The bioload and aflatoxin content of market garri from some selected states in southern Nigeria: public health significance.

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Abstract

Background: Garri is consumed by several millions of people in the West African sub-region and in Nigeria in particular regardless of ethnicity and socio-economic class. However production and handling methods have not been standardized resulting in a product with varying quality and safety indices hence varying public health concern.

Objectives: To investigate the microbial contamination level, presence, prevalence and distribution of Aflations B_1, B_2, G_1 and G_2 in market garri with the aim of developing useful indices for safe handling and acceptable public health standards.

Methods: A total of 300 samples comprising of 30 samples each from various market in both urban and rural settings were randomly collected using sterile polyethylene bags. These were analysed for microbiological quality and aflatoxins content using standard procedures.

Results: Eight bacteria genera *(Bacillus, Staphylococcus, Streptococcus, Pseudomonas, Clostridium, Salmonella Klebsiella* and *Coliforms* groups) genera and six fungi genera (*Aspergillus, Penicillium, Rhizopus, Botrytls, Fusarium and Cladosporium)* were detected and isolated. Aflatoxins B_1, B_2, G_1 and G_2 were detected in varying concentrations amongst the samples analysed within and amongst the states investigated with an average occurrence rate of 17.5%

Conclusion: Market garri was found to contain high bioload with vast array of micro-organisms and Aflatoxins in all the states investigated. Results are useful in developing and establishing public health standards for the production and safe handling of garri.

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Introduction

Garri, a roasted granular hygroscopic starchy food product, produced from cassava (*Manihot esculenta*-Crantz) is the most popular form in which cassava is consumed in the West Africa sub region. It is consumed by several millions of people regardless of ethnicity and socio-economic class, making it the commonest meal amongst the rich and the poor. Garri available in the market can be consumed directly without further processing in the dry form with pea nut, coconut, smoked fish, soaked in water (sometimes with milk and beverage) or processed minimally using boiled water to form stiff paste popularly called "eba" and eaten with various types of African soups.[1]

Cassava for garri production is harvested manually in the farm with the aid of a cutlass, hoe and flat iron sheet (digger), which occasionally inflicts various degrees of injuries on the root tubers. After harvesting, the root tubers are hauled to the market where they are heaped in 20s, 40s, 50s, or 100s for sales under humid and warm topical conditions. These practices predispose the root tubers to contamination and infestation by various groups of microorganisms (especially moulds), mites and insects which potentiates biodeteriaration [2, 3, 4, 5]

Following processing, garri is spread on the bare floor or on a mat to allow it to cool before final sieving and packaging for marketing. In the open market, garri is displayed in open basins, bowls, bags and mats. These practices potentiate contamination by various groups of microorganisms and may predispose public health hazards [1]

Various groups of moulds have reported to be associated with garri during storage and distribution[6, 7, 8]. Moulds, if present can grow and affect the nutritional and sensory properties of garri , and species if toxigenic may produce mycotoxins. Aflatoxins B_1,B_2,G_1,G_2 are the most frequently encountered mycotoxins because they are produced by ubiquitous fungal genera such as *Aspergillus* and *Penicillium*. The cancerous and neurological associations of these toxins reinforce the need for continous and regular search for their presence in foods[9, 10].

Furthermore, the presence of aflatoxins in market food items such as yam flour, plantain flour, corn flour and others destined for consumption in Nigeria has been reported in previous studies[10, 11].

In line with the foregoing, this work was designed to investigate the microbial contamination level,

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the presence, prevalence and distribution of aflatoxins B_1,B_2,G_1 and G_2 in market garri destined for consumption with the aim of developing useful indices for safe handling of garri and protection of public health.

Materials and Methods

Source of samples and sampling: - Garri samples used for this study were obtained from sellers in the open markets from ten selected states (Anambra, Cross Rivers, Delta, Edo, Enugu, Imo, Lagos, Ogun, Ondo and Rivers) in Southern Nigeria. A total of thirty samples were obtained form each state comprising of white and yellow garri (commercially available types). While a grand total of three hundred samples were collected and analysed in the ten states investigated. The samples (approximately 500g/pack) were collected in sterile polyethelene bags adopting standard procedures and transported to the laboratory. These were analysed within twenty four hours.

Analyses

Microbiological : - Twenty five grams proportion of each sample was asceptically taken (after thorough mixing) and weighed into a beaker containing 225ml of 0.1% sterile peptone water (w/v) and allowed to soak for 2-3 minutes with occasional stirring with a sterile glass rod. Ten-fold serial dilution was subsequently prepared by transferring 1ml aliquot of the supernatant into 9ml of sterile peptone water as diluent. Further serial dilution was carried out and thereafter, 1ml of appropriate dilution was aseptically plated using pour plate technique for total viable bacterial count on plate count agar (Oxiod), *Staphylococcus aureus* on manitol salt agar (Oxiod), total coliforms on McConkey agar (Oxiod) and viable fungi count on potatoe dextrose agar (Biotech) supplemented with chlorampenicol. The media used were prepared and incubated according to the manufacturer's instructions. The numbers of viable microorganisms that developed were counted, calculated and expressed as colonidophores forming units per gram (cfu/g). Isolation, characterization, and identification of the bacteria groups were carried out for qualitative determination using colonial, morphological and biochemical characteristics[12]. The fungal isolates were identified based on examinations of the conider heads, phialides, conidiophores and presence and absence of foot cells or rhizoids[13].

Aflatoxin Extraction and Detection

The aflatoxin(s) content of the various market garri samples was extracted and detected according to the method previously described9. Briefly, 10g of the various market garri samples was homogenised and added into sterile, clean Erlenmeyer flask containing 40ml of methanol and water (11:9) and shaken at 2000 rpm on a mechanical shaker (Griffin and George, England) for 1min. The resultant slurry was filtered through Whatman number 1 filter paper. The filtrate was extracted three (3) times each with 20ml of petroleum ether (May & Baker Ltd, Dagenham, England) with (boiling point 60-80^{0}c) in a separating funnel to remove the lipid fractions. The pooled petroleum ether extract was re-extracted with 40ml of methanol and water (11:9). The aqueous methanol extracts were combined and transferred to a separating funnel and extracted three times with 25ml of chloroform (BDH Chemicals Ltd, Poole, England) to extract the aflatoxin(s) present. The pooled chloroform extract was passed through a bed of anhydrous sodium sulphate. The bed was re-washed with additional 20ml of chloroform. Aflatoxins extracted were detected by thin-layer chromatography against aflatoxin B_1,B_2,G_1 and G_2 (Aldrich chemicals, Milwankee) and quantitated using a spectrophotometer (Coleman instrument).

Data analysis

The various data obtained were subjected to statistical analysis of mean, standard and the significant differences of mean determined at ($p< 0.01$, 0.01 and 0.05).

Results

Results of the investigation of the bioload and aflatoxins content of market garri destined for consumption from ten selected states in southern Nigeria are shown in tables 1-4 and figure 1 respectively. Average range count of 3.13 – 5. 91 $\log_{10}$cfu/g for viable bacteria, 0.70-4.30 $\log_{10}$cfu/g, for fungi, 0.78-3.83 $\log_{10}$cfu/g for Staphylococcus and 0.48-1.55 $\log_{10}$cfu/g for coliforms counts were recorded respectively in all the samples analysed from all the states investigated (table1). Slight variations were observed amongst the groups of microorganisms within each state and from one state to another. The average rate of occurrence and distribution of some members of the fungi group were significantly different from the bacteria group at ($p< 0.05$) and were in the order, *A.niger* (40.85%) > *A.flavus* (38.96%)> *Fusarium* (35.31%)> *Penicillium* (32.96%)> *A. fumigatus* (25 – 97%) > *S. aureus* (21.87%)> Coliforms (12.97%) (table 2). In addition, figure 1 shows the mean percentage distribution of the various groups of micro-organisms isolated and detected.

Table1 **Mean range viable count (log_{10}cfu/g) of market garri**

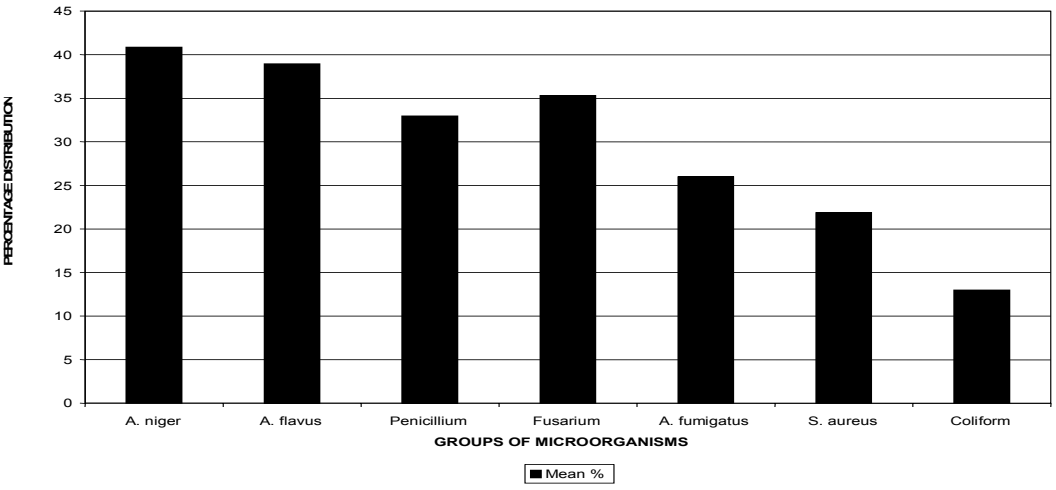
State	Types of microorganisms in range (log_{10}cfu/g)			
	Bacteria	*S.aureus*	Coliform	Fungi
Anambra	3.61±0.2-6.02±0.2	0.84±0.01-4.11±0.3	0.30±0.02-2.14±0.2	0.78±0.01-5.26±0.1
Cross River	3.79±0.1-5.20±0.3	0.77±0.01-4.14±0.2	0.61±0.01-1.04±0.3	0.69±0.01-3.49±0.01
Delta	1.80±0.1-6.38±0.2	0.69±0.01-3.25±0.2	0.30±0.01-1.11±0.1	0.69±0.02-4.18±0.01
Edo	2.95±0.4-6.02±0.1	0.60±0.01-3.27±0.2	0.47±0.01-1.00±0.2	0.69±0.01-4.20±0.01
Enugu	3.81±0.5-5.01±0.3	0.47±0.01-3.53±0.1	0.44±0.02-0.90±0.3	0.95±0.02-5.29±0.01
Imo	3.01±0.2-6.08±0.1	0.95±0.02-5.20±0.2	0.64±0.01-2.20±0.1	0.85±0.01-4.36±0.01
Lagos	4.07±0.2-6.89±0.01	0.85±0.01-4.04±0.1	0.43±0.02-2.11±0.1	0.90±0.01-4.20±0.01
Ogun	2.31±0.3-5.30±0.2	0.90±0.03-4.17±0.2	0.45±0.03-0.90±0.2	0.47±0.02-3.25±0.02
Ondo	3.92±0.1-5.32±0.3	0.78±0.01-3.40±0.2	0.51±0.01-2.04±0.1	0.30±0.01-3.61±0.02
Rivers	2.02±0.2-6.83±0.2	0.95±0.02-3.17±0.3	0.60±0.02-2.09±0.02	0.69±0.01-5.17±0.02

NB: Each value is the overall mean ± standard deviation for duplicate determinations.

Table 2 **Percentage distribution of viable groups of microorganisms in market garri**

State	Types of microorganisms (%)						
	A.niger	*A.flavus*	*Penicillium*	*Fusarium*	*A.fumigatus*	*S.aureus*	Coliform
Anambra	40	46.6	36.6	33.3	26.6	23.3	13.3
Cross River	46	43.3	26.6	36.6	23.3	20	13.3
Delta	33.3	36.6	33.3	30	30	30	16.6
Edo	40	30	36.6	33.3	20	13.3	13.3
Enugu	50	40	30	33.3	26.6	16.6	16.6
Imo	50	36.6	33.3	30	23.3	13.3	10
Lagos	36.6	46.6	36.6	40	33.3	26.6	20
Ogun	33.3	36.6	26.6	36.6	20	23.3	10
Ondo	33.3	30	30	40	26.6	20	6.6
Rivers	40	43.3	40	40	30	33.3	13.3

Figure 1: Mean percentage distribution of viable groups of microorganisms in market garri.



Vast array of microorganisms were detected and isolated. *B. subtilis, Streptococcus faecalis, Staphylococcus aureus, Pseudomonas aruginosa, Clostridium spp, Escherichia coli* and *Salmonella spp.* were among the bacteria group while the fungi group included, *A niger, A. flavus, A. fumigatus, Fusarnium moniliforme, Pennicillium citrinum, Rhizopus stolonifer, Botrytis cinerae* and *Cladosporium spp.* Amongst the bacterial group, *B. subtilis, S. faecalis and S. aureus* were most prevalent while *A. niger*, *A. flavus*, *Fusarium moniliforme* and *A. fumigatus* were most prevalent among the fungi group (table 3).

Table 3 Microorganisms isolated from market garri

Bacterial group	Fungi group
Bacillus subtilis	*Aspergillus niger*
Streptococcus faecalis	*Aspergillus flavus*
Staphylococcus aureus	*Aspergillus funigatus*
Pseudomonas aeruginosa	*Penicillium citrinum*
Clostridium spp	*Rhizopus stolonifer*
Escherichia coli	*Botrytis cinerae*
Salomonella spp	*Fusarium moniuforme*
Klebsiella spp	*Cladosporium spp.*

Table 4, shows the range and rate of occurrence of aflatoxins in market garri in all the states investigated and were in the order Enugu (0.37 – 5.71 μg/kg)>Cross Rivers (0.32-4.57 μ g/kg) >Edo (0.13-4.46 μg/kg) > Anambra (0.44-3.69 μg/kg) >Delta (0.26-3.64 μg/kg) > Imo (0.14-3.16 μg/kg) > Rivers (0.17-3.14 μg/kg) > Lagos (0.012-2.54 μg/kg) > Ondo (0.18-2.41 μg/kg) > Ogun (0.25-1.66 g/kg). While the rate of occurrence were in the order of Lagos (30%)> Rivers (26.6%) > Imo (23.3%) > Anambra (20%)> Cross Rivers (16.6%) = Enugu (16.6%)> Edo (13.3%)> Delta (10%) = Ogun (10%) > Ondo (6.6%). Aflatoxins B_1,B_2,G_1 and G_2 were variously detected and quantified from some of the samples in all the states investigated. However, slight variation was observed in the type of aflatoxins from one state to another.

Table 4 Range and percentage occurrence of Aflatoxins in market garri

State type.	No of Samples	Aflatoxin range (μg kg)	% occurrence	Aflatoxin(s)
Anambra	30	0.44-3.69	20	B_1,B_2, G_2
Cross River	30	0.32-4.57	16.6	B_1,B_2, G_1, G_2
Delta	30	0.26-3.64	10	B_1,B_2
Edo	30	0.13-4.46	13.3	B_1,B_2, G_2
Enugu	30	0.37-5.71	16.6	B_1,B_2, G_1
Imo	30	0.14-3.16	23.3	B_1,B_2, G_1, G_2
Lagos	30	0.12-2.54	30	B_1,B_2, G_1, G_2
Ogun	30	0.25-1.66	10	B_1,B_2
Ondo	30	0.18-2.41	6.6	B_1,B_2, G_2
Rivers	30	0.17-4.14	26.6	B_1,B_2, G_2

Discussion

The high viable bacteria, fungi and *Staphylococcus aureus* count recorded may be associated with inadequate post processing handling practices such as spreading on the floor, mat and sometimes on high density polyethene spread on the floor after frying to allow it to cool before sieving into finer grains; and the open display in bowls and basins in the market, measurement with the aids of bare hands, coughing and sneezing while selling and the use of non microbiologically determined hessian bags for packaging and haulage. These may also be responsible for the vast array of microorganisms detected and isolated. These finding corroborate some other reports[14, 15, 1]. Low count of *Coliforms* and *Salmonella* were detected. However, their presence appeared transient since no growth was detected on agar plate following analysis after 24hours. This may be due to their inability to withstand the micro environmental conditions, hence may not be of public health concern. But consumption habits such as soak and travel with milk and beverages may enhance proliferation and potentate health hazards

The high rate of occurrence and distribution of moulds such as *Aspergillus, Penicillium*, *Fusarium* and others may be traced to the inadequate post processing handling practices, the ubiquitious nature of these moulds, and their ability to withstand and tolerate harsh environmental conditions such as low pH and low moisture content of garri. Previous reports support these findings[9, 1].

The slightly high rate of occurrence and prevalence of aflatoxins B_1,B_2,G_1 and G_2 observed and recorded from all the samples investigated may be related to the high rate of occurrence and distribution of moulds such as *A. niger* (40.85%), *A.flavus* (38.96%) A. *fumigatus* (25.97%) *Penicillium* (32.96%) and Fusarium (35.31%). These groups of moulds have been variously linked with the

production of various types of aflatoxins under various conditions and supportrs previous reports[11, 9, 10, 16]. Exposures to aflatoxins through ingestion of contaminated foods and inhalation of toxins have been linked to acute and chronic toxicity in animals. Effects such as acute liver damage, liver cirrhosis, induction of tumors and teratogenic and other genetic effects in animals and humans are well documented[17, 18, 19, 20]. Further more, since market garri require little or no further processing or treatment prior to consumption, there is the possibility of ingesting large dosage over a period of time with possible health hazards. Hence the need to develop adequate processing and handling techniques for this relish food item.

In summary, the present work revealed high bioload and vast array of microorganisms in market garri and high rate of occurrence and prevalence of aflatoxins B_1,B_2,G_1 and G_2 respectively. These are threatening and alarming and suggest early warning signals indicating the level of safety of available market garri. It also warrants renewed vigillance on the efficacies of food processing conditions, handling techniques and handlers technical know how, hygiene practices and safety of finished products. In addition, strict application and implementation of quality control, quality assurance, good manufacturing practice and the hazard analysis critical control point principles will help to ensure the safety of garri consumed by several millions of people in Africa.

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