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Effectiveness of Traditional Processing Techniques in Reducing Cyanide Levels in Selected Cassava Varieties in Zombo District, Uganda

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Abstract: The root tubers of cassava (*Manihot esculenta* Crantz) contain varying amounts of cyanogenic glucosides which liberate toxic hydrogen cyanide with the resultant effect of poisoning especially the highly cyanogenic cultivars. These include some of the local and improved cassava varieties grown in Zombo district, Uganda among others. Therefore, this study explored the effectiveness of some traditional processing techniques used singly and in combination to reduce cyanide levels in highly toxic root tubers of selected local (*Nyar-anderiano, Nyar-papoga, Nyar-udota, Nyar-pamitu, Bisimwenge, Nyar-matia*) and improved (NASE 3, NASE 9, NASE 14, NASE 19, TME 14 and TME 204) cassava varieties grown in Zombo District, Uganda. The processed and the fresh cassava tubers were analysed for Hydrogen cyanide (HCN) by the standard titration method. The results indicated that heap fermentation followed by sun-drying reduced the cyanide levels in all the selected improved varieties to WHO safe levels for consumption (below 10mg/kg) in the dried chips. Mixed processing methods combining soaking, fermentation and sun-drying reduced the cyanide concentrations in the local varieties (*Nyar-udota, Nyar-anderiano*) and improved variety (NASE 19) to World Health Organisation (WHO) safe levels. None of the processing techniques used singly reduced the HCN levels in the root tubers of the selected cassava varieties to safe levels. Hence mixed strategies combining several traditional cassava processing techniques should be adopted to detoxify the selected cassava varieties of cyanogens. The local cassava varieties (*Bisimwenge*, *Nya-matia*, *Nya-pamitu*, *Nya-papoga*) would require modifications of the combined traditional processing techniques or other more complex processes to detoxify them of HCN before human consumption.

Keywords: Detoxification, Food Safety, Hydrogen Cyanide, Traditional Methods

1. Introduction

Cassava (*Manihot esculenta* Crantz) is a staple food in tropical Africa and its production is on rapid rise in order to feed the fast increasing population [1]. Cassava is now a major food security crop in Uganda and is grown by about 72% of the households in Zombo district [2, 3] with most of the people still depending on the bitter varieties. Unfortunately, the roots and leaves of cassava plant contain cyanogenic glucosides, linamarin (93%) and lotaustralin (7%) which liberate toxic hydrogen cyanide (HCN) on hydrolysis [4-6]. Over 5000 cassava cultivars have been identified with differing cyanogenic glucoside concentrations in the roots ranging from 10 to 500 mg/kg fresh weight and the bitter varieties have higher cyanogens compared to sweet varieties [7-10].

Chronic and acute cyanide toxicity in humans following long-term consumption of cassava with high cyanogenic glycoside content are well recognized problems [11, 12]. The intoxication presents as tropical neuropathy, konzo –an irreversible paralysis of the legs, goitre and cretinism [13-15]. Symptoms of vomiting, stomach pains, nausea, diarrhea, dizziness, weakness, headache and sometimes death also occur [16, 17]. Cyanide poisoning is associated with the consumption of poorly processed cassava and death occurs at lethal HCN dosage of 0.5 to 3.5 mg/kg body weight [5, 11].

Fortunately, cassava roots can be processed by a range of traditional methods to reduce cyanide levels in the cassava products [5, 18]. The methods involve different combinations of drying, soaking, boiling and fermentation of whole or fragmented roots [12, 19]. The common ones used to produce flour in eastern Africa involve either sun drying of the peeled root followed by pounding and sieving, or heap fermentation [20]. Sun drying of peeled cassava roots is a necessary step before processing it to make cassava flour while fermentation of roots is either done under water or mould fermentation in heaps [9]. However, the effectiveness of the various traditional processing methods is low when used singly compared to combined techniques [21, 22]. Therefore, cassava processing for the future should improve the traditional methods [23]. One strategy is to use combination of several traditional processing techniques [22, 24].

Oloya et al. [25] found out that total HCN concentrations were much beyond the FAO/WHO recommended safe value of <10 mg/kg in the peeled fresh root tubers of local cassava varieties (*Nyar-anderiano, Nyar-papoga, Nyar-udota, Nyar-pamitu, Bisimwenge, Nyar-matia*) and improved varieties (NASE 3, NASE 9, NASE 14, NASE 19, TME 14 and TME 204) grown in Zombo District, Uganda. As a result, adequate detoxification of the varieties before consumption was recommended. Therefore, this study explored the effectiveness of some traditional processing techniques used singly and in combination to reduce cyanide levels in the root tubers of the above selected local and improved cassava varieties grown in Zombo District, Uganda.

2. Materials and Methods

2.1. Sampling, Processing and Analysis of Cyanide in Cassava

The description of the study area, sampling process including the traditional processing techniques are presented in Andama and Lejju [21], Andama et al. [22] and Oloya et al. [25]. The processed cassava tubers as well as the fresh tubers were analysed for HCN in the Government Analytical Laboratory (GE058/07), Kampala using the standard titration method [26]. The details of the analysis are found in Andama, and Lejju [21] and Oloya et al. [25]. The cyanide levels in peeled fresh root tubers of local cassava cultivars (*Nyar-anderiano, Nyar-papoga, Nyar-udota, Nyar-pamitu, Bisimwenge, Nyar-matia*) and improved cultivars (NASE 3, NASE 9, NASE 14, NASE 19, TME 14 and TME 204) are presented in Oloya et al. [25] while the extent to which the various traditional processing techniques (single and combined) removed HCN are detailed in Andama and Lejju [21] and Andama et al. [22]. The amounts of HCN in the local and improved cassava varieties on subjecting them to the various traditional processing techniques were then obtained.

2.2. Data Analysis

The amounts of the HCN levels in tubers under the various processing techniques were tabulated with variations among them compared using one-way ANOVA (F test). Least Significant Difference (LSD) multiple tests were used to determine significant differences in HCN levels between the processing methods at 5% level of significance. The data analyses were done by using Microsoft Excel 2007 and SPSS 20 computer packages.

3. Results and Discussion

The traditional processing techniques (single and combined) are summarized in Table 1.

Table 1. *The Traditional Cassava Processing Techniques.*

Number Code	Processing Techniques
1	[a]Aquatic fermentation for 4 days
2	[a, b]Sun drying small slices for 5 days at 28 to 40°C.
3	[b] Soaking for 4 days
4	[a] Heap fermentation for 4 days
5	[a]Soaking for 4 days, sun drying for 5 days at 28 to 40°C.
6	[a]Soaking and aquatic fermentation for 4 days, sun drying for 5 days at 28 to 40°C.
7	[a]Four days heap fermentation of partially sun-dried tubers for 3 hours, sun drying for 5 days at 28 to 40°C.

Source: [a][21]; [b][22]

The mean cyanide (total HCN) in the root tubers of the local and improved cassava varieties under the various traditional cassava processing techniques (Table 1) significantly varied from one another ($F_{(6, 77)}$=18.670, p = 0.000). The mean cyanide (total HCN) in the tubers of the local and improved cassava varieties under the combined traditional cassava processing techniques (Table 2) were significantly lower than those under the single processing methods (p<0.00) using the Least Significant Difference (LSD) post hoc multiple comparisons.

Aquatic fermentation of the root tubers for 4 days was the least effective single technique in detoxifying root tubers of the local and improved cassava varieties while heap fermentation of root tubers for 4 days followed by sun drying for 5 days at 28 to 40°C was the most effective combined technique (Table 2).

Heap fermentation of partially sun dried peeled root tubers for 4 days followed by sun drying at 28 to 40°C for 5 days reduced HCN concentrations in all the improved cassava varieties (NASE 19, TME 14, NASE 9, TME 204, NASE 3, NASE 14) and the local varieties (*Nyar-udota, Nyar-anderiano*) with higher initial HCN concentrations (88.5 to 116.51 mg/kg DW) to WHO safe level of less than 10 mg/kg [27]. In general, the HCN concentrations in all the selected cassava varieties in Zombo district (tribe Alur) with initial total HCN levels in the range 88.5-316.69 mg/kg were reduced to 8.43-30.15 mg/kg in the dried chips using the combination of heap fermentation and sun-drying method. These values are supported by previous studies of Montagnac et al. [24] and Essers [28]. According to Montagnac et al. [24], heap fermentation process involving drying superficially peeled cassava roots, heaping, and covering them for 3 days of incubation, and then crushing, sun-drying, and pounding into flour after prior removing of fungal mycelium resulted in a high loss of cyanogens. Using the same processing method, Essers [28] obtained 8 to 41 mg HCN equivalents/kg DW in the final product (flour) from tubers with initial total cyanogen levels in the range 237 to 550 mg HCN equivalents/kg DW in cassava flour of 6 Alur households in Uganda. The extent of removal of HCN from the root tubers in this study by heap fermentation of partially sun dried peeled root tubers for 4 days followed by sun drying at 28 to 40°C for 5 days (i.e. 93%) was close to values obtained by the previous researchers. For example, Dufour [18] reported 95% effectiveness of combined processing methods (sun-dry, ferment, crush, sun-dry, pound, sieve for 6 days) in removing cyanide in cassava tubers in East Africa while Lambri et al. [29] recorded 90% breakdown of the cyanogens content in cassava roots processed by a combination of fermentative treatment and a single stage of drying.

Table 2. *Total HCN (mg/kg) in Cassava Varieties Under the Traditional Cassava Processing Techniques.*

Cassava Type	Cassava Variety	[a]Initial HCN (mg/kg)	HCN in tubers under Processing techniques (coded 1 to 7)							Guideline [27]
			Single				Combined			
			1	2	3	4	5	6	7	
Improved	NASE 19	101.84	100.59	58.48	54.31	50.38	10.95	9.70	7.22	< 10
	TME 14	105.60	104.30	60.64	56.32	52.24	11.35	10.05	7.49	
	NASE 9	107.33	106.01	61.63	57.24	53.10	11.54	10.22	7.61	
	TME 204	107.97	106.64	62.00	57.58	53.41	11.61	10.28	7.66	
	NASE 3	113.25	111.86	65.03	60.40	56.02	12.17	10.78	8.03	
	NASE 14	116.51	115.08	66.90	62.13	57.64	12.52	11.09	8.26	
Local	*Nyar-udota*	88.50	87.41	50.82	47.20	43.78	9.51	8.43	6.27	
	Nyar-anderiano	90.00	88.89	51.68	48.00	44.52	9.68	8.57	6.38	
	Bisimwenge	181.48	179.25	104.21	96.78	89.78	19.51	17.28	12.87	
	Nya-matia	257.28	254.12	147.73	137.21	127.28	27.66	24.49	18.24	
	Nya-pamitu	275.97	272.58	158.46	147.17	136.52	29.67	26.27	19.57	
	Nya-papoga	316.69	312.79	181.84	168.89	156.67	34.04	30.15	22.45	
Mean			153.29	89.12	82.77	76.78	16.68	14.78	11.00	

[a][25]; Refer to Table 1 for the number codes of the processing techniques.

Furthermore, soaking and aquatic fermentation for 4 days followed by sun drying for 5 days at 28 to 40°C reduced the HCN levels in the root tubers of the improved variety (NASE 19) and local varieties (*Nyar-udota, Nyar-anderiano*) with higher initial HCN values of 101.84, 88.50 and 90.0 mg/kg respectively by 90.48% to 9.70, 8.43 and 8.57mg/kg respectively, all below 10 mg/kg (safe level). On the other hand, HCN levels were reduced to 9.51 and 9.68 mg/kg (89.25% decrease) in the dried chips of root tubers of *Nyar-udota* and *Nyar-anderiano* respectively on subjecting them to soaking for 4 days followed by sun drying for 5 days at 28 to 40°C. According to Montagnac et al. [24], soaking fresh cassava roots for 3 days followed by 3 days of drying resulted in the removal of 85.9% of total cyanogens. The slight differences in the total HCN removed from the root tubers in the present study as opposed to values obtained by other researchers in the previous studies could be associated to the additional processing methods and number of days employed among other factors. Montagnac et al. [24] further stated that the residual level of cyanogens in cassava products will differ depending on the nature and duration of the processing methods. Oke [30] earlier on reported that increasing the soaking and fermentation times improves the cyanogens removal process in cassava. For the fermentation of cassava roots soaked in water, microbial growth is essential for the efficient elimination of cyanogens [31].

All the investigated combined traditional processing methods reduced the cyanide levels in the tubers of the local (sweet) varieties (*Nyar-udota, Nyar-anderiano*) to WHO safe levels in the dried chips while the rest of the local (bitter) varieties (*Bisimwenge, Nya-matia, Nya-pamitu, Nya-papoga*) retained slightly higher HCN levels (> 10mg/kg) despite the processing. This is associated with the higher levels of cynanide in the bitter local varieties than the sweet local varieties. According to Sundaresan et al. [32], cassava varieties with HCN ranging from 100 to 450 mg HCN equivalents/kg fresh weight (FW) are categorized as bitter varieties while those with cyanide <100 mg HCN equivalents/kg FW are non-bitter (sweet) varieties. Furthermore, Westby [9] stated that bitter root tubers are associated with high cyanogens while sweet roots have low cyanogens. Hence subjecting the sweet local varieties to the combined processing techniques reduced the HCN levels to FAO/WHO safe levels but not the bitter local varieties.

According to Lancaster and Coursey [33], "sweet" cassava varieties usually require simpler preparation methods like boiling or roasting, or sometimes consumed while raw although they can also be processed by more complex methods. The selected sweet varieties in this study contained higher HCN (> 10mg/kg, safe level) in their fresh root tubers hence not suitable for consumption in raw form and thus required some complex processing techniques (like the combinations of soaking, fermentation and sun-drying) to reduce their cyanide concentrations. The local cassava varieties (*Bisimwenge, Nya-matia, Nya-pamitu, Nya-papoga*) require more modifications of the combined traditional processing techniques or some other complex processing methods to reduce the HCN concentrations to safe levels for consumption. Lancaster and Coursey [33] further reported that "bitter" varieties are usually subjected to more complex processing methods due to their higher HCN levels.

The high HCN in local cassava cultivars (*Bisimwenge, Nya-matia, Nya-pamitu, Nya-papoga*) compared to the improved cultivars even after processing by similar methods is associated with genetic factors. This is supported by previous studies by Salvador et al. [11], Oloya et al. [25], Ravindran [34], Gleadow and Woodrow [35] who reported that the variation in HCN content in cassava is strongly influenced by genetic factors. According to Hahn et al. [36], low HCN content in cassava is controlled by minor recessive gene complex, and this probably explains the variation in HCN content in the cassava varieties with improved varieties most likely having more of the gene complex than the local varieties except in *Nyar-udota* and *Nyar-anderiano*.

None of the traditional processing techniques used singly (i.e., aquatic fermentation for 4 days; sun drying small slices at 28 to 40°C for 5 days; soaking for 4 days; heap fermentation for 4 days) reduced the HCN levels in the root tubers of all the selected cassava varieties to safe levels (< 10mg/kg). This is attributed to the ineffectiveness of these single processing techniques compared to the combined methods. Generally, drying is an inefficient means of detoxification of cassava varieties with high initial cyanogen glucoside content [24]. Ernesto et al. [14] obtained significantly lower total cyanogens contents in cassava flour processed by heap fermentation followed by drying than cassava flour processed by sun-drying only while Hahn [23] reported a low decrease (10-30%) in total cyanide content of cassava chips processed through fast air drying. Furthermore, Oloya et al. [37] obtained relatively low detoxification of *Nyar-udota* cassava variety of HCN (64.7%) after ten days of fermentation. According to Montagnac et al. [24], high efficiency of cyanogen removal can be attained through combination of the efficient and the less efficient processing techniques. Hence combined strategies decrease the total cyanide levels much further as they are effective in removing the different forms of the HCN. Cyanide in cassava can be found as bound glucosides, cyanohydrins, and free cyanide [38] with each reacting differently to processing techniques that remove cyanide [12]. For example, sun-drying is effective in removing free HCN while soaking and heap fermentation are good at removing bound HCN (cyanogenic glycosides) [21, 22].

4. Conclusions

Mixed traditional cassava processing techniques combining heap fermentation and sun drying are effective in detoxifying all the selected improved cassava cultivars including the local varieties (*Nyar-udota*, *Nyar-anderiano*) with low cyanide amounts to safe levels for human consumption. Combinations of soaking, aquatic fermentation and sun drying are also safe in reducing cyanide levels in the local varieties (*Nyar-udota*, *Nyar-anderiano*) and improved variety (NASE 19).

However, all the investigated mixed strategies are unsafe for processing most of the local cassava cultivars (*Bisimwenge*, *Nya-matia*, *Nya-pamitu*, *Nya-papoga*) with high cyanide levels. Hence these local cassava varieties would require modifications of the combined traditional processing techniques or other more complex processes to detoxify them of HCN before human consumption.

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