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Research Article

Microbiological Safety of Ijebu Garri (Fermented *Manihot esculenta Crantz* Flour) Produced and Sold in Abeokuta Metropolis, Nigeria and Its Public Health Implications

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Abstract

Garri is a fermented cassava (*Manihot esculenta Crantz*) product which is widely consumed as staple in Nigeria and West Africa. Cassava processing into garri in Nigeria lack quality control. Also, during sales in the markets, garri is displayed in open basins and as a result they are exposed to contamination with dust and microorganisms. The aim of this study was to assess the microbiological safety of Ijebu garri sold in Abeokuta metropolis, Ogun State, Nigeria. Three hundred (300) grams of Ijebu garri was purchased from three major markets in Abeokuta metropolis namely; Lafenwa, Kuto and Itoku markets. Samples were purchased from 10 traders within each market using random sampling. These samples were pooled into a composite per market resulting in 3 composite samples. One (1) gram from each sample was used for fungal and bacteria isolation. Five (5) grams of the sample was sent for complete mycotoxin analysis using LC-MS/MS technique. The total mold counts in garri samples were: 2.5 x 10^4 CFU/mL (Lafenwa); 6.0 × 10^3 CFU/mL (Itokun), and 4.0 × 10^4 CFU/mL (Kuto). No bacteria colony was isolated from garri. Molecular analyses and characterization identified the presence of toxigenic fungi namely *Penicillium* spp. (2), *Penicillium singorense* (3), and *Curvularia lunata* (3). Mycotoxin results showed all mycotoxins analyzed were less than reporting limits (<RL). The presence of mycotoxigenic fungi in garri suggests a potential public health risks as these can produce multi-mycotoxins under favorable conditions during storage, market display and distribution, which can lead to mycotoxicosis, organ failures, and cancers in humans and animals on consumption.

Keywords

Garri, Mycotoxigenic Fungi, Mycotoxin, Cassava, Public Health

1. Introduction

Garri is a creamy-white, granular flour with a slight fermented flavor and sour taste, made from fermented, gelatinized fresh cassava (*Manihot esculenta Crantz*) roots. Cassava is a major root crop in the tropics and the main source of carbohydrate for more than 500 million people globally, thus playing an essential role in food security [1]. Garri is staple in Nigeria, where it provides about 70% of the daily calories of over 50 million people [2]. Apart from its role in food security, cassava contributes significantly to rural economies by generating income to farmers and creating employment opportunities along its value chain. Nigeria is the world largest producer of cassava [3], with about 45 million metric tonnes, and its cassava transformation is the most advanced in Africa [4]. It is a major food crop in Nigeria [5]. Nutritionally, cassava is

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essentially a carbohydrate food with low protein and fat. Cassava roots contains 32–35% carbohydrate, 2-3% protein, 0.1% fat, 1.0% fibre, and 0.70–2.50% ash, and 75–80% moisture [2].

Garri is obtained from Cassava by grating cassava roots, fermenting the obtained pulp, dewatering the pulp, drying, sieving and frying (Figure 1). *Garri* is traditionally made at home in Africa, using mechanized means. However, before the advent of machines, the cassava was hand grated. The roots are harvested, peeled and the white pulp is grated in a *garri* grater. The grated produce is then put into a jute sack and the sack tied and pressed to remove the water. Traditionally, this is left to ferment for three to seven days depending on the type of *garri* being made [6]. The poisonous juice containing hydrogen cyanide is removed during the pressing or dewatering step by placing a heavy object on the bag and the contents of the bag are allowed to undergo spontaneous solid-state fermentation for several days at ambient temperatures [7]. Sieving to remove fibers and lumps, and frying with or without palm oil result in the final product known as garri.

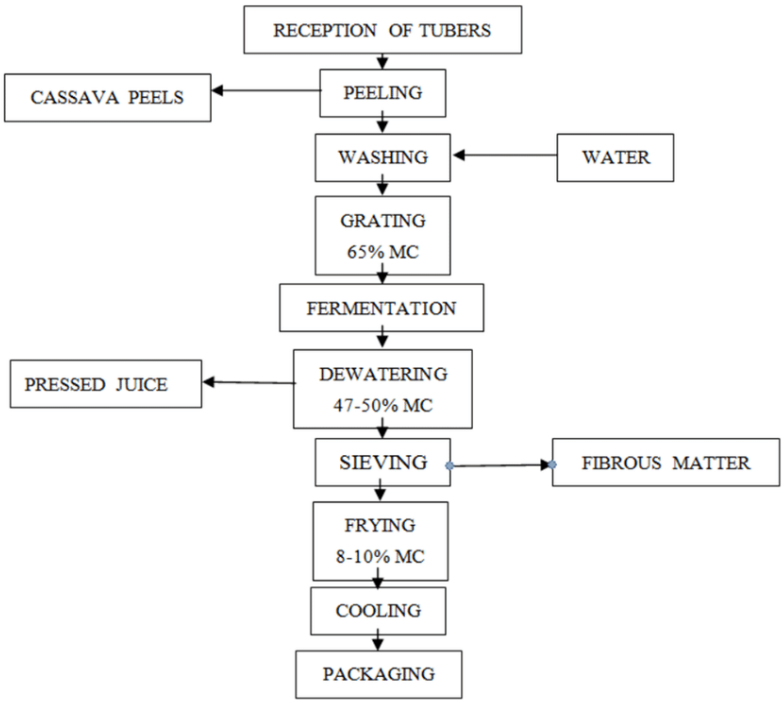

Figure 1. *Flow chart for production of garri [8].*

Garri is widely consumed in Nigeria mostly by people from the mid-western part of Nigeria who consume it as Red-garri and White garri, while the western part consumes it as Ijebu garri (Figure 2). Ijebu garri is a special type of garri produced by Ijebu people of Ogun State, Nigeria, and is so called because of the length of fermentation applied during the production process which takes as long as six- seven (6-7) days, resulting in a distinctively textured and flavored garri. It is commonly consumed either dry or soaked in cold water with sugar, coconut, roasted groundnuts or dried fish as accompaniments, or as a paste made with hot water called “Eba” which is eaten with varieties of African soups. It can also be eaten dry without any accompaniments. When properly stored, it has a shelf-life of six months or more [9].

Figure 2. *Pictorial representation of (a) Ijebu garri (b) White garri and (c) Red garri sold in Abeokuta, Ogun State.*

Cassava processing into garri especially by small holders in Nigeria lack quality control. However, during frying in an open pan, heat is applied which aids in the reduction of microbial load. Nevertheless, various microbes have been associated with garri, which could emanate from the raw cassava from the farm or recontamination due to exposure to market conditions. Post-process problems of garri include loss of microbial stability and spoilage during storage, distribution or marketing. The sale and distribution of garri in local markets is associated with practices such as display of product in open sacs, bowls and mats at points of sale and the use of bare hands during handling and sales [10]. These unhygienic practices, which may lead to microbial contamination due to deposition of bio aerosols on exposed products, transfer of microbes from dirty hands and utensils and frequent visits by animals and fomites (which may carry infectious agents), can contribute to the post-process problems of this product. The objective of this study was to investigate the microbiological safety of Ijebu garri sold in Abeokuta metropolis, Ogun state, and its possible public health concerns.

2. Materials and Methods

2.1. Sample Collection

This study was conducted in Abeokuta metropolis, Ogun state, Nigeria and was limited to only three major markets namely Lafenwa, Kuto and Itokun markets. Three hundred grams (300g) of Ijebu garri were purchased from three major markets in Abeokuta metropolis namely; Lafenwa, Kuto and Itoku market. Using simple random sampling within each market, 1 cup (30 g) of Ijebu *garri* was purchased from ten (10) different traders within each market. All samples purchased were pooled together as composite per market. Samples were stored in sterile airtight zip-locked bags and taken for microbiological analysis.

A market survey during sampling was carried out to determine the length of storage from production date and length of fermentation of Ijebu garri in order to determine their effect on the microbial load of the product.

2.2. Sample Analysis

2.2.1. Moisture Analysis

The garri samples were subjected to moisture content analysis by oven drying to a constant weight [11]. Five (5) grams of the pooled garri samples were weighed (initial weight) and dried in an oven at a temperature of 105 ℃ for 1-3 hours. The samples were then weighed hourly until a constant weight was achieved (the final weight). Readings were taken in triplicates. Moisture content was determined by subtracting the final weight (oven dry weight) from the initial weight, and the percentage calculated.

2.2.2. Isolation and Enumeration of Fungi in Ijebu Garri

The dilution plating technique as described by [12] was applied for the isolation and enumeration of fungi present in Ijebu garri. Ten (10) grams of the Ijebu *garri* samples from each of the markets was weighed into test tubes containing 90 ml of diluent (distilled water). After thorough mixing with vortex mixer, 0.1 ml was transferred onto already poured and set Potato Dextrose Agar (PDA) plates containing chloramphenicol (500 mg/l) and streptomycin (500 mg/l) in duplicates and cultured using the spread plating technique. The plates were incubated at 25 ℃ for 5 days. After 5 days, fungal colonies from plates were counted and reported as colony forming units per gram (CFU/g) of analyzed garri samples. All distinct colonies were sub-cultured on fresh PDA plates. The plates were incubated at 25 ℃ for 7 days, for good sporulation to take place, and then used for morphological identification according to [13]. Pure cultures of the isolates were sub-cultured onto cryovials containing PDA and incubated again for 7 days, after which the pure cultures were aseptically covered with sterile distilled water, and maintained at 4 ℃. These cultures were sent for molecular characterization and identification.

2.2.3. Isolation and Enumeration of Bacteria in Ijebu Garri

The dilution plating technique was also used for bacterial isolation and enumeration. Ten (10) gram of each sample from the three markets was weighed into test tubes which contains 90 mL of diluent (distilled water) and used to perform 10-fold serial dilution. after thorough mixing with vortex mixer, 1 ml was transferred from each dilution into a sterile petri dish and 15-20 mL molten violet red bile glucose (VRBG) agar was poured onto the plate, mixed and allowed to solidify. the plates were incubated at 37 ℃ for 24 hours. Another set of diluted samples were cultured using nutrient agar, and plates incubated at 30 ℃ for 24 hours.

2.2.4. Mycotoxin Analysis of Garri Samples

Five (5) grams of garri flour were sent to Trilogy laboratory Washington DC, USA for mycotoxin analysis. Mycotoxins associated with garri samples were analyzed using the dilute and shoot LC-MS/MS technique as described by [14]. Five grams (5 g) of garri samples were homogenized with 20 ml of extraction solvent (acetonitrile/water/acetic acid 79: 20: 1 v/v/v) in a 50 ml polypropylene tube. All samples were extracted for 90 minutes on a GFL 3017 rotary shaker and diluted with the same volume of the extraction solvent. The diluted extracts were directly injected into the LC-MS/MS instrument. Apparent recoveries of the analytes were determined by spiking 0.25 g of the five different samples. The spiked samples were stored overnight at ambient temperature

to allow evaporation of the solvent and to establish equilibrium between the analytes and samples.

3. Results

The fungal species isolated from garri were mainly molds with total counts of 6 ×10 ^{3}CFU/mL in Itokun market, 2.5 × 10^4 CFU/mL in Lafenwa market, and 4 × 10^4 CFU/mL in Kuto market (Table 1). From the result, Lafenwa and Kuto markets had higher counts than Itohun which had comparatively lower counts. The moisture content of the garri samples ranged from 10% in Lafenwa and Itokun markets to 11% in Kuto market (Table 1).

Table 1. *Total mold counts and moisture content in garri samples.*

S/No	Location	Total mold counts (CFU/mL)	% Moisture
1.	Lafenwa	2.5 x 10^4	10%
2	Kuto	4.0 x 10^4	11%
3	Itokun	6.0 x 10^3	10%

The fungal species were identified based on colony morphology and molecular characterization as *Penicillium spp.*, *Penicillium singorense*, and *Curvularia lunata* (Figures 3-5), all of which are known producers of mycotoxins.

Figure 3. *Penicillium spp.*

Figure 4. *Penicillium singorense.*

Figure 5. *Curvularia lunata.*

The DNA sequencing results of the mold isolates, the accession numbers and percent relatedness are shown in Table 2.

Table 2. *DNA sequencing result for the mold isolates obtained from garri.*

S/N	Species	Strain	Accession number	% Relatedness
Figure 3	*Penicillium spp*	FO5_17	JN624900.1	99.82%
Figure 4	*Penicillium singorense strain*	GO5_20	PP385020.1	99.27%
Figure 5	*Curvulari lunata strain*	HO5_23	KV806118.1	99.62%

Bacterial culture plates showed no growth in both VRBG and NA media, indicating absence of Enterobacteriaceae and other food poisoning and food spoilage bacteria.

The mycotoxin results of garri samples using LC-MS/MS technique showed all the mycotoxins analyzed as less than reporting limits (<RL) (Table 3).

Table 3. *Mycotoxin profile of garri sample.*

S/No.	Analyte	Results	Units	Analysis date	Reporting limit	Method	Reference
1.	15 Acetyl deoxynivalenol	<RL	ppm	05/16/2025	0.1 ppm	LC-MS/MS	Internal SOP.14-168
2.	3-Acetyl Deoxynivalenol	<RL	ppm	05/16/2025	0.1 ppm	LC-MS/MS	Internal SOP.14-168
3.	Aflatoxin B1	<RL	ppm	05/16/2025	1 ppm	LC-MS/MS	Internal SOP.14-168
4.	Aflatoxin B2	<RL	ppm	05/16/2025	1 ppm	LC-MS/MS	Internal SOP.14-168
5.	Aflatoxin G1	<RL	ppm	05/16/2025	1 ppm	LC-MS/MS	Internal SOP.14-168
6.	Aflatoxin G2	<RL	ppm	05/16/2025	1 ppm	LC-MS/MS	Internal SOP.14-168
7.	Citrinin	<RL	ppm	05/16/2025	50 ppm	LC-MS/MS	Internal SOP.14-168
8.	Diacetoxyscirpenol	<RL	ppm	05/16/2025	100 ppm	LC-MS/MS	Internal SOP.14-168
9.	Deoxynivalenol	<RL	ppm	05/16/2025	0.1 ppm	LC-MS/MS	Internal SOP.14-168
10.	Fumonisin B1	<RL	ppm	05/16/2025	0.1 ppm	LC-MS/MS	Internal SOP.14-168
11.	Fumonisin B2	<RL	ppm	05/16/2025	0.1 ppm	LC-MS/MS	Internal SOP.14-168

S/No.	Analyte	Results	Units	Analysis date	Reporting limit	Method	Reference
12	Fumonisin B3	<RL	ppm	05/16/2025	0.1 ppm	LC-MS/MS	Internal SOP.14-168
13.	Fusarenon -X	<RL	ppm	05/16/2025	0.5 ppm	LC-MS/MS	Internal SOP.14-168
14.	HT-2 Toxin	<RL	ppm	05/16/2025	5 ppm	LC-MS/MS	Internal SOP.14-168
15.	Neosolaniol	<RL	ppm	05/16/2025	20 ppm	LC-MS/MS	Internal SOP.14-168
16.	Nivalenol	<RL	ppm	05/16/2025	0.5 ppm	LC-MS/MS	Internal SOP.14-168
17.	Ochratoxin A	<RL	ppm	05/16/2025	1 ppm	LC-MS/MS	Internal SOP.14-168
18.	T-2 Toxin	<RL	ppm	05/16/2025	5 ppm	LC-MS/MS	Internal SOP.14-168
19.	Zearalenone	<RL	ppm	05/16/2025	12.5 ppm	LC-MS/MS	Internal SOP.14-168

4. Discussion

The total fungal counts found in the garri samples across three major markets ranged from 6 × 10 ^{3}CFU/mL in Itokun market to 4 × 10^{4} CFU/mL in Kuto market. The total fungal counts in Lafenwa was 2.5 × 10^{4} CFU/mL. From the result, Lafenwa and Kuto markets had higher counts than Itohun. This could be due to the level of exposure of the product to market environment and unhygienic handling by the traders. Lafenwa and Kuto markets are located close to major roads and vehicular movement is high, making the environment very dusty and highly susceptible to fungal contamination. The fungal counts from these markets were moderate because of the freshness of the gari (2-3 days after production) but could go higher as the gari ages and is continuously exposed to environmental dust and spores, and could lead to moldiness in taste. Fungal contamination is a public health concern as fungi are capable of causing cutaneous, subcutaneous, and deep-seated mycoses especially in immunocompromised individuals. Globally, 6.5 million invasive fungal infections are recorded annually, and about 2.5 million deaths are attributed to fungal diseases [15]. The moisture content of the garri samples ranged from 10% in Lafenwa and Itokun markets to 11% in Kuto market. According to [16], the low moisture levels of the garri are critical for fungal contamination and mycotoxin accumulation. Molds also grow better in acidic environment [17] and garri is highly acidic due to the fermentation step in its processing.

The fungal isolates identified in this study through morphological and molecular techniques were *Penicillium spp.*, *Penicillium singorense*, and *Curvularia lunata*, all of which are known producers of mycotoxins. The detection of these genera, especially *Penicillium*, raises concerns as they are capable of producing ochratoxins, which are nephrotoxic and carcinogenic [18]. Additionally, the detection of *Curvularia lunata*, a known opportunistic pathogen, suggests the potential for exposure to infection, particularly among immunocompromised individuals. *Curvularia lunata* produces mycotoxins called curvularin which has been linked to pheo-hyphomycosis and mycotoxicosis, as well as causing lesions in various organs in animal studies [19]. No bacteria were isolated in this study. This could be due to the low moisture content, the acidic nature, and the freshness of the product. The survey report showed that the Garri samples were just 2-3 days old. However, [20] reported a high load of microorganisms in garri sold in the markets in Nigeria, and suggested the source of contamination to either emanate from the raw cassava roots coming from the farm, or recontamination due to exposure in the market place.

The prolonged fermentation process typical of Ijebu garri reduces the cyanide levels but may not effectively limit fungal contamination if post-processing hygiene is not maintained. The open market display of garri, often in uncovered containers, and exposure to dust, insects, and human handling has been identified as the major sources of recontamination, a finding consistent with [21]. Previous studies on cassava derived products indicate that they are prone to fungal invasion and mycotoxin accumulation during post-harvest storage and distribution [22, 23]. Moreover, the isolation of mycotoxigenic fungi with potential to produce mycotoxins and other secondary metabolites add weight to the proposition that market practices need urgent reforms to protect public health. The fact that mycotoxins analysis results were all below reporting limits does not indicate safety, because consuming low levels of mycotoxins for a long time can lead to biomagnification in humans and animals, leading to chronic mycotoxicosis, organ failures and cancers [24].

5. Conclusion

The results from this study indicate that Ijebu garri sold in Abeokuta metropolis is exposed to significant microbial contamination, including the presence of mycotoxigenic fungi which could produce dangerous mycotoxins. Despite the reduction in microbial load through fermentation and frying, post-processing contamination during market sales remains a critical issue. The isolation of mycotoxigenic fungi from garri

such as *Penicillium spp.* and *Curvularia lunata*, signals a pressing public health risk especially in communities where garri is consumed without heat application or any further processing. This study contributes valuable insight into the microbiological safety of a widely consumed staple and calls for urgent intervention in terms of market hygiene, improved packaging, and public awareness.

Abbreviations

LC-MS/MS	Liquid Chromatography Tandem Mass Spectrophotometry
CFU	Colony Forming Units
<RL	Less Than Reporting Limit
VRBG	Violet Red Bile Glucose Agar
PDA	Potato Dextrose Agar
NA	Nutrient Agar
USA	United States of America

Author Contributions

Annabella Ademuyiwa Adewunmi: Conceptualization, Methodology, Supervision, Writing – review & editing

Nifemi Paul Sanyaolu: Data curation, Formal Analysis, Writing – original draft

Conflicts of Interest

The authors declare no conflicts of interest.

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