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Potential of Fermentation in Detoxifying Toxic Cassava Root Tubers

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Abstract: Quantitative determination of hydrogen cyanide (HCN) content in a toxic local cassava variety "Rutuga" with an initial total HCN of 16.65%, free HCN of 9.19% and bound HCN (cyanoglycosides) of 7.46% in the fresh peeled root tubers was done to assess the effectiveness of aquatic and terrestrial (heap) fermentation in detoxifying cassava root tubers for obtaining dried product used in making flour. This was indirectly done by getting the difference in HCN content that remained after processing the root tubers using some traditional processing techniques. The findings indicated that aquatic fermentation in water from river Rwizi for 4 days only removed 1.23% of total HCN, 0.05% of free HCN and 2.68% of Cyanoglycosides (bound HCN) while terrestrial (heap) fermentation for 4 days removed 50.33% of total HCN, 20.84% of free HCN and 86.66% of Cyanoglycosides (bound HCN). Therefore, terrestrial (heap) fermentation has a higher potential in removing total HCN, free HCN and cyanoglycosides (bound HCN) than aquatic fermentation, especially in water from river Rwizi.

Key words: Aquatic fermentation, cassava, hydrogen cyanide, terrestrial fermentation.

1. Introduction

Cassava is widely cultivated in many parts of the world [1]. The carbohydrate rich but low in protein storage roots represent an important energy source and are a staple foodstuff for more than 500 million people throughout tropical Africa, Latin America and parts of Asia [2].

Cassava is the second most important staple crop in Africa after maize and a major staple for more than 200 million people (over 70% of the population in the Eastern and Central Africa region) [3]. The crop is grown in 39 African countries of which Nigeria, DR Congo, Angola, Ghana, Mozambique, Tanzania and Uganda are among the top ten producers in the world [3]. Both cassava roots and leaves can be used as food, but economically the roots are usually more important, although in parts of some African countries, the leaves may be as important as or more important than the roots [4]. The Food and Agriculture Organisation (FAO) estimated that Africa harvested over 117 million metric tons of cassava in 2007 [3]. This importance is largely a consequence of cassava's agronomic advantages, particularly its high yield of carbohydrate even on poor soils, good tolerance to drought, and it is relatively resistant to pest infestation and disease, and because it can be stored in the ground until required [5].

In Uganda, the crop is grown in most parts of the country and about 80% of the population depends on cassava for their livelihoods [3]. Percentage contribution of cassava roots to the total energy intake in populations of Uganda in the period 1990-1992 was 17.1% [6].

Total world cassava use is expected to increase from 172.7 million ton to 275 million ton in the period 1993-2020 by using the International Food Policy Research Institute's (IFPRI's) baseline data [4]. A higher prediction of demand and production growth

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puts the 2020 production at 291 million ton [7]. In both projections cassava use in Africa is equivalent to 62% of total world production [4].

However, if the contribution that cassava can make to the livelihoods of poor people is to be increased, there is also a need to consider its post harvest handling, processing and marketing [4].

The tubers of cassava (*Manihot esculenta*), a high-carbohydrate, staple food in many tropical countries, contain high levels of cyanogenic glycosides which are not toxic in themselves but are readily broken down to give off volatile poisons when the plant is crushed [8]. Cyanogenic glycosides release the well-known poisonous gas Hydrogen Cyanide (HCN) [8]. All cassava tissues, with the exception of seeds, contain the cyanogenic glycosides linamarin (> 90% total cyanogen) and lotaustralin (< 10% total cyanogens) [9]. Leaves have the highest cyanogenic glycoside levels (5.0 g linamarin/kg fresh weight), whereas roots have approximately 20-fold lower linamarin levels. Total root linamarin levels range between 100 and 500 mg linamarin/kg fresh weight for low- and high-cyanogenic cultivars, respectively. No cassava cultivars, however, lack cyanogenic glycosides [10] and cyanogenic glucosides are responsible for the toxicity of unfermented roots and leaves of cassava [11, 12].

According to Gershenzon [8], cyanogenic glycosides are not normally broken down in the intact plant because the glycoside and the degradative enzymes are spatially separated, in different cellular compartments or in different tissues. Linamarin is stored in vacuoles of the cassava cells [9] and linamarase is situated in the cell wall [13] physically separated from linamarin. Cyanogenesis is initiated in cassava when the plant tissue is damaged. Rupture of the vacuole releases linamarin, which is hydrolyzed by linamarase, a cell wall-associated β-glycosidase [9].

According to Westby [4], chemically, linamarin is stable, soluble in water and resists boiling in acid. Acetone cyanohydrin is also soluble in water and has a boiling point of the order of 82 °C. Free HCN is volatile at 25.7 °C and so is rapidly volatilized at tropical ambient temperatures.

The essential features of good processing are sufficient tissue disruption to allow endogenous linamarase to react with linamarin and then favourable conditions for the breakdown of acetone cyanohydrin, or, conditions under which the compound will volatize spontaneously [4]. Various health disorders are associated with the consumption of cassava, which contains residual cyanogens including goitre, dwarfism, tropical ataxic neuropathy [14], hyperthyroidism and konzo [15, 16]. The traditional detoxification methods employed to remove cynogenic glycosides from cassava are therefore not completely effective [8]. One strategy to reduce the cyanide content of processed cassava is to improve processing methods used for conversion of roots to storable cassava products such as flour [1].

In this study, the contribution of aquatic and terrestrial (heap) fermentation in removing total HCN, free HCN and bound HCN (cyanoglycosides) was indirectly determined by getting the difference in concentrations of HCN left in root tubers of a toxic cassava variety "Rutuga" processed by some traditional techniques used in making flour. "Rutuga" is literally interpreted as strangler or killer in English.

2. Materials and Methods

2.1 Materials

The research required the use of materials such as airtight polythene bags, a knife, thermometer, refrigerator, containers (basins), river water, distilled water, distillation flask, microbiuret and other distillation apparatus. The main reagents used during laboratory analysis were sodium hydroxide, 5% potassium iodide solution, 0.02 N silver nitrate, and distilled water.

2.2 Methods

The cassava was obtained from Bwambara

sub-county in Rukungiri District, Uganda. Packed in airtight polythene bags and transported to Mbarara University of Science and Technology laboratory for processing and Government analytical laboratory for analysis. The cassava samples were processed by the following traditional processing techniques.

A. Fresh cassava sample (peeled raw cassava root tubers). Cassava roots are usually peeled prior to processing [4].

B. Sun drying peeled root tubers cut into small slices at temperature range of 28 to 40 °C for 5 days.

The cutting of the peeled root tubers into small slices was done using a knife by hand and then sun dried. According to Westby [4], size reduction of fresh roots is usually by grating, where machines are not available, grating is done by hand but this is a very labour-intensive process [4].

The major methods of flour made in Africa involve sun drying of peeled roots followed by crushing in a pestle and mortar and sieving. This method retains 25% to 33% of the original linamarin present [17]. Sun-dried products are the most common types of processed products in Africa [4]. Drying over fire is practised in some places. One problem of sun drying is that drying time is long [4]. In a study conducted by Wareing et al. [18] on a dried product in Ghana, kokonte, it took 7-12 days to dry during the dry season and 8-14 days during the rainy season. Various methods are available for improving drying to produce a better quality product. These include modifications to the size and shape of cassava pieces, use of inclined trays or concrete drying floors [19]. For this study, peeled cassava root tubers were cut into small pieces using a knife to facilitate faster drying. Therefore, 5 days were sufficient for complete sun drying of the cassava root tubers during the rainy season.

C. Soaking peeled root tubers in cold distilled water for 4 days followed by sun drying at temperature range of 28 to 40 °C for 5 days.

D. Soaking peeled root tubers in cold undistilled water (river water) for 4 days during which time aquatic fermentation occurred and then sun dried at temperature range of 28 to 40 °C for 5 days.

According to Westby [4], fermentation of cassava roots under water is conducted across Africa from Sierra Leone to Tanzania and a variety of products are produced including wet paste and dried flours. Roots are soaked in water with or without peeling for typically 3-5 days [4]. The fermentation causes the roots to soften [20], which means that they can be easily broken up by hand into small pieces and sun dried or passed through a sieve to remove fibre, leaving a smooth paste [4]. At the start of the fermentation there is a mixed microbial flora consisting of *Bacillus* spp., *Leuconostoc* spp., *Klebsiella* spp., *Corynebacterium* spp., *Lactobacillus* spp., *Aspergillus* spp., *Candida* spp. and *Geotrichum* spp. The final fermentation is, however, dominated by lactic acid bacteria and yeasts [21]. *Clostridium* is thought to be the origin of butyrate, which imparts a typical odour to the product [22].

E. Partial sun drying of peeled root tubers at temperature range of 28 to 40 °C for 3 hours followed by heaping them for 4 days to enable terrestrial fermentation by growth of moulds and then sun dried at temperature range of 28 to 40 °C for 5 days. The cassava samples were removed from sun drying 3 hours earlier on the fifth day in order to compensate for the 3 hours partial sun drying before subjecting them to terrestrial fermentation.

According to Bradbury [23], other methods (such as heap fermentation) are known to remove twice as much linamarin as sun drying does, but still 12.5% to 16.5% of linamarin is retained because of the lack of intimate contact between the linamarin and the hydrolyzing enzyme linamarase. Fermentation is therefore a detoxication process [14]. Heap fermented cassava products are produced in Tanzania [24], Uganda and Mozambique [25]. This type of fermentation is achieved by heaping peeled roots and leaving them to ferment naturally. Essers and M.J.R Nout [26] reported the isolation of *Rhizopus* spp.,

Mucor spp., *Penicillium* spp. and *Fusarium* spp. on fermented cassava roots. According to Kobawila et al. [14], many strains of isolated lactic bacteria possess linamarase activity including *Lactococcus lactis*, *Leuconostoc mesenteroides*, *Lactobacillus plantarum* and one strain of *Lactobacillus* sp. They contended that elimination of cyanogenic glucosides is thus ensured, at least partially, by the action of the bacterial enzymes. Some of the lactic acid bacteria such as *Lactobacillus coprophilus*, *Lactobacillus delbrueckii*, *Lactobacillus fermentum*, *Leuconostoc mesenteroides*, *Lactobacillus plantarum* and *Lactococcus lactis* have the capacity to resist to strong concentrations of free cyanide, from 200 to 800 ppm and these lactic bacteria have a selective advantage with regard to the others in the fermentation medium [14].

The efficiency of these processes and of any post-fermentation processes in reducing cyanogens dictates the safety of the product [4]. Market studies in the Lake Zone of Tanzania have indicated that different moulds can have an impact on the value of the commodity. When growth of potentially mycotoxigenic fungi occurs, there is the possibility of mycotoxin formation [4]. Mycotoxins are extracellular zootoxic metabolites (exotoxins) produced by filamentous fungi (moulds) in foods consumed by man or animals [4]. According to Westby [4], mould growth on fermented cassava products has so far not been associated with mycotoxin formation. Essers [25] reported that in his investigation of the solid substrate fermentation of cassava in Uganda, the Ames test for mutagenicity and cytotoxicity was negative in all of the tested flours and aflatoxins were absent in ten screened samples.

The cassava samples were analysed in the Government Analytical Laboratory (GE058/07), Kampala by the standard method [27] described as follows;

Crushed root material (10 to 20 g) was put in a distillation flask, about 200 mL of water was added and allowed to stand two to four hours, in order to set free all the bound hydrocyanic acid, the flask meanwhile was being kept connected with an apparatus for distillation. It was distilled with steam and 150 to 200 mL of distillate collected in a solution of 0.5 g of sodium hydroxide in 20 mL of water. To 100 mL of distillate (it is preferable to dilute to a volume of 250 mL and titrate an aliquot of 100mL) was added 8 mL of 5% potassium iodide solution and titrated with 0.02 N silver nitrate (1 mL of 0.02 N silver nitrate corresponds to 1.08 mg of hydrocyanic acid) using a microbiuret. The end point was indicated by a faint but permanent turbidity, which was easily recognized, especially against a black background.

2.3 Data Analysis

Tables and bar graphs were generated from the results of laboratory analysis by using computer packages, Microsoft Excel, OriginPro 7.5 and SPSS 16. The results are presented below.

3. Results and Discussion

The percentage of HCN left in the cassava samples were reported since the absolute amounts would not be established because of the inevitable losses of HCN due to its high volatility amidst the care during the handling of the samples. However, the losses were uniform for all the samples as they were handled in similar manner. The results of analysis are shown in Table 1.

Values for A were taken as the initial levels of total HCN, free HCN and cyanoglycosides (bound HCN) in the fresh peeled cassava root tubers. Percentage of hydrogen cyanide removed by the traditional cassava processing techniques represented by the letters B, C, D and E were calculated by subtracting each of the values of B, C, D and E from the initial value (A) and expressing the differences as a percentage of the initial values. The results are shown in Table 2.

The percentage HCN removed by only aquatic fermentation of the peeled root tubers for 4 days in water from river Rwizi was obtained by subtracting

Table 1 HCN contained (%) in the cassava samples.

Samples	HCN (%)		
	Total HCN	Free HCN	Cyanoglycosides (bound HCN)
A (Initial values)	16.650	9.190	7.460
B	9.560	2.350	7.210
C	1.790	0.435	1.355
D	1.585	0.430	1.155
E	1.180	0.435	0.745

Table 2 HCN removed (%) by the traditional processing techniques.

Technique	HCN removed (%)		
	Total HCN	Free HCN	Cyanoglycosides (bound HCN)
B	42.583	74.429	3.351
C	89.249	95.267	81.837
D	90.481	95.321	84.517
E	92.913	95.267	90.013

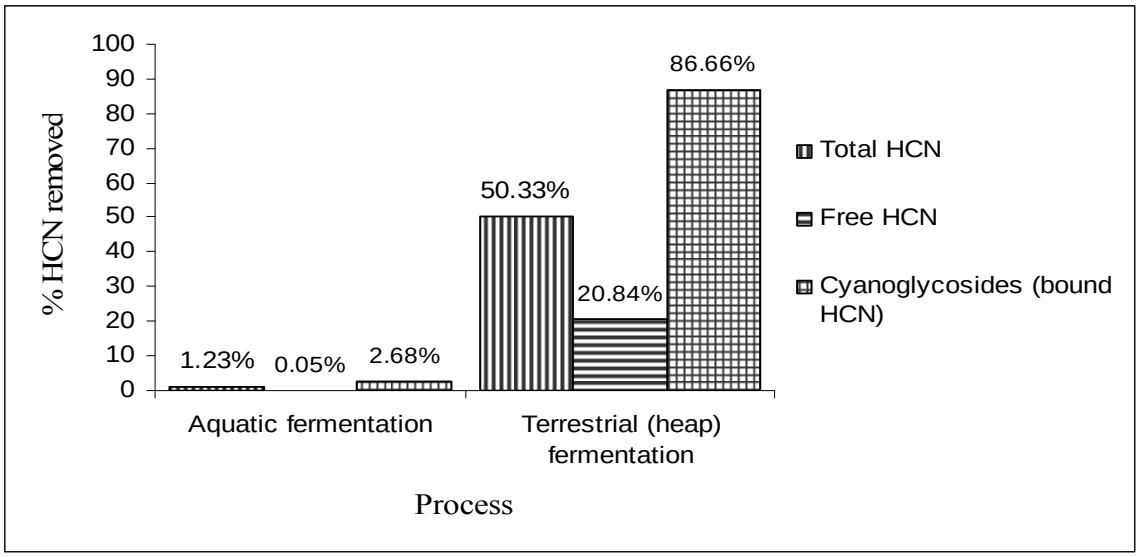

Fig. 1 % HCN removed by aquatic and terrestrial (heap) fermentation.

the amounts of HCN removed by technique C from that by technique D. Similarly, the percentage HCN removed by only terrestrial fermentation (heap-fermentation) of peeled root tubers for 4 days was obtained by subtracting the amounts of HCN removed by technique B from that by technique E. These results are graphically represented in Fig. 1.

Heap fermentation removed 86.66% of cyanoglycosides and maintained 13.34% of cyanoglycosides in the processed cassava root tubers. This amount (13.34%) of cyanoglycosides left in cassava root tubers processed by heap fermentation fits in the range of linamarin retained in heap fermented cassava of 12.5% to 16.5% reported by Bradbury [23]. Since cassava contains the cyanogenic glycosides linamarin (> 90% total cyanogen) and lotaustralin (< 10% total cyanogens) according to McMahon et al. [9], the 86.66% of cyanoglycosides removed by heap fermentation was mainly the removal of linamarin.

Fermentation possibly causes more cell rupture which easily brings about contact between substrate cyanoglycosides and the enzymes, thus leading to the breakdown of cyanoglycosides to liberate free HCN.

In the case of the heap-fermented products, microbial growth contributes to cyanogens reduction by softening the cassava roots which enhances the contact between endogenous linamarin and linamarase [25]. According to Kobawila et al. [14], the linamarase produced by the cassava lactic acid bacteria, notably *Leuconostoc mesenteroides* and *Lactococcus lactis*, and the endogenous linamarase contribute to the process of detoxification.

When roots are soaked in water, the fermentation enables softening of the roots which has the combined effect of enabling linamarin and linamarase to mix and also to enable leaching of the cyanogens [20]. For the heap-fermented cassava, heat energy generated as one of the by-products of fermentation an anaerobic respiration process also removed some of the free HCN by volatization. Heat energy generated as one of the by-products of fermentation was less felt for aquatic fermentation due to the cold temperature of the water and possibly removed little of the free HCN by volatization compared to heap-fermented cassava.

However, there was little aquatic fermentation of the cassava samples put in cold water from river Rwizi probably due to less aquatic fermenters such as microbial flora like *Bacillus* spp., *Leuconostoc* spp., *Klebsiella* spp., *Corynebacterium* spp., *Lactobacillus* spp., *Aspergillus* spp., *Candida* spp. and *Geotrichum* spp., lactic acid bacteria and yeasts among others due to the poor quality of river Rwizi because of pollution. According to Mukwaya [28], the river has declined both in quantity and quality over the past ten years as seen by brown water.

4. Conclusions

Terrestrial (heap) fermentation has a higher potential in removing total HCN, free HCN and cyanoglycosides (bound HCN) than aquatic fermentation especially in water from river Rwizi.

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