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Molecular Detection of Mycological Content in Ready to Eat Garri in Bayelsa State

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Authors' contributions

This work was carried out in collaboration among all authors. Author AT designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors ISE and OAO managed the analyses of the study. Author LD managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Background and Objective: Garri is a powdery carbohydrate-based food material of cassava root tubers (*Manihot esculenta Crantz*) consumed predominantly in West African countries. It can be processed with palm oil rich in carotenoid (Light-yellow) or without palm oil (Creamy-white). In Nigeria, garri is widely acceptable and consumed by both the poor, the middle men or average Nigerian, and also the rich because it serves as a major source of carbohydrate.
The study aimed at detecting fungal strains that produce mycotoxins in garri sourced from Southern Ijaw Local Government Area of Bayelsa State.
Materials and Methods: A total number of fifty freshly prepared and market displayed-garri samples were collected and processed using standard mycological techniques and Polymerase Chain Reaction from the 23 villages that constitute the LGA.
Results: Results showed that the moulds isolated in yellow garri samples in this study were; *Aspergillus spp* 9(37.50%),*Cladosporium spp* 5(20.83%), *Fusarium spp* 4(16.67%), *Penicillus spp* 3(12.50%), Rhizopus *spp* 2(8.30%) and Mucor *spp* 1(4.17%), while those isolated in the white garri

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samples were; Aspergillus *spp* 6(25%), *Penicillium spp* 8(33.3%), *Cladosporium spp* 4(16.7%), *Rhizopus spp* 2(8.3%), *Mucor spp* 1(4.1%), *Alternaria spp 2*(8.3%) and *Fusarium spp 1*(4.1%) with predominance of *Penicillium* and *Asperigellu*s s*pecies*. Twenty samples subjected to molecular analysis to determine the Internal Transcribed Spacer region (ITS) and characterization of the fungal strains were all positive (100%).

Conclusion: Fungal contamination on garri mostly results from unhealthy post-processing activity basically from poor packaging and storage. Mycotoxins from fungal strains have serious health implications on humans therefore it is paramount that proper packaging and storage of this product is publicized to reduce its mycological contamination.

Keywords: Garri; cassava root tubers; molds; PCR; Bayelsa State.

1. INTRODUCTION

Garri is a powdery carbohydrate-based food material of cassava root tubers (*Manihot esculenta Crantz*) consumed predominantly in West African countries. It can be processed with palm oil rich in carotenoid (Light-yellow) or without palm oil (Creamy-white). Although, cassava (*Manihot esculenta* Crantz), a woody shrub native to South America and of the *spurge* family-*Euphorbiaceae* is a major food crop in developing world [1], Cassava is a known perennial plant, but extensively cultivated as annual crop in tropical and sub-tropical regions for its edible starchy tuberous root, a major source of carbohydrates. Garri is a very valuable source of food energy for millions of people in the tropics especially in sub-Sahara Africa where it ensures food security for large number of people living under unpredictable socio political and ecological circumstances [2]. There is also a conjecture that pre-harvest pruning of vitamin A cassava roots can have the effect of further reducing the dry matter content and modify the starch composition of the roots thus bringing about potentially favorable modifications that could reduce postprandial blood glucose [3].

Lawani et al. [4] writes that cassava based dishes are consumed daily in Africa in various forms such as garri, fufu, starch, achaba, cassava flour, cassava chips, cassava beer, commercial sugar etc. Cassava flakes, a local cheap snack commonly known as Tapioca and kpo-kpo garri, are widely consumed as a staple food in Bayelsa State. Eba is a Nigerian staple food eaten all over the country and beyond. It is made from fried grated cassava flour, commonly called garri. The garri is sprinkle into hot water and stirred to form a stiff paste which can be eaten with indigenous soups or stew [4].

Garri has a slightly sour taste and it could be white or cream depending on the variety of cassava used and the processing method adopted. Its particle size may vary from 0.6 to1.1 mm depending on the method of production and the preferences of the targeted consumers. Nearly 75% of cassava produced in Nigeria is processed into garri.

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 [5]. Frying at high temperatures dries the fermented pulp to about 10% moisture content which may result in partial dextrinization of starch [6] also revealed that frying destroys enzymes and micro-organisms and aids in eliminating cyanide gas from the product. 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 [7,8].

Agarose gels is used for the separation of DNA fragments of varying sizes ranging from 100bp to 25kb and it has proven to be the most useful and versatile technique in biological science research for the separation of DNA. Because DNA has a uniform mass/charge ratio, DNA molecules are separated by size within an agarose gel in a pattern such that the distance travelled is inversely proportional to the log of its molecular weight. The DNA standard contains a mixture of DNA fragments of pre-determined sizes that can be compared against the unknown DNA samples. Supercoiled plasmid DNA, because of its compact conformation, moves through the gel fastest, followed by a linear DNA fragment of the same size with the open circular form travelling the lowest.

The ITS region is the most widely sequenced DNA region in molecular ecology of fungi [9] and

has been recommended as the universal fungal barcode sequence [10]. ITS1 and ITS4 are universal primers [11].

The most important aspect of food spoilage is the formation of mycotoxins that may cause food poisonings. In Nigeria, some researchers have characterized the fungal contaminants of this food item using the classical microbiological technique [12]. But there are limited information regarding the molecular characterization of fungal contaminants of this commodity (Garri), no study on mycological strain typing of these fungal contaminants in processed *Manihot esculenta Crantz* in circulation in Amassoma, Bayelsa State has been done. Hence the need to identify the fungal contaminants in Garri using sequencing of the hyper-variable 18S rRNA gene and providing information on strain diversity using the Random amplified polymorphic DNA markers.

2. MATERIALS AND METHODS

2.1 Study Area

Amassoma, a community surrounded by river hence the name Wilberforce Island is situated in Southern Ijaw local government area of Bayelsa state with latitude of 4°58'13.15''N and a longitude of 6°06'32.94'' E. Amassoma community has about twenty-three compounds having a population of about twenty thousand people mainly of farmers, fishermen, traders and civil servant. Amassoma constitutes one of the largest areas in garri production and consumption in Bayelsa state.

2.2 Sampling Method

A random sampling was made as both freshly prepared garri and market garri samples were collected from every compound and shop in Amassoma community.

2.3 Sample Collection

A total of 50 freshly prepared and market displayed-garri samples in shades of light yellow and creamy white were collected from different processing centers in the 23 compounds in the community. Okori-Ama, OGOUN –Ama, Tantua - Ama, Ayaogbo - Ama, Wapere-Ama, Efeke -Ama, Okori -Ama, Oweidei –Ama ,Ogoun –Ama , Okuloba -Ama, Adula-Ama, Ikoki –Ama, Sadeimo-Ama, Ebilade-Ama, Agbedi-Ama, Oporo-Ama, Goin-Ama, Ogbopina-Ama, Ogbo-Ebiama-Ama, Ibenikiri-Ama, Foro-Ama, Bietebi-Ama, Waduwei-Ama, Azene-Ama and Ebitimikondei-Ama. The Samples were collected in a sterile polythene bags in duplicate of freshly prepared and market displayed and labelled adopting standard procedures, transported to the laboratory for analysis within twelve hours. The Garri samples were labeled with abbreviations from the compound names corresponding with their appropriate shade of color representing the 23 compounds in Amassoma.

2.4 Sample Processing

The garri samples were processed and cultured by weighing 1 g aseptically of each sample with a weighing balance after thorough mixing into 3 ml of sterile peptone water (w/v) in a plain container and allowed to stand for 5 minutes with occasional stirring using a sterile wooden applicator stick as described by Ogiehor and Ikenebomeh [7,13]. Then a drop of the prepared garri suspension was dispensed with a sterile pipette into the centre of the sterile culture media of sabouraud dextrose agar. The plates were incubated at 25°C for 7 days and were observed each day for possible fungal growth. When growth was observed the identification of the fungi was carried out both macroscopically, microscopically and molecularly using Polymerase Chain Reaction.

2.5 Microscopical Examination

Fungi were isolated and identified according to the protocol of Samson and Reennen-Hoekstra (1998), which was based on microscopic examination of their conidial heads, philiades, conidiophores and presence or absence of Rhizoids.

2.5.1 Cultural characteristics

Cultural characteristics of fungi were based on the colour (obverse), size, exudate, soluble pigment.

2.5.2 Fungi confirmation

Small portions of the fungal pure culture were teased and mounted in lactophenol cotton blue dye and stained on a clean slide, covered with a clean cover slip and observed under the microscope with 10x objective lens and confirmed with 40x objective lens.

2.6 Molecular Analysis

2.6.1 DNA extraction (Boiling method)

The organism was cultured overnight in Luria Bertani broth and the broth culture of about 1.5ul was transferred into an eppendorf tube and centrifuged at 12000rpm for about 3-5 minutes in a Denville 260D brushless micro-centrifuge, then supernatant was decanted and 1ml normal saline was added into the eppendorf tube, vortexed to mix, then the tube was placed in a heating block at a temperature of 95ºC for 20 minute after which the tube was ice-cooled for 20minutes. The tubes were then spun again at 14000rpm for 3 minute and 200ul of the supernatant was extracted into a 1.5 ml eppendorf tube and stored in a freezer at a temperature of -20ºC for further analysis.

2.6.2 DNA quantification

The extracted fungal DNA was quantified using a Nanodrop 1000 with its corresponding software installed in a computer system. Two (2ul) of the extracted fungal DNA was used to determine the quantity and quality of the extracted DNA.

2.6.3 Amplification of ITS1 gene

The PCR amplification of the ITS1 gene was performed on the 20 selected isolates in 20ul reaction mixture containing 2X master mix (Taq polymerase, dNTPs, MgCL, Buffer) forward and reverse primers 0.5 m concentration of water and fungal DNA was used as the template, the genes were amplified using set conditions as 94°C for 5 minutes, 94°C for 30 seconds, 54°C for 30 seconds, 72°C for 30 seconds and a 72°C for 5 minutes for 35 cycles.

2.6.4 Agarose gel electrophoresis

The amplicons were resolved on Agarose gel electrophoresis using 1.5% agarose with ethidium bromide and was visualized on an ultraviolet Transilluminator. Then the size of the DNA were determined using a mass ruler high range molecular ladder from 500bp and above for fungal.

The sequences for the forward and reverse primers are CTTGGTCATTTAGAGGAAGTAA& TCCTCCGCTTATTGATATGC respectively.

3. RESULTS

From the total number of (50) garri samples collected from the open market at Ogoun -Ama, the processing center at Okiri-Ama and from shops in the different compounds, the yellow garri were altogether 23, coupled with 1 each from the market and the freshly processed yellow garri making a total of 25. For the white garri, one (1) sample was collected each from the open market at Ogoun-Ama and the processing center at Okiri-Ama also 23 white garri samples was collected from the individual shops in each compounds making a total of 25 white garri samples, in Amassoma Bayelsa State. The tables below encapsulate the details of the sample collections and the respective molds isolated. The agarose gel electrophoresis result and the cultural characteristics of the isolates are all represented in Table 1.

Table 1 showed a total of six fungi genera from the freshly prepared yellow garri with *Aspergillus species* most frequently occurring and *Mucor spp* being the least.

Table 2 showed a total of six fungi genera from the freshly prepared yellow garri with *Aspergillus species* most frequently occurring and *Mucor spp* being the least.

Table 3 showed a total of seven fungi genera from the freshly prepared yellow garri with *Penicillium species* (34%) most frequently occurring and the duo of *Fusarium spp* & *Mucor spp* (4%) each being the least.

Table 4 showed a total of seven fungi genera from the freshly prepared yellow garri with *Penicillium species* (34%) most frequently occurring and the duo of *Fusarium spp* & *Mucor spp* (4%) each being the least.

4. DISCUSSION

The isolated moulds from each compound from the yellow garri samples had the distributions and percentage as; *Cladosporium* 5(20.83%), *Aspergillus* 9(37.50%), *Mucor spp* 1(4.17%), *Fusarium spp* 4(16.7%), *Rhizopus spp 2*(8.30%) and *Penicillium spp 3*(12.50%) as seen in Table 2. *Aspergillus species* were recorded in the following compounds as; Wapere-Ama, Ogoun-Ama, Okuloba-Ama, Oporo-Ama, Ogbo-ebi-Ama, Ibenikiri-Ama, Bietebi-Ama and Azene-Ama, with the highest occurrence. *Cladosporium species* were found in samples from; Tantua-Ama, Oweidei-Ama, Sadeimo-Ama, Ogbopina-Ama and Agbedi-Ama was recorded as the second in occurrence. Fusarium *species* were isolated from samples from Efeke-Ama, Okori-Ama, Adula-

Ama and Goin-Ama. *Penicillium species* were found in compounds as; Ebitimikondei-Ama, Ebilade-Ama and Ayagbo-Ama. *Rhizopus species* were isolated from the samples collected from; Waduwei-Ama and Foro-Ama compound while Mucor was isolated from Ikoki-Ama alone. The yellow garri sample collected from the open market also grew *Aspergillus species* and the freshly processed yellow garri collected from the processing center at Okiri-Ama had no growth the market yellow garri grew Aspergillus, while the freshly processed yellow garri sample yielded no growth. The sum total of the isolates gotten from the yellow garri was six.

The isolated moulds in the white garri samples from the individual compounds had a distribution of *Aspergillus spp* 6(25%) *Penicillium spp* 8(33.3%); *Cladosporium spp* 4(16.7%); *Rhizopus spp* 2(8.3%); *Mucor spp 1*(4.1%); *Alternaria spp* 2(8.3%) and *Fusarium spp* 1(4.1%) as shown in Table 4. The market white garri samples grew *Penicillium* but no fungal growth was seen in the freshly processed white garri. A total of 7 moulds were isolated from the white garri sample in this study while those isolated from the yellow garri sample was 6. *Cladosporium spp* were isolated from compounds as; Ebitimikondei-Ama, Ibenikiri-Ama, Oweidei-Ama andTantua-Ama. *Penicillium spp* were isolated from; Ayaogbo-Ama, Wapere-Ama, Ikoki-Ama, Ebilade-Ama, Oporo-Ama, Goin-Ama and Foro-Ama compounds .Aspergillus spp were isolated from; Azene-Ama, Ogbopina-Ama, Agbedi-Ama, Sadeimo-Ama, Okuloba-Ama and

Table 1. Molds isolated from freshly prepared yellow garri samples

S/N	Compounds in Amassoma	Sample	Mould isolated
1.	TANTUA-AMA	TAN-Y	*Cladosporium spp*
2.	AYAOGBO-AMA	AYA-Y	*Penicillium spp*
3.	WAPERE-AMA	WAP-Y	*Aspergillus spp*
4.	EFEKE-AMA	EFE-Y	*Fusarium spp*
5.	OKORI-AMA	OKO-Y	*Fusarium spp*
6.	OWEIDEI-AMA	OWE-Y	*Cladosporium spp*
7.	OGOUN-AMA	OGO-Y	*Aspergillus spp*
8.	OKULOBA-AMA	OKU-Y	*Aspergillus spp*
9.	ADULA-AMA	ADU-Y	*Fusarium spp*
10.	IKOKI-AMA	IKO-Y	*Mucor spp*
11.	SADEIMO-AMA	SAD-Y	*Cladosporium spp*
12.	EBILADE-AMA	EBE-Y	*Penicillium spp*
13.	AGBEDI-AMA	AGB-Y	*Cladosporium spp*
14.	OPORO-AMA	OPO-Y	*Aspergillus spp*
15.	GOIN-AMA	GOI-Y	*Fusarium spp*
16.	OGBOPINA-AMA	OGP-Y	*Cladosporium spp*
17.	OGBO-EBIAMA-AMA	OGB-Y	*Aspergillus spp*
18.	IBENIKIRI-AMA	IBE-Y	*Aspergillus spp*
19.	FORO-AMA	FOR-Y	*Rhizopus spp*
20.	BIETEBI-AMA	BIE-Y	*Aspergillus spp*
21.	WADUWEI-AMA	WAD-Y	*Rhizopus spp*
22.	AZENE-AMA	AZE-Y	*Aspergillus spp*
23.	EBITIMIKONDEI-AMA	ETK-Y	*Penicillium spp*

NB: One isolate-Aspergillus spp from the market displayed yellow garri was recovered from the Ogoun-Ama market

Table 2. Frequency distribution and the percentage of molds isolated from yellow garri

S/N	Moulds in yellow garri	Frequency occurrence	Percentage (%)
1.	*Fusarium spp*	4	16.67
2.	*Aspergillus spp*	9	37.50
3.	*Penicillium spp*	3	12.50
4.	*Rhizopus spp*	2	8.30
5.	*Cladosporium spp*	5	20.83
6.	*Mucor spp*	1	4.17
	Total	24	100

Table 3. Molds isolated from freshly prepared white garri samples

S/N	Compounds in Amassoma	Sample	Mould isolated in white garri sample
1.	TANTUA-AMA	TAN-W	*Cladosporium spp*
2.	AYAOGBO-AMA	AYA-W	*Penicillium spp*
3.	WAPERE-AMA	WAP-W	*Aspergillus spp*
4.	EFEKE-AMA	EFE-W	*Fusarium spp*
5.	OKORI-AMA	OKO-W	*Fusarium spp*
6.	OWEIDEI-AMA	OWE-W	*Cladosporium spp*
7.	OGOUN-AMA	OGO-W	*Aspergillus spp*
8.	OKULOBA-AMA	OKU-W	*Aspergillus spp*
9.	ADULA-AMA	ADU-W	*Fusarium spp*
10.	IKOKI-AMA	IKO-W	*Mucor spp*
11.	SADEIMO-AMA	SAD-W	*Cladosporium spp*
12.	EBILADE-AMA	EBE-W	*Penicillium spp*
13.	AGBEDI-AMA	AGB-W	*Cladosporium spp*
14.	OPORO-AMA	OPO-W	*Aspergillus spp*
15.	GOIN-AMA	GOI-W	*Fusarium spp*
16.	OGBOPINA-AMA	OGP-W	*Cladosporium spp*
17.	OGBO-EBIAMA-AMA	OGB-W	*Aspergillus spp*
18.	IBENIKIRI-AMA	IBE-W	*Aspergillus spp*
19.	FORO-AMA	FOR-W	*Rhizopus spp*
20.	BIETEBI-AMA	BIE-W	*Aspergillus spp*
21.	WADUWEI-AMA	WAD-W	*Rhizopus spp*
22.	AZENE-AMA	AZE-W	*Aspergillus spp*
23.	EBITIMIKONDEI-AMA	ETK-W	*Penicillium spp*

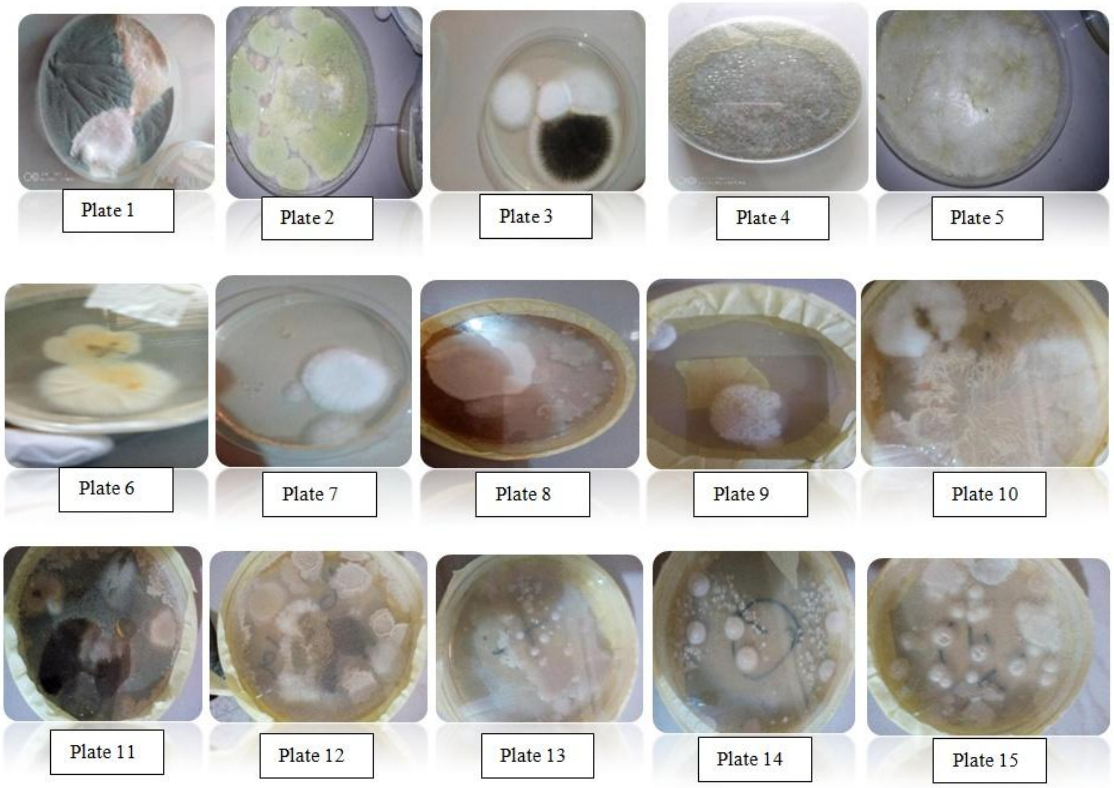
NB: one isolate-Aspergillus spp from the market displayed white garri was recovered from the Ogoun-Ama market

Table 4. Frequency distribution and percentage of molds isolated from white garri

S/N	Mould	White garri	Percentage (%)
1.	*Fusarium spp.*	1	4.1
2.	*Aspergillus spp.*	6	25
3.	*Penicillium spp.*	8	33.33
4.	*Rhizopus spp.*	2	8.3
5.	*Cladosporium spp.*	4	16.7
6.	*Mucor spp.*	1	4.1
7.	*Alternaria spp.*	2	8.3
	Total	24	100

Table 5. Prevalence comparison of fungi strains isolated from yellow and white garri

S/N	Moulds isolated from both yellow and white garri	Prevalence fungi strains in yellow garri	Percentage prevelance of fungi strains in yellow garri	Prevalence fungi strains in white garri	Percentage prevalence of fungi strains in white garri
1.	*Aspergillus* spp	10	40%	6	24%
2.	*Penicillium* spp	2	12%	9	36%
3.	*Cladospori-um* spp	5	20%	4	16%
4.	*Fusarium* spp	4	16%	1	4%
5.	*Mucor* spp	1	4%	1	4%
6.	*Alternaria* spp	-	-	2	8%
7.	*Rhizopus* spp	2	8%	2	8%
	Total	25	100	25	100%



Plates 1-3: Culture of *Aspergillus* spp.; Plates 4-6: Culture of *Rhizopus* spp.; Plates 6-8: Culture of *Penicillium* spp.; Plates 9-10: Culture of *Cladosporium* spp.; Plate 11: *Fusarium* spp.; Plate 12: *Alternaria* spp.; Plates 13-15: Culture of *Mucor* spp.

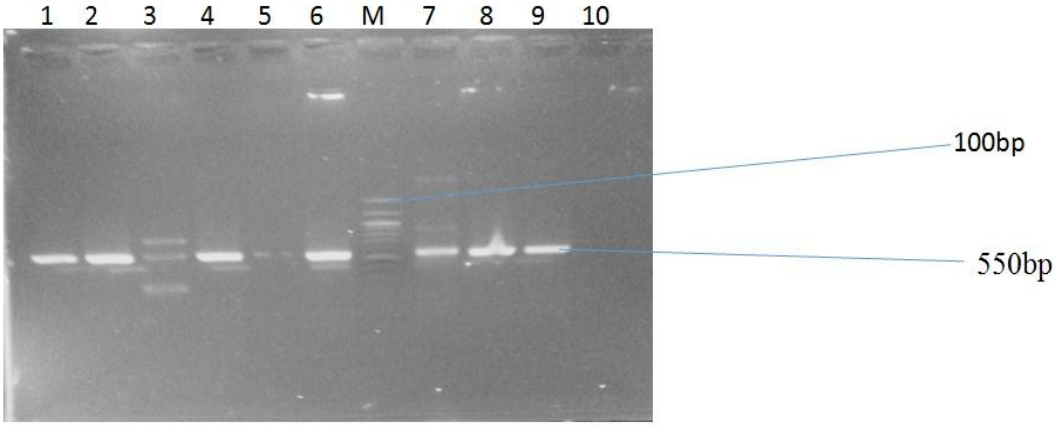


Plate 16. Agarose Gel Electrophoresis showing ITS gene of some fungi isolates from garri. Lanes 1-9 represent ITS gene (550 bp). Lanes 3, 6, 7 and 8 represent spurious amplifications; Lane M represents molecular ladder 100bp

Ogoun-Ama compounds. Alternaria spp were isolated from Efeke- Ama compound. *Rhizopus spp* were isolated form Okiri-Ama and Adula-Ama, Mucor *spp* from Bietebi-Ama and lastly *Fusarium spp* from Ogboebi-Ama. The white garri from the open market at Ogun-Ama grew *Penicillium,* while the freshly processed white garri sample collected from the processing center

at Okori -Ama grew nothing. The collected from the processing center at Okori- Ama grew nothing.

The result of this study shows that garri samples (yellow and white) obtained from open markets and shops contains fungal contaminants. This is in agreement with Efiuvwevwere and Isaiah [14]; but palpably absent in freshly prepared garri, this again is in accordance with Egbebi et al., 2012. However similar moulds as; *Aspergillus spp, Mucor spp* and *Penicillium species* were also recorded from other study on garri from cassava assessment in product fermentation, in Ibadan, Akure and Ado-Ekiti, several authors have also isolated and identified numbers of similar mould species in garri under various storage conditions [5] and Agoura et al., 2015). The number of moulds obtained in the different compound in Amassoma community results from their different storage conditions in the marketed garri product which may be due to their relative permeability to oxygen, carbon dioxide and water vapour [15]. The permeability characteristics and oxygen transfer rate (OTR) are favourable factors that aids the growth of moulds in stored market garri and fungal growth in food product are associated with organoleptic, micro-biological and nutritive quality changes that leads to spoilage (Ogeihor and Ikenebomeh, 2015), that possess an imminent public health danger due to their metabolite production (mycotoxins) when ingested in food [16] may lead to serious devastating clinical conditions in consumers.

Nevertheless, the freshly processed garri (yellow and white garri) glaringly showed no fungal growths. Lawani et al. [4] also gave a similar report that freshly processed garri contained no viable mould. The absence of mould in freshly processed garri of both shades of yellow and white may be due to the inability of the fungi to resist the frying stage of processing. However, some unhygienic post processing handling as pouring out the fried garri on the floor or mats after frying, exposing the garri in an uncovered bowel or bucket as it is commonly sampled in our markets and shops for sales coupled with the use of various packaging materials to transfer the finished products of garri from the rural to urban areas may all lead to the contamination resulting in fungal spore or mycelia from fly deposited (Ogugbe et al., 2011). *Aspergillus species, Cladosporium species and Penicillium species* had the highest frequency occurrence in the garri samples(yellow and white) due to unhygienic handling or poor storage of the garri in high moisture content in the compounds [5].

Twenty samples subjected to molecular analysis to determine the Internal Transcribed Spacer region (ITS) and characterization of the fungal strains were all positive (100%) as evidenced in the agarose gel electrophoresis.

5. CONCLUSION

From this study, it was shown that freshly prepared garri yielded no fungal growth. Therefore, fungal contamination of garri are as a result of the post-processing activities of the individual producers of the product leading to its contamination of which some are associated with; poor packaging and storage of the products, displaying in uncovered containers as bowl or buckets in the markets during sales and handling the product with bare hand hereby exposing it to both bacteria and fungi contaminants that results in fungal spores formation.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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