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Aflatoxin Contamination of Garri Sold in Some Selected Markets in Benue State, North Central, Nigeria

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Abstract

The presence of aflatoxin-producing fungi in foods consumed by humans and animals has often resulted in the health hazards and even death. Aflatoxin contaminations of garri sold in some markets of Benue State, Nigeria, were studied, to ascertain the health implications on the consumers. Sixty garri samples comprising of 30 white garri and 30 yellow garri respectively were studied. The garri samples were ground to a particle size of 250 µm using a sterile blender. The total aflatoxin was extracted using 70% (v/v) methanol. The total aflatoxin concentration was detected and quantified using the Enzyme-Linked Immunosorbent Assay technique. The results showed that overall, 76.67% of the white garri samples and 80% of the yellow garri samples were aflatoxin positive. The total aflatoxin concentration in white garri ranged from 0.3 µg/kg to 2.4 µg/kg and 0.2 to 2.4 µg/kg in yellow garri respectively. The total mean aflatoxin across the States recorded was 2.96 µg/kg in white garri and 3.07 µg/kg in yellow garri. All the aflatoxin-positive garri samples of both the white and yellow garri were within the NAFDAC permissible aflatoxin level. Even though the aflatoxins are within the approved standard consumable limits, the continuous consumption of these doses over a long period of time could lead to the accumulation of these toxins in the body. These may eventually constitute a toxic health challenge.

Keywords

Aflatoxin, Garri, Enzyme-Linked Immunesorbent Assay (ELISA), Benue State

1. Introduction

Aflatoxins have been reported to pose a significant economic burden, causing an estimated 25% or more of the world's crops to be destroyed yearly [1]. Aflatoxigenic moulds under favourable conditions typically found in tropical and subtropical regions, including high temperatures and high humidity, normally found on dead or decaying vegetation, can invade food crops [1].

It has also been reported that drought stress, insect damage and poor storage can contribute to higher occurrence of moulds in these regions. The occurrence of aflatoxin is influenced by certain environmental factors; hence the extent of contamination will vary with geographical location, agricultural and agronomic practices, and the susceptibility of commodities to fungal invasion during pre-harvest, storage and/or processing periods [1]. Some authors have estimated that more than five billion people in developing countries globally are at risk of chronic exposure to aflatoxins through contaminated food [2] [3]. Aflatoxins have been considered to be unavoidable contaminants of food/feed and could occur at any of the stages of pre- and post-harvest conditions such as storage, transportation and food processing, even when the best practices are followed [4].

According to Ogiehor and Ikenebomeh [4], aflatoxin causes liver cancer: responsible for 5% - 28% of liver cancer cases. Aflatoxin causes stunted growth in children; it also causes immunosuppression and decreases resistance to infectious agents (e.g., HIV, tuberculosis). In animals, aflatoxins cause a variety of adverse effects including liver damage, impaired productivity and reproductive efficiency, and increased susceptibility to diseases. It has been estimated that 7761 out of 10,130 liver cancer cases in Nigeria in 2010 were attributable to aflatoxins [5]. The US Food and Drug Administration (FDA) has set limits of 20 μg/kg for total aflatoxins for human and animals, and 0.5 μg/kg for milk and its products [6]. The Nigeria's National Agency for Food and Drugs Administration and Control (NAFDAC) enforces a standard of 4 μg/kg for ready-to-eat foods and 10 μg/kg for raw food items, for packaged goods and export-bound products [7].

It has been reported by Wacoo *et al.* [8] that the toxicity and potency of the aflatoxins make them to be the primary health hazard as well as responsible for losses associated with contamination of processed foods and feed. The determination of aflatoxins concentration in food stuff and feeds is thus very important. However, due to their low concentration in foods and feedstuff, analytical methods for detection and quantification of aflatoxins have to be specific, sensitive, and simple to carry out. Several methods such as the thin-layer chromatography (TLC), high performance liquid chromatography (HPLC), mass spectroscopy, enzyme-linked immune-sorbent assay (ELISA), among others, have been described for detecting and quantifying aflatoxins in food [8]. The ELISA technique is one of the rapid test methods developed for the detection of aflatoxins in agricultural products [8] [9]. The ELISA technique is a safer, more suitable alternative to radio-immunoassays and uses non-radio-active signals; and works

by labeling either the antigens or the antibodies with enzymes, rather than isotopes [8] [9]. This reaction of the special antigen-antibody of the analyte can be detected by various markers [9]. Many markers have been developed, and these include enzymes, radioisotopes, fluorophores, gold nanoparticles, and some other sensitive optical and electrochemical components [10].

Garri is the most popular fermented cassava product in Africa. The production process of garri involves peeling, washing, grating, fermenting and toasting fresh cassava tuber (*Manihot esculenta* Crantz) [11] [12]. Palm oil is added according to preference (to make it a yellow garri). Palm oil added to the cassava mash gives the garri an aesthetic value and the palm oil also serves as source of vitamin A. Yellow garri is more nutritious and preferably cherished than the white garri [13]. Garri is stored and marketed in a ready-to-eat form and prepared into stiff paste or dough-like form called "eba" by adding the granules into hot water and stirring to make a paste of varied consistencies. The eba can be consumed with local soups or stews of various types by chewing or swallowing in morsels [14] [15]. Garri can also be consumed directly (without cooking) with groundnut, smoked fish, coconut, cowpeas, moimoi, or taken as fast food when soaked in cold water [16]. However, some unhygienic practices involved in production, processing of cassava to garri and post-processing handling such as spreading on the floor and mats after frying, displaying in open bowl or buckets in the markets during sales; the use of various packaging materials to transfer finished products from rural to urban areas and the use of bare hands during handling and sales may lead to microbial contamination due to deposition of bioaerosols on exposed products and transfer of infectious agent during handling [15] [16] [17]. Moulds such as *Aspergillus*, *Penicillium*, *Fusarium*, *Rhizopus*, *Cladosporium* and *Mucor* have been implicated to be associated with garri during storage and distribution [17] [18].

However not all moulds are implicated in the production of aflatoxin. The present study investigated aflatoxin contamination of garri sold in some selected markets in Benue State, North Central, Nigeria. Aflatoxin was extracted from garri samples using methanol. The total aflatoxin concentration was detected and quantified using the Enzyme-Linked Immunosorbent Assay technique (ELISA). The result was related to set food safety standards.

2. Materials and Methods

2.1. Collection of Samples

Two hundred and sixteen (216) garri samples were purchased from different markets across the state. Sixty garri samples comprising of 30 white garri and 30 yellow garri respectively, out of the 216 were used for the aflatoxin detection after preliminary determinations. These were from different markets from 12 Local Government Areas (LGA) across the three senatorial zones of Benue State. These markets are: Daudu, Abinsi and Gbajimba in Guma LGA; Tyowane, Gbaragboghol and Abwa in Buruku LGA; Gboko Main market, Yandev and

Tse-Kucha in Gboko LGA; Adikpo, Jato-Akaa and Ikyogen in Kwande LGA; Ushongo, Lessel and Sati Ikov in Ushongo LGA; Vandeikya, Ihugh and Tsar in Vandeikya LGA. Other markets are: High Level, Wadata and North Bank in Makurdi LGA; Tse Agberagba, Korinya and Tongo in Konshisha LGA; Edumoga, Okpoga and Ugbokolo in Okpokwu LGA; Ihigile, Ihiejwo and Ihiobila in Oju LGA; Orokam, Otukpa and Owukpa in Ogbadigbo LGA; Otukpo Main market, Adoka and Ogobia in Otukpo LGA.

2.2. Preparation of Garri Samples and Aflatoxin Extraction

The sample extraction was done according to the manufacturer's instructions from R-Biopharm AG (RIDASCREEN Aflatoxin Total test kit). The garri samples were ground to a particle size of 250 micrometers (μm) using a sterile blender. Then, 2 g aliquot of ground portion of the garri samples were weighed out and added to 10 mL of solvent for extraction. The solvent was 70% methanol that was obtained by adding 30 mL of distilled water to 70 mL of absolute methanol in a very sterile bottle with cover. The mixtures were stirred for ten minutes using a shaker at room temperature and allowed to settle down. The corresponding suspensions were filtered through Whatman No. 1 filter paper. One hundred microliters (μL) of the filtrate were diluted with 600 μL distilled water. Then, 50 μL of the diluted filtrate were used per well in the tests. Thereafter, the filtrate was kept in a tight container for the detection and quantification of the total aflatoxins present in the garri samples.

2.3. Detection and Quantification of Aflatoxin

The total aflatoxin was detected and quantified using the Enzyme-Linked Immunosorbent Assay (ELISA) kits from the R-Biopharm AG (RIDASCREEN® Aflatoxin Total) test kit. The instructions of the manufacturer were followed strictly. Briefly, a sufficient number of microtiter wells were inserted into the microwell holder for all standards and samples to be tested. Standard and sample positions were recorded. Fifty (50) microliters (μL) of the standard or prepared sample were added to separate duplicate well. Also, 50 μL of the conjugate was added to each well. Then, 50 μL of the antibody was added to each well. These were gently mixed by shaking the plates manually and incubated for 30 minutes at room temperature of 20˚C - 25˚C. The liquid was poured out of the wells and the microwell holder tapped upside down vigorously against absorbent paper; this was done three times in a row to ensure complete removal of liquid from the wells. The wells were filled with 250 μL wash buffer. The wells were emptied again. This was repeated two more times. Then, 100 μL of substrate/chromogen was added to each well and mixed gently by shaking the plate manually and incubated for 15 minutes at room temperature of 20˚C - 25˚C. Also, 100 μL of the stop solution was added to each well. In addition, 100 μL of the stop solution was added to each well. These were gently mixed by shaking the plates manually. Finally, the absorbance (optical density, OD) was measured at 450 nm, and read

within 30 minutes after addition of stop solution with a micro titer plate reader.

3. Results

The mean aflatoxins levels according to the studied Local Government Areas (Figure 1) showed the distribution of the aflatoxins. Kwande LGA has the highest mean aflatoxin concentration of 1.68 μg/kg (equivalent to 1.68 parts per billion, ppb) (14.26%). Ogbadigbo LGA has the lowest mean aflatoxin concentration of 0.44 μg/kg (3.74%); Others are: Vandeikya: 0.72 μg/kg (6.11%); Otukpo: 1.24 μg/kg (10.53%); Oju: 0.82 μg/kg (6.96%); Makurdi: 0.58 μg/kg (4.92%); Ushongo: 1.16 μg/kg (9.85%); Okpokwu: 0.92 μg/kg (7.81%); Guma: 1.12 μg/kg (9.51%); Konshisha: 1.38 μg/kg (11.71%); Buruku 0.64 μg/kg (5.43%); and Gboko 1.08 μg/kg (9.17%).

As could be seen in Figure 2, aflatoxin concentration according to the markets showed that white garri sample from Tongo market in Konshisha Local Government Area (LGA) has the highest concentration of 2.4 μg/kg and Jato-Akaa market in Kwande LGA has 2.4 μg/kg in yellow garri, both in Zone A. senatorial area. There were no aflatoxins seen in some yellow garri sold in some markets like Ihugh market in Vandeikya LGA, and Sati Ikov market in Ushongo LGA, in Zone A.

The highest aflatoxin concentration of 2.4 μg/kg as could been seen in Figure 3 was recorded also in Gboko Main market in Gboko LGA in white garri, and 2.0 μg/kg in yellow garri from Abinsi market in Guma LGA. However, there were no aflatoxins in white garri sample from Wadata market in Makurdi LGA, yellow garri sample from Gbajimba market in Guma LGA, yellow garri sample from Tse-Kucha market in Gboko LGA, and Abwa market in Buruku LGA, all in Zone B.

The highest aflatoxin concentration from the Zone C markets as shown in Figure 4 were 2.1 μg/kg from a white garri sample from Ihiobile market in Oju LGA and 2.1 μg/kg from Adoka market for the yellow garri in Otukpo LGA. No aflatoxins were also recorded in some markets in Zone C such as in white garri from Otukpo Main market in Otukpo LGA, in yellow garri from Ihiobila and Ihiejwo markets in Oju LGA, white garri from Owukpa market in Ogbadigbo LGA and in white garri from Ugbokolo market in Okpokwu LGA.

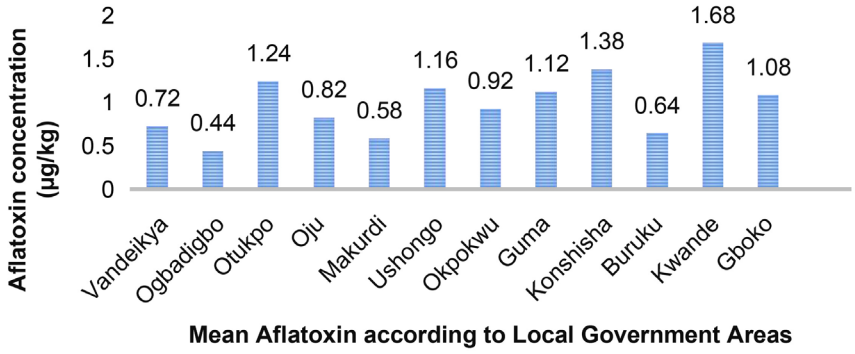

Figure 1. Mean aflatoxin concentration of garri samples by LGA.

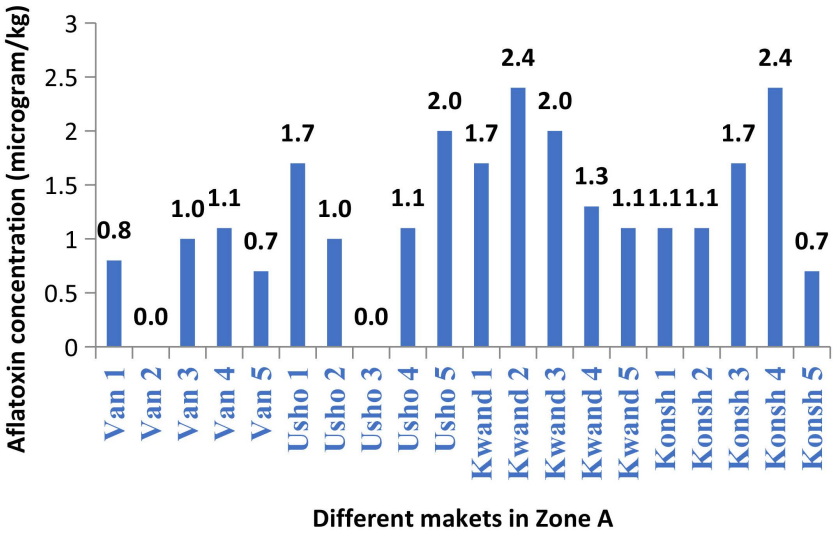

Figure 2. Aflatoxin levels from different Market Locations in Zone A. Keys: 1, 3, and 5 are white garri samples; 2 and 4 are yellow garri samples. Van = Vandeikya LGA: (1 and 2 = From Ihugh market, 3and 4 from Tsar market, 5 from Vandeikya market); Usho = Ushongo LGA: (1 and 2 from Ushongo market, 3 and 4 from Sati Ikov market, 5 from Lessel market); Kwand = Kwande LGA: (1 and 2 are from JatoAkaa market, 3 and 4 are from Ikyogen market, 5 is from Adikpo market).

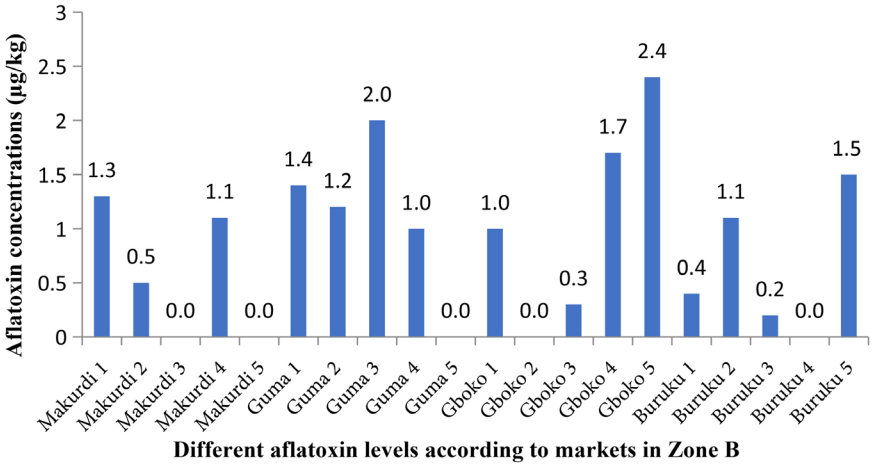

Figure 3. Aflatoxin levels from different Market Locations in Zone B. Key: 1,3, and 5 are white garri samples; 2 and 4 are yellow garri samples.Makurdi LGA: (1 and 2 are from North Bank market, 3 and 4 are from Wadata market, 5 is from High Level market) Guma LGA: (1 and 2 are from Daudu market, 3 and 4 are from Abinsi market, 5 is from Gbajimba market) Gboko LGA: (1 and 2 are from Tse-Kucha market, 3and 4 are from Yandev market, 5 is from Gboko Main market). Buruku LGA: (1 and 2 are from Tyowane market, 3 and 4 are from Abwa market, 5 is from Gbaragbogol market.

The highest mean aflatoxin concentration of 1.29 µg/kg (as shown in **Table 1**) was found in Zone A senatorial district and lowest was 0.83 found in Zone B for the white garri. For the yellow garri samples, the highest mean aflatoxin concentration of 1.20 µg/kg was found in Zone A and the least of 0.88 µg/kg was found in Zone B senatorial district.

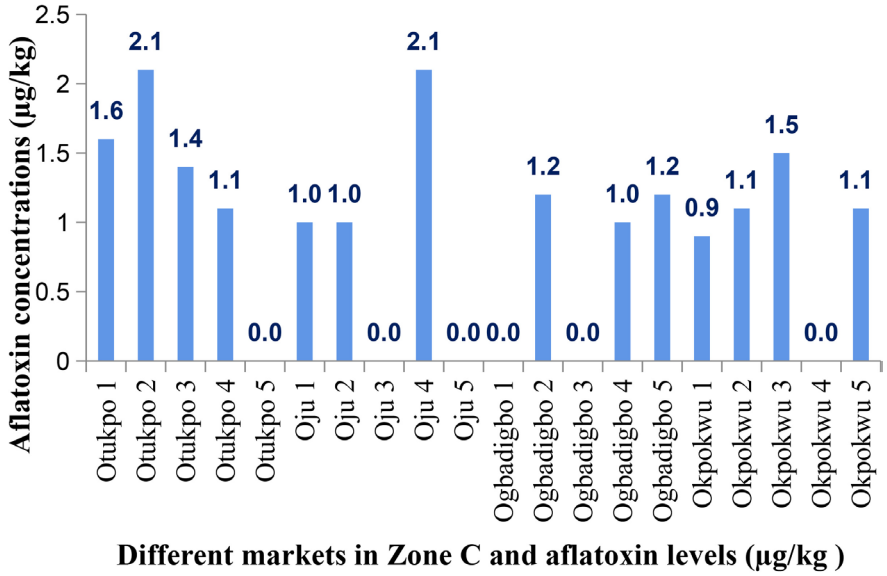

Figure 4. Aflatoxin levels from different Market Locations in Zone C. Key: 1, 3, and 5 are white garri samples; 2 and 4 are yellow garri samples Otukpo LGA: (1 and 2 are from Adoka market, 3 and 4 are from Ogobia market, 5 is from Otukpo Main market). Oju LGA: (1 and 2 are from Ihigile market, 3 and 4 are from Ihiobila market, 5 is from Ihiejwo market). Ogbadigbo LGA: (1 and 2 are from Orokam market, 3 and 4 are from Owukpa market, 5 is from Otukpa market). Okpokwu LGA: (1 and 2 are from Edumoga market, 3 and 4 are from Ugbokolo market, 5 is from Okpoga market.

Table 1. Mean aflatoxin concentration of garri according to the 3 senatorial zones.

	Total Samples tested	White garri	Positive (%)	Negative (%)	Mean aflatoxin concentration (µg/kg)	Yellow garri	Positive (%)	Negative (%)	Mean aflatoxin concentration (µg/kg)
Zone A	20	10	10 (100)	0 (0)	1.29	10	8 (80)	2 (20)	1.20
Zone B	20	10	7 (70)	3 (30)	0.83	10	8 (80)	2 (20)	0.88
Zone C	20	10	6 (60)	4 (40)	0.84	10	8 (80)	2 (20)	0.99
Total	**60**	**30**	**23 (76.67)**	**7 (23.33)**	**2.96**	**30**	**24 (80)**	**6 (20)**	**3.07**

4. Discussion

The results showed that 76.67% of the white garri samples and 80% of the yellow garri samples were aflatoxin positive. The total aflatoxin concentration in white garri ranged from 0.3 µg/kg to 2.4 µg/kg and 0.2 to 2.4 µg/kg in yellow garri respectively. These ranges are in agreement with the previous studies reported by [15] (in Rivers: 0.17 - 3.14 µg/kg; Lagos: 0.012 - 2.54 µg/kg; Ondo: 0.18 - 2.41 µg/kg; and Ogun: 0.25 - 1.66 µg/kg); [7] in Katsina and Zaria metropolis, where the aflatoxin levels were 1.0 - 1.4 µg/kg and [19] in Njaba, Imo State, where the levels of aflatoxins of the garri samples were ranged between 0.26 and 0.55 µg/kg.

The aflatoxin levels in these garri samples in this study might not be unconnected with the array of aflatoxigenic organisms isolated in the garri samples sold in markets in Benue State. These isolated organisms included: *Aspergillus fumigatus*, *A. niger*, *A. aculeatinus*, *A. flavus*, *A. aculeatus.* Others are *Penicil-*

lium digitatum, *Rhizopus stolonifera*, *Mucor mucedo*, and *Fusarium oxysporum*. Previous studies have also implicated moulds such as *Aspergillus*, *Penicilium*, *Fusarium*, *Rhizopus*, *Cladosporium* and *Mucor* to be associated with garri spoilage during storage and distribution [17] [18].

The *Aspergillus aculeatinus* and *A. aculeatus* belong to the group of "cryptic" *Aspergillus* species that were not formerly known, and use of cultural and traditional methods of characterization were not able to categorize and differentiate them from the other *Aspergillus* strains, but have now been successfully characterized by use of DNA sequence. Gautier *et al.* [20] had noted that recently, these organisms have been a major cause of life-threatening infections, especially in individuals whose immunities have been compromised. These organisms belong to the Nigri section of the *Aculeatus* clad, and included are *Aspergillus aculeatinus*, *A. aculeatus*, *A. indologenus*, *A. japonicus*, *A uvarum*. [20]. The *Aspergillus aculeatinus* had previously been isolated in beach sand in Malaysia in 2015 [21]. These organisms cause aspergillosis, with various clinical presentations such as aspergilloma also known as fungus ball of the lung, asthma exacerbations and severe asthma with fungal sensitization, allergic bronchopulmonary aspergillosis, cutaneous and wound infections, osteoarticular infections, chronic pulmonary aspergillosis, chronic invasive and granulomatous sinusitis, *Aspergillus* bronchitis, invasive pulmonary aspergillosis, and disseminated aspergillosis [22] [23]. These organisms have been reported somewhere to be very resistant to antifungal agents [24].

The total mean aflatoxin across the State recorded was 2.96 µg/kg in white garri and 3.07 µg/kg in yellow garri. On the average, the yellow garri recorded higher number of aflatoxins of 3.07 µg/kg across the State, as compared to the white garri that recorded total mean aflatoxin concentration of 2.96 µg/kg across the State. This was in contrast to the findings of [19] in Njaba, Imo state, that reported a reduction in aflatoxin contents in yellow garri as against a higher aflatoxin level in the white garri. Chikezie and Orjiako [19] attributed the reduction of aflatoxin in the yellow garri to the presence of red oil in the yellow garri. However, the differences in the present study may be due to the processing methods of the garri in Benue State, differences in geographical locations and also contamination by the garri handlers/sellers.

The highest total aflatoxin concentration found in garri in the markets according to zones were: 2.4 µg/kg in Jato-Akaa market in Kwande LGA, and also 2.4 µg/kg in Tongo market in Konshisha LGA, both in Zone A. In zone B senatorial district, 2.4 µg/kg in Gboko Main market in Gboko LGA, and 2.0 µg/kg in Abinse market in Guma LGA were recorded. Also, 2.1 µg/kg both in Adoka market in Otukpo LGA and Ihiobila market in Oju LGA respectively were recorded. Both LGAs are in Zone C. However, all the aflatoxin concentrations were within the approved aflatoxin level in the ready-to-eat food contents (such as garri) approved by NAFDAC in Nigeria, EU and USFDA. There were no aflatoxins seen in some garri sold in some markets like Ihugh market in Vandeikya

LGA, and Sati Ikov market in Ushongo LGA, in Zone A. Also, from Wadata market in Makurdi LGA, Gbajimba market in Guma LGA, Tse-Kucha market in Gboko LGA, and Abwa market in Buruku LGA, all in Zone B. No aflatoxins were also recorded in some markets in Zone C such as Otukpo Main market in Otukpo LGA, Ihiobila and Ihiejwo markets in Oju LGA, Owukpa market in Ogbadigbo LGA and Ugbokolo market in Okpokwu LGA.

The mean concentration and absorbance of the total aflatoxins were 0.98 ± 0.7226 and 1.29 ± 0.177798, respectively. All the aflatoxin-positive garri samples of both the white and yellow garri were within the NAFDAC permissible aflatoxin level. The concentration of the total aflatoxins level in garri was statistically significant at $P \leq 0.01$. Even though the aflatoxins are within the approved standard consumable limits, the continuous consumption of these doses over a long period of time can lead to the accumulation of these toxins in the body. These may eventually constitute a toxic health challenge.

5. Conclusion

Aflatoxin contaminations of the garri quantified using the Enzyme-Linked Immunosorbent Assay technique showed that 76.67% of the white and 80% of the yellow garri samples were aflatoxin positive. The total mean aflatoxin across the States recorded was 2.96 μg/kg in white garri and 3.07 μg/kg in yellow garri. All the aflatoxin-positive garri samples of both the white and yellow garri were within the NAFDAC permissible aflatoxin level.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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