Original Article

Cyanide and Aflatoxin Loads of Processed Cassava (*Manihot esculenta*) Tubers (Garri) in Njaba, Imo State, Nigeria

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ABSTRACT

Objectives: The present study sought to investigate the role of palm oil, in conjunction with the duration of fermentation, on cyanide and aflatoxin (AFT) loads of processed cassava tubers (Garri). **Materials and Methods:** Matured cassava (*Manihot esculenta* Crantz) tubers were harvested from three different locations (Akunna, Mkporo-Oji and Durungwu) in Njaba Local Government Area, Imo State, Nigeria. The cassava tubers were processed into Garri according to standard schemes with required modifications and measured for cyanide content using titrimetric methods. Samples of Garri for determination of AFT levels were stored for 30 days before the commencement of spectrophotometric analysis. **Results:** Cyanide content of peeled cassava tubers was within the range of 4.07 ± 0.16-5.20 ± 0.19 mg hydrocyanic acid (HCN) equivalent/100 g wet weight, whereas the various processed cassava tubers was within the range of 1.44 ± 0.34-3.95 ± 0.23 mg HCN equivalents/100 g. For the 48 h fermentation scheme, Garri treated with palm oil exhibited marginal reduction in cyanide contents by 0.96%, 3.52% and 3.69%, whereas 4 h fermentation scheme is in concurrence with palm oil treatment caused 4.42%, 7.47% and 5.15% elimination of cyanide contents compared with corresponding untreated Garri samples ($P > 0.05$). Levels of AFT of the various Garri samples ranged between 0.26 ± 0.07 and 0.55 ± 0.04 ppb/100 g. There was no significant difference ($P > 0.05$) in AFT levels among the various samples in relation to their corresponding sources. **Conclusion:** The present study showed that the 48 h fermentation scheme for Garri production caused significant ($P < 0.05$) reduction, but did not obliterate the cyanide content of cassava tubers. Conversely, the 48 h fermentation scheme promoted the elevation of AFT levels, but was relatively reduced in Garri samples treated with palm oil.

Key words: Aflatoxin, cyanide, Garri, *Manihot esculenta,* Njaba

INTRODUCTION

Cassava (*Manihot esculenta* Crantz) is a dicotyledonous plant and widely grown root crop in tropical regions of Africa, Latin America and Asia.[1] Two varieties of cassava are known; the sweet cassava known for low cyanide content and the bitter cassava with its high characteristic content of cyanogenic glycosides (CGs) that is highly toxic when consumed.[1-3] Total cyanide in cassava products exists in form CGs (linamarin and lotaustralin), cyanohydrin and free hydrocyanic acid (HCN).[4] The CGs notwithstanding, cassava meal provides dietary energy to over 500 million people in the world.[5,6] According to FAO,[7] 172 million tons of cassava was produced world-wide in 2000 with Africa accounting for 45%, Asia 28% and Latin America and the Caribbean 19%. The five main producing countries are Nigeria, Brazil, Thailand, Congo (DRC) and Indonesia. The crop plays a prominent

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role in daily subsistence of many indigenous communities in Southern Nigeria. Some commonly proceeded cassava meals include chips, "abacha","fufu", "lio-lio", tapioca, cassava flour and "Garri."[1,8] Nevertheless, the dynamism in food habits coupled with industrial food processing and marketing needs have directed research attention toward new products. Cassava is also a source of feed to farm animals and raw materials for industries.[9]

Garri is a granular starchy food prepared from cassava mash in a manner similar to Farinha de Mandioc. The cassava meal is in the form of paste made with hot water ("eba") is eaten with vegetable sauce or soaked in cold water with sugar, coconut, roasted groundnuts, dry fish or boiled cowpea as complements. The characteristic taste and flavor of Garri is mainly from its lactic acid content produced during fermentation.[10] Traditional production of Garri involves peeling of the cassava roots and grating into fine pulp. Next, the pulp is transferred into hessian sacks and compressed to drain and ferment for 4 days. The fermented and relatively dewatered pulp was sieved to remove fibrous materials and palm oil could be added according to preference. Roasting is carried out in large frying pan to yield gelatinized Garri granules of reduced moisture content, which can be stored for relatively long-time. Palm oil is added to cassava mash to give the Garri an esthetic value and source of vitamin A. Therefore, yellow Garri is more nutritious and preferably cherished than white Garri.[11]

The FAO estimates that 25% of the world food crops are contaminated by mycotoxin, of which the most notorious are the aflatoxins (AFTs).[12-14] AFTs are metabolites produced primarily by the fungi *Aspergillus flavus* and *Aspergillus parasiticus*. There are four major naturally produced AFTs, referred to as B1, B2, G1 and G2.[15,16] The B1 is the most toxic of the AFTs and potent naturally occurring liver carcinogen.[16] Reports estimated that more than 5 billion people in developing countries world-wide are at risk of chronic exposure to AFTs through contaminated foods.[17,18] AFTs affect livestock and poultry causing reduced feed efficiency, subtle immunosuppression, growth rate and death of animals.[14,19] Other economic adverse effects of AFTs include low yields of food and fiber crops.[20]

AFTs are considered to be unavoidable contaminants of food/feed and could occur at the pre- and post-harvest conditions: Storage, transportation and food processing, even when best practices are followed.[21] AFT contamination is a particular problem in wide variety of food commodities including maize, oilseeds, spices, groundnuts, tree nuts, milk (in the form of AFT B1's metabolite AFT M1) and dried fruits.[13,17,18,22] Maize and peanuts are the main sources of human exposure to AFT.[23] The Food and Drug Administration (FDA) has established specific guidelines on acceptable levels of AFTs in human food and animal feeds.[24] The acceptable level for human and animal is total (AFT) <20 ppb with the level of (AFT M1) <0.5 ppb for milk and its products;[20] http://www.uaex.edu.

The etiology of liver malignancy and renal dysfunction[16,25] alongside with the reproductive concerns[13,26] has been linked to AFT metabolites in humans. Given their seemingly unavoidable occurrence in foods and feeds, prevention and detoxification of mycotoxins pose an enormous challenge on toxicological issues in present time. Furthermore, incidences of an acute and chronic toxicity associated with the consumption of cassava meal are common and are often linked to cyanide content of the meal consumed by affected individuals. However, the implication of AFTs in cassava meal poisoning and toxicity are often not considered, poorly reported and taken for granted. Therefore, it has become imperative to study and document levels of cyanide and AFT in Garri produced in Njaba; a hub of Garri production in Imo State, Nigeria. The present study sought to investigate the role of palm oil, in conjunction with adjustments in duration of fermentation, on cyanide and AFT loads in Garri, in efforts to bring these toxic substances to innocuous levels.

MATERIALS AND METHODS

Collection/preparation of samples

Matured cassava (*M. esculenta* Crantz) tubers were harvested from private farms in three[3] different locations (Akunna, Mkporo-Oji and Durungwu) in Njaba Local Government Area (LGA), Imo State, Nigeria. Samples of the tubers were authenticated by Dr. F.N. Mbagwu at the Herbarium of the Department of Plant Science and Biotechnology, Imo State University, Owerri, Nigeria. A voucher specimen was deposited at the Herbarium for reference purposes. The tubers were washed with clean water and the rind was removed with kitchen knife. Samples of the tubers were measured for cyanide content.

Processing of cassava tubers/experimental design

The cassava tubers were processed into Garri according to standard schemes described elsewhere[21,27] with minor modifications outlined as follows:

- Scheme X_1: Garri roasted 4 h post fermentation (GRF_{4h})
- Scheme X_2: Garri roasted 4 h post fermentation + 100 mL palm oil/kg (GRF_{4h} + PO)
- Scheme X_3: Garri roasted 48 h post fermentation (GRF_{48h})
- Scheme X_4: Garri roasted 48 h post fermentation + 100 mL palm oil/kg (GRF_{48h} + PO).

Garri samples produced using the four different schemes were collected in sterile polyethylene bags (≈500 g/pack) adopting standard procedures and transported to the laboratory. The samples were stored at room temperature 25 ± 5°C of 30-55% relative humidity. Samples for

determination of AFT level were stored for 30 days before the commencement of analysis.

Cyanide content

Cyanide content of fresh cassava tubers and GRF_{4h}, GRF_{4h} + PO, GRF_{48h} and GRF_{48h} + PO samples were measured by alkaline titration method as described by Kamalu and Oghome,[28] with minor modifications. About 15 g sample was measured into 800 mL Kjedahl flash containing 200 mL of distilled water and allowed to stand for 3 h at 25 ± 5°C. Autolysis was carried out with the apparatus connected to a distiller. A 150 mL of distillate was collected in 20 mL 25% of NaOH solution and further diluted to 250 mL with distilled water. Next, 100 mL of the diluted distillate was mixed with 8.0 mL of 6.0 N NH_4OH and 2.0 mL of 5% KI indicator solution and titrated against 0.02 N $AgNO_3$. The end point was indicated by a faint permanent turbidity appearance. The cyanide content (mg/100 g cassava wet weight) of the sample was evaluated from the expression: 1.0 mL 0.02 N $AgNO_3$ ≡ 1.08 mg HCN.

AFT content

The GRF_{4h}, GRF_{4h} + PO, GRF_{48h} and GRF_{48h} + PO samples were measured for AFT content by methods according to Ibeh *et al*.[29] as reported by Ogiehor and Ikenebomeh,[21] but with minor modifications. A 10 g Garri sample was homogenized in a clean Erlenmeyer flask containing 40 mL MeOH and water mixture (*v/v* ratio 11:9). The resultant slurry was filtered using Whatman No 1 filter paper. To remove the lipid fractions, the filtrate was extracted 3 times with 20 mL boiling petroleum ether (60-80°C) in a separating tunnel. Once more, the pooled petroleum ether extracts were extracted with 40 ml MeOH and water mixture (v/v ratio 11:9). The aqueous MeOH extract were pooled and transferred to separating funnel and extracted 3 times with 25 mL of CCl_4 (BDH, England). The pooled CCl_4 extract was passed through the bed of anhydrous Na_2SO_4. The bed was rinsed with 20 mL of CCl_4. AFTs were detected by a thin layer chromatography against standard AFT B1, B2, G1 and G2 (Aldrich Chemicals, Milwaukee, USA) and quantitated as described by Sekhon *et al.*,[30] using a spectrophotometer λ_{max} = 492 nm (Jenway, Model 6400, Essex, CM6 3LB, England).

Statistical analyses

The data were analyzed by the use of Students' *t*-distribution test of significance as described by Pearson and Hartley.[31]

RESULTS

Table 1 shows that cyanide contents of peeled cassava tubers was within the range of 4.07 ± 0.16-5.20 ± 0.19 mg HCN equivalent/100 g wet weight. The variable levels of cyanide contents of cassava tubers harvested from the three locations in Njaba showed no significant difference ($P > 0.05$) and were in the order: Akunna > Mkporo-Oji > Durungwu.

An overview of Figure 1 shows comparative reduction in cyanide contents of the various processed cassava tubers (GRF_{4h}, GRF_{4h} + PO, GRF_{48h} and GRF_{48h} + PO). Their cyanide contents ranged between 1.44 ± 0.34 and 3.95 ± 0.23 mg HCN equivalents/100 g. In addition, GRF_{4h} sourced from Durungwu gave the relatively highest level of cyanide concentration of 90.90%, compared with fresh unprocessed cassava tubers. Table 2 shows relative reduction in cyanide contents of Akunna and Mkporo-Oji GRF_{4h} by 24.04% and 21.76% respectively. Akunna, Mkporo-Oji and Durungwu. GRF_{4h} + PO showed marginal reductions in cyanide contents compared with GRF_{4h}, corresponding to 4.42%, 7.47% and 5.15% of

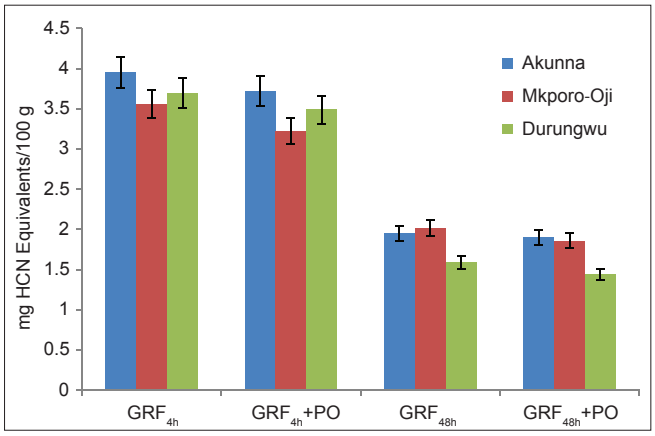

Figure 1: Cyanide contents of proceeded cassava tubers from Akunna, Mkporo-Oji and Durungwu

Table 1: Cyanide contents of peeled cassava tubers

Sample source	mg HCN equivalents/100 g wet weight
Akunna	5.20±0.19
Mkporo-Oji	4.55±0.31
Durungwu	4.07±0.16

The results are means (X)±SD of nine (*n*=9) determinations, SD = Standard deviation, HCN = Hydrocyanic

Table 2: Relative cyanide contents eliminated in processed cassava tubers from Akunna, Mkporo-Oji and Durungwu

	Relative cyanide contents eliminated (%)		
Samples	Akunna	Mkporo-Oji	Durungwu
GRF_{4h}	24.04	21.76	9.10
GRF_{4h}+PO	28.46	29.23	14.25
GRF_{48h}	62.50	55.60	60.93
GRF_{48h}+PO	63.46	59.12	64.62

GRF_{4h} = Garri roasted 4 h post fermentation, GRF_{4h}+PO = Garri roasted 4 h post fermentation+100 mL palm oil/kg, GRF_{48h} = Garri roasted 48 h post fermentation, GRF_{48h}+PO = Garri roasted 48 h post fermentation+100 mL palm oil/kg

reduction in cyanide contents. The relative cyanide content of GRF_{4h} + PO was within the range of 71.54-85.75%.

Comparatively, cyanide content in GRF_{48h} was significantly reduced; specifically, Akunna GRF_{48h} = 37.50%, Mkporo-Oji GRF_{48h} = 44.40% and Durungwu GRF_{48h} = 39.07% of mg HCN equivalents/100 g compared to corresponding unprocessed cassava tubers. These values represented an average of 59.68% of total eliminated cyanide content of GRF_{48h} samples. As compared with corresponding GRF_{48h} samples, Akunna, Mkporo-Oji and Durungwu. GRF_{48h} + POs showed marginal reduction in cyanide contents by 0.96%, 3.52% and 3.69% respectively. The mg HCN equivalents/100 g of GRF_{48h} + POs showed no significant difference ($P > 0.05$) with values of 1.90 ± 0.41, 1.86 ± 0.44 and 1.44 ± 0.34 mg HCN equivalents/100 g for Akunna, Mkporo-Oji and Durungwu Garri respectively. In general, the total cyanide content of the processed cassava tubers was in the order: GRF_{4h}, > GRF_{4h} + PO > GRF_{48h} > GRF_{48h} + PO irrespective of the source of the processed cassava.

In addition, an overview of Figure 2 indicated that AFT levels of the various Garri samples ranged between 0.29 ± 0.07 and 0.55 ± 0.04 ppb/100 g. There was no significant difference ($P > 0.05$) in AFT levels of the various Garri samples in relation to their corresponding sources.

DISCUSSION

Occurrence of CGs in diverse plant species[32] including cassava[33-35] has been widely reported. Two major CGs of cassava origin are linamarin and lotaustralin, which are the sole contributors of cyanide contents in cassava tubers as reported in the present study and elsewhere.[32,35] During processing, cassava tuber tissues are disrupted and CGs is brought in contact with β-glucosidases (linamarase; pH > 5) and α-hydroxynitrile lyases; engendering hydrolysis of CGs into cyanohydrins, HCN and ketones.[9,36,37] Conventional

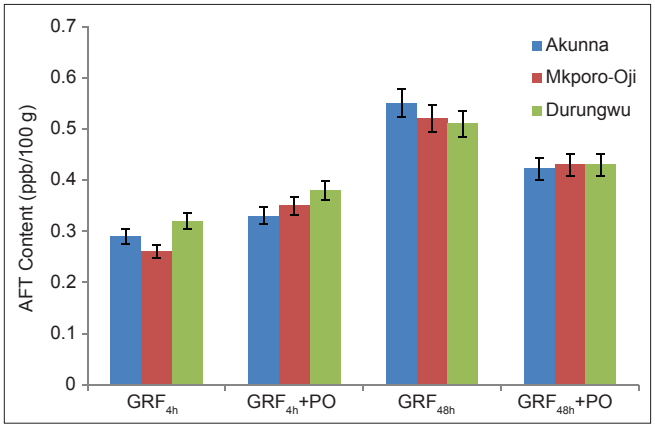

Figure 2: Aflatoxins contents of proceeded cassava tubers from Akunna, Mkporo-Oji and Durungwu

breeding has generated varieties of cassava with a wide range of cyanide contents. Previous investigations showed that cassava tubers contained HCN within the range of 10-500 mg/kg fresh tuber.[32,35,38,39] Notwithstanding, Jørgensen *et al.*,[34] reported the depletion of transgenic cassava plants of CG content in leaves and tubers using ribonucleic acid interference technology to block expression of CYP79D1 and CYP79D2 genes that is responsible for the biosynthesis of CGs. However, no cassava cultivar totally devoid of cyanide has been produced.[40] From our findings, cyanide contents of fresh cassava tubers harvested from the three[3] selected locations in Njaba LGA showed variable values ($P > 0.05$). In related study, Mburu *et al.*,[35] reported variability of cyanide contents ($P < 0.05$) in cassava tubers obtained from five[5] regions in Kenya. They noted that the basis for the disparity in cyanide content was the difference in ecological factors and soil chemistry in the various regions. Furthermore, they observed that key components of soil such as potassium, calcium and magnesium adversely affected the biosynthesis and translocation of cyanide to storage organs, which invariably contributed to inconsistencies in cyanide content in the plant tissues harvested from the various regions in Kenya. Therefore, in the opinion of our present findings, the non-significant ($P > 0.05$) difference in cyanide content of the various cassava tubers analyzed was an indication that Akunna, Mkporo-Oji and Durungwu are situated within the same ecological zone with similar soil chemistry.

WHO recommended 10 mg HCN/kg body weight as the maximum safe intake of cyanide containing food/feed for humans and animals.[41] The present study showed that the scheme used for processing cassava tubers defined the cyanide levels in finished Garri products [Figure 2 and Table 2]. This finding has been corroborated by previous authors.[28,42,43] The reports presented here indicated that fermentation of cassava tubers was a major determining factor to achieving a significant reduction in cyanide content in Garri, which was in conformity with earlier reports.[44,45] Specifically, the cassava products fermented for 48 h (GRF_{48h} and GRF_{48h} + PO) exhibited comparative lower residual levels of cyanide than those fermented for 4 h (GRF_{4h} and GRF_{4h} + PO) [Figure 2 and Table 2]. However, the addition of palm oil in the production scheme did not cause significant ($P > 0.05$) reduction in residual levels of cyanide in corresponding Garri samples (i.e., $[Cyanide]_{mg/100\,g}$ of GRF_{48h} vs. GRF_{48h} + PO and GRF_{4h} vs. GRF_{4h} + PO; $P > 0.05$). This finding corroborated the observations of Asegbeloyin and Onyimonyi,[46] who noted that the addition of palm oil in the Garri processing scheme did not significantly ($P > 0.05$) cause reduction in cyanide content. Notwithstanding, nutritional studies and animal feed experiments have showed that the addition of palm oil to cassava meal impeded the release and absorption of cyanogens and cyanide, signified by normal levels of

serum thiocyanate in experimental animals.[47,48] The 48 h non-significant ($P > 0.05$) cyanide lowering effect of palm oil was contradicted by a couple of previous reports.[37,48-50] These previous reports showed that the cyanide lowering effect was more profound in production schemes designed to ferment the cassava mash over a longer duration; $t > 72$ h than the relatively shorter period ($t \leq 48$ h) presented here. Therefore, the seemingly conflicting reports presented here as against those of previous authors may not be unconnected with the relatively longer duration of the fermentation process coupled with the effect of certain physicochemical properties of palm oil on the cassava mash during roasting.[37]

The detection of AFTs in the various Garri samples (GRF_{4h}, GRF_{4h} + PO, GRF_{48h} and GRF_{48h} + PO) conformed to previous reports by several authors[15,19,51] including other food crops and animals products.[13,52,53] Although there are no specific FDA standards for Garri in terms of AFT load, the present study showed that the presence of AFT were below the minimum recommended index for general food products designed for human consumption.[24] The biosynthesis of AFTs is strongly dependent on growth conditions such as substrate composition or physical factors such as pH, water activity, temperature or modified atmospheres and enzyme activity.[54,55] Accordingly, the comparative high levels of AFTs in GRF_{48h} samples are not unconnected to biochemical properties of fermented cassava products. Studies have shown that increase in mycotoxin production occurred at pH < 6.0.[56] Specifically, other mycotoxin producing fungi-*Aspergillus umbrosus* exhibited maximum growth phase at pH = 5.0,[57] whereas *Alternaria alternate* cultured under acidic pH in the range of 4.0-4.5 gave optimal mycotoxin production.[58] From these observations, it was not unexpected that the present findings reported maximum AFT load in GRF_{48h} samples [Figure 2]. According to Kobawila *et al.*,[45] fermentation process promoted the reduction in levels of HCN with concomitant buildup of lactic acid propionate and acetate and related volatile organic compounds.[10,59] The low pH encouraged the production of AFTs[15,29] in the finished cassava product, especially the samples that were allowed to ferment over a relatively longer period. Furthermore, the poorly fermented product GRF_{4h} and GRF_{4h} + PO samples served as poor media for the propagation of the causative fungi agents and production of AFTs compared with the GRF_{48h} and GRF_{48h} + PO samples. Nawaz,[60] had previously observed that palm oil did not suffer AFT contamination compared to other types of oil and its products. Therefore, the reduced levels of AFTs in palm oil treated samples (GRF_{4h} + PO and GRF_{48h} + PO) were indications of the capacity of palm oil to retard the production of AFTs in fermented Garri. However, it is worthwhile to note that the level of AFT in food in a given geographical location is dependent on certain environmental factors such as agricultural and agronomic practices and the susceptibility of commodities to fungal invasion during pre-harvest, storage and/or processing periods.[13,61]

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