may accelerate post-harvest deterioration and lead to increase in CNP [41]. Also, storage of peeled roots tends to greatly increase CNP level [41]. In case the roots are not processed immediately, their CNP may increase by 33% in 48 h for unpeeled roots and by 100% in 24 h for peeled roots. Grating of peeled cassava roots from large pieces to small dices to a mash damaged cells, thus exposing their linamarin content in contact with hydrolytic enzymes and leading to a rapid decrease in CNP. In a recent survey some "gari" samples were found to have a CNP as high as 6 mg HCN/100 gm. The length of fermentation for producing "gari" cannot be blamed for the high level of residual cyanogens. It should rather be the extent of hydrolysis. In the process of baking, frying and steaming, the heat may reduce bursting of the cells by the cyanogenic glucosides remaining inside the cassava roots [27] have estimated at losses of cyanogenic

glucoside 14.2, 1.3 and 15.7% from cassava upon baking, frying and steaming respectively. Previous studies revealed that much work have been done in the area of removal of cyanogens in cassava foods processing, but have not been applied in a non-formal centralized "gari" processing industry. An effective food control is needed to see that large plants making or processing foods comply with Good Manufacturing Practices (GMP) which will prevent contamination or reduce the final CNP in processed "gari". This study was conducted in an informal "gari" processing industry to examine the effect of manufacturing practices on cyanide (toxicity) level in each processing step and stage from farm to table.

Materials and Methods

Sample collection and interview

A multi-stage sampling technique was used to select one "gari" processor out of the (20) twenty processors screened.

A "gari" processor identified for this study was followed through "gari" processing chain twice (for two samples) from raw tuber to ready to eat "gari". The study made use of the same cassava tubers purchased by the processors from the industry market and their method of production. The processed steps were identified for sample collection. Samples were collected from points in the "gari" processing chain for cyanide (toxicity) level. Based on direct participatory observation and frequent visits to the industry for verification of points of poor manufacturing practices, control points were established. The key informant that has worked in the industry for more than three years was selected for interview to collect information on industry background, sources of cassava tubers, knowledge, attitude and practices. These were carefully discussed on individual basis with each informant and their information collaborated and elaborated on each other's contribution. In-depth interview was also used in the study. One expert in "gari" processing industry that had been there from its inception was interviewed.

Information on manufacturing practices of "gari" was also collected. All the samples were immediately stored in the refrigerator awaiting analysis. Each sample was clearly labeled with date and time of collection and the name of sample.

Cyanogen was determined in triplicate in cassava roots, identified processing step and food products by improved automated enzymatic assay [42] method. Cyanogens were extracted from fresh cassava tuber samples from the identified processing steps and foods using 0.1 M orthophosphoric acid. For the extraction of cyanogens from cassava tuber, peeled cassava root was cut into 1^{-cm} cubes and mixed thoroughly. About 15 g of cassava cubes was homogenized in 250 ml refrigerated 0.1 M of orthophosphoric acid in a blender for 15 seconds at low speed, followed by 60 seconds at high speed, 50 seconds of rest and another 60 seconds at high speed. For cassava mash, effluent, "gari", 30 g samples of each product were homogenized in 250 ml of refrigerated 0.1 M orthophosphoric acid as for fresh tubers. The homogenates were centrifuged at 6000 x g for 10 minutes and the supernatant used for the quantification of cyanogens.

The supernatant liquid was chlorinated with chloramines-T and coloration was done by a modification of Konig reaction using 1.3-dimethyl barbituric acid and isonicocidic acid [42]. Separate determinations were made for the total non-glycosides and free HCN fractions of the cyanogens. The glucoside and cyanohydrins content were calculated from these determinations. All extractions and determinations of cyanogens were done in triplicate. Crude linamarase extracted from cassava roots cortex [43] was used for hydrolysis of glucoside.

Statistics

The major interest in this study was to determine the differences in cyanide levels and changes along the processing steps in the "gari" processing chain. Analysis of Variance (ANOVA) and T-Test were perhaps the most suitable tests to determine significant differences in cassava processing steps and stages for cyanide-level changes associated with "gari" processing. In the study, T-Test was used to test the significant difference in cyanide level and Analysis of Variance (ANOVA) was used to test the significant differences within and between groups (raw and fermented) processing. Pairwise Multiple Comparison Test (Post HOC Test) was used to determine which step was significantly different from other steps within the processing chain. Changes in cyanide levels associated with "gari" processing steps and stages were composed at 5% confidence level.

Results

The cyanide levels at every step in "gari" processing was measured and recorded using bound non-glu free CN expressed in mg HCN eq. average/100. Two samples were made and the mean of the two samples found and recorded in table 1.

The mean cyanide level along the processing "gari" steps

Table1: Cyanide level in gari processing steps.

Processing Steps	Sample I mg HCN eq. Average/100 g	Sample II mg HCN eq. Average/100 g	Mean ± SD mg HCN eq. Average/100 g
Unpeeled cassava	3. 31	4.01	3.62 ± 0.50
Peeled cassava	9.20	9.50	9.35 ± 0.21
Peeled and washed cassava	9.99	10.37	10.16 ± 0.23
Grated mash	0.41	3.73	2.30 ± 2.35
Pressed mash before sieving	7.50	7.45	7.48 ± 0.04
Sieved mash	6.48	7.18	6.83 ± 0.50
Fried gari from fry pan	1.32	1.31	1.32 ± 0.01
Cooled gari (hold)	2.05	2.12	2.09 ± 0.05

F = 32.332 P = 0.000

from raw cassava tuber is characterized by fluctuating rise and fall. In raw unpeeled cassava tuber, the HCN recorded was 3.62 mg HCN eq. Average/100 g. This rose to 10.16 mg HCN eq. Average/100 g in peeled and washed cassava tuber and a steep reduction to 2.30 mg HCN eq. Average/100 g in the grated cassava mash, it is immediately followed with a sharp increase to 7.48 mg HCN eq. Average/100 g in pressed mash before sieving. A reduction through sieved mash from 6.83 to 1.32 mg HCN eq. Average/100 g in fried "gari" from pan was recorded. The final product cooled "gari" (hold) increased to 2.09 mg HCN eq. Average/100 g.

Figure 1 is the graphic representation of mean cyanide levels in the processing steps of the two samples in "gari". The observations in table 1 were analyzed assuming a completely randomized design of two observations per processing step. Analysis of Variance (ANOVA) was used. F calculated is equal to 32.332 corresponding to a P-value of 0.000 < 0.05 tabulated at 95% confidence level. Therefore, there is statistically significant difference in cyanide level (toxicity) among "gari" processing steps.

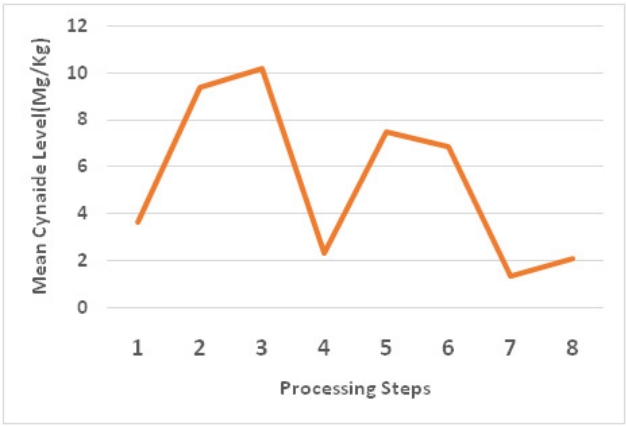

Figure 1: Mean cyanide level in "gari" processing chain. Processing steps: 1. unpeeled cassava. 2. peeled cassava, 3. peeled and washed cassava, 4. grated mash, 5. pressed mash before sieving, 6. sieved mash, 7. fried "gari" from pan, 8. cooled "gari" and hold.

Explanation on the post Hoc analysis

The mean cyanide level at the different "gari" processing chain was significantly different ($F = 32.328$, $P < 0.0001$). A post hoc analysis reveals that the major differences occurred in three processing steps where there was a least significance of just a pair of the processing step and these are; unpeeled cassava, peeled and washed cassava, and sieved mash cassava. For example, cyanide level in unpeeled cassava was similar to the level in grated mash cassava, peeled and washed cassava cyanide level was similar to that obtained in peeled cassava while the level of cyanide in sieved mash cassava was similar to that in pressed mash before sieving cassava.

Table 2 indicates processing step(s) that contributed to the statistical significance difference in the cyanide level or toxicity in "gari" processing stages. The mean difference of peeled cassava, peeled and washed cassava, pressed mash before sieving, sieved mash and fried "gari" from pan are significant to unpeeled cassava. At step of peeled cassava, the mean difference of unpeeled cassava, grated mash (fermented), sieved mash, fried "gari" from pan and cooked "gari" and hold are significant to peeled cassava. The mean difference of unpeeled cassava, grated mash (fermented), pressed mash before sieving, sieved mash, fried "gari" from pan and cooked "gari" and hold are significant to peeled and washed cassava. The mean difference of peeled cassava, peeled and washed cassava, pressed mash before sieving and sieved mash are significant to grated mash (fermented). At step of pressed mash before sieving, the mean difference of unpeeled cassava, peeled and washed cassava, grated mash (fermented) fried "gari" from pan and cooked "gari" and hold are significant. The mean difference of unpeeled cassava, peeled cassava, peeled and washed cassava, grated mash (fermented), sieved mash and cooked "gari" and hold are significant to sieved mash. At step of fried "gari" from pan, the mean difference of unpeeled cassava, peeled cassava, peeled and washed cassava, pressed mash before sieving, fried "gari" from pan are significant. The mean difference of peeled cassava, peeled and washed cassava, pressed mash before sieving and sieved mash are significant to cooked "gari" and hold at the 0.05 level.

Discussion

Bruises during harvesting and transportation accounted for CNP level of 3.66 mg HCN eq. Average/100g for unpeeled cassava [41]. Normally, the grating of peeled cassava does not take place immediately as processors have to take turns according to first come first serve at the grating plant. The storage of peeled cassava tuber and time lapse before grating and laboratory test accounts for the increase in CNP to 9.35 mg HCN eq. Average/100 g before grating for peeled cassava and 10.18 mg HCN eq. Average/100 g for peeled and washed cassava respectively [41]. Grating of cassava tuber reduced the tuber from small dices to mash. In the process, more or less cells are damaged, thus exposing their linamarin contents to be in contact with hydrolytic enzymes leading to a more or less rapid decrease in CNP. The smaller the final particle size the greater the decrease [41]. This accounted for the decrease of CNP from 9.35 mg HCN eq. Average/100 g for peeled cassava to 2.01 mg HCN eq. Average/100 g for grated cassava mash. Pressed mash after mash fermentation recorded a CNP of 7.48 mg HCN eq. Average/100 g.

Fermentation of cassava mash is the second step in the processing of cassava, the first step being a considerable size reduction. It has been shown that most of the hydrolysis of cyanogenic glucosides is a consequence of the size reduction step and not of microbial activity [44]. In fact, it is thought that the low pH (around 4.0) rapidly achieved during fermentation is inhibitory to linamarase activity and stabilizes cyanohydrins, thus slowing down linamarin hydrolysis and cyanohydrin breakdown. In a recent survey, some "gari" samples were found to have a CNP as high as 6 mg HCN eq. Average/100 g. However, the conversion of the pressed mash to fine size through sieving brought down the CNP to 6.83 mg HCN eq. Average/100 g for sieved mash [41]. In the process of baking, roasting, frying and steaming, bursting of the cells is induced to release the cyanogenic glucosides remaining inside

Table 2: Post Hoc test for significant difference in cyanide level in gari processing steps.

					95% Confidence Level	
Group 1	Group 2	Mean Difference	Standard Error	Sig	Lower Bound	Upper Bound
Unpeeled cassava	Peeled cassava	-5.6900 *	.8735	.000	-7.7044	-3.6756
	Peeled and washed cassava	-6.4950*	.8735	.000	-8.5094	-4.4806
	Grated (mash fermented)	1.5900	.8735	.106	.4244 -	3.6044
	Pressed mash before sieving	-3.8150 *	.8735	.002	5.8294	-1.8006
	Sieved mash	-3.1700 *	.8735	.007	-5.1844	-1.1556
	Fried "gari" from pan	2.3450 *	.8735	.028	.3306	4.3594
	Cooked "gari" and hold	1.5750	.8735	.109	-.439	3.5894
Peeled cassava	Unpeeled cassava	5.6900 *	.8735	.000	3.6756 -	7.7044
	Peeled and washed cassava	-.8050	.8735	.384	2.8194	1.2094
	Grated mash (fermented)	7.2800 *	.8735	.000	5.2656 -	9.2944
	Pressed mash before sieving	1.8750	.8735	.064	1394	3.8894
	Sieved mash	2.5200 *	.8735	.020	.5056	4.5344
	Fried Gari from pan	8.0350 *	.8735	.000	6.0206	10.0494
	Cooked gari and hold	7.2650 *	.8735	.000	5.2506	9.2794
Peeled and	Unpeeled cassava	6.4950 *	.8735	.000	4.4806	8.5094
washed cassava	Peeled cassava	.8050	.8735	.384	-1.2094	2.8194
	Grated mash (fermented)	8.0850 *	.8735	.000	6.0706	10.0994
	Pressed mash before sieving	2.6800 *	.8735	.015	.6656	4.6944
	Sieved mash	3.3250 *	.8735	.015	1.3106	5.3394
	Fried "gari" from pan	8.8400 *	.8735	.000	6.8256	10.8544
	Cooked "gari" and hold	8.0700 *	.8735	.000	6.0556	10.0844
Grated mash (fermented)	Unpeeled cassava	-1.5900	.8735	.106	-3.6044	.4244
	Peeled cassava	-7.2800 *	.8735	.000	-9.2944 -	-5.2656
	Peeled and washed cassava	-8.0850 *	.8735	.000	10.0994	-6.0706
	Pressed mash before sieving	-5.4050 *	.8735	.000	-7.4194	-3.3906
	Sieved mash	-4.7600 *	.8735	.001	-6.7744	-2.7456
	Fried "gari" from pan	.7550	.8735	.413	-1.2594 -	2.7694
	Cooked gari and hold	-1.5000E02	.8735	.987	2.0294	1.9994
Pressed mash before sieving	Unpeeled cassava	3.8150 *	.8735	.002	1.8006	5.8294
	Peeled cassava	-1.8150	.8735	.064	-3.8894	.1394
	Peeled and washed cassava	-2.6800 *	.8735	.015	-4.6944	-.6656
	Grated mash (fermented)	5.4050 *	.8735	.000	3.3096	7.4194
	Sieved mash	.6450	.8735	.481	-1.3694	2.6594
	Fried "gari" from pan	6.1600 *	.8735	.000	4.1456	8.1744
	Cooked "gari" and hold	5.3900 *	.8735	.000	3.3756	7.4044
Sieved mash	Unpeeled cassava	-3.1700 *	.8735	.007	-1.1556	-.5.1844
	Peeled cassava	-2.5200 *	.8735	.020	-4.5344	-.5056
	Peeled and washed cassava	-3.3250 *	.8735	.005	-5.3394	-1.3106
	Grated mash (fermented)	-4.7600 *	.8735	.001	2.7456	6.7744
	Pressed mash before sieving	-.6450	.8735	.481	-2.6594	-1.3694
	Sieved mash	-5.5150 *	.8735	.000	-3.5006	-7.5294
	Cooked "gari" and hold	-4.7450 *	.8735	.001	-2.7306	-6.7594
Fried gari from pan	Unpeeled cassava	-2.3450 *	.8735	.028	-4.3594	.3306
	Peeled cassava	-8.0350 *	.8735	.000	-10.0494	-6.0206
	Peeled and washed cassava	-8.8400 *	.8735	.000	-10.8544	-6.8256
	Grated mash (fermented)	-.7550	.8735	.413	-2.7694	1.2594

	Pressed mash before sieving	-6.1600 *	.8735	.000	-8.1744	-4.1456
	Fried "gari" from pan	-5.5150 *	.8735	.000	-7.5294	-3.5006
	Cooked "gari" and hold	-.7700	.8735	.404	-2.7844	1.2444
Cooked gari and hold	Unpeeled cassava	-1.5750	.8735	.109	-3.5894	.4394
	Peeled cassava	-7.2650 *	.8735	.000	-9.2794	5.2506
	Peeled and washed cassava	-8.0700 *	.8735	.000	-10.0844	-6.0556
	Grated mash (fermented)	1.500E -02	.8735	.987	-1.9994	2.0294
	Pressed mash before sieving	-5.3900 *	.8735	.000	-7.4044	-3.3756
	Sieved mash	-4.7450 *	.8735	.001	-6.7594	-2.7306
	Fried "gari" from pan	.7700	.8735	.404	-1.2444	2.7844

*The mean difference is significant at the 0.05 level.

the cassava [45] estimated 14.2 mg HCN eq. Average/100 g, 11.3 mg HCN eq. Average/100g and 15.7% loss of cyanogenic glucoside from cassava upon baking, frying and steaming respectively. The fried "gari" from fry pan recorded a CNP of 1.32 mg HCN eq. Average/100 g. The cooled "gari" (hold) had a CNP of 2.09 mg HCN eq. Average/100 g higher than the fried "gari" from pan (hot). This may be due to the absorption of the evaporated cyanogenic glucoside during frying by the emitting moisture of the spread "gari" to cool as it is readily dissolved in water [46].

Conclusion

In conclusion this study shows that there is statistically significant difference in cyanide level (toxicity) changes among "gari" processing steps (Post HOC Test). It also shows that many step(s) contributed to the significant level differences in the cyanide level or toxicity. These findings expose the limiting problems of toxic effect in "gari" in spite of its wide use. Adherence to appropriate Good Manufacturing Practices (GMP) especially along critical points in the "gari" processing chain is essential measure to avoid toxic effects. Cassava promotion in form of short-term mass-campaign focusing only on introducing new varieties and processing methods without regard to the traditional processing methods and effective control points strategies in the processing chain may induce toxic effect.

Conflict of Interest

The authors declare no conflict of interest.

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