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Hydrogen cyanide and mycotoxins: Their incidence and dietary exposure from cassava products in Anyigba, Nigeria

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ABSTRACT

While cassava products are important in addressing food security crises in Africa, they are not exempted from recurring food safety concerns. This research determined consumption, incidence, and concentrations of hydrogen cyanide (HCN), total aflatoxins (AFs), ochratoxin A (OTA) and fumonisins (FBs) in cassava product (casava flour, fufu and gari) among residents of Anyigba, Nigeria, and also estimated dietary exposure to the toxins from the foods. Consumption data from 82 households showed that 89%, 68% and 94% of the residents consume cassava flour, fufu, and gari, respectively. Average daily intake for individuals was 0.751, 0.111, and 0.120 kg of cassava flour, fufu, and gari respectively. The highest incidence and concentration of HCN were found in cassava flour, with %TDI (EDI) of 33.3% (0.03 mg/kg.bw/day). Weekly, residents are exposed to 0.0371, 0.0077 and 0.0224 μg/kg.bw of OTA, and daily to 0.0315, 0.0043, 0.0060 μg/kg.bw of AFs and 0.0000, 0.0025, 0.0004 μg/kg.bw of FBs from consumption of cassava flour, fufu, and gari, respectively. The mycotoxins also co-occurred in the food products.

1. Introduction

Cassava (*Manihot esculenta Crantz*) is a very important staple in Africa. It is already established that after sugarcane, maize, rice, wheat and potatoes, cassava is the sixth most produced and consumed crop in the world (FAO, 2017), it has been reported that cassava is the third most important source of calories in the tropics (Gacheru et al., 2016) and it significantly contributes to food security, incomes and employment in the rural areas of sub-Saharan Africa. Apart from Africa where about 70% of the cassava produced is for human consumption, between 35% and 41% of cassava produced in Latin America, the Caribbean, and Asia are also used for direct human consumption (Anyanwu et al., 2015), as a result, cassava is a matter of global importance with respect to food safety. Nigeria is the world's largest producer of cassava, giving the world 21% of the world total cassava produced in 2016 (FAO, 2016; Githunguri et al., 2007).

In Nigeria, there are three major forms in which cassava intended for consumption are processed into. These are: gari, fufu and cassava flour. Cassava flour is further processed into a stiff dough called 'tuwo' by the Hausa' and 'oje' by the Igala tribes. The processing steps of cassava into gari, fufu or its flour are available in literatures. However, to distinguish between the three produce, gari involves fermentation for 1–5 days and roasting resulting in a dry granular product, production of fufu involves boiling of fermented cassava and pounding thereafter and forming it into a stiff dough, while cassava flour processing involves soaking fresh cassava for about 3 days until the root is soft, the root is air/sun dried and then milled into flour, this flour is eventually eaten after it is formed into dough with boiling water. A larger part of cassava processing in Africa is more traditional than mechanized. Cassava are generally classified into sweet and bitter varieties according to the cyanogenic glucoside contents of their roots, while the sweet ones have ≤50 mg/kg HCN content, the bitter ones have ≥100/mg/kg HCN content (CAC, 2019).

Hydrogen cyanide which have toxic potentials when ingested by human and animals, can be produced when cyanogenic glucosides such as linamarin and a small amount of lotaustralin naturally present in cassava are hydrolyzed. The extent of toxicity of HCN is dependent on the route, concentration and time of exposure (Bhattacharya & Flora, 2009). Acute HCN poisoning results in rapid breathing rate, dizziness, restlessness, headache, weakness, and nausea, while chronic HCN

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poisoning result in breathing difficulties, chest and/or heart pain, eye irritation, vomiting, headaches, loss of appetite, nosebleeds, enlargement of the thyroid gland (goitre) and death (Mburu, 2013). Survivors of long-term cyanide exposure may develop CNS, brain and heart damage due to oxygen deprivation to the organ system (Baskin et al., 2008). In Ososa, Southwest Nigeria, occurrence of ataxic polyneuropathy suspected to be from exposure to HCN in cassava was reported in 6.4% of the population in 2003 (Oluwole et al., 2013). In Kogi state Nigeria where this study was carried out the death of six people from exposure to cassava product was reported in the year 2016 (Nigerian Punch Newspaper, 2 Nov 2016). In Malaysia, a mother and three daughters were poisoned following consumption of cassava (ubikayu) resulting in death of one daughter with symptoms such as nausea, abdominal cramp, diarrhoea, vomiting and drowsiness reported (Arriffin et al., 1992).

Mycotoxins are poisonous secondary metabolites produced on food and feeds by several moulds. Of the over 300 mycotoxins that occur in nature aflatoxins, fumonisins and ochratoxins are among the first five listed with respect to their toxicity, high incidence and wide occurrence. Aflatoxins are produced majorly by *Aspergillus flavus* and *A. parasiticus*. They are poisonous carcinogens prevalent in staple foods (Fratamico et al., 2008). They are classified as aflatoxin B (blue-B_1, B_2) and aflatoxin G (green – G_1, G_2) based on the colour of fluorescence they emit when their crystals are subjected to UV-light (Wacoo et al., 2014), and jointly referred to total aflatoxins when more than one of the various types appear together on a commodity. Aflatoxins have been included in the group 1B carcinogens by IARC due to their carcinogenicity, teratogenicity and mutagenicity effects in humans and farm animals, with AFB_1 as the most potent. Ochratoxin A (OTA) is mainly produced in foods by *Aspergillus ochraceus* in the tropics and *Penicillium verrucosum* in the temperate regions (Kumar et al., 2008). Ochratoxin A (OTA) was discovered in 1965 in South Africa (Van der Merwe et al., 1965). It is a potent nephrotoxin that causes kidney and liver impairment in animals (especially in pigs) and human beings (Hussein & Brasel, 2001). Ochratoxin A is potentially carcinogenic to humans and belongs to group 2B list of carcinogens (IARC, 1976). It has been associated with endemic Balkan nephropathy (BEN) that is often accompanied by upper urinary tract urothelial cancer (Grollman & Jelakovic, 2007). Fumonisins are produced by different species of *Fusarium* fungi (Surai, 2008). Fumonisin B_1 (FB_1) and B_2 are of toxicological significance, while the others (B_3, B_4, A_1 and A_2) occur in very low concentrations and are less toxic (Peraica, 1999). An alternative mechanism of action of FB_1 involves the disruption of the de novo sphingolipid biosynthesis pathway by inhibition of the enzyme ceramide synthase (Doi & Uetsuka, 2011; Merrill et al., 2001). The inhibition of sphingolipid biosynthesis disrupts numerous cell functions and signalling pathways, including apoptosis and mitosis, thus potentially contributing to carcinogenesis through an altered balance of cell death and replication particularly on the liver and kidney in humans, also, causing oesophageal cancer and birth defects (Hussein & Brasel, 2001; Lauren et al., 1996; Norred, 1993). Fumonisins is potentially carcinogenic to humans and belongs to group 2B list of carcinogens (IARC, 1976).

The objective of this study was to assess the levels of contamination of cassava products by hydrogen cyanide, total aflatoxins, ochratoxin A and fumonisins, as well as to determine the dietary exposure of the residents to the toxins.

2. Methodology

2.1. Determination of food consumption

A questionnaire was used as a tool for the collection of data, and same administered in the study location. The questionnaire focused on estimating the dietary exposure of residents of Anyigba to hydrocyanic acid and major mycotoxins present in cassava products by determining the frequency of consumption and serving size of the foods. Eighty-two (82) households with the total of 485 individuals were selected at random and were interviewed.

The average daily intake (ADI) of the food commodities per person was calculated by the formula below;

$$\text{Average daily intake} = \frac{\sum(fx^{*}SS^{*}W)}{N}$$

where *fox* = number of times food item is consumed per day *W* = average weight of food item *SS* = Serving size (number of cups cooked or number of wraps served per seating) *N* = total number of individuals.

2.2. Sampling of cassava products and sample preparation

Thirty cassava products, ten each of gari, fufu and cassava flour were randomly collected in February 2019 from 30 vendors in Anyigba town of Kogi state, Nigeria. The samples were collected in Ziploc bags and transported to the Central Research and Diagnostic Laboratory, Ilorin, Kwara State, Nigeria where analyses were carried out. Sample preparation involved blending of 50 g each of gari. Cassava flour was already in powdered form. While the fufu samples were oven dried at 50 °C for 48 h and then blended to powder. Three gari samples containing palm oil were defatted using the Soxhlet method.

2.3. Determination of hydrogen cyanide concentration

The analysis was carried out as described by Surleva et al. (2016). The cyanide concentration of cassava products (gari, cassava flour and fufu) were determined using the ninhydrin-based spectrophotometric method described by Surleva et al. (2016) with little modifications. Calibration level were taken at of 0.02, 0.04, 0.08, 0.1 and 0.2 mg/l of standard solution of CN^- respectively in 2% Na_2CO_3, while 0.5 ml ninhydrin solution (5 mg/ml in 2% Na_2CO_3) was added to each standard cyanide concentration. The blank contained 0.5 ml of ninhydrin in 1 ml 2% NaOH. The mixture was homogenized and incubated for 15 min to allow colour development. For the analyses of cassava product samples, 0.01g of each sample was scooped into a volumetric flask and made up to 5 ml with 0.1% $NaHCO_3$, after sonication (20 min) and centrifugation (10,000 rpm for 10 min), the supernatant was pipetted twice (20 μl + 20 μl) and added to 0.5 ml ninhydrin in NaOH, allowed for 15 min for colour development and the absorbance was measured. UV–Visible absorption of the reaction product (cyanide-ninhydrin adduct) of the standards and samples were measured using UV/Vis Spectrophotometer (SURGISPEC SM 735, Surgical Medical, England) at a wavelength of 485 nm.

To determine the limit of detection (LOD) and the limit of quantification (LOQ), blank solutions containing 1 ml of 2% Na_2CO_3 and 0.5 ml of 5 mg/ml ninhydrin was prepared thrice, the absorbance was measured to obtain triplicate reading. The calibration curve was used to establish the various concentrations; and the mean blank concentration ($C_b = 0.101$) was recorded. The LOD was then determined by adding C_b to the standard deviation of the blank signal ($S_b = 0.020$) multiplied by 3 ($LOD = C_b + 3S_b$). The LOQ was ($C_b + 10S_b$). LOD = 0.161; LOQ = 0.301. Recovery of 97 ± 2.1% was obtained. Calibration curve had a linearity value; $r^2 = 0.9913$.

2.4. Extraction of total aflatoxins, ochratoxin A and fumonisins

The method described in Onyedum et al. (2020) was used for quantification of the various mycotoxins. In summary, AgraQuant® Total Aflatoxin (48 wells; part number = 10,002,101), AgraQuant® Ochratoxin (48 wells; part number = 10,002,103), AgraQuant® Fumonisin (48 wells; part number = 10,002,105) from Romer Labs, Getzersdorf Austria was used for reaction, while STAT FAX Elisa Reader MODEL: 303 PLUS was used for quantification. The quantification parameters were; for AFs, the extraction solution was methanol: water (70:30), range of quantification was 1–20 ppb, recovery rate was 93 ±

2% while the LOD was 1 ppb. For FBs, extraction solution was methanol: water (70:30), range of quantification was 250–5000 ppb, recovery rate was 91 ± 2% while the LOD and LOQ were 200 ppb and 250 ppb, respectively. For OTA, the extraction solution was methanol (100%), range of quantification was 2–40 ppb, recovery rate was 88 ± 5% while the LOD and LOQ were 1.9 ppb and 2 ppb respectively.

2.5. Determination of average weight of individuals

A weighing scale was used to take the weight of individuals within a household. This was done for adult males, females and children. The data was harmonised and the averages were calculated.

2.6. Determination dietary exposure and percentage tolerable daily intake (%TDI)

The estimated daily intake (EDI) of HCN and major mycotoxins present in cassava products was calculated using the formula suggested by Saladino et al. (2017):

2.7. EDI (mg/kg.bw/day) for cassava products =

$$\text{EDI (mg/kg.bw/day) for cassava products} = \frac{\left[\text{mean conc. of toxin}\left(\frac{mg}{kg}\right)\text{x daily intake (kg/day)}\right]}{\text{average body weight } (kg)}$$

Percentage tolerable daily intake (%TDI) of HCN and major mycotoxins in cassava products was calculated by dividing the EDI of the toxins by established Tolerable Daily (TDI/PMTDI) or weekly (PTWI) intake (in the case of Ochratoxin A), and multiplying the value by 100.

$$\%TDI = \frac{EDI}{TDI} X\ 100$$

2.8. Data analyses

Data obtained were analysed using MS Excel 2016, to determine mean and standard deviation data. They were also subjected to IBM SPSS version 20 to determine test for significance using Duncan method. Thereafter, results are presented in figures and tables.

3. Results and discussion

3.1. Food consumption

In the study as depicted in Fig. 1, the data derived from 82 households (485 individuals) interviewed, revealed that 68%, 89% and 94% of households consume fufu, cassava flour and gari, respectively. Considering the individuals, 16%, 21% and 22% of men consume fufu, cassava flour and gari, respectively, 19%, 24% and 25% of women consume fufu, cassava flour and gari, respectively, while 38%, 49% and 51% of children consume fufu, cassava flour and gari, respectively. A report by the Nigerian National Bureau of Statistics in collaboration with the Federal Ministry of Agriculture and Rural Development, and the world bank in 2014 estimated that 83.1% of households in Nigeria and 88.4% of households in North-Central Nigeria where Kogi state is a part of, consume starchy roots, tubers and plantains, the South-South region has the highest (98.6%) consumption while the North-East region had the lowest (49.3%) consumption of the produce (LSMS, 2014).

Our findings on household consumption is in agreement with the early reports from the national survey.

Table 1 shows the daily intake of cassava products per individual from the study area. All cassava products considered in this study are reported to be consumed once a day on average. Cassava flour had the highest mean daily consumption value of up to 0.751 kg/day compared to other cassava products consumed in the study area. In comparison with the WHO GEMS database, estimated consumption of raw or boiled roots and tuber was given as 0.166 kg/day while that for processed root and tuber was 0.024 kg/day for G13 countries in 2012. Nigeria is categorized under G13 countries. This report presents a relatively higher intake level of cassava flour compared to the WHO/GEMS data.

Table 1
Food intake of cassava products in Anyigba, Kogi State, Nigeria.

Parameter	Cassava flour	Gari	Fufu
Average number of times consumed daily	1	1	1
Maximum number of times	2	1	1
Average serving size (wraps or cups)	2.22	1.78	1.5
Mean weight of food sample (kg)	0.528	0.121	0.483
Daily consumption (kg)	0.751	0.110	0.120

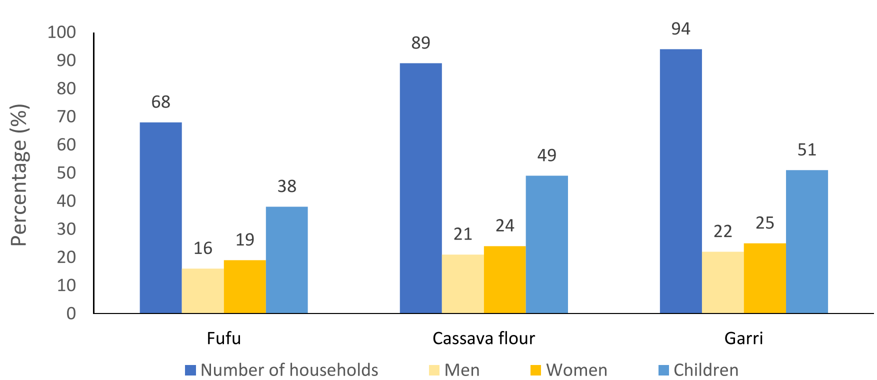

Fig. 1. Household and individual consumption of various cassava products.

Table 2
HCN levels (mg/kg) in cassava products in Anyigba, Kogi State, Nigeria.

Parameter	Cassava flour	Gari	Fufu
Total Samples	10	10	10
Contaminated Samples	9	0	5
Contamination (%)	90	0	50
Range	0.309–6.294	–	0.441–1.838
Mean ± SD (mg/kg)	2.371 ± 1.92[b]	–	0.553 ± 0.69[a]
Number of samples exceeding ML	Nil	Nil	Nil

Mean ± SD values with different superscript alphabets across a row are significantly different.

From Table 2, it is seen that 90% of the cassava flour samples contained cyanide with mean value of 2.371 ± 1.92 mg/kg and a maximum value of 6.29 mg/kg. No HCN was detected in all the 10 fufu samples analysed and about 50% of the gari samples were contaminated with HCN between 0.00 and 1.84 mg/kg, with mean of 0.553 ± 0.69 mg/kg.

The percentage occurrence of HCN in cassava flour, gari and fufu has been reported to be high in fermented cassava products but appeared at low levels (CAC, 2019). The research showed the occurrence of HCN in gari and cassava flour, but none in fufu. The individual mean HCN concentrations of cassava flour (2.371 ± 1.92 mg/kg) and gari (0.553 ± 0.69 mg/kg) were below the 10 mg/kg and 2 mg/kg legislated by JECFA (CAC, 2013) as the permissible level of HCN in the two respective cassava products. On the contrary, other literatures reported higher occurrences of HCN and higher levels of HCN from the same cassava products analysed. This may be attributed to similar factors determining the presence or absence of HCN in cassava products, such as processing methods or variety of the cassava cultivar. A study carried out in Sierra Leone by Blanshard et al. (1994) reported 100% incidence of HCN in 51 and 36 samples of 'foofoo' (28.200 ± 21.2 mg/kg) and gari (8.60 ± 3.45 mg/kg), respectively. Orjiekwe et al. (2013) reported 100% incidence of HCN in samples of gari (5.000 ± 0.10 mg/kg) and fufu (10.000 ± 0.13 mg/kg) in Edo, Nigeria, accordingly, all samples were reported to have values above respective MLs. Yet another report (Babalola, 2014) recorded 100% incidence of HCN in 6 samples of gari (8.410 ± 4.75 mg/kg) from Ekiti, Nigeria with all samples having HCN level greater than MLs. The National Agency for Food, Drug Administration and Control reported 0.02% (4/154) occurrence of HCN in gari (39.150 ± 38.75 mg/kg) from Lagos, Nigeria (CAC, 2019). Olorunnado et al. (2019) reported the incidence of HCN in gari from samples across Nigeria to have HCN level ranging from 0.056 to 2.463 mg/kg. The rate and extent of HCN removal depended on the processing method or the combination of methods employed, being a common observation that cassava processed by fermentation are reported to have lower levels of HCN (Kamalu & Oghome, 2012). From this report, the mean value of 2.371 ± 1.92 mg/kg for cassava flour suggests that methods employed in the processing of the cassava tuber to cassava flour is less effective in removing HCN compared to methods used in the processing of cassava to gari with mean value of 0.553 ± 0.69 mg/kg. Further processing as in the case of fufu reduced HCN to the minutest level or totally remove it (CAC, 2019).

3.2. Occurrence of mycotoxins in cassava food products

It was earlier insinuated that cassava is not susceptible to mycotoxin contamination (Essono et al., 2009) and more so because it undergoes milling and fermentation which are procedures that are known to reduce mycotoxins in processed products (Hell & Mutegi, 2011). This claim may have been over taken with more recent findings, since Ingenblek et al. (2019) reported an incidence of 13% and the mean concentration of OTA contamination at 0.65 μg/kg in unfermented fresh cassava from Cameroon.

Considering the important role cassava play in food security in Africa, its contamination by AFs, OTA and FBs is of serious food safety consequences. According to existing literature, *Aspergillus flavus* and *A. parasiticus* are the major fungi that produces AF, *Fusarium verticillioides* and *F. proliferatum* are major producers of FBs while *A. ochraceus* is the major producer of OTA in tropical regions (Abbas et al., 2009; Adebajo et al., 1994). Awosusi and Oriye (2015) reported the mean monthly temperature of Anyigba town in Kogi, Nigeria as ranging between 21 °C and 32 °C. The optimum temperature and water activity range for production of mycotoxins by various fungi are given as follows; *A. flavus* (30–33 °C/0.82), *A. parasiticus* (33 °C/0.99), *A. ochraceus* (25–31 °C/0.98), *F. verticillioides* (15–30 °C), and *F. proliferatum* (15–30 °C/0.92) (CAST, 2003; Sanchis & Magan, 2004), the AF producers require higher temperature and low water activity, OTA producers require lower temperature than AF producers and high water activity, FB production is favoured by a lower temperature range and relatively lower water activity than OTA production require. It is evident that the temperature of the study area supports not just the growth of fungi but also mycotoxin production, however it is worthy of note that temperature is not the only factor for mycotoxin production, other factors are water activity, pH, availability of oxygen, presence of other fungi, strain variability, insects and pest presence, nutritional factors and antifungal agents.

Based on the findings of this work, total aflatoxin was found in gari, fufu and cassava flour with a mean value of 3.371 ± 0.25 (0.921–5.307), 2.201 ± 0.78 (0.913–3.634) and 2.596 ± 1.07 (0.363–3.680) respectively (Table 3). These levels are within the MLs guiding the United State (10 μg/kg) and EU (4 μg/kg), however among fufu samples, one was found above the EU limit. The MLs sometimes serve as barrier to international trade when a toxin level in an export crop exceeds their values, Nigeria mostly adopts the EU MLs. In Nigeria, studies carried out have revealed incidences of total aflatoxins in cassava products. In Anambra State, the concentration of total aflatoxin in gari was reported to be within a range of 0.44–3.69 μg/kg, while in Cross River, a range of 0.32–4.57 μg/kg was reported (Ogiehor et al., 2007). Ogiehor et al. (2007) reported the incidence of total aflatoxins in gari in some southern Nigeria states within the range 0.12–5.71 μg/kg.

In Ogun state, cassava flour has been reported to contain a mean value of 0.05 μg/kg of total aflatoxin (Adejumo et al., 2013). Report on total aflatoxin contamination in cassava flour carried out in Kenya, Nairobi and Mombasa reveals a mean contamination of 6.11 ± 3.05 μg/kg (Gacheru et al., 2015). Generally, the occurrence of total aflatoxins in fermented cassava products have been established and values that are above MLs are also established. However, based on the range reported in most studies, it is suggestive that unlike other staples especially cereal based ones, cassava products maintain lower levels of AFs, hence the predisposing factors if properly addressed can halt the incidence of aflatoxins in cassava products. This has been very difficult to achieve with other staples.

This survey as depicted in Table 4 has shown that OTA contaminates all the studied products with the highest concentration in gari followed by fufu, and cassava flour. This variation in concentrations and therefore

Table 3
Total aflatoxin levels (μg/kg) in cassava products in Anyigba, Kogi State, Nigeria.

Parameter	Cassava flour	Gari	Fufu
Total Samples	10	10	10
Contaminated Samples	10	8	9
Contamination (%)	100	80	90
Range	0.363–3.680	0.921–5.307	0.913–3.634
Mean ± SD (μg/kg)	2.596 ± .1.07[c]	3.371 ± 0.25[b]	2.201 ± 0.78[a]
Number of samples exceeding EU limits	0	4	0
Number of samples exceeding JECFA ML	Nil	3	Nil

Mean ± SD values with different superscript alphabets across a row are significantly different.

Table 4
Ochratoxin A (OTA) levels (μg/kg) in cassava products in Anyigba, Kogi State, Nigeria.

Parameter	Cassava flour	Gari	Fufu
Total Samples (N)	10	10	10
Contaminated Samples (n)	2	1	5
OTA Contamination (%)	20	10	50
Range (μg/kg)	1.813–4.337	0–12.44	0.354–9.480
Mean ± SD (μg/kg)	3.075 ± 1.79[c]	12.44 ± 0.00[a]	3.927 ± 3.81[b]
Number of samples exceeding EU limits	1	3	0
Number of samples exceeding JECFA ML	Nil	Nil	Nil

Mean ± SD values with different superscript alphabets across a row are significantly different.

vulnerability to the toxin is fermentation and processing dependent with OTA levels reducing with decreasing fermentation and processing (i.e. gari > fufu > cassava flour). The current study revealed low prevalence (26.7%) of OTA in cassava products from Kogi State and four of the samples analysed had OTA contents above the EU regulatory limit of 5 μg/kg for cereals and cereal-based products (CEC, 2006). Previously, Ezekiel et al. (2019) found out that 98% of cassava and gari samples from northern Nigeria contained OTA and more than 74% samples had values greater than 5 μg/kg while Ediage et al. (2011) reported the occurrence of OTA in 4 samples of cassava flour from the Republic of Benin. From Duala, Cameroun, 13% of fresh cassava analysed were contaminated with OTA with a mean value of 0.65 μg/kg (Ingenblek et al., 2019). Makun et al. (2013) analysed OTA in eighteen (18) samples of gari from markets in Minna, Nigeria and found 98.2% contamination with mean concentration of 3.28 ± 22.73 μg/kg. Ediage et al. (2014) did not find OTA in 420 samples of cassava products. In a more recent research, Olorunnado et al. (2019) reported the incidence of OTA in gari from Nigeria to have a mean of 0.028 ± 0.024 μg/kg.

The incidence of fumonisins in cassava products studied is reported in Table 5, while cassava flour did not have fumonisins, gari and fufu had 30% (0.006–0.15 μg/kg) and 40% (0.831–2.05 μg/kg) contamination respectively. This could be explained based on the earlier stated observations that fumonisin production is optimal under lower temperature and higher water activity. The processing and eventual market display of cassava flour requires high temperature and non-moist environment unlike fufu that had the highest incidence and level of FBs. Chilaka (2018) reported 25% (45–80 μg/kg) contamination of Nigerian gari with FB_1, Olorunnado (2019) reported that only one sample out of 41 analysed had FB1 and the value was 6.117 μg/kg from Benin republic and Cameroun, 25% (3/12) of dried cassava products analysed had FB_1 within the range of 19.90–91.61 μg/kg (Ingenblek et al., 2019). According to some earlier reports, Ediage et al. (2011) reported FB_1 (4–21 μg/kg) in cassava flour samples from the Republic of Benin, while Abass et al. (2017) upon investigating 373 processed cassava products in Nigeria, found in FB_1 in lafun (88.1 μg/kg) and fufu flour (102.7 μg/kg) however at very low incidences of 1/30 and 1/36 samples, respectively.

Table 5
Total Fumonisins levels (μg/kg) in cassava products in Anyigba, Kogi State, Nigeria.

Parameter	Cassava flour	Gari	Fufu
Total Samples	10	10	10
Contaminated Samples	0	3	4
FB_1 + FB_2 Contamination (%)	0	30	40
Range	0.363–3.680	0.006–0.146	0.831–2.054
Mean ± SD (μg/kg)	2.596 ± 1.07[c]	0.249 ± 0.31[a]	1.289 ± 0.541[b]
Number of samples exceeding EU limits	0	0	0
Number of samples exceeding JECFA ML	Nil	Nil	Nil

Mean ± SD values with different superscript alphabets across a row are significantly different.

Table 6
Dietary exposure to HCN from consumption of cassava products.

Food Sample	Mean total of HCN (mg/kg)	Estimated Daily intake (mg/kg. bw/day)	% TDI
Fufu	0	0	0
Cassava Flour	2.37	0.03	33.3
Gari	0.553	0.001	5

3.3. Dietary exposure of Anyigba residence to HCN and mycotoxins

Table 6 shows the level of dietary exposures to HCN from the consumption of fufu, cassava flour and gari. From the study, it is shown humans are more exposed to HCN poisoning from consumption of cassava flour used for Oje with an EDI value of 0.03 mg/kg.bw/day and % TDI of 150% when compared with gari having an EDI of 0.001 mg/kg. bw/day and %TDI of 5%. There was no measurable threat from the consumption of fufu with no appreciable level of the toxin.

The PTDI or PTWI was allocated for contaminants not cleared rapidly from the body and have capacities of accumulating in the body while the PMTDI is used for contaminants that are not known to accumulate in the body (Herrman & Younes, 1999). The PMTDI of HCN for cassava flour and gari were put at 0.09 and 0.02 mg/kg.bw/day (CAC, 2019) and exceedance of the endpoint may cause chronic toxicities in human population, most especially in populations with high consumption of cassava-based products and low protein diet (CAC, 2019). The present study indicates that %TDI levels of HCN from cassava products is considered safe on a long-term basis. Most reports of HCN toxicity in Nigeria are on acute toxicity effects, incidence such as sudden death of families who consumed cassava meal containing lethal dose of cyanide (Dufour, 2011; Nigerian Punch Newspaper, 2 Nov 2016). Chronic, low-level of cyanide exposure is associated with the development of goitre and with tropical ataxic neuropathy, a disorder that damages the nerves and render a person unsteady (Wagner, 2010). Severe cyanide poisoning, particularly during famines, is associated with outbreaks of konzo (*tied legs*) an irreversible paralytic disorder, and death. The incidence of konzo and tropical ataxic neuropathy can be as high as 3% in some areas according to Wagner (2010). A total of 1237 cases of konzo

Table 7
Dietary exposure to major mycotoxins from consumption of some major staples in Anyigba.

Food Sample	Mycotoxin	Mean total of mycotoxin (μg/kg)	Estimated Daily Intake (μg/kg.bw/day)	%TDI
Fufu	Total aflatoxins	2.201	0.0043	NA
	Ochratoxin A	3.927	0.0011 (0.0077 μg/kg.bw/wk.)	1.10 (7.7)
	Fumonisins	1.289	0.0025	0.13
Cassava Flour	Total aflatoxins	2.596	0.0315	NA
	Ochratoxin A	3.075	0.0053 (0.0371 μg/kg.bw/wk.)	5.32 (37.1)
	Fumonisins	0	0.0000	0.00
Gari	Total aflatoxins	3.371	0.0060	NA
	Ochratoxin A	12.435	0.0032 (0.0224 μg/kg.bw/wk.)	3.17 (22.4)
	Fumonisins	0.249	0.0004	0.02

*Note: The PTWI for ochratoxin A in common staples is 0.*1 μg/kg.*bw/week, while the PMTDI for fumonisins is 2.*0 μg/kg.*bw/day*. NA: Not Applicable.

were reported from the Democratic republic of Congo before 1975, 919 cases from 1975 to 1993, and 1303 cases from 1994 to 2009 (Nzwalo & Cliff, 2011). In Mozambique, Tanzania, and Central African Republic, 2123, 121, 16 cases of konzo were reported from 1975 to 1993; 281, 238, 18 and 469 cases of konzo were also reported in Mozambique, Tanzania, Central African Republic and Cameroon respectively, between 1994 and 2009. An occurrence of ataxic polyneuropathy was recorded in Ososa, Southwest Nigeria with 64 per 1000 in 2003 (Oluwole et al., 2003).

Dietary exposure to major mycotoxins such as aflatoxins, fumonisins and ochratoxins is among the leading contemporary health concerns. Estimated daily intake of total aflatoxins, ochratoxin A and fumonisins in fufu, gari, and cassava flour are given in Table 7. Although the percentage TDI from consumption of these toxins is insignificant. There is higher risk of dietary exposure to aflatoxin from cassava flour with EDI of 0.0315 μg/kg.bw/day. The cassava flour seems to be more susceptible to AFs contamination and least susceptible to FB contamination. Due to their ability to initiate carcinoma of the liver cells, aflatoxins are classified as group 1 carcinogen by IARC. Exposure to aflatoxins should be as low as reasonably achievable (ALARA); this is very critical (Herrera et al., 2019). According to American Cancer Society (2011) even EDI level as low as 0.001 μg/kg bw/day may induce liver cancer. Eventually, no threshold of exposure and tolerable daily intake (TDI) is established for aflatoxins. Although holistic exclusion of the aflatoxins from food produce may be very hard, reduced exposure to aflatoxins can be achieved by applying good agricultural and good management practices.

The consumption of gari and cassava flour with EDIs of 0.0032 and 0.0053 μg/kg.bw/week show highest tendencies towards contributing to Ochratoxin A poisoning compared to fufu (0.0011 μg/kg.bw/week). Eventually, no cassava product exceeded the accepted PTWI for OTA as such, the general public in Anyigba is unlikely to experience major toxicological effects of OTA on a short term based on consumption of cassava products. EDI and %TDI of OTA from roots and tuber in Niger state, Nigeria was reported between a range of 1.524–17.75 μg/kg bw/day and 1.067%–12.42% respectively (Onyedum, 2020), this is higher when compared with the EDI range of 0.0011–0.0053 μg/kg bw/day and %TDI range of 1.10–5.32%, respectively, in cassava products from Kogi state. In Kogi state, cassava is produced and processed at commercial quantity and transported to other states, among which Niger state is one of them. It is possible that product handling was a predisposing factor to having higher levels of OTA in cassava root and tuber product in Niger state than in Kogi state.

For all cassava products analysed, dietary exposure to FBs are minimal. Although the levels are low with %TDI of 0.13% and 0.02% in fufu and gari, respectively, fufu consumption affords higher exposure compared to other cassava products. Percentage TDI of 0.518% was reported for gari in Niger state (Onyedum et al., 2020).

One important aspect of this work is the observation that the various mycotoxins occur not only singly but in twos and threes in a few cases. The consequences of such toxin combinations on human health could be additive, synergistic or antagonistic (Miller, 1995). Interface between AFB_1 and FB_1 had an additive effect in mice, and amplified wounds in liver and kidneys of the investigational animals (Gelderblom et al., 2002). The concurrent exposure rabbits to OTA and AFB_1 demonstrated an antagonistic interaction between the toxins with regards to teratogenic effects (Wangikar et al., 2005), synergistic effects have been established for FB_1 and OTA combinations (Creppy et al., 2004). Speijer and Speijer (2004) postulated that combined exposure to several classes of mycotoxins generally results in an additive effect with a few minor exceptions, indicating synergistic interaction.

4. Conclusion

Hydrogen cyanide, total aflatoxin, ochratoxin A and fumonisins are reported as contaminants of cassava products in Anyigba, Kogi state Nigeria. The levels of the food contaminants found are of low toxicological consequences. Fermentation is helpful in reducing HCN level to a relatively safe level. Cassava flour stored under dry condition is less prone to fumonisins contamination but other mycotoxins were found to contaminate it.

The results infer that if good manufacturing practices and good handling practices are applied along the cassava value chain, HCN and mycotoxins will not be associated with the products. On the part of regulation, there should be enforcement of maximum permissible limits by concerned bodies, this will further strengthen the drive towards having contaminant 'safe' cassava products.

Author contribution

Daniel Ojochenemi Apeh: Conceptualization, Supervision, Writing - review & editing. Omachoko Mark: Investigation, Writing - original draft; food consumption aspect. Victor Ojogbane Onoja: Investigation, Writing - review & editing. Michael Awotunde: Investigation, Writing - original draft, fumonisins aspect. Taiwo Ojo: Investigation, Writing - original draft, total aflatoxin aspect. Precious Christopher: Investigation, Writing - original draft, ochratoxin aspect. Hussaini Anthony Makun: Supervision, Validation.

Declaration of competing interest

The authors declare no conflict of interest.

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Appendix A. Supplementary data

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