Original Research Article

Study of Aflatoxin Content and Bacteriological Assay of Garri Collected From Ado–Ekiti in Ekiti State

ABSTRACT

Introduction: The majority of people in Nigeria consume Garri, as it is known there. It can be consumed by mixing it with cold water, sugar, or milk to make a snack, or by reconstituting it with hot water to make a thick paste that can be eaten with soup or stew. The lack of standardization in production and handling procedures, however, has led to a product with a range of quality and safety indices.
Aims: This study examined the level of microbiological contamination, the presence of aflatoxin, and its dispersion in Garri procured in Ado-Ekiti.
Methodology: Altogether 14 samples were collected into sterile ziplock bags. Isolation of fungi was carried out using direct plating method.
Results: The result showed six (6) genera of fungi viz: *Aspergillus* spp., *Penicillium* spp., *Rhizopus* spp., *Fusarium* spp., *Mucor* spp., and *Yeast*. The bacteria isolated and identified from the samples include; *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Bacillus* spp., and *Pseudomonas* spp. Presence of these organisms pose risk on both Human and Animals. These were analyzed for aflatoxin content using standard procedures. Aflatoxins B1 (AFB1) was detected in varying concentrations ranging from 13ppm-259ppm while the concentration of aflatoxin B2 (AFB2) was between 3ppm-16ppm. The concentration of Aflatoxin G1 and G2 were zero.
Conclusion: High levels of aflatoxins pose a great risk to human health. It is hereby recommended to implement steps like prioritizing sanitation in the state to protect the lives of the populace. Also handlers/vendors should be educated on proper handling of food.

Keywords: aflatoxin, assessment, bacteriological

1. INTRODUCTION

Most Nigerians consider garri, as it is commonly called, to be a basic cuisine. It is made by fermenting freshly grated *Manihot esculenta* (rantz) cassava roots. This cassava was first grown in tropical America and is now grown around the world [1]. It is a by-product of cassava. Regardless of ethnicity or socioeconomic status, most Nigerians include this crucial item on their menus, making it the most popular supper for both the wealthy and the underprivileged. It is very well-liked because it is ready to eat [2]. According to reports, aflatoxins are a substantial financial burden, destroying 25% or more of the world's crops each year. Aflatoxigenic molds, which are often found on dead or decaying plants and are typically found in tropical and subtropical climates with high temperatures and high humidity, can attack food crops under favorable conditions [3].

Additionally, it has been noted that these areas may see more mould outbreaks due to drought stress, insect damage, and inadequate storage. According to the Food and Agriculture Organization (FAO), mycotoxins affect one-fourth of the world's crop [4, 5].

Mycotoxins are produced mostly by the three genera: *Aspergillus*, *Fusarium*, and *Penicillium* of fungi [6]. Aflatoxins (AFs), one form of very poisonous mycotoxins, are known to infect a variety of foods, including maize, groundnuts, dried fruits, cassava, beef, and dairy-based products [7, 8, 9].

Aflatoxins are made by the *Aspergillus* species. *A. Flavus A. Nomius* and In addition to being produced by other *Aspergillus* species like *A. parasiticus* [10]. Aflatoxin production is regulated by a number of environmental conditions. Therefore the degree of contamination will differ depending on the region, the farming and agronomic methods used and the susceptibility of the commodities to fungal invasion before harvest, during storage, and/or during processing [3]. According to some authors, there are more than five billion people in underdeveloped nations who run the risk of being chronically exposed to aflatoxins because of tainted food. Even when the finest standards are followed, aflatoxins have been thought to be unavoidable pollutants of food and feed that can appear throughout any step of pre- and post-harvest settings, including storage, transportation, and food processing.

Aflatoxin causes liver cancer, accounting for 5% to 28% of instances, claim Ogiehor and Ikenebomeh [11]. Children's growth is stunted as a result of aflatoxin. Garri has been linked to molds like *Aspergillus*, *Penicillium*, *Fusarium*, *Rhizopus*, *Cladosporium*, and *Mucor* during storage and distribution [12]. As a result, this study aims to look at the distribution, prevalence, and fungal contamination levels of Aflations B1, B2, and G1 in market garri marketed in Ado Ekiti.

2. MATERIAL AND METHODS

2.1 Gathering of samples

At seven separate marketplaces in Ado Ekiti (Oja Ago, Oja Bisi, Mojere market, Shasha market, Ojido, Irona, and Oja Aba-Erifun), a total of fourteen (14) samples of gari were acquired from food vendors. The Samples were correctly labeled with the names of the markets, the date, and the time of collection. Sterile ziplock bags were used to carry samples to the lab for analysis.

2.2 Sample setting up and cleaning up the immunoaffinity

Using a lab homogenizer, five grams (5g) of finely ground sample were extracted with 25 milliliters (7 mL) of 70% aqueous methanol before being filtered through filter paper (Whatman no.1). 50 liters of the diluted sample were applied to an immunoaffinity column (RBiopharm Ag, Darmstadt, Germany) together with 100 liters of each filtrate to clean up the samples. Sample washing was based on an antigen-antibody response. the gel suspension-filled column to which covalently bound monoclonal antibodies were present. Specific Aflatoxin B1, B2, G1, G2, and M antibodies exist. 0.5mL of HPLC grade methanol was used to elute the total aflatoxins, which were then measured using an ELISA microplate reader model 680. (Bio-Rad India).

2.3 Measurement of the total amount of aflatoxin

Using the RIDASCREEN total aflatoxin kit from Darmstadt, Germany, competitive ELISA was used to provide a quantitative study of total aflatoxin. In duplicates, 50 mL of the aflatoxin standard solution and the cleaned, eluted sample were added to the wells of the microtiter plate. Each well of the microtiter plate received 50 mL of the peroxidase enzyme

conjugate and 50 mL of the mouse monoclonal anti-aflatoxin antibodies, which were then incubated for 30 minutes at room temperature and in the dark. Following three complete washes with 250 liters of distilled water, each well received 50 liters of urea peroxidase (the substrate) and 50 liters of tetramethylbenzidine (the chromogen), which were properly mixed before being incubated for 30 minutes at room temperature in the dark. Add 100 l of 1M sulfuric acid (the stop reagent) to the reaction to halt it, and then use an ELISA microplate reader Model 680 to detect the absorbance at 450 nm (Bio-Rad India). With total aflatoxin standards ranging from 0 ppt to 4050 ppt, a calibration curve was created [13].

2.4 Microbiological evaluation

Each sample was weighed out at ten grams (10 g) and homogenized in fifty milliliters (50 ml) of sterile distilled water. The homogenates were diluted tenfold to a concentration of 10-4 before being replicatedly plated on MacConkey agar and Eosin Methylene blue agar using the pour plate method. The plates were then incubated for 24 to 48 hours at 37°C. On Nutrient Agar, a total count of aerobic bacteria was done. Colonies were counted using an illuminated colony counter at the conclusion of the incubation period (Gallenkamp, England). The count was given as colony forming units per gram (CFU/g) for each plate [14]. Gram's response, catalase test, coagulase test, citrate test, and sugar fermentation test are a few of the biochemical and morphological traits used to identify the microorganisms present.

2.5 Analytical Statistics

Using Microsoft Excel Package 2010, the sensory scores were subjected to an analysis of variance (ANOVA), and Fisher's LSD test was used to differentiate the treatment means.

3. RESULTS AND DISCUSSION

3.1 Results

Table 1 shows the concentration of aflatoxin in the garri sample. Aflatoxin B1 was abundance in the entire samples, except for sample DG7 (Irona market). B2 was also present in all the samples in varying concentration. Aflatoxin G1 and G2 were absent.

Table 2 shows the morphological characteristics of fungi isolated from the garri samples. The identified fungal isolates include *Aspergillus flavus*, *A. niger*, *Rhizopus* spp., *Penicillium* spp., *Fusarium* spp., *Mucor* spp., and Yeast.

Morphological and biochemical characteristics of the bacteria isolates from the garri samples are presented in table 3. The isolates were flat in elevation and mostly even in margin while some are wavy. Gram's reaction shows the isolates to be positive cocci and positive rod. The suspected bacterial isolates include; *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Bacillus* spp., and *Pseudomonas* spp.

The total bacterial count ranged from 2.3 to 4.2 x 10^3 CFU/g. DG6 (Ago market) sample had the highest (4.2 x 10^3 CFU/g) bacterial count, while DG1 (Mojere market) sample had the least (2.3 x 10^3 CFU/g) bacterial count among the samples analyzed.

Table 1: Concentration of aflatoxin in garri samples from Ado Ekiti

Sample type	Aflatoxin concentration (ppb)			
	B1	B2	G1	G2
DG1a	24.00±0.0.01^{d}	7.00±0.01^{d}	0	0
DG1b	35.00±0.01^{g}	16.00±0.01^{e}	0	0

DG2a	28.00±0.00[f]	16.00±0.01[e]	0	0
DG2b	26.23±0.02[e]	6.00±.0.00[c]	0	0
DG3a	38.00±0.00[h]	6.00±0.00[c]	0	0
DG3b	259.00±0.01[i]	0	0	0
DG4a	38.00±0.00[h]	6.00±0.00[c]	0	0
DG4b	16.00±0.01[c]	0	0	0
DG5a	38.00±0.01[h]	5.00±0.00[b]	0	0
DG5b	13.00±0.02[b]	3.00±0.00[a]	0	0
DG6a	29.00±0.01[f]	6.00±0.00[c]	0	0
DG6b	9.00±0.02[a]	3.00±0.00[a]	0	0
DG7a	0	6.00±0.00[c]	0	0
DG7b	0	0	0	0

DG1, DG2, DG3, DG4, DG5, DG6 and DG7 represent dry gari samples from Mojere, Oba, Bisi, Shasha, Ojido, Ago, and Irona markets respectively. Values are means of duplicate sample

Table 2: Morphological characteristics of fungi isolates from garri samples

Microscopy	Microscopy	Suspected organisms
Colonies are dark brown to black	Conidiophores are smooth-walled	*Aspergillus niger*
Velvety, wooly, whitish but later turned black fungal colony with yellowish reverse side.	Septate with Unbranched conidiophores. Double sterigmata covered the entire vesicles to form radiate head.	*Aspergillus flavus*
Colonies light grey, growing extremely rapidly and filling the petridishes with dense cottony mycelia producing mass of sporangia.	Sporangia contain spores, have rhizoids	*Rhizopus* spp.
Powdery whitish surface but later turned bluish-green with whitish reverse side and edges.	Branched septate hyphae with flask shaped sterigmata. The conidia are unbranched with a penicillate or bluish appearance.	*Penicillium* spp.
Fluffy creamy growth that later turned pinkish with a yellowish reverse side.	Septate with branched conidiophore and oblong conidia.	*Fusarium* spp.
Colonies are typically colour white to grey, older colony become grey to brownish colour due to development of spores	The hyphae are non-septate and the sporangiophores are erect	*Mucor* spp.
Off white to cream, dry smooth colonies, heavy growth around the plate.	Oval to convex, budding yeast cells, purple coloured colonies.	Yeast

Table 3: Morphological and biochemical characteristics of the bacteria isolates from the samples

Samples	Gram rxn	Elevation	Margin	Cat	Cit	Coa	Ferm	Suspected organism
1	+ve cocci	Flat	Even	+	+	+	+	*Staphylococcus aureus*
2	+ve cocci	Flat	Even	+	+	+	+	*Streptococcus pneumonias*
3	+ve rod	Flat	Wavy	–	+	–	+	*Bacillus* spp.

4	+ve rod	Flat	Even	+	+	–	+	*Pseudomonas* spp.

1, 2, 3, 4, represent different isolates from dry gari samples from differet market
+ = positive
– = negative
Cat = Catalase
Cit = Citrate
Coa = Coagulase
Ferm = Fermentation

Table 4: Total bacteria count

Sample code	Total bacteria count (cfu/g)
DG1	2.3×10^3
DG2	4.0×10^3
DG3	3.2×10^3
DG4	3.0×10^3
DG5	2.5×10^3
DG6	4.2×10^3
DG7	3.2×10^3

DG1, DG2, DG3, DG4, DG5, DG6 and DG7 represent dry gari samples from Mojere, Oba, Bisi, Shasha, Ojido, Ago, and Irona markets respectively

3.1 Discussion

The high frequency of occurrence and distribution of fungi species like *Aspergillus*, *Fusarium*, *Penicillium*, *Yeast*, *Mucor*, and *Rhizopus* can be attributed to poor post-processing handling procedures, their ubiquity, and their capacity to withstand and tolerate difficult environmental conditions like the low pH and low moisture content of garri [12].

The prevalence and slightly higher frequency of aflatoxins B1 was observed. This result correlate with the research of Sanyaolu *et al.* [16] in which aflatoxin B1was prevalent in all the samples collected in Akwa Ibon. The highest value was recorded in sample six. This might be due to the location where the sample was collected (Ago). These fungus groups support earlier reports and have been variously associated with the formation of various forms of aflatoxins under diverse settings. It is well known that exposure to aflatoxins through contaminated food consumption and toxin inhalation in animals can cause acute liver injury, liver cirrhosis, tumor induction, teratogenic effects, and other genetic impacts in both animals and people.

Additionally, as market garri requires little to no additional processing or treatment prior to ingestion, there is a chance that consuming significant doses over time could result in health risks. Therefore, it is important to establish suitable processing and handling methods for these relish food items [12].

Bacteria including *Staphylococcus aureus*, *Bacillus* spp., *Streptococcus* spp., and Pseudomonas spp. were isolated for this investigation. Most of them were cream in color, rounded, and elevated bluntly. Microorganisms cause food to deteriorate and can have a negative impact on people's health. Additionally, it affects the biochemical qualities and flavors of the product (garri), and because of their unattractive appearance from a commercial standpoint, the product is frequently devalued.

Additionally, bacteria can stick to grain dust particles and travel through the air. Due to the organic elements in grain dust, which offer vital nutrition to airborne microbes adhering to their surfaces during secondary processing of grains (sacking, milling, handling of powdered

grains, sorting, etc.) in markets, grain dust can function as an efficient infectious aerosol [12]. It is not surprising that the atmospheric bio-loads of these markets were high given that they were close to grain milling facilities, farms, refuse dumps, road construction sites, abattoirs, and grain processing facilities, despite the apparent weaknesses of the gravitational sampling technique used in this study compared to the use of air sampling impellers.

The overall plate count is frequently a sign of the hygienic quality, safety, and usefulness of foods; it may also be an indicator of the manufacturing conditions of the product, such as contamination of raw materials, processing efficiency, and hygienic conditions of tools and utensils at the processing facilities [16].

4. CONCLUSION

The present work revealed high occurrence and prevalence of aflatoxins B1, B2, G1 and G2 respectively. The presence of bacteria such as *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Bacillus* spp., and *Pseudomonas* spp. in the garri samples obtained from markets in Ado-Ekiti were also established. These are concerning and disturbing, and they may be early warning signs of the level of safety of the garri on the market. It also calls for more attention to the effectiveness of food processing settings, handling methods, handler technical proficiency, hygienic practices, and completed product safety. The safety of the garri that is consumed by several million people in Africa will also be helped by the strict application and implementation of quality control, quality assurance, good manufacturing practices, and the risks analysis key control point principles.

According to the result of the current study, I hereby recommend that useful indices for safe appropriate public health standards for handling should be developed. More creation of awareness and training should be given to farmers and retailers on better and improved storage techniques. Investigation of toxins produced by these fungi should be carried out and in relation to the health status of human being in the study area.

COMPETING INTERESTS

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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