

Prevalence and colonization of fungi in dried food (garri) obtained from Ikere-Ekiti

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Abstract

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 garri product with varying mycological contamination. The objective of this study was to assess the mycological safety and mycological contamination of garri marketed in Ikere-Ekiti, Ekiti State. A total of eight (8) samples of garri displayed in the open at Ikere-Ekiti markets were used for this study. The samples were collected with sterile polythene bags adopting standard procedures and transported to the laboratory for analysis. The result of this study clearly showed fungal contamination resulting from its display in the open market, *Aspergillus* spp. and *Mucor* sp. had the highest frequency of occurrence and *Aspergillus* spp. was the fungi most frequently isolated. Other fungi species isolated in the garri samples were *Absidia* spp., *Botrytis* spp., *Penicillium* spp. and *Rhizopus* spp. Since this product harbor arrays of fungi, strategy to antagonize their growth and survival in this commodity in order to neutralize the potential of these organisms serving as agents of food borne diseases should be adopted.

Keywords: Contamination; Fungi; Food sample; Garri

1. Introduction

Fungi are non-photosynthetic protists growing as a mass of branching, interlocking filaments (hyphae) known as mycelium. Although the hyphae exhibit cross walls, the cross walls are perforated and allow free passage of nuclei and cytoplasm. The entire organism is thus coenocyte which is a multinucleated mass of continuous cytoplasm confined within a series of branching tubes (Ogiehor and Ikenebomeh, 2005).

All fungi are eukaryotic organisms and each fungi cell has at least one nuclear membrane, endoplasmic reticulum, mitochondria and secretory apparatus. Most fungi are obligate or facultative aerobes. They are chemotrophic, secretory enzymes that degrade wide variety of organic substrates into solute nutrients which are then passively absorbed on taken into the cell by active transport (Abee *et al.*, 2005). Fungal contaminant are also responsible for substantial effects in stored foodstuffs including discoloration, losses of nutritional value, product of odors, deterioration in technological quality and contamination of mycotoxin (Magnoli *et al.*, 2006).

Cassava production is primarily used for human consumption. It represents staple food and a major contributor to food security in producing areas and Africa consume almost its entire production. Cassava products fermented or not, are also devoted to foodstuff (Akoroda, 2007). Cassava tubers are processed into an amazing variety of foods. Traditionally processing techniques varies from region and ethnic group with a given area. In Africa, the two common products of Cassava tubers are farina (gari) and fufu (sticky dough made by pounding cooked of fermented tubers into paste. Garri

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is the product of Cassava tubers obtained from peeled, grated, fermented and roasted *Cassava* tubers. It is consumed by several millions of people of West Africa (Ogiehor and Ikenebomeh, 2005).

Garri is made from peeled, washed, grated, fermented and toasted fresh cassava tuber (*Manihot esculenta* Crantz). It is the most popular fermented cassava products in Africa (Oluwole *et al.*, 2004). It is consumed by several millions of people in West Africa where it forms a major part of their diet (Kostinek *et al.*, 2005; Ogiehor *et al.*, 2007). In Nigeria, its acceptability cuts across the various ethnic and socio-economic classes, making it the commonest food item (Jekayinfa and Olajide, 2007). Garri is stored and marketed in a ready-to-eat form and prepared into a stiff paste or dough-like called 'Eba' by adding the granules into hot water and stirring to make a paste of varied consistency. Eba can be consumed with local soups or stews of various types by chewing or swallowing in morsels (Ogiehor *et al.*, 2007). Garri can also be deliciously consumed directly (without cooking) with groundnut, smoked fish, coconut, cowpeas, moi-moi, or taken as a fast food when soaked in cold water (Ogugbue and Obi, 2011). Sometimes, it is taken with beverages mixed with cold water or warm water with salt depending on the choice of the individual (Aguoru *et al.*, 2014).

Microbial growth, deterioration and spoilage of garri are major cause of food borne illnesses and threat to public health. 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 (Ogugbue and Obi, 2011; Ogugbue *et al.*, 2011).

The main biological agents that contaminate and spoil garri are moulds, insects and mites (Ogiehor *et al.*, 2005). Garri is rich in carbohydrate and therefore, suitable for fungal growth. Moulds such as *Aspergillus*, *Penicillium*, *Fusarium*, *Rhizopus*, *Cladosporium* and *Mucor* have been associated with garri during storage and distribution (Ogugbue *et al.*, 2011). The growth of moulds in garri results in changes in the organoleptic, microbiological and nutritive quality which lead to spoilage of the food product (Efiuvwevwere and Isaiah, 1998). Some moulds such as *Aspergillus flavus*, *Aspergillus parasiticus* and *Penicillium* spp. can also produce aflatoxins (Frazier and Westhoff, 2000; Ogiehor *et al.*, 2007), which can have serious effects depending on the dosage consumed (Aguoru *et al.*, 2014).

Garri is a major food consumed among students in Africa, especially in Nigeria; hence, the present study was based on the need to assess the microbiological safety of garri produced in Ikere-Ekiti, Ekiti State, Nigeria.

2. Material and methods

2.1. Collection of samples

Eight (8) garri samples were collected from eight different location in Ikere-Ekiti, Ekiti State, Nigeria for the experimental study. The samples were aseptically collected into sterile plastic bag from different spots and carried to the laboratory for the isolation and identification of fungal species.

2.2. Preparation of agar

7.8 gram of Potato Dextrose Agar (PDA) was properly mixed with 200 mL distilled water and boiled to dissolve the medium completely. It was sterilized using autoclaving at 121°C for 15 minutes and mixed well before dispensing aseptically and with proper care so that any contamination is avoided.

2.3. Isolation of fungal organisms

1 gram of sample was mixed with 1% hypochlorate solution. The aliquot was placed at equidistance on Potato dextrose agar (PDA) and incubated for 5 days at room temperature. Pure culture of the different colonies (based on morphology) was obtained by sub-culture of the isolates on potato dextrose agar plates. The fungal isolates were identified to the genus/species level based on macroscopic and microscopic characteristics of the isolates obtained from pure cultures.

2.4. Preparation of pure culture

In order to make a pure culture, spores from initial culture was transferred to sterilized medium in petri dishes using sterilized inoculating needle and were incubated at 25 °C for 3 – 4 days at room temperature until the fungal growth were observed.

2.5. Identification of fungus

From pure culture, fungal colony was taken with the help of an inoculating needle on a sterilized and grease-free glass slide containing two drops of lactophenol cotton blue stain. The fungal colony was covered with a cover slip and the slides were examined under the microscope. The fungus was identified on the basis of its cultural and morphological characteristics (Rippon, 1998).

3. Results

The results of this study are shown below. Table 1 showed the morphological characteristics of fungi isolated which are *Absidia* spp., *Aspergillus fumigatus*, *Aspergillus niger*, *Botrytis* spp., *Mucor* spp., *Penicillium oxalicum*, *Rhizopus* spp. The macroscopy examination revealed that some of the colonies were cream powder, velvety, wooly, yellowish and greenish spores. Also the microscopy of the isolates showed aseptate and septate hyphae while some of the sporangiophores ranged from erect to branch.

Table 2 showed the occurrence of fungi isolated in garri samples collected from different locations in Ikere-Ekiti. Garri samples from Oja-Oba, Oke-Oja, Ado garage, and Akure road had the highest incidence of fungal isolates. Other locations; Ese-Odo, Ise Junction, and Iloro had single occurrence of different fungi. It was observed that the same fungi (*A. niger*) was isolated in samples from Ise junction and Iloro.

Table 1 Morphological characteristics of fungi isolated from different garri samples

S/N	Macroscopy	Microscopy	Organism
1.	white to a greyish-brown in colour with a woolly texture	hyphae are aseptate (rare septa) and relatively wide	<i>Absidia</i> spp.
2.	Colonies are typically blue-green with a suede-like surface consisting of a dense felt of conidiophores.	Conidial heads are typically columnar Conidiophore stipes are short, smooth-walled and have conical-shaped terminal vesicles which support a single row of phialides on the upper two thirds of the vesicle.	<i>Aspergillus fumigatus</i>
3.	Colonies are dark brown to black	Conidiophores are smooth-walled	<i>Aspergillus niger</i>
4.	Colonies are frequently white and fluffy at first. The conidia are grey or greenish-grey in mass, hyaline and 1-celled	Conidiophores are irregularly branched, and the apical cells are frequently enlarged	<i>Botrytis</i> spp.
5.	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	<i>Mucor</i> spp.
6.	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.	<i>Penicillium oxalicum</i>
7.	Creamy powdery growth that later turned black	Aseptate hyphae, unbranched sporangiospores are from the foot of rhizoids that enlarged in a cup-shaped form with the mycellial region	<i>Rhizopus</i> spp.

Table 2 Occurrence of fungal isolates in garri sample from different locations

Sample location	Fungal isolates
Oja-Oba	<i>Aspergillus fumigatus</i> , <i>Penicillium oxalicum</i> , <i>Aspergillus niger</i>
Ese-Odo	<i>Mucor</i> spp.
Ise junction	<i>Aspergillus niger</i>
Oke-Oja	<i>Botrytis</i> spp., <i>Aspergillus niger</i> , <i>Mucor</i> spp.
Iloro	<i>Aspergillus niger</i>
Ado garage	<i>Aspergillus niger</i> , <i>Mucor</i> spp., <i>Rhizopus</i> spp.
Akure road	<i>Absidia</i> spp., <i>Mucor</i> spp., <i>Aspergillus niger</i>

4. Discussion

A total of seven fungal species (*Absidia* spp., *A. fumigatus*, *A. niger*, *Botrytis* spp., *Mucor* spp., *P. oxalicum* and *Rhizopus* spp.) were isolated from garri displayed in the open market at Bisi market from different sellers. *A. niger* and *Mucor* spp. had the highest occurrence in the entire sample locations. Location of garri that was exposed to unhygienic post production processes was more likely to be contaminated with fungi.

In their metabolic process, fungi produce mycotoxins. These natural products, poisonous to humans and animals, are created as the result of a secondary metabolic process of fungi when grown on organic substrates. Chemical structure of these metabolites varies; however, they are largely of small molecular mass, which conditions their varied toxic characteristics. So far over 400 metabolites produced by fungi have been identified from different genus of fungi: *Aspergillus* spp., *Penicillium* spp., *Fusarium* spp., *Alternaria* spp. and *Trichothecium* spp. (Bräse *et al.*, 2009; Krzyściak *et al.*, 2011).

Fungi isolated from garri samples in this study are not different from isolates from other states in Nigeria. In the western part of Nigeria, an assessment of some fermented cassava products obtained from three states reported the isolation of *Aspergillus* spp., *Mucor* spp. and *Penicillium* spp. from garri (Lawani *et al.*, 2015) while Ogugbue *et al.* (2011) isolated *Aspergillus* spp., *Penicillium* spp., and *Mucor* spp. from garri stored under various storage conditions while a study carried out in Makurdi isolated similar fungi subspecies from garri (Ogugbue *et al.*, 2011). In this study, *Absidia* spp. was found in garri samples from seller from Iyin-Ekiti and this differs from other report as documented by Lawani *et al.* (2015) in Bayelsa. Its presence in garri samples might be due to its exposure to an environment harbouring the fungi during post production processes. The result of this study shows that garri from the open market harbor arrays of fungal contamination which conforms to reports from other parts of Nigeria but the fungi subspecies isolated from the different locations might be different due to varying post production processes.

Aspergillus spp. and *Mucor* spp. had the highest frequency of occurrence in the different garri samples from the entire sellers. This high frequency of occurrence of these fungi in garri could have been as a result of the unhygienic handling of the garri or poor storage conditions of the garri in compounds where they were isolated. High moisture content favours the growth of these fungi and significant fungi count has been obtained from garri samples kept in basins exposed to air (Ogugbue *et al.*, 2011).

Aspergillus species which had the highest occurrence in garri samples in this study are among the most abundant and widely distributed organisms on earth (Klich, 2002). Most members of this genus being saprophytic fungi are found in the environment without causing disease (Cheesborough, 2005). Fungi of the *Aspergillus* species are typical isogenic opportunistic fungi, which for the most part fail to trigger an infection with a healthy person; however, they constitute a threat predominantly to persons with immunity disorders (Türel, 2011).

It is one of the most commonly reported fungi isolated from foods and indoor environments with ability to produce aflatoxin as its major mycotoxins (Pitts and Hocking, 1997; Klich, 2002). *Mucor* spp. commonly found in soil, digestive systems, plant surfaces, cheese, rotten vegetable matter and iron oxide residue in the biosorption process. Most species of 'Mucor' are unable to infect humans and endothermic animals due to their inability to grow in warm environments close to 37°C. Thermotolerant species such as *Mucor indicus* sometimes cause opportunistic, and often rapidly spreading, necrotizing infections known as zygomycosis (Klich, 2002).

The variation in fungi obtained from the different sellers could be due to their different storage conditions in the market and also their relative permeability to oxygen, carbon dioxide and water vapour. Permeability characteristics and oxygen transfer rate (OTR) has been reported to be factors responsible for differences obtained in fungi count progression in stored market garri (Amadi and Adebola, 2008). The growth of fungi in any food results in changes in the organoleptic, microbiological and nutritive quality which leads to spoilage (Ogei and Ikenebomeh, 2005) and its presence in food suggest an imminent public health danger since their metabolites (mycotoxins) if produced in food (Klich, 2002) like garri may lead to serious and devastating clinical conditions in the consumers.

5. Conclusion

Fungal contamination of garri is as a result of practices associated with post processing of this product. These processes are spreading on the floor to cool, poor packaging, storage conditions, displaying in open bowl or buckets in the markets during sales and some customers antics before purchase.

Some of the fungi isolated in this study can cause diseases or produce mycotoxins that can have serious health effect in man; it is henceforth important to develop a strategy to properly package and store this product to reduce fungal contamination. Also, handling of this product with bare hand, displaying it in open bowl and buckets in the market during sales should be avoided to prevent the introduction of fungi spores to the product.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

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