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Mycological analysis of commercially-marketed guarri in selected markets within Lokoja metropolis, Kogi State, Nigeria

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Abstract

Garri is a commonly consumed staple food in Nigerian homes. However, its handling and processing expose it to several contaminants, including microorganisms. This study identified and determined the prevalence of moulds in garri sold at different marketplaces within Lokoja, Kogi State, Nigeria. Thirty (30) garri samples from 3 markets underwent proximate analysis and aerobic culture on chloramphenicol-supplemented Sabouraud dextrose agar. Fungal identification used morphological characteristics and lactophenol cotton blue stain. The moisture content of the gaari samples ranged between 13.2±0.01 - 17.1±0.02, Protein content (0.90±0.15 - 1.0±0.00), Ash content (1.32±0.13-1.53±0.02), Fat content (1.00±0.02 - 1.04±0.13), Fibre content (1.22±0.01 - 1.27±0.00) and carbohydrate content (84.5±0.03 -85.8±0.14). The total viable fungal count (CFU/g) of garri samples ranged from 2.8 x 103 – 8.3 x 105 in Old Market, 1.3 x 103 – 5.0 x 105 in Adankolo Market, and 3.9 x 103 – 6.6 x 105 in International Market. A total of seventy (70) moulds were recovered from the sampled garri, 33 (47.14%) from Old Market, 21 (30.0%) from International Market, and 16 (22.86%) from Adankolo Market. Of the 70 mould isolates, Aspergillus spp (34.29%) was the most prevalent, followed by Alternaria spp (25.71%), Mucor spp (15.71%), Rhizopus spp (11.43%) and Microsporum spp (10.0%). There was no statistically significant variation ($p > 0.05$) in fungal prevalence within and across the studied markets. The high prevalence of moulds in commercially-marketed garri is significant, as they can produce mycotoxins. Hence, hygienic practices should be practiced by garri sellers to reduce the risk of microbial contamination of their produce.

Keywords: Fungal Contamination; Proximate; Mycological Analysis; Commercially-Marketed Garri; Lokoja Metropolis; Fungal Identification

1. Introduction

Cassava (*Manihot esculentum*) is a woody shrub popularly cultivated in the tropics and belongs to the family Euphobiaceae. The plant is a perennial crop with highly perishable enlarged tuberous roots that are only stored for a short period of time post-harvest. The crop is drought-resistant and is able to flourish under minimal soil nutrients. Nigeria, being one of the leading producers of Cassava globally, produces about 45 million tonnes annually [1]. However,

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increase in the production of Cassava in the region is attributable to increasing demands for quality value-added processed products of Cassava such as garri, flour, dough, and starch.

Garri is the most common fermented cassava product and it is produced by fermenting grated cassava and roasting the cassava fibres [2]. Garri is commonly eaten with beans, moin moin, groundnut, kuli kuli, sugar, and beverages depending on individual preferences. Garri has high fibre content, contains essential vitamins, and is rich in starch. However, because the manufacturing and processing of garri is not standardized, the final product of the processing is mostly heavily contaminated with different types of microorganisms, especially bacteria and fungi that could be potentially pathogenic to humans [3].

Garri is rich in carbohydrates, fibres, and essential vitamins, thereby making it a suitable medium for the growth of bacteria and fungi [4,5]. Microorganisms contaminate garri through various means, including unsanitary practices in the production, processing, and post-processing handling of cassava into garri. These practices encompass spreading on floors and mats post-frying, exposing the garri in open containers in the markets during sales, utilising diverse packaging materials for transferring the finished products from rural to urban areas and employing bare hands during handling and sales [6–8]. According to Mohammed *et al.* [9], fungal contamination of garri leads to diverse effects including discoloration, loses to nutritional value, and contamination with mycotoxins.

Garri is among the most widely eaten food products in many Nigerian communities and microbial contamination of this product can be of significant public health challenge. Also, the fact that fungal contaminants are able to produce and secrete mycotoxins into garri is of significant concern. Hence, this study aimed to identify the moulds infecting commercially sold garri in Lokoja city, Kogi State, Nigeria.

2. Material and methods:

2.1. Study area

Lokoja is the capital of Kogi State and it lies at the confluence of rivers Niger and Benue. It has a total land area of 3,180km^2, and a total population of about 692,050. Lokoja lies about 7.8023^{o}N and 6.7333^{o}E and is about 170 km Southwest of Abuja. Residents of Lokoja are predominantly Igala, Ebira, and Nupe, Bassa Nge. The city has an average temperature of 32.4^{o}C, an average rainfall of 45.95 inches, and an average relative humidity of 74%. For this study, three different markets within Lokoja metropolis were selected including the International Market, Old Market, and Adankolo Market.

2.2. Sample collection

A total of thirty (30) commercially-marketed white garri samples (10 samples per market) were purchased in well-labeled sterile plastic containers using conventional protocols. Samples were thereafter tagged accordingly and conveyed to the laboratory for analysis.

2.3. Proximate Analysis of the Garri

Proximate analysis of Garri was carried out which includes the determination of percentage moisture content, ash, crude protein, crude fibre, crude fat, and carbohydrate using the Association of Official Analytical Chemists (AOAC) [10] procedures as described by Ajifolokun and Adeniran [11].

2.4. Sample preparation

Sample preparation was conducted utilising the spread plate count technique, wherein 10 g of each garri sample was weighed and combined with 90 mL of 0.1% (w/v) sterile peptone water, followed by stirring for 5 minutes [6]. Ten-fold serial dilution of the suspension was then carried out for individual samples.

2.4.1. Fungal isolation and identification

mL aliquot from each dilution factor of the garri samples were cultured individually on freshly prepared Sabouraud Dextrose agar (SDA) supplemented with 30 µg chloramphenicol and incubated at 28^{o}C for 7 days. After incubation, total viable fungal counts for individual samples were carried out and expressed as colony-forming units per gram (CFU/g). Fungal identification was conducted through the microscopic morphology of the isolates observed at x10 and x40 magnifications, as well as the macroscopic colonial characteristics, including colony texture, size, pigmentation, colouration on the reverse side of the plates, and colony margins [12]. Confirmatory identification of fungal isolates was done using the lactophenol cotton blue stain.

2.5. Statistical analysis

statistical analysis was conducted with IBM SPSS version 21. Significant difference in the prevalence of individual mould between and within the selected markets was computed using one way analysis of variance at 95% confidence interval. Computed p-value greater than 0.05 was determined not to be statistically significant.

3. Results and discussion

3.1. Proximate Composition of Garri Samples

Table 1 shows the proximate composition of Garri samples from the different markets. The moisture content of the gaari samples ranged between 13.2±0.01 - 17.1±0.02, Protein content (0.90±0.15 - 1.0±0.00), Ash content (1.32±0.13 - 1.53±0.02), Fat content (1.00±0.02 - 1.04±0.13), Fibre content (1.22±0.01 - 1.27±0.00) and carbohydrate content (84.5±0.03 -85.8±0.14). No statistically significant difference was seen among the values of the three marketplaces studied.

3.2. Fungal Count of Garri samples at selected markets

Table 2 shows the result of the total viable fungal count (CFU/mL) of selected garri sold at Lokoja market. The total viable fungal count (CFU/g) of garri samples ranged from 2.8×10^3 – 8.3×10^5 in Old Market, 1.3×10^3 – 5.0×10^5 in Adankolo Market, and 3.9×10^3 – 6.6×10^5 in International Market.

3.3. Morphological characteristics of mould isolated from commercially-marketed garri

Table 3 shows the structural features of moulds isolated from commercially-marketed garri in Lokoja metropolis, Kogi State. The fungal isolates were *Aspergillus* spp, *Alternaria* spp, *Mucor* spp, *Rhizopus* spp, *Microsporum* spp, and *Cladosporium* spp.

3.4. Frequency of Occurrence of Fungal Isolates from Garri

A total of seventy (70) moulds were recovered from the sampled garri, of which 33 (47.14%) were from Old Market, 21 (30.0%) were from International Market, and 16 (22.86%) were from Adankolo Market. Of the 70 mould isolates, *Aspergillus* spp (34.29%) was the most prevalent, followed by *Alternaria* spp (25.71%), *Mucor* spp (15.71%), *Rhizopus* spp (11.43%), *Microsporum* spp (10.0%), and *Cladosporium* spp (2.86%). Nonetheless, there was no statistically significant variation ($p > 0.05$) in fungal prevalence both within and across the studied markets (Table 4).

Table 1 Proximate Composition of Garri Samples

Parameters	**Sampling location (Market)**		
	Old	Adankolo	International
Moisture	11.2±0.02	11.4±0.11	10.2±0.01
Protein	0.90±0.15	1.0±0.00	0.94±0.01
Ash	1.53±0.00	1.32±0.13	1.42±0.02
Fat	1.02±0.02	1.00±0.02	1.04±0.13
Fibre	1.27±0.00	1.25±0.01	1.22±0.01
Carbohydrate	84.5±0.03	85.8±0.14	84.6±0.04

Table 2 Total viable fungal counts of commercially-marketed garri in Lokoja metropolis

Market	**Sample**	**Total Viable Fungal Count (CFU/g)**		
		10^{-1}	10^{-2}	10^{-3}
Old market	1	3.4×10^3	4.4×10^4	6.0×10^5
	2	2.8×10^3	3.2×10^4	6.3×10^5
	3	5.7×10^3	3.2×10^4	4.8×10^5
	4	6.9×10^3	5.0×10^4	2.3×10^5
	5	5.1×10^3	4.7×10^4	5.0×10^5
	6	7.4×10^3	8.9×10^4	5.9×10^5
	7	2.8×10^3	3.3×10^4	4.9×10^5
	8	4.4×10^3	5.7×10^4	NG
	9	5.5×10^3	5.8×10^4	8.3×10^5
	10	NG	NG	NG
Adankolo market	1	8.3×10^3	7.8×10^4	5×10^5
	2	3.3×10^3	2.2×10^4	3.5×10^5
	3	3.2×10^3	1.4×10^4	3.4×10^5
	4	5.7×10^3	5.6×10^4	5.0×10^5
	5	NG	NG	NG
	6	NG	NG	NG
	7	1.3×10^3	2.7×10^4	3.3×10^5
	8	NG	NG	NG
	9	NG	NG	NG
	10	NG	NG	NG
International market	1	9.7×10^3	5.6×10^4	1.2×10^5
	2	8.6×10^3	8.3×10^4	6.6×10^5
	3	7.4×10^3	2.3×10^4	1.9×10^5
	4	8.6×10^3	8.1×10^4	2.6×10^5
	5	3.9×10^3	3.3×10^4	4.1×10^5
	6	NG	NG	NG
	7	6.1×10^3	2.9×10^4	NG
	8	NG	NG	NG
	9	NG	NG	NG
	10	NG	NG	NG

NG: No Growth.

Table 3 morphological characteristics of mould isolated from commercially-marketed garri

Mould	Colony characteristics	Hyphae	Sporangia/ conidia	Rhizoid	Colour
Alternaria spp	Colonies are fast growing, appear white woolly surface	Septate	Conidia	Absent	Black to greyish
Aspergillus spp	Colonies grow fast mostly consisting of dense and erect conidiophores	Septate	Conidia	Absent	White, yellow-brown, brown to black, green
Cladosporium spp	Colonies are slow growing, often becoming powdery due to the production of abundant colonies	Septate	Conidia	Absent	olivaceous-brown to blackish-brown but also sometimes grey, buff or brown,
Rhizopus spp	Fast growing colonies that rapidly filled the petri dish with grey mycelium	Non septate	Sporangia	Present	At first white to grey or yellowish brown
Mucor spp	Fast growing colonies that fill up the petri dish with abundant woody mycelium	Non septate	Sporangia	Present	White to yellow become dark grey
Microsporum spp	Colonies are fast spreading	Septate	Conidia	Absent	Greyish white to light tan white in colour

Table 4 Prevalence of moulds isolated from commercially-marketed garri in Lokoja

Mould	Old market	International Market	Adankolo Market	Total	*f*-ratio	p-value
	n (%)	n (%)	n (%)	n (%)		
Cladosporium spp	2 (6.06)	0 (0.00)	0 (0.00)	2 (2.86)	0.7747	.478431
Microsporum spp	0 (0.00)	4(19.05)	3 (18.75)	7 (10.00)		
Mucor spp	2 (6.06)	5(23.8)	4 (25.00)	11 (15.71)		
Rhizopus spp	5 (15.2)	0 (0.00)	3 (18.75)	8 (11.43)		
Alternaria spp	8 (24.24)	4 (19.05)	6 (37.5)	18 (25.71)		
Aspergillus spp	16 (48.5)	8(38.09)	0 (0.00)	24 (34.29)		
Total	33 (47.14)	21 (30.00)	16 (22.86)	70(100.00)		

Garri is a major staple food that is commonly consumed in different Nigerian homes. Although some people mix the garri flour with hot water to make a mound that can be swallowed, a vast majority of individuals simply either eat the flour dry or mix it with cold water and eat. Hence, commercially-marketed garri flour within Lokoja metropolis, Kogi State, Nigeria was examined for their proximate and fungal compositions. The proximate analysis of the chosen garri samples indicated differing percentages. The moisture content of garri samples in this investigation exceeded the established safe threshold of 12.0% for white garri [13]. Also, higher moisture content was reported by Ogbonna *et al.,* [14]. This could be attributed to the traditional method of processing of the Garri; gratification, extent of dry-frying/roasting and storage condition. Moisture is a critical factor in the preservation of cassava flour. Excessive levels over 12% promote proliferation of microbes, making lower levels preferable for an extended shelf life [15].

The crude ash content usually reflects inorganic elements (minerals such as K, Zn, and Ca) and, when included cassava, varies from 1% to 2%. Ash content denotes the aggregate mineral composition of food following combustion at elevated temperatures. The ash concentration of the garri samples in this research was below 1.90 – 2.84% [16]. The variances may be ascribed to variations in the genotypic composition of the raw cassava roots' dry matter and their proximate composition. Higher dry matter concentrations have been correlated with lower ash contents [17].

In this study, protein content (0.90±0.15 - 1.0±0.00) was relatively low. Similarly, Okolo and Makanjuola, [18] reported low content of protein in garri sold within Ahmadu Bello University main campus, Samaru, Zaria, Kaduna State. The percentage crude fat, crude fiber and carbohydrate contents of garri found in this study fell within the regulation range as reported by Bamidele *et al.* [19].

The total viable fungal count (CFU/g) of garri samples ranged from $2.8 \times 10^3 – 8.3 \times 10^5$ in Old Market, $1.3 \times 10^3 – 5.0 \times 10^5$ in Adankolo Market, and $3.9 \times 10^3 – 6.6 \times 10^5$ in International Market. These fungal counts are comparable to those reported in similar studies [9,20–22]. Several factors can account for these high fungal counts including high moisture contents, nutritional composition, storage conditions, storage duration, and physical handling [8,9,23]. According to Aguoru *et al.* [23], the longer the storage time of garri, the higher its microbial load, pH, and moisture content.

Fungal contamination of garri is not only significant because it causes the spoilage of garri which incurs significant economic loss, it is also significant because such contaminating moulds can be of significant public health concern. *Mucor* spp, *Aspergillus* spp, *Rhizopus* spp, *Microsporum* spp, *Alternaria* spp, and *Cladosporium* spp were isolated from commercially-marketed garri in this study. In earlier studies in Kogi State, Akoma *et al.* [21] reported *Aspergillus* sp, *Penicillium* sp, *Rhizopus* sp, *Fusarium* sp, *Mucor* sp, *Cladosporium* sp, *Alternaria* sp, *Montospora* sp in Lokoja metropolis and Mofolorunsho *et al.* [20] reported *Aspergillus* spp, *Rhizopus* spp, *Penicillium* spp, *Mucor* spp, and *Neurospora* spp in Anyigba, Kogi State, Nigeria.

The fungal isolates recovered in this study have also been reported in similar studies in several Nigerian states including Kaduna, Ogun, Benue, Bayelsa, and Katsina [9,22,24–27]. Minor variations in the frequency and species of fungi isolated and reported in other studies can be associated with individual location-specific factors as well as difference in the robustness of the method employed for the identification of fungi [26]. However, Numerous fungal species are often identified in outdoor and indoor air samples in diverse locations and are common environmental contaminants generally linked to the contamination of ready-to-eat foods [21,26,28,29].

Aspergillus spp was the most prevalent fungi in this study. Most species of *Aspergillus* produce aflatoxins, a type of mycotoxin, and some are associated with invasive diseases such as *aspergillosis* [27]. Other than *Aspergillus* spp, *Alternaria* spp have also been reported as potent mycotoxin producers [30,31]. Mycotoxins are poisonous secondary metabolites produced by fungi that often contaminate food and adversely affect human and animal health upon ingestion [32]. The occurrence of mycotoxin in food has been widely reported and exposure can lead to broad toxic effects [33,34]. Furthermore, several outbreaks have been reported due to mycotoxin contamination of food and they are significant threats to global food security and human health [23,35].

Also, *Alternaria* spp are important allergens and aetiologic agents of phaeohyphomycosis in immunocompromised patients [36], *Mucor* spp and *Rhizopus* spp are associated with mucormycosis, an opportunistic human infection that ranges from chronic cutaneous to rhinocerebral [37], *Microsporum* spp is associated with superficial dermatomycosis [38]

4. Conclusion

This study has shown the excessive moisture content of garri marketed in Lokoja, which promotes fungal development in some samples, underscoring the necessity for adequate drying to reduce moisture levels. Nevertheless, certain garri satisfied the stipulated criterias concerning ash, fat, fibre, and carbohydrate levels. However, selected garri complied with acceptable standards with respect to ash, fat, fibre and carbohydrate contents. Also, this study has identified the different contaminating fungi in commercially-marketed garri in Lokoja metropolis, Kogi State, Nigeria. Hence, sellers of garri should be properly sensitized on the need to properly handle the product to reduce the risks of contamination.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest is to be disclosed.

Statement of informed consent

Informed consent was obtained from all individual participants included in the study.

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