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**Incidence and health risk assessment of hydrogen cyanide and multi-mycotoxins in Nigerian garri**

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ABSTRACT

Garri is a granular, starchy food prepared by the fermentation of mashed cassava. Hydrogen cyanide (HCN) and mycotoxins are contaminants in certain foods at different points along the food value chain. The incidence and contamination levels of HCN and multi-mycotoxins in garri from five agroecological zones of Nigeria were determined using a spectrophoto-metric method and ultra-high-performance liquid chromatography-tandem mass spectros-copy (UHPLC-MS/MS), respectively. The health risk associated with the consumption of contaminated garri was assessed. The health risk assessment model was used to calculate the dietary exposure of humans to the mycotoxins in garri. This was done by estimating the daily intake (EDI), the percentage tolerable daily intake (%TDI), the annual hepatocellular carcinoma (HCC) cases attributable to exposure to aflatoxins (AFs) in garri, as well as the HCC risk. The average intake of garri was estimated at 0.303kg/day for a Nigerian adult. The incidence of HCN was 98.3% (0.056–2.463mg/kg), and fermentation reduced the HCN level in garri more than other processing steps. The twenty-one mycotoxins identified and quanti-fied were all within maximum levels, as applicable to those that are regulated by the EU. The %TDI for the other mycotoxins, with the exception of AFs, showed no alarming health risk with garri consumption. Annual HCC cases resulting from AF in garri were estimated at 10–60 cases for HBsAgþve individuals and 4–23 cases for HBsAg−ve individuals based on 8.1% hepatitis B virus (HBV) incidence. Results further revealed no interdependence between HCN levels and mycotoxin content. This work suggests an unlikely chance of acute toxicity from HCN and major mycotoxins from a garri-based diet in Nigeria. Hence, it is recom-mended that concerned regulatory bodies maintain the existing permissible limits for HCN in Garri.

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KEYWORDS

Hydrogen cyanide; multi-mycotoxins; garri; risk assessment; Nigeria

**Introduction** people around the world (FAO 2004). Nigeria

Cassava (Manihot esculenta crantz) is a perennial plant with conspicuous fan-shaped leaves resem-bling those of castor oil. It is grown for many reasons, which include food, industrial starch and filler, animal feed, making medications, papers, fabrics, and building materials. It is one of the most abundant staple crops grown in tropical Africa, Asia, and South America and provides a basic source of food for about 200 to 300 million

produces the largest tonnage of cassava annually; production was estimated at 59 million metric tons in the fiscal year 2018 (FAO 2019). According to data obtained from FAO, about 80% of Nigerians consume various cassava products all year round owing to the long shelf life of processed cassava (FAO 2019). Among the cassava products, G garri is the most consumed processed form of cassava products in West Africa. It is a granular in nature,

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starchy food. It is prepared from fermented cassava mash. It is consumed either directly by soaking it in water or by adding it to protein-rich foods like beans, or it is further processed with hot water into a pudding called ‘eba’, usually eaten with vegetable sauce or soups. Garri is rich in fiber, carbohydrates, copper, and magnesium and has a unique taste and flavor that is attributed to the fermentation of the lactic acid content (Akintonwa and Tunwashe 1992). However, cases of abdom-inal upset, stomach discomfort and death have been reported following the consumption of garri in some Nigerian communities (Akintonwa and Tunwashe 1992). Lung function abnormalities such as restrictive ventilatory function defects and obstructive defects were observed among garri processors, and it was reported that ventilatory function parameters of peak expiratory flow (PEF), forced expiratory volume in one second (FEV1), forced vital capacity (FVC), and FEV1/ FVC ratio were significantly lower among garri workers compared to the control population. It was concluded that the garri production process pre-dis-posed workers to increased respiratory symptoms and abnormal spirometry which was attributed to the presence of hydrogen cyanide (HCN) (Akintonwa and Tunwashe 1992). Hydrogen cyanide is a highly toxic substance obtained from the hydrolysis of cyanogenic glycosides (linamarin and lotaustralin), which are highly concentrated (about 130–200mg HCN/kg) in raw cassava. They could cause symptoms like calcific pancreatitis, apnea and cardiac arrest, permanent paralysis, goiter, and trop-ical ataxic neuropathy (Coursey 1973). These com-pounds must be removed by processing the cassava before consumption in any form (Oboh 2011). Aside from the concerns with HCN, moulds can be formed in exposed, improperly dried, or improperly stored cassava or its products. These mould mostly release toxic secondary metabolites, generally referred to as mycotoxins (Ogiehor et al. 2007; Makun et al. 2011).

It is estimated that 25% of the world’s food crops are contaminated by mycotoxins, of which the most common and toxicologically potent type are aflatoxins (AFs) (Ogiehor et al. 2007). Other common mycotoxins are ochratoxin, deoxynivale-nol, HT-2, and zearalenone. Generally some myco-toxin is associated with urothelial urinary tract

tumors, hemorrhage, diarrhea, emesis, leucopenia, immunosuppression, decreased reproductivity, and bone marrow damage (Sweeney and Dobson 1998). The International Agency for Research on Cancer (IARC 2002) has established the carcino-genicity of AFs and classified aflatoxin B1 (AFB1) as a Group 1 human carcinogen. The association between high exposure to AFB1 and increased human liver cancer has been established (Liu and Wu 2010). The NAIIS survey was a national, household-based survey that assessed the preva-lence of HIV and related health indicators, includ-ing the national prevalence of two additional blood-borne viruses, i.e. hepatitis B and C viruses, based on an estimated Nigerian population of 190 million. Meanwhile, 13.2% prevalence has been previously reported (Fasola et al. 2008; Liu and Wu 2010). The research carried out by Babalola (2014) in some communities in Nigeria showed some ‘garri’ samples contain residual cyanide with a concentration above 10mg HCN/kg maximum recommended by the WHO. It was found that ‘garri’ fried with palm oil has the lowest cyanide content. It was also found that the ‘garri’ samples with the least cyanide content were the ones fer-mented for a longer period of time (2–3days), while higher cyanide content was found in the ‘garri’ samples that were fermented before frying for a maximum of 48h. However, with the new insight and innovation of this research, the five agro-ecological zones of Nigeria were captured so as to extend the study area. This is because proc-essing techniques and procedures differ from one country to another and from localities to localities within a country according to food cultures. Moreover, the processing technique for cassava has an influence on the HCN level. Also, the research investigates if there is any effect of HCN in cassava on its mycotoxins level. Also in this research, twenty-four different types of mycotoxins were identified and quantified.The United Nations Food Association Organization and World Health Organization Coordinating Committee for Africa (FAO/WHO CCAfrica) of the Codex Alimentarius Commission in 2017 requested to know if it is appropriate to extend the existing maximum limit for HCN of 2mg/kg in garri to fermented cassava products and whether mycotoxins were of public health concern in these products. The request was

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against the background that cassava is a staple for more than one billion people worldwide, especially in Africa, Asia, and South America. Hence, this study was carried out to establish the incidence of HCN and mycotoxins in Nigerian garri, estimate the dietary exposure to the toxins from garri con-sumption, and characterize associated risks with the consumption of garri in Nigeria.

**Materials and methods**

***Sampling area and sample collection***

Garri samples were collected from five out of the seven agroecological zones (AEZs) of Nigeria, namely: Humid Forest (HF) (*n*¼11), Derived Savanna (DS) (*n*¼24), Mid-altitude (MA) (*n*¼5), Southern Guinea Savanna (SGS) (*n*¼12), and Northern Guinea Savanna (NGS) (*n*¼9). While the sixty-one (61) samples were analyzed for HCN, only forty-one (41) garri samples were analyzed for multi-mycotoxins. This is because twenty-four different types of mycotoxins were quantified in the samples as a goal, hence a comprehensive data on the level of the mycotoxins were achieved which further reduces the cost and ambiquity of the the table. Samples were collected in the rainy season (June) of the year 2017. The samples were collected in accordance with the method of the National Veterinary Laboratory (NVL 2016). Each garri sample (1kg) was collected in sterile polythene bags from markets and process-ing factory sites and transported to the biochemistry laboratory of the Federal University of Technology, Minna, Nigeria, for sample preparation. Data collec-tion Questionnaires were administered as a tool for the collection of data in the study. location (see Appendix I). The questionnaire focused on getting data on the processes used in garri production, the frequency of consumption of garri, and serving size. Participants included market vendors, the owners of garri processing factories, and random consum-ers. A digital Omron BF212 body composition monitor was used to obtain the weight of the respondents.

***Determination of hydrogen cyanide level and its incidence in Nigerian garri***

Hydrogen cyanide levels in garri were quantified according to the ninhydrin-based spectrophotometric

method described by Surleva et al. (2016) with little modification. A standard solution of CN- in 2% Na2CO3 was used to make calibrations at 0.02, 0.04, 0.08, 0.1, and 0.2mg/L, with 0.5mL ninhydrin solu-tion (5mg/mL in 2% Na2CO3) added to each stand-ard concentration. The blank contained 0.5mL of ninhydrin in 1mL 2% NaOH. The mixture was homogenized, then incubated for 15min for color development. For the garri samples analyzed, 0.01g each was added into a volumetric flask and made up to 5mL with 0.1% NaHCO3, after which it was soni-cated for 20min and centrifuged for 10min at 10,000rpm. The supernatant (40ml) was pipetted and added to 0.5mL ninhydrin in NaOH, then allowed for 15min for colour development. The absorbance was measured using a UV/Vis spectrophotometer (SURGISPEC SM 735, Surgical Medical, England) at a wavelength of 485nm.

The limit of detection (LOD) and limit of quan-tification (LOQ) of the setup were determined. LOD ¼ 0.01mg/kg; LOQ ¼ 0.03mg/kg. A recovery of 98±0.2% (98.0±0.2%) was obtained. The cali-bration curve had a linearity value of r2¼0.9038. Based on the calibration curve, the absorbance val-ues for each sample were used to determine their respective concentrations.

***Determination of multi-mycotoxins levels and incidence***

***Sample preparation and extraction procedures***

The extraction of mycotoxins from the ‘garri’ samples was carried out according to the method described by Abia et al. (2013) and Monbaliu et al. (2010). Each of the garri samples (5g) were extracted with 20mL of acetonitrile/water/formic acid (79/20/1 v/v/v) for 90min on a GFL 3017 rotary shaker at 180 revolutions per min. The extract was centrifuged for 5min at 300rpm, and the supernatant was filtered through a 0.22mm syringe filter into a 1.5mL amber vial bottle. The supernatant was analyzed for 23 mycotoxins using UHPLC-MS/MS.

***Mycotoxin screening using UHPLC-MS/MS***

Multi-mycotoxin screening was carried out at the analytical laboratory of the Food, Environment, and Health Research Group, University of Johannesburg, South Africa. The analytical method employed had

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**Table 1.** Incidence and concentrations of HCN in garri from five agroecological zones of Nigeria.

Agroecological zone/Location

Humid forest (HF) Derived savanna (DS)

Southern Guinea Savanna (SGS) Mid- altitude (MA)

Northern guinea savanna (NGS) Cumulative

*n*/*N* % contamination

11/11 100 24/24 100 11/12 91.67

5/5 100 9/9 100

60/61

Range (mg/kg)

0.221–1.935 0.056–1.593 0.168–0.174 1.315–2.463 0.734–1.508 0.056–2.463

Mean ± SD (mg/kg)

1.289 ± 0.603a 1.048 ± 0.388a 1.198 ± 0.428a 1.613 ± 0.480a

1.129 ± 0.240a

Samples above ML of 2 mg/kg (%)

0 0 0 20 0

Key: Values with the same superscript alphabets down a column are not significantly (*p>*0.05) different. *n* ¼ Number of samples contaminated, *N* ¼ Number of samples analyzed, ML ¼Maximum limit

been previously optimized by Rubert et al. (2012) and Chen et al. (2012). Shimadzu UHPLC-MS 8030 equipment (Shimadzu Corporation, Tokyo, Japan) was used. Data was acquired by a multiple reaction monitoring (MRM) method at optimized MS condi-tions for the analytes (Table 1). The interface nebu-lizing gas flow rate was 3L/min, the DL temperature was 250�C, the heat block temperature was 400�C, and the drying gas flow rate was 15L/min. The opti-mized parameters are listed in Table 1.

***Validation of UHPLC-MS/MS method for multi-myco-toxin extraction***

The recovery experiment was performed by spik-ing blank garri samples (previously confirmed to be free of any mycotoxins) with a known concen-tration (25ng/kg) of mycotoxins as described by Gbashi et al. (2019). The spiked samples were then extracted and analyzed by UHPLC-MS/MS, and the results were presented as the ratio of the recovered concentration to that of the spiked concentration. For linearity, standard calibration curves were plotted for all mycotoxins within the concentration range of 0.98ng/kg to 2,000ng/kg, and the coefficient of determination (R2) between the instrumental responses and the analyte con-centrations was determined. The limit of detection (LOD) and limit of quantification (LOQ) were determined using the signal-to-noise ratio (SN) of the matrix-matched standards as described by Gbashi et al. (2019) by first measuring the limit of blank (LOB). This was done by finding the mean of the blank samples and the standard deviation of the blank samples, after which the LOD was then calculated using this formula:

LOD ¼ 3*:*33 � ðr*=*SÞ (1) LOQ ¼ 10 � ðr*=*SÞ

Where r ¼ standard deviation of response; S¼Slope of calibration curve (2).

***Risk assessment***

***Hazard identification and dose response analyses*** The International Programme on Chemical Safety (IPCS) and WHO arrived at two different P-cancer factors for aflatoxin: the first one was for individu-als that do not have HBV infection being 0.01 cases/100,000/year/ng/kg bw/day and the second was for individuals which are infected with HBV being 0.30 cases/100,000/year/ng/kg bw/day (IPCS/ WHO 1998; Liu and Wu 2010)

***Dietary exposure assessment***

The level of human exposure to HCN and vari-ous mycotoxins was determined by calculating the estimated daily intake (EDI) using the for-mula below described by Saladino et al. (2017). The average garri consumption level was estab-lished for adult Nigerians based on the question-naires administered. The average body weights of male and female adult Nigerians were also deter-mined using a weighing scale.

*EDI* ¼

Mean conc*:* of toxins ðmg*=*kgÞ � Mean garri consumption ðkg*=*dayÞ Average body weightðkgÞ

(3)

***Health risk characterization***

***Determination*** ***of*** ***percentage*** ***tolerable*** ***daily intake (%TDI).*** The health risk characterization of each food contaminant, with the exception of AFs, which are carcinogens, was determined by calculating the percentage tolerable daily intake (TDI) of each mycotoxin. %TDI was calculated using the formula:

%TDI ¼ EDI � 100 (4)

The respective %TDI of the mycotoxins were also determined based on existing provisional maximum tolerable daily intakes (PMTDI/TDI)

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adopted from JECFA (JECFA 2001). The PMTDI is the reference value established by the Joint FAO/ WHO Expert Committee on Food Additives to indicate the safe level of intake of a contaminant with no cumulative properties. For contaminants with cumulative properties, a PTWI or PTMI is a more appropriate representation (IPCS 2009). The values obtained for OTA and OTB multiplied by 7 will give the estimated PTWI (provisional tolerable weekly intake).

***Determination of the burden of aflatoxin-induced hepatocellular carcinoma from garri.*** The formu-las used to arrive at estimates of both annual HCC cases per 100,000 for HBsAgþve and HBsAg−ve individuals, as well as those used to estimate the annual HCC cases based on populations that are HBsAgþve and HBsAg−ve, are given below.

Annual HCC cases*=*100,000 for HBsAg negative individual ng

¼ Aflatoxin exposure kgbw x 0*:*01

(5)

Annual HCC cases*=*100,000for HBsAg positive individuals ng

¼ Aflatoxin exposure kgbw x 0*:*30

(6) Annual HCC cases (7)

Aflatoxin exposure x AFB1potency factorð0*:*01 or 0*:*3Þ

100,000

x NðHBsAg − ve or þ veÞ

where is N the total population of the individu-als; 0.01 and 0.30 have been defined earlier.

***Determination of HCC risk.*** Taking into consider-ation the prevalence of HBsAgþve (8.1% or 13.2%) individuals in Nigeria’s total population, the carcinogenic potency (Pcancer) of AFB1 was calcu-lated as previously reported in the literature (Udovicki et al. 2019) as *Pcancer*¼0.01 *x* %*HBs*,*Ag*-−*ve*.þ 0.3%*x* %*HBs*,*Ag*-þ*ve*. (8) The risk of HCC incidence per year, resulting from dietary exposure to AFB1 through garri consumption, was calculated as follows: HCC risk¼EDI *x* P-cancer (9)

Data analysis A one-way analysis of variance (ANOVA) was used to determine statistically sig-nificant differences between the means of: (1) HCN levels obtained from the 5 AEZs; and (2)

mycotoxin concentration obtained from the AEZs. Regression analysis was performed to determine the interdependence between levels of HCN and the individual mycotoxins using SPSS version 20.

**Results and discussion**

***Incidence and concentrations of hydrogen cyanide in Nigerian garri***

The mean concentration of the HCN in all garri samples in each zone was calculated and reported in Table 2. The value was highest in samples from MA and least in DS samples, however, there was no statistically significant difference (*p<*0.05) among the values obtained across the agroecological zones. The study also showed that sixty of sixty-one garri samples analysed were contaminated with HCN within a range of 0.056 to 2.463mg/kg.

***Effect of duration of fermentation and de-watering steps of garri processing***

Slight variations were observed in the processing methods used for garri production, which may influence the level of hydrogen cyanide. The bar chart in Figure 1 reflects the mean and standard deviation of HCN based on the durations of fer-mentation and de-watering during garri process-ing. The samples that underwent fermentation for �3days had significantly lower HCN values (*p<*0.05) than those subjected to fermentation for just �1day. Also, the effect of de-watering was in such a way that garri samples drained for �1day had a higher HCN than those de-watered for �3 or �5days. No significant difference (*p>*0.05) was observed when garri was either de-watered for �3days or �5days.

***Multi-mycotoxin incidence and concentrations in garri from the five agroecological zones of Nigeria***

Forty-one garri samples were analyzed for the presence of mycotoxins, and about twenty-one mycotoxins were recovered from all the samples, which included the following having the highest incidences, which were greater than 95%: b-ZEN (100%), AME (100%), ZEN (97.6%), a-ZEN (97.6%), CIT (97.6%), KA (95.1%), and those with the lowest incidences *<*5% were AFB1

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**Figure 1.** Hydrogen cyanide levels based the duration of fermentation and dewatering.

**Table 3.** Estimated annual burden of HCC cases and Risk of HCC/year attributable to aflatoxin exposure from garri in HBsAg posi-tive and HBsAg negative populations in Nigeria.

Estimated annual HCC (per 100,000)

Annual HCC cases (HBsAg prevalence ¼ 13.2%)

HCC risk/ year (13.2%)

Annual HCC cases (HBsAg prevalence ¼ 8.1%)

HCC risk/ year (8.1%)

Types of aflatoxin

HBsAg þve Range (mean)

HBsAg −ve Range (mean)

�HBsAg þve Range (mean)

��HBsAg −ve Range (mean)

���HBsAg þve Range (mean) Range (mean)

����HBsAg −ve

Range (mean) Range (mean)

AFB1 0.063–0.388 0.002–0.013 16–97 (0.225) (0.008) (57)

AFB2 0.041–2.919 0.001–0.097 10–732 (0.599) (0.02) (150)

AFG1 0.051–0.111 0.002–0.004 13–28 (0.08) (0.003) (20)

AFG2 0.102–0.102 0.003–0.003 26–26 (0.102) (0.003) (26)

AFG ¼Aflatoxin G AFG ¼AflatoxinG , AFB ¼Aflatoxin *N*¼174,610,000.

3–21 1.01–6.24 10–60 4–23 (12) (3.63) (35) (13)

2–160 0.66–46.97 6–449 2–170 (33) (9.63) (92) (35) 3–6 0.82–1.79 8–17 3–6 (4) (1.28) (12) (5) 6–6 1.65–1.65 16–16 6–6 (6) (1.65) (16) (6)

B1, AFB2 ¼Aflatoxin B2 �*N*¼25,080,000, ��*N*¼164,920,000,

0.70–4.33 (2.52)

0.46–32.58 (6.68) 0.57–1.24 (0.89) 1.14–1.14 (1.14)

���*N*¼15,390,000,

(4.9%), AFG2 (2.4%), FB1 (2.4%), FB2 (2.4%), FB3 (2.4%), and 15-ACDON (2.4%). The mean concentrations, range of contamination, and inci-dence per AEZ of the analyzed mycotoxins are shown in Tables 2 and 3. Some mycotoxins were not detected in samples from some zones but were detected in those from other zones. For example, AFG2, FB1, FB2, FB3, and 15ACDON were found only in one zone each, namely: SGS, NGS, DS, and MA, respectively. AFB1 was detected in samples from two AEZs (NGS and SGS), HT-2 was detected in DS and NGS, while AFB2, OTB, ZEN, A-ZEN, b-ZEN, KA, TA, CIT, CPA, and AME were detected across the five AEZs. Mycotoxins detected in four of the five zones were AFG1, OTA, 3ACDON, and STECY. DON and NIV, commonly produced by Fusarium species, were not detected in any of the samples across the five agro-ecological zones. Concentrations of AFB1 in sam-ples from the NGS were higher compared to the levels in the SGS, though not statistically significant (*p>*0.05). OTA contamination in NGS was signifi-cantly higher (*p<*0.05) than that in HF, DF, and

SGS. The mean levels of HT-2 in NGS were signifi-cantly higher (*p<*0.05) than those in DS. Also, the levels of CPA detected varied across zones, with the highest level reported in NGS and the lowest level in SGS.

***Dietary exposure to HCN and mycotoxins from garri and risk characterization***

The daily intake of 13 mycotoxins and their metabo-lites was determined as shown in Table 3. Exposure to these mycotoxins was determined based on their mean concentrations and range. Daily intakes were high in KA, FB, A-ZEN, and 15ACDON compared to other mycotoxins. From Table 3, A-ZEN had the highest %TDI, followed by HT-2 toxin. HCN had a higher TDI compared to all the studied fungal tox-ins. 3.63.5 Annual Burden and HCC/year attribut-able to aflatoxin from Garri Estimates of the annual burden of HCC cases in Nigeria for populations that are HBsAgþve and HBsAg−ve based on either 8.1% or 13.2% prevalence were established. The risk of hepatocellular carcinoma among those that

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consume garri was also estimated (Table 3). When HBsAgþve values of 8.1% and 13.2% were consid-ered, cancer was calculated to be 3.35 and 4.83, respectively.

***Relationship between the level of HCN and various mycotoxins***

From the results of the regression analysis, with mycotoxins as the dependent variable and HCN lev-els as the independent variable, no interdependency was found between HCN levels and mycotoxins.

**Discussion**

In an attempt to address the question posed by CCAfrica 2017: seeking to know whether myco-toxins were of public health concern in fermented cassava products and to know if it is appropriate to extend the existing maximum limit for HCN of 2mg/kg in garri to other fermented cassava prod-ucts, this work has provided the HCN and multi-mycotoxin profile of garri produced in Nigeria, including their health risks, for policy making. Twenty-one mycotoxins were found in garri, and the estimates of dietary exposure to these contami-nants and cancer-related health risks from afla-toxin in garri in the country were determined. In this report, the EDI, which is a quantitative evalu-ation of the likely intake of chemicals *via* food on a daily basis (IPCS 2009), is the parameter for reporting the dietary exposure assessment. We observed some statistically significant variations (*<*95%) in the contamination levels of HCN and mycotoxins in different AEZs in Nigeria.

***Hydrogen cyanide content***

Hydrogen cyanide concentration was highest in MA and least in DS, being in the order of MA*>*HF*>*SGS*>*NGS*>*DS (range ¼ 0.056– 2.463mg/kg) (Table 2). Generally, HCN levels in all the samples were below the recommended CODEX Alimentarius Commission (CAC) maximum limit (2mg/kg in cassava flour) except for one sample from MA. The concentrations of HCN reported in this study were quite low when compared to those (range¼ 2.10–15.3mg/kg) reported by Babalola (2014). The authors reported HCN concentrations

in garri from the DS region to be higher than the maximum limit regulated in fermented cassava prod-ucts. Akinsola et al. (2015) obtained concentrations of 31.51mg/kg (42%) for white garri, 25.24mg/kg (34%) for light yellow garri, and 17.64mg/kg (2%) for deep yellow garri in Damaturu, in the Sahel savanna region of Nigeria. Some years ago, Blanshard et al. (1994) reported a 100% incidence (36/36) of HCN in garri with a mean concentra-tion of 8.60mg/kg in Freetown, Sierra Leone. An increasing awareness of the poisoning potency of unproperly processed garri due to pockets of epi-sodes of garri poisoning may have been influential towards the reduced level of HCN in garri in the current study when compared to previous studies (Aregheore and Agunbiade 1991). In this study, it was observed that the duration of fermentation had a greater impact on reducing HCN levels when compared to the duration of de-watering (Figure 1). This observation agrees with Iwuoha et al. (2013) and Babalola (2014), who also documented further reductions in cyanide levels during prolonged fer-mentation. Hence, more public enlightenment on the role of proper processing in food safety is encouraged and may be directed towards Garri value chain actors. Generally, the HCN levels of most samples obtained from the five AEZs were considered safe; however, dietary exposure among people to trace amounts of HCN over a long period of time can have a negative impact on health. One of the mechanisms of toxicity of HCN is that it causes inhibition in the flow of electrons at Complex IV in the electron transport chain, which results in a limited supply of oxygen and adenosine triphosphate (ATP); hence, HCN is regarded as a major respiratory poison (Eisler 1991). In this study, dietary exposure to HCN *via* garri consumption was estimated to be within the range of 273 to 12,017ng/kg bw/day (mean: 6125ng/kg bw/day). This is below the PMTDI of cyanide of 20,000ng/ kg bw/day. Also, the associated risk (%TDI) was between 1.37 and 60.09% (mean: 30.63%), hence HCN exposure can be regarded as being of low health risk to garri consumers.

***Aflatoxin content***

The level of AFB1 that contaminated Nigerian garri in this study was less than the EU

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maximum level of 2mg/kg for foods, being one of the most stringent regulations existing. The levels of other AF types found were also within the ML of AFB1. In Benin, Nigeria. Ibeh et al. (1991) reported an incidence of 30% (*N*¼10) contamin-ation of the garri sample by AFB1, with contam-ination ranging from 1500 to 2000mg/kg. Also, Adejumo et al. (2013) reported an AFB1 inci-dence of 72.2% (*N*¼18) with a mean contamin-ation value of 0.25mg/kg in garri samples. Ibeh et al. (1991) presented AFB1 concentrations in garri that were above the ML. Furthermore, it is worthy to note that the method used by Ibeh et al. (1991) may not have been very selective, and many other compounds may have contrib-uted to the intensity of the analyte fluorescence under UV light. In the current report, a more sensitive and selective method has been employed for the estimation of the target analytes. Indeed, contamination of food commodities by AFs is of great concern to food safety and public health. Aflatoxin is a unique mycotoxin in assessing health risk. This is due to its highly carcinogenic nature and because no level of exposure is con-sidered safe. The burden of aflatoxin-induced hepatocellular carcinoma was determined, and the risk of HCC per year was estimated for the AFs (Table 3). According to Liu and Wu (2010), sub-Saharan Africa, Southeast Asia, and China are the most burdened regions with respect to HCC induction by aflatoxin. They estimated that aflatoxin plays a role in 4.6–28.2% of all HCC cases worldwide and attributed the large range to uncertainty in cancer factors, HBV prevalence, exposure to aflatoxin, and other risk factors. Their estimations were based on exposure to AF from maize and peanut, and for Nigeria, they used a HBsAgþve prevalence of 13.2%, a popu-lation of 149 million, and EDI of 139–227ng/kg bw/day. Eventually, Liu and Wu (2010) arrived at an estimated annual HCC cases in Nigeria for hepatitis B-positive individuals to range between 8,200 and 13,400 and that for hepatitis B-negative individuals to range between 1,800 and 2,940 based on exposure to AF from maize and peanut. In the current study, we arrived at annual HCC cases based on 13.2% HBsAgþve prevalence to range between 16–97 and 3–21 for HBsAgþve and HBsAg−ve individuals, respectively. The

annual HCC cases based on the latest prevalence of 8.1% for HBsAgþve individuals were between 10 and 60 and 4 and 13 for HBsAg−ve individu-als. The study also determined the risk of HCC per year to be 1.01–6.24 HCC cases/year/105 based on 13.2% incidence and 0.70–4.33 HCC cases/year/105 based on 8.1% prevalence. Cancer risk estimates based on total AFs intake in 17 WHO GEMS/food consumption cluster diets were estimated at 0.189 HCC cases/year/105 indi-viduals for Cluster 6, 0.121 HCC cases/year/105 individuals for Cluster 10, and 0.084 HCC cases/ year/105 for Cluster 15 (Fasola et al. 2008).

***Zearalenone***

Zearalenone (ZEN) and its derivatives, a-ZEN and b-ZEN, were determined. The results show that ZEN, a-ZEN, and b-ZEN had the highest incidences among all studied mycotoxins. ZEN in dried cassava products sampled in the dry season from Cameroon had a mean value of 7.6lg/kg (Ingenbleek et al. 2019) which was higher than that detected in our samples, which were col-lected in the rainy season. The differences in con-centration in our study may result from seasonal variations during sampling, which is a factor in fungi’s production of mycotoxins. Seasonal varia-tions often come with changes in temperature and humidity in the environment. Despite the high incidence of ZEN and its metabolites, their EDI was far below their PMTDI, which means there is no severe health risk associated with con-sumption of ZEN and its derivatives in Nigerian garri. The average concentrations of 15-ACDON and 3-ACDON were 5.098mg/kg and 0.276mg/kg, respectively, which is lower than the maximum permissible level of 1250lg/kg set by the EU for DON and its metabolites. Chilaka et al. (2018) reported incidence and mean concentrations of 9/24 (57) and 3/24 (16) for DON and DON-3G, respectively, as well as incidence and mean con-centrations of 10/36 (62) and 3/36 (30) for DON and 15ACDON, respectively, in garri samples. The fungi that produce ZEA and its derivatives, F. *culmorum*, grows optimally between 20�C and 25�C, and F. *graminearum* grows optimally between 24�C and 26�C; both produce myco-toxin optimally between 29�C and 30�C. Is it

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therefore expected that DF, MA, and SGS would support the growth of these fungi and the pro-duction of mycotoxin at extreme temperatures, probably in all studied AEZs as found in this study (Sweeney and Dobson 1998; Sanchis and Magan 2004). Daily exposure to 15ACDON and 3ACDON from garri and the % TDI obtained in this study pose no health threat. DON and NIV, which are both type B trichothecenes, were not detected in the garri samples across Nigeria in this study.

***Fumonisins***

Fumonisin B1 was detected at relatively trace lev-els in the garri sample, with a mean concentra-tion of 6.117mg/kg (incidence ¼ 2.4%) in SGS only. Chilaka et al. (2018) reported higher inci-dences of FB contamination in Nigerian garri (FB1 incidence ¼ 25%; mean ¼ 6mg/kg, FB2 incidence ¼ 20.83%; mean ¼ 40mg/kg). From the sub-Saharan Africa TDS by the NRC in 1996, the authors presented the following FB incidences: FB1 (75.4%), FB2 (14.1%), and FB3 (6.2%). Just like in our study, Adebayo et al. (2017) got a low incidence of 3.3% (1/30) for FB1 in dried cassava flour, however, with a higher concentration than all other reports (88.1mg/kg). According to the literature, producers of fumonisins F. *verticil-lioides* have an optimum growth temperature between 23�C and 30�C, and F. *proliferatum* grows optimally at 30�C. Both of them produce FB optimally within a wide range of temperatures from 15�C to 30�C; DF, MA, and SGS present a more convenient temperature range for FB pro-duction. Based on our report, DF and SGS pre-sented FBs, but DF did not; NGS also presented FB (Sweeney and Dobson 1998; Sanchis and Magan 2004). The estimated daily intake of FBs from garri (19.38–39.52ng/kg bw/day) and their TDI (0.97–1.98%) were low and do not pose alarming food safety issues.

***Ochratoxins content***

Ochratoxins are common mycotoxins found in wide varieties of agricultural commodities; ochra-toxin A (OTA) had an incidence ¼ 22.0% and mean ¼ 0.028mg/kg and OTB had an incidence

¼ 51.2% and mean ¼ 0.0044mg/kg) are reported in this study. Our report can be considered to have observed lower concentrations when com-pared to an older publications, but with fewer samples and a different method of analysis (HPLC/UV). In the report, OTA was present in 100% of garri samples within a range of 3.28 to 22.73mg/kg and had a mean concentration of 7.63mg/kg. Makun et al. (2013) found A. *ochra-ceus*, a major OTA producer, had an optimum growth temperature of 24�C to 37�C and an optimum temperature for production of OTA of 25�C to 31�C; OTA concentration was higher in NGS and not reported in MA. DF and SGS are expected to present higher incidences of OTA due to their temperature, but that was not the case; however, both reported some levels of con-tamination by OTA (Sweeney and Dobson 1998; CAST 2003; Sanchis and Magan 2004). On the basis of EDI (0.01–0.434ng/kg bw/day) and TDI (0.07%–3.04%) estimated for ochratoxin A from garri, the mycotoxin does not present potential for acute toxicity or raise any health alarm. Acute and chronic exposure to ochratoxin A can pose health risks that include immunosuppression, teratogenesis, liver necrosis, potential nephro-toxin, liver damage, mutagenicity, and a weak genotoxic effect.

***Citrinin content***

Citrinin, a secondary metabolite of fungi associ-ated with stored food, was detected in 97.6% (*N*¼41) of garri samples in Nigeria. Citrinin is known for its nephrotoxicity; based on existing reports, it leads to necrosis of the distal tubule epithelium, degeneration, and alteration of renal tubule function (JECFA 2001). New information from Commission Regulation (EU) No. 212/2014 also suggests that CIT is both carcinogenic and genotoxic (EU 2014). A maximum level of 2000mg/kg exists for some rice-based fermented foods (EU 2014). The EDI (2.86ng/kg bw/day) obtained for CIT from Garri in this study is neg-ligible compared to the PMTDI (200ng/kg bw/ day). Kojic acid (a 5-hydroxy-2-hydroxymethyl-4-pyranone) had the highest mean concentration of 110.40mg/kg in Nigerian garri. Adebayo et al. (2017) obtained similar results of 102.65g/kg for

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white garri and 183g/kg for yellow garri. KA is present in high amounts in most of the samples, probably due to the growth of Aspergillus species being favored by environmental conditions. Exposure to KA has health consequences; although considered a weak carcinogen, it causes skin irrita-tion, genotoxicity, and ataxia. The health risk asso-ciated with KA as found in this study cannot be quantified due to a lack of established PMTDI for KA. Individuals were exposed to a range of 14.896 to 1860.853 (mean ¼ 538.621) ng/kg bw/day of KA daily from consumption of garri.

***Tenuazonic content***

Tenuazonic acid (TA) in this study had an incidence of 9.8%, a mean concentration of 0.1917mg/kg, and a range of 0.069 to 0.600mg/kg. Since no PMTDI has been fixed for TA, the EDI range of 0.337 to 2.927ng/kg bw/day determined in this study cannot be further characterized for its associated health risk. TA is an Alternaria mycotoxin mainly produced by *Alternaria alternata* (Chełkowski and Visconti 1992); some reports suggest it as a cause of hemorrhages in several dog and chicken organs. Mice fed TA were reported to have exhibited precancerous changes in the esophageal mucosa (Chełkowski and Visconti 1992). Alternariol monomethyl ether (AME), which is an emerging mycotoxin, has an average concentration of 0.3139–0.314mg/kg; this represents an average of the 97.6% contaminated samples. No PMTDI has been set for AME, hence the difficulty in estimating the risk of exposure to the mycotoxin. A PMTDI of 60ng/kg bw/day has been set for HT-2 toxin; this is far above the val-ues Nigerian garri consumers are daily exposed to, between 1.713 and 13.505ng/kg bw/day. The %TDI determined, which ranged from 2.85% to 22.51%, is indicative that there is low health risk due to the consumption of HT-2 toxin from Nigerian garri. The toxin was detected in only 14.6% of the studied samples in this study, and the values obtained ranged from 0.008 to 0.069mg/ kg. Since no PMTDI has been arrived at for CPA, the risk to human health when exposed to the toxin from garri cannot be quantified. The esti-mated daily intake of CPA from Nigerian garri ranged from 0.039 to 0.337ng/kg bw/day.

***Sterigmatocystin content***

Sterigmatocystin (STECY) is a precursor of AFB1. It is a carcinogenic mycotoxin (O’Brian et al. 2003), but up to 10 to 150 times less hepatocarcinogenic than AFB1 (Scudamore et al. 1996). Not many Aspergillus species produce AFs, unlike STECY, which is produced by more Aspergillus species (A. *flavus*, A. *parasiticus*, and A. *nidulans*) and even other fungi genera (Bensassi et al. 2011). In the cur-rent study, 17.1% of the incidence was recorded within the range of 0.057 to 0.324mg/kg (mean ¼ 0.125mg/kg). Since no PMTDI has been assigned for STECY, the dietary exposure, which ranged from 0.278 to 1.581ng/kg bw/day, may not be cat-egorically placed as safe or not. However, since there is similarity between STECY and AFB1, it is OK to have levels that are as low as reasonably pos-sible. A ‘no significant risk’ level for humans of 8000ng/kg bw/day was estimated by the State of California (1993). Based on this, there is little or no health risk associated with the consumption of STECY from Nigerian garri. The regression analysis between the cyanide level and each mycotoxin level shows that there was no interdependence between the cyanide level and the mycotoxin lev-els. However, research conducted by Chikezie et al. (2013) showed that low pH encouraged the production of AFs in fermented cassava products, especially the samples that were allowed to fer-ment. The study proposed that samples with high cyanide content present a poor medium for fungi. For all toxins with established PMTDIs (Table 3, footnote), the EDI estimates established in this study were lower than the PMTDIs’. This is a good indication that the risk assessment of HCN and mycotoxin exposure from garri is not alarming.

**Conclusions**

Hydrogen cyanide (HCN) was found in 98.3% of the samples analyzed, with concentrations across the AEZs depending largely on the duration of fermentation. Based on the risk characterization, the levels of HCN contaminating garri are unlikely to cause any acute toxicity. All the 41 garri sam-ples analyzed had contamination with at least one mycotoxin. Twenty-one mycotoxins, except DON

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and NIV, were detected at different concentra-tions. Also, no interdependence between HCN and mycotoxin levels was found. The mycotoxins quantified were all below their respective max-imum levels, as applicable to those that have exist-ing legislated limits. The %TDI determined for other mycotoxins, with the exception of AFs, showed no serious health risk associated with garri consumption. Annual HCC cases resulting from aflatoxin in garri were estimated at 10–60 cases for HBsAgþve individuals and 4–23 cases for HBsAg−ve individuals based on 8.1% HBV inci-dence. Based on the highly sensitive instrumentation used (UHPLC-MS/MS) and a wide representative sampling of the seven agro-ecological zones of Nigeria in this work, we therefore recommend that CCAfrica and CAC maintain the existing permis-sible limits for HCN in garri and extend them to other fermented cassava products, and we also advise that mycotoxins are not of great public health significance in garri from Nigeria.

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**Disclosure statement**

No potential conflict of interest was reported by the author(s).

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