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Comparative Study of Different Processing Methods for the Reduction of Cyanide from Bitter Cassava Flour

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ABSTRACT
Bitter cassava (*Manihot esculanta*) is one of the most important staple root crops planted in Nigeria. Substantial quantity of anti-nutrient factor cyanogenic glucoside that interferes with digestion and injurious to human health is present in it. This work is aimed at comparing different processing methods in the reduction of Hydrogen cyanide from bitter cassava flour. The fermentation and mechanical pressing-fermentation processing methods were used in the reduction of cyanide cassava flour. The flour was produced from bitter cassava root obtained from the three geo-political zones of Niger state, Nigeria labelled as zone A (Bida), B (Minna) and C (Kontagora) respectively. Twenty samples from each zone were used for the study. The initial average cyanide concentrations in the roots from each political zone were 106.44mg/kg, 94.99mg/kg and 102.59mg/kg respectively. The fermentation processing method reduces the cyanide concentration level from zone A, B, and C to 6.35mg/kg, 5.57mg/kg and 7.16mg/kg while the mechanical pressing-fermentation processing method decreases it to 4.31mg/kg, 4.48mg/kg and 4.15mg/kg from the aforementioned zones respectively. The result of the two processing methods reduced the cyanide concentration to the barest minimum level required by World Health Organization (10mg/kg). The mechanical pressing-fermentation method removed more cyanide when compared to fermentation processing method.

Keywords: Cyanide, Fermentation, *Manihot esculenta*, Mechanical press-fermentation

INTRODUCTION

Bitter cassava (*Manihot esculenta*) also called *yucca* or *manioc*, is a woody shrub of the *euphorbiaceae* or spurge family. It is one of the most important staple food crops in Tropical Africa. It plays a major role in efforts to alleviate the African food crisis because of its efficient production of food energy, year-round availability and tolerance to stress condition (Hahn and Keyser, 1985). Cassava is a major source of carbohydrate. The flour made of the roots is called *tapioca*. Cassava is the third largest source of carbohydrates for human food in the world in terms of food calories produced per unit area per unit of time, significantly higher than other staple crops. Cassava is classified as sweet or bitter depending on the level of toxic cyanogenic glucosides (Oyewole and Afolabi, 2001). The sweet cultivars can produce as little as 20 milligrams of cyanide per kilogram of fresh roots, whereas bitter ones may produce more than 50 times as much (Kamalu and Oghome, 2012).

Cassava, like other foods, also has anti-nutritional and toxic factors. The most important is the cyanogenic glucosides of cassava, which are linamarin and lotaustralin. The hydrolysis of these compounds releases hydrocyanic acid (HCN) which is of great concern for human and animal consumption. The concentration of cyanide varies considerably between varieties and climatic conditions (Kamalu and Oghome, 2012).

Improper preparation of bitter cassava causes a disease called *konzo* and it is linked to chronic pancreatitis. Furthermore, cassava roots and products that contain cyanogenic glycoside have also been linked to cyanide poisoning (Essers *et al.*, 1996; Kamalu and Oghome, 2012). Nevertheless, farmers often prefer the bitter varieties because they deter pests, animals and thieves (Oke, 1982). Cassava grown during drought and direct sun-drying of the roots are high in cyanide levels (Muzanial *et al.*, 2000). The combination of techniques such as crusting, blanching, pressing and cooking is an efficient way to produce safe bitter cassava products such as cassava flour and *gari* for consumption (Agbor and Mbome, 2006).

The processed bitter cassava can be used in various ways such as glucose, syrup, glue, production of ethanol, *tapioca*, composite bread, starch production among others (IITA, 2005). Cyanide is a very poisonous chemical which is usually found jointly with other chemicals to form cyanide compounds. Examples of cyanide compounds are: Hydrogen cyanide (HCN), Potassium cyanide (KCN), and some cyanide compound are produced by the action of some cyanide compound bacterial, fungi and algae and it is found in a number of foods and plants (Collard and Levis, 1959). The concentration of HCN in cassava tubers ranges from 10 – 490 mg/kg tuber (Conn, 1969). The hydrocyanic acid (HCN) is lethal if more than 0.1 g of it is contained in the food eaten by an individual at any time (Owueme, 1978). The bitter variety of *manihot* root is used to treat diarrhoea and malaria. The leaves are used to treat hypertension, headache and pain. Cubans commonly use cassava (bitter) for treating irritable bowel syndrome. As cassava is a gluten-free natural starch, there have been increasing incidences of its appearance in western food as a wheat alternative for celiac disease patients (FAO/WHO, 1991).

Owing to the presence of the cyanogenic glycoside in cassava, various processing methods are employed to bring about a reduction in the toxicity of the roots. Studies on a wide variety of traditional cassava processing methods have been adopted. Some of these methods include maceration, soaking, boiling, fermentation, drying and combination of these methods. Soaking of cassava for at least four hours is sufficient for cyanide removal, but soaking for 18 to 24 hours can remove half the level of toxin.

Improvement of cassava processing and utilization techniques would greatly increase income and living standards of peasant farmers and the urban poor, help improved human and animal nutrition. This work is aimed at comparing the different processing methods in the reduction of hydrogen cyanide (HCN) from bitter cassava flour using the fermentation and mechanical pressing-fermentation processing methods.

MATERIALS AND METHODS

20 tuberous root samples per zone were obtained from the local market situated in each of the three geo-political zones (Bida, Minna and Kontagora) in Niger State, Nigeria. Randomized sampling technique was adopted in selecting the representative sample for the research. All chemicals used were of analytical grade. Other equipment used includes a mechanical crusher (Model: ED-5, Thomas Mill), a digital weighing balance (Seaforx, England) and a spectrophotometer (Model: DX521, Delux, England).

Sample Preparation

Cassava root tubers from the three geo-political zones A, B and C were washed with clean water in a bowl to remove the dirt material such as sand, debris, mould that may cause contamination. The roots were peeled to remove the unwanted outer skin. The peeling process was done manually using knife. After the peeling process, they were washed and sliced and made ready for cyanogenic reduction using fermentation method while for the mechanical press-fermentation processing method, the sample was crushed using mechanical crusher.

Fermentation Processing Method

The sliced roots from zone A were soaked in a container containing water to enhance microbial growth necessary to effect cyanogenic reduction. The soaked roots were left for 5 days for the fermentation of the pulp to take place. The central fibres of the fermented roots are manually removed and the recovered pulp was mashed using laboratory pestle. The supernatant containing soluble cyanide was decanted at every 24 hours interval for 5 days fermentation period. After the pulp has been dewatered using a sack, it become lumpy. The lumps were broken manually and sun dry for further fermentation and draining of the excess moisture. This process effectively reduces the cyanide content. The same procedure was applied for samples from zone B and C respectively.

Mechanical Press - Fermentation Processing

The crushed roots were packed into a clean sack and subjected to mechanical press to leach out the juices which contain larger percentage of cyanide. The pressed roots were fermented in sack for 24 hours (overnight) at room temperature to activate the activities of microbial growth. The activities of microbial growth reduce the hydrocyanide content of the bitter cassava through leaching out cyanide content into the water. The fermentation was carried out overnight (24 hours) to retain all the necessary nutrients embedded in it. The resultant pulp was sifted in a sieved to remove fibrous material and then sundried. The final product was pulverised into cassava flour. The same procedure was applied to samples from zone B and C respectively.

Determination of Potassium Cyanide (KCN) Concentration Curve

1g of picrate and 5g of sodium carbonate were weighed using a digital weighing balance (Seaforx, England) into a beaker containing 10cm^3 of warm water. The solution was stirred and made up to 200 ml with distilled water. The resultant yellowish solution is known as the alkaline picrate solution (Oke, 1982). 2.494g of potassium cyanide (KCN) was weighed using digital weighing balance (Seaforx, England) and dissolved in 1000cm^3 distilled water using volumetric flask. The standard solution was measured into a 100cm^3 volumetric flask. Distilled water was added to each standard solution and made up to 100cm^3 to give a concentration of 5, 10, 20, 60, 80 and 100mg/kg respectively. The Potassium Cyanide calibration curve was prepared where 20cm^3 of each insipid sample of KCN was measured into a test tube and 80cm^3 of alkaline picrate was added to each sample. The mixture was boiled for 20 minutes and there was a colour change from yellowish to reddish brown, the colour change indicates the presence of cyanide. There was colour variation due to variation in concentration. After the colour development the absorbance of each concentration were read using a spectrophotometer (Model: DX521, Delux, England) at a wavelength of 490nm. The absorbance values were plotted against concentration to obtain cyanide concentration curve.

Determination of Cyanogenic Glycoside in Fresh Cassava Roots and Cassava Flour from Fermentation and Mechanical Press-Fermentation Processing Methods

Fresh cassava roots and 20 different cultivars flour products from each zone using different processing methods were analysed for total cyanogens using picrate test method. The fresh bitter cassava root samples from zone A were peeled and washed to remove debris. The roots were grated and 2g of the sample was weighed and dissolved in $20cm^3$ of distilled water. The mixture was left for 24 hours for cyanide extraction. The mixture was filtered using filter paper and $80cm^3$ of alkaline picrate solution was added to the filtrate and boiled in a test tube inside a water bath. Change in coloration from yellowish to reddish brown was observed indicating the presence of cyanide. The absorbance of the sample was read using spectrophotometer at a wavelength of 490nm. The cyanide concentrations were determined by interpolating its absorbance value on the cyanide concentration curve. This procedure was adopted for fresh cultivars samples from zone B and C respectively.

The pulverised cassava flour from the two processing methods were also analysed using the picrate method. 2g of flour sample from zone A processed using fermentation method was weighed and dissolved in $20cm^3$ of distilled water in a flat bottom flask. The sample was allowed to stay for 24 hours to enhance cyanide extraction. $80cm^3$ of alkaline picrate was added to the sample filtrate in a test tube and boiled in a water bath for 20 minutes. The change in colour from yellow to reddish brown indicates the presence of cyanide. The absorbance of the solution was read using spectrophotometer at 490nm wavelength and the cyanide concentration of the flour was determined by interpolating its absorbance value on the standard cyanide concentration curve. This procedure was adopted for all the 20 samples from zones A, B and C and the concentrations in miligram per kilogram (mg/kg) were recorded and the average for each zone calculated.

RESULTS AND DISCUSSION

The standard calibration curve for the determination of cyanide concentration is as presented in Figure 1. The curve guides the determination of cyanogenic glucoside concentration in fresh and cassava flour produced using fermentation and mechanical press-fermentation processing methods (Kobawila *et al.*, 2005; Oboh and Elusiyan, 2007).

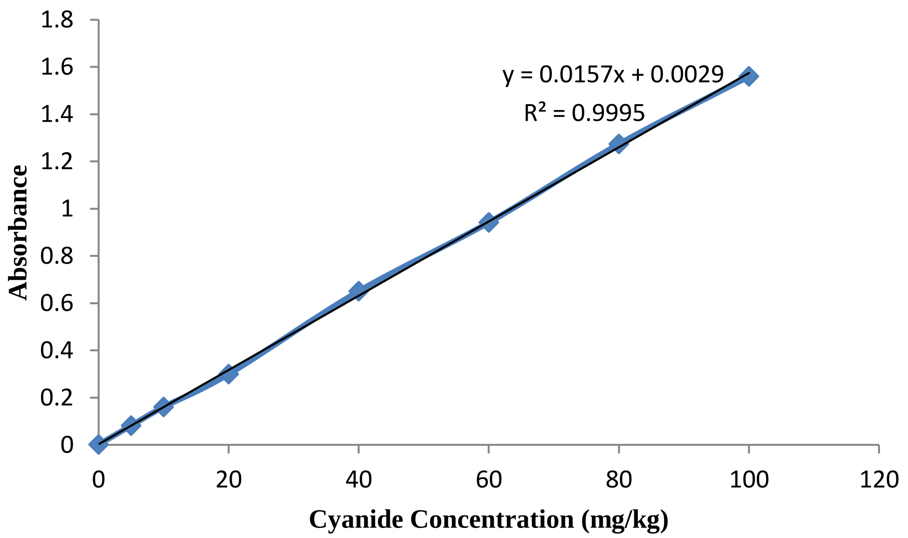

Fig 1: Concentration curve for standard cyanide solution

Figures 2, 3 and 4 showed the comparative cyanide content in parts per million (ppm) for fresh and processed flour from three geo-political zones in Niger State designated as **A**, **B** and **C**. The results showed that the cassava varieties differ in their total cyanide content in their tuberous roots.

The differences may be attributed to environmental factors. It has been shown that cyanide content is a function of both genetic composition of cassava and environmental factors such as soil and temperature condition (Gomez *et al.,* 1980). A comparison of the data in Figure 2, 3 and 4 showed that the cyanide contents in the fresh samples from zone **A** ranges from 98.45 to 118.24 mg/kg, while for zones **B** and **C** ranges from 81.33 to 115.56 mg/kg and 86.39 to 122.49 mg/kg respectively. These cyanide values far exceed the Food and Agriculture Organization/World Health Organization (1991) recommendation of 10 mg/kg, which makes the cassava roots acutely toxic for human and animal consumption (Montagnac *et al.*, 2008). Long term consumption of these roots (Bitter Cassava) from these zones may cause severe health problems such as tropical neuropathy, konzo (Cardoso *et al.,* 2005), and fibrocalculous pancreatic diabetes (FCPD).

A comparative cyanide content in *mg/kg* of the flour products processed using fermentation and mechanical press-fermentation methods from the three zones are presented in Figure 2, 3 and 4. Generally, the results showed lower cyanide contents in the flour produced using both processing methods. The cyanide content for fermentation and mechanically press-fermentation methods ranges from 2.4 to 10.2 mg/kg and 1.2 to 8.5 mg/kg for zone **A**, 1.49 to 9.2 mg/kg and 1.2 to 8.1 mg/kg for zone **B** and for zone **C**, it ranges from 3.2 to 10.5 ppm and 0.19 to 7.99 ppm respectively. The results showed that the cyanide content in the processed flour from the three zones falls below the FAO/WHO (1991) food standard programme. Thus, the processed flour obtained from the two processing methods is satisfactory for consumption. When cassava was crushed, fermented and sun dried, cyanide loss was high. The high percentages of losses were expected since grinding allows endogenous linimarase to come in contact with linimarin releasing HCN. Also, soaking improved cyanide removal by hydrolysis and leaching. Drying removed certain percentage of cyanide content of bitter cassava. Rajaguru (1975) earlier reported that drying of chips resulted in 50% loss of cyanide. The remaining cyanide diffused into the air as HCN. Therefore, the rate of diffusion of HCN is directly proportional to the thickness of the dried flour.

In addition, the results depict lower cyanide content in flour processed using mechanical press-fermentation method from the three zones investigated (Ikediobi *et al.,* 1980). This might be attributed to high loss of cyanogenic glucoside when tissue was crushed, mechanically pressed, fermented and finally sun dried. Crushing and mechanical pressing ruptures cell compartment, which allows free cyanide removal by damaging cassava tissue due to extraction with water or volatilization into the atmosphere (Montagnac *et al.,* 2008).

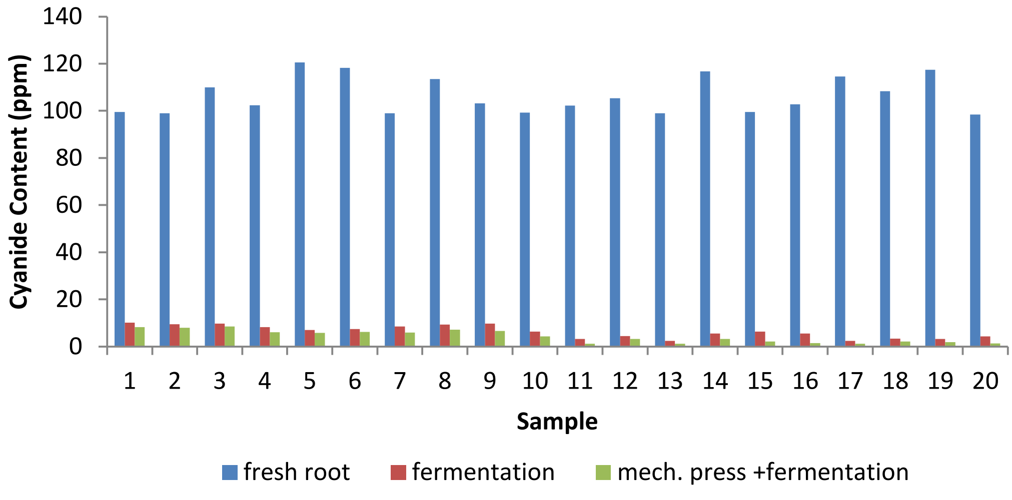

Fig 2: The Total Cyanide Content (mg/kg) in Cassava from Zone A

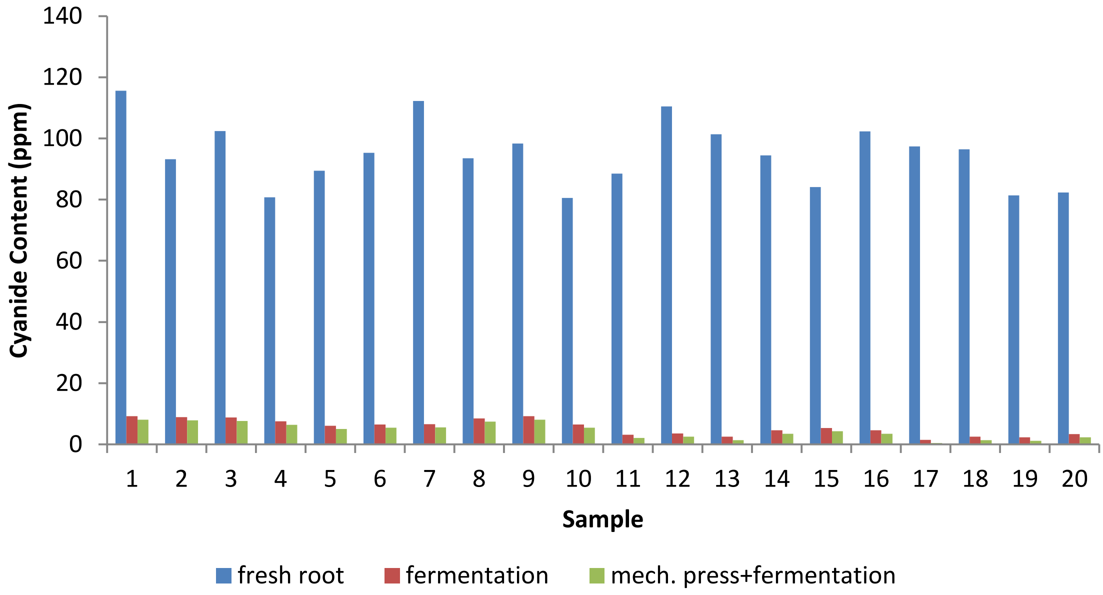

Fig 3: The Total Cyanide Content (mg/kg) in Cassava from Zone B

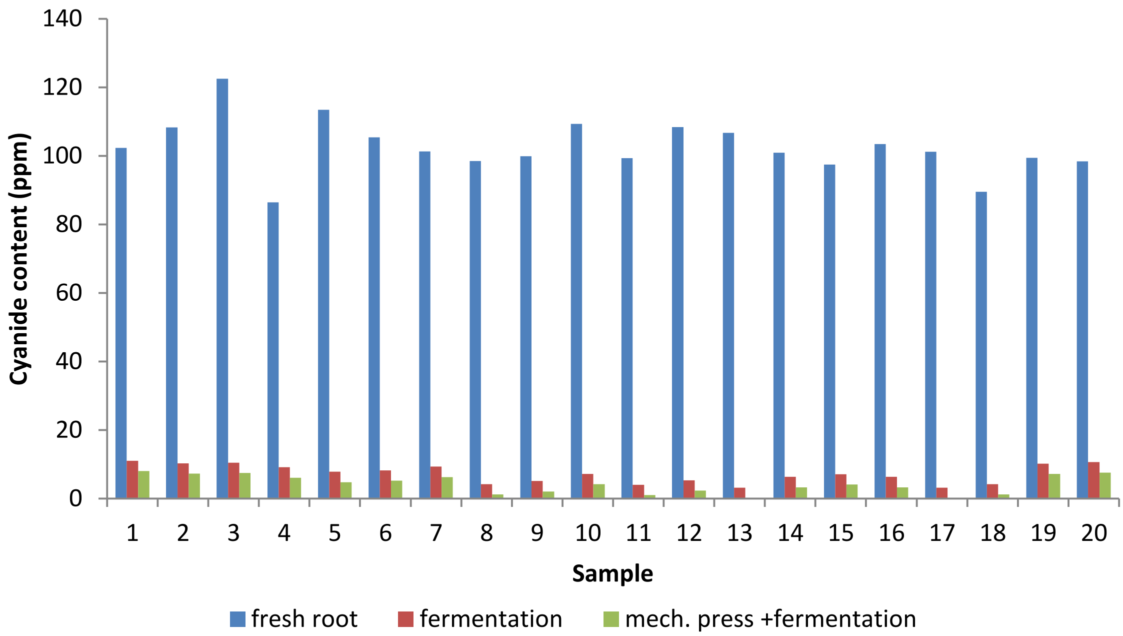

Fig 4: The Total Cyanide Content (mg/kg) in Cassava from Zone C

The average cyanide concentration of fresh roots in zones **A**, **B** and **C** shown in Table 1 were reduced from 106.45mg/kg, 94.99mg/kg and 102.59mg/kg to 6.35mg/kg, 5.56mg/kg and 7.16mg/kg respectively for fermentation processing method. While for the mechanical pressing – fermentation method, the average cyanide concentration of fresh roots from zones A, B and C were equally reduced from 106.45mg/kg, 94.99mg/kg and 102.59mg/kg to 4.31mg/kg, 4.48mg/kg and 4.15mg/kg respectively. The results showed that the two processing methods were effective in reducing the cyanide content in bitter cassava irrespective of the zone it was cultivated and conformed to the acceptable limit required by FAO/World Health Organization (1991) standard. This is in agreement with that reported by Kamalu and Oghome (2012).

It was also observed that cassava from zone A has the highest average level of HCN in the fresh tuberous roots (106.44mg/kg) when compared with other zones. This might be attributed to variations in environmental factors such as soil and temperature conditions coupled with genetic factor (Gomez *et al.*, 1980).

CONCLUSION

In conclusion, it has been discovered that the major factor that influences the level of residual cyanide in bitter cassava can be attributed to the length of fermentation period. This is because, during fermentation, the cyanogenic glucosides will be broken down into cyanide which is soluble in water. Constant de-watering by applying pressure helps to remove the dissolved cyanide in the liquid water. And all the two methods used in the reduction of cyanide content yielded a good result but mechanical pressing-fermentation processing method reduces the cyanide contents in the bitter cassava more as compared to the fermentation processing method.

Table 1: Average Cassava Cyanide Content (mg/kg) before and after Processing for the three Zones

Zone	Cyanide Content (mg/kg) of fresh root	Cyanide Content (mg/kg) after processing	
		Fermentation	***Mechanical Press + Fermentation***
A	106.44	6.35	4.31
B	94.99	5.57	4.48
C	102.59	7.16	4.15

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