The mycological content of ready to eat garri in Amassoma, Bayelsa State

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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 Amassoma, Bayelsa State. A total of forty-four samples comprising of both freshly prepared garri and displayed garri in the open market were used for this study. The samples were collected with sterile polythene bags adopting standard procedures and transported to the laboratory for analysis within 12 h. The result of this study clearly showed fungal contamination resulting from its display in the open market, Aspergillus sp. and Penicillium sp. had the highest frequency of occurrence (33.3%) in white garri while in yellow garri, Aspergillus sp. (33.3%) was the fungi most frequently isolated. Other fungi species isolated in the garri samples were Fusarium sp, Mucor sp, Alternaria sp, Cladosporium sp and Rhizopus sp. The relationship between fungi spp. and type of garri (white or yellow) was not statistically significant as the calculated value was greater than the p value of 0.05. 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.

Key words: Disease, fungi, garri, microbiology, production, occurrence.

INTRODUCTION

Garri is made from peeled, washed, grated, fermented and roasted fresh cassava tuber (Manihot esculenta Crantz) (Ernesto et al., 2000). It is the most popular fermented cassava products in Africa (Oluwole et al., 2004; Ernesto et al., 2000) and it is consumed by several millions of person in West Africa where it forms major part of their diet (Edem et al., 2001; 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 among the rich and the poor (Jekayinfa and Olajide, 2007; Ogiehor et al., 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
swallowed in morsels (Asegbeloyin and Onyimonyi, 2007; Ogiehor et al., 2007). Garri can also be deliciously consumed directly (without cooking) with groundnut, smoked fish, coconut, cowpeas, moi- moi, kuli-kuli, 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 or sugar depending on the choice of the individual. Garri is rich in starch, has high fibre content and contains some essential vitamins (Adetugba et al., 2010). Its high fibre content makes it very filling and in the prevention or at least in reducing the likelihood of constipation and bowel diseases (Adetugba et al., 2010). Microbial growth, deterioration and spoilage of garri are major causes of foodborne illnesses and threat to public health (Ogiehor et al., 2007). 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 bowls 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 bio-aerosols on exposed products and transfer of infectious agent during handling (Ogiehor et al., 2007; Ogugbue and Obi, 2011; Ogugbue et al., 2011).

The main biological agents that contaminate and spoil garri are moulds, bacteria, insects and mites (Igbeka, 1987; 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 (Ekundayo, 1984; Ogugbue et al., 2011). Several reports have revealed high occurrence of microorganisms in market samples of garri (Ijabadeyi, 2007; Amadi and Adebola, 2008; Ogiehor et al., 2007). The growth of moulds in garri results in changes in the organoleptic, microbiological and nutritive quality which lead to spoilage of the food product (Ejiuwuweere and Isaiah, 1998). Some moulds such as Aspergillus flavus, Aspergillus parasiticus and Penicillium sp. can also produce aflatoxins (SubbaRao, 2000; Frazier and Westhoff, 2000; Ogiehor et al., 2007), which can have serious effects on human health depending on the dosage consumed. The objective of the study was to assess the mycological contamination of garri freshly prepared and marketed in Amassoma community which is a major producer of garri in this part of the country and this information will help in the formulation of policy that will ensure the mycological safety of the product.

MATERIALS AND METHODS

Sample collection

The study was carried out in Amassoma located in Niger Delta region of Bayelsa State, Nigeria. Amassoma is a community with a population of about twenty thousand people who are mainly farmers, fishermen, traders and civil servant and constitute one of the largest areas production and consumption of garri in Bayelsa state.

Garri samples used for this study were obtained from sellers in the open markets and from local garri processors. A total of 44 garri samples were collected for the analysis. Freshly prepared garri samples and garri displayed in the market were collected without bias from every first compound and shop in the community.

Microbiological analyses of garri samples

The garri samples were processed by weighing 1 g proportion of each sample aseptically (after thorough mixing) into 9 ml of (w/v) sterile peptone water in a beaker, and allowed to stand for 30 min, stirring occasionally using a sterile wooden applicator stick as described by Ogiehor and Ikemebomeh (2005). A drop of the garri suspension was dispensed with a sterile pipette onto the centre of the sterile Petri dish containing Saboroud dextrose agar and spread with a sterile hockey stick (Lugauskas, 2005). The plates were incubated at 25°C for 7 days and observed each day for fungal growth (Appendix plates 1 to 7). Identification of fungi was done by both macroscopic and microscopic methods (Reenen-Hoekstra, 1998).

Macroscopic examination of fungi were based on the following characteristics; colony colour (obverse/reverse), colony size, exudate and soluble pigment. Microscopical examination of fungi was done by teasing small portions of the fungal pure culture and mounting in lactophenol cotton blue dye on a clean slide, covered with a clean cover slip and observed under the microscope (Fawole and Osho, 1995) with 10 x objective lens and confirmed with 40x objective lens. Royal Horticultural Society (RHS) mini colour chart was used in this study as a guide for morphology identification.

Statistical analysis

Statistical analysis was performed with the SPSS version 17 statistical software package. Comparisons between groups were analyzed by Pearson’s Chi-square to determine the relationship between fungi contamination and garri type (yellow or white) at the significance level, p<0.05.

RESULTS

A total of seven mould species (Aspergillus sp, Penicillium sp, Fusarium spp, Mucor sp, Alternaria sp, Cladosporium sp and Rhizopus sp) were isolated from both yellow and white garri displayed in the open market. The fungi in white garri were Alternaria sp. (16.7%), Aspergillus sp. (33.3%), Penicillium sp. (33.3%) and Rhizopus sp. (16.7%) as shown in Table 1.

In yellow garri, the distribution of occurrence were Aspergillus sp. (33.3%), Cladosporium sp. (11.1%), Fusarium sp. (11.1%), Mucor sp. (11.1%), Penicillium sp. (22.2) and Rhizopus sp. (11.1%) as shown in Table 2.

In white garri, Aspergillus sp. and Penicillium sp. had the highest occurrence in 33.3% each, while Aspergillus sp. was found more in yellow garri (33.3%). Both freshly processed garri yielded no fungal growth in this study. The relationship between fungi species and type of garri (white or yellow) was examined using a Chi square test.
Table 1. The frequency of occurrence of mould species isolated from white garri.

<table>
<thead>
<tr>
<th>Fungi</th>
<th>Frequency</th>
<th>White garri (market)</th>
<th>White garri (fresh)</th>
<th>Percentages (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alternaria sp.</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>16.7</td>
</tr>
<tr>
<td>Aspergillus sp.</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>33.3</td>
</tr>
<tr>
<td>Penicillium sp.</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>33.3</td>
</tr>
<tr>
<td>Rhizopus sp.</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>16.7</td>
</tr>
<tr>
<td>Total</td>
<td>6</td>
<td>0</td>
<td>0</td>
<td>100</td>
</tr>
</tbody>
</table>

Table 2. The frequency of occurrence of fungi species isolated from yellow garri.

<table>
<thead>
<tr>
<th>Fungi</th>
<th>Frequency</th>
<th>Yellow garri (Market)</th>
<th>Yellow garri (fresh)</th>
<th>Percentages (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aspergillus sp.</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>33.3</td>
</tr>
<tr>
<td>Cladosporium sp.</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>11.1</td>
</tr>
<tr>
<td>Fusarium sp.</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>11.1</td>
</tr>
<tr>
<td>Mucor sp.</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>11.1</td>
</tr>
<tr>
<td>Penicillium sp.</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>22.2</td>
</tr>
<tr>
<td>Rhizopus sp.</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>11.1</td>
</tr>
<tr>
<td>Total</td>
<td>9</td>
<td>0</td>
<td>0</td>
<td>100</td>
</tr>
</tbody>
</table>

and the association was not statistically significant as the calculated value was greater than the p value of 0.05. Garri type that was exposed to unhygienic post production processes was more likely to be contaminated with fungi.

DISCUSSION

In their metabolic process, moulds 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 moulds have been identified from different genus of fungi: Aspergillus sp., Penicillium sp., Fusarium sp., Alternaria sp. and Trichothecium sp. (Krzyściak et al., 2011; Bräse et al., 2009).

The mould isolated from both yellow and white garri sample were Aspergillus sp., Penicillium sp., Fusarium sp., Mucor sp., Alternaria sp., Cladosporium sp. and Rhizopus spp (Appendix). Fungi isolated from garri in this study is slightly 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 (Egbebi et al., 2012) while Ogugbue et al. (2011) isolated Aspergillus sp., Penicillium sp., Fusarium sp. and Mucor sp. from garri stored under various storage conditions while a study carried out in Makurdi isolated similar fungi subspecies from garri (Agoura et al., 2014). In this study, Cladosporium sp. was found in yellow garri only and differs from the isolation of it in white garri as documented by Agoura et al. (2014) in Makurdi. Its absence in yellow garri might be due to its non exposure to an environment harbouring the mould 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.

The result of this study clearly showed no fungal growth in freshly produced yellow and white garri. The absent of moulds in freshly prepared garri may be due to the inability of these fungi to resist the frying stage during garri processing. Since, there was no fungal growth in freshly prepared garri, the mould recorded in this study may be due to contamination as a result of varying factors. These factors may include unhygienic practices involved in 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
production site to storage area and also the use of bare hands by different customers during the buying process. Also, buyers hand may contain fungal particle which they use to feel the garri, or sometimes put it in their mouth to test the quality of the garri before buying and the left over is put back into the selling bowl. These activities may lead to contamination due to deposition of fungal spores or mycellia on the exposed garri products and transfer of infectious agent (Ogeihor et al., 2007; Ogugbue and Obi, 2011; Ogugbue et al., 2011; Ogeihor and Ikenebomeh, 2005).

Aspergillus species and Penicillium species had the highest frequency of occurrence in the different garri samples, while Aspergillus species had the highest occurrence in yellow garri. This high frequency of occurrence of these moulds in white and yellow 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 moulds and significant mould count has been obtained from garri samples kept in basins exposed to air (Ogugbue and obi, 2011).

Aspergillus species which had the highest occurrence in both yellow and white garri in this study are among the most abundant and widely distributed organisms on earth (Bennett and Klich, 2003). Most members of this genus being saprophytic moulds, are found in the environment without causing disease (Cheesborough, 2005). Fungi of the Aspergillus species are typical isogenic opportunistic moulds, 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). Penicillium sp. commonly found in carpet, wall paper and in the interior may cause hypersensitivity, pneumonitis, allergicalveolitis in susceptible individuals and is also a common cause of extrinsic asthma (John et al., 1995).

Though not a common pathogens to humans, it has however, been reported as a common opportunistic pathogen causing Peniciliosis among HIV positive individuals in Southern Asia (Skoulidis et al., 2004). The presence of Penicillium species in food puts the consumer at risk of ingesting citrinin which had been reported to be nephrotoxic in pigs and in broilers (Ojo, 2003).

These three moulds, Aspergillus sp. (isolated from the compound Okori-Ama, Okulobo-Ama, Adule-Ama), Penicillium sp. (isolated from Wapere-Ama, Ogoun-Ama) and Rhizopus sp. (isolated from Ebilade-Ama) were present in yellow garri and also white garri. While in white garri, Aspergillus sp. was isolated from Agbedi-Ama, Goin-Ama, Penicillium sp. was isolated from the compounds, Bietebi-Ama, Ebitimikondei-Ama and Rhizopus was isolated from Ogbopina-Ama compound. The isolation of these three moulds in both white and yellow garri may be due to poor storage of the garri sample in shops of these compounds. Also, Alternaria sp. was present in white garri obtained in Foro-Ama compound but absent in yellow garri, while Fusarium sp., Mucor sp. and Cladosporium sp. were absent in white garri in the market but present in yellow market garri samples obtained from Eleke-Ama, Sadeimo-Ama and Ayaogbo-Ama compound, respectively. The variation in moulds obtained from the different compound 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 mould 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 (Ogeihor 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.

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.

Conflict of interests

The authors did not declare any conflict of interest.

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Appendix: Photo plates of fungi isolated

Plate 1. *Cladosporium* sp.

Plate 2. *Penicillium* sp.

Plate 3. *Rhizopus* sp. (obverse side and reverse side).
Plate 4. *Mucor* sp.

Plate 5. *Aspergillus* sp.

Plate 6. Culture of *Fusarium* sp.
Plate 7. *Alternaria* sp.