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Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) of fungal contaminant of garri

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ABSTRACT

Garri, a roasted granular product from peeled, grated and fermented cassava roots, is consumed by several millions of people in West Africa. Hence, this research was carried out to investigate the fungi that are associated with contamination of garri. A total of 100 samples of both white and yellow garri were collected for analysis. The isolation and identification of the fungal isolates were conducted by standard microbiological techniques. Fungal contamination of commercially collected garri were *Aspergillus niger* (26%), *Aspergillus flavus* (28%), *Penicillium* species (39%), *Aspergillus ochraceous* (1%) for white garri while in yellow garri *A. niger* (30%), *A. flavus* (15%), *Penicillium* spp. (28%) *and A. ochraceous* (27%). The frequency of occurrence of the isolated fungal for both the white and yellow garri was compared and it was observed that the frequency and occurrence of *A. flavus* and *Penicillium* spp. were significantly higher in white garri than in yellow garri while *A. niger* and *Penicillium* spp. were the predominant isolated in yellow garri. This study has revealed that this food is mostly contaminated by xerophilic mould and hence should be viewed with great concern because these organisms are threat to public health.

Keywords: Garri, fungal contaminant, microbial growth factor, preservation.

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INTRODUCTION

Cassava is an important root crop often processed into various food products in tropical countries. In West Africa, the most widely known cassava derived food is garri (ICP, 2005). In Nigeria, the consumption pattern varies according to ecological zones. Garri is a roasted granule consumed without any additive or can be consumed with variety of additive such as groundnut, fish, meat and stew (FAO, 2010).

Garri is the most popular fermented food product made from cassava (*Manihot esculenta Crantz*) and in west Africa where it forms a significant part of their diet (Edem et al., 2001; Oduro et al., 2000; Ogiehor et al., 2007). It is preferred by urban consumers irrespective of ethnicity and socio-economic class as it is a pre-cooked food product with good flavor (Jekayinfa and Olajide, 2007).

The dry form of the post processed garri as obtained in the market is commonly consumed without further cooking (soaked in water) with sugar, smoked fish, roasted groundnut, cooked cowpea, and coconut, and sometimes with milk and beverages as complements. It can also be prepared into a stiff paste called “Eba” by adding the granules into hot water and stirring to make the paste of varied consistency which can be consumed with local soups or stews of various types by chewing and swallowing in morsels (Asegbeloyin and Onyimoyin, 2007).

Garri processing covers a series of procedures such as peeling, washing, grating and packing into closely knot bags. The bag is fixed inside a machine specially made to squeeze out some of the liquid and the content of the

bags are then left to undergo spontaneous solid-state fermentation for several days at ambient temperature (Huch et al., 2008; Ray and Sivakumar, 2009). Fermentation of the grated tubers helps in product preservation, flavor development, cyanide reduction, and changes in functional properties (Akindahunsi et al., 1999). The fermented pulps is then dried to about 10% moisture content by frying at high temperatures which probably resulting in partial dextrinization of starch (Osho and Dashiell, 2002), destruction of enzymes and microorganisms and the expulsion of cyanide gas from the product (Asegbeloyin and Onyimonyi, 2007; Harbor and Ogundun, 2009).

The fermentation of cassava to produce garri provides this starchy food in a wide variety of flavours, aromas, and texture that enrich the human diet (Steinkraus, 1997). Distribution and marketing is associated with practices such a display of product in open buckets, bowls and mats at points of sale and the use of bare hands during handling and sales.

These unhygienic practices, which may lead to microbial contamination due to disposition of bio aerosols on exposed products, transfer of microbes from dirty hand and utensils and frequent visits by animals and formites- in animate objects (which may carry infections agents), can contributes to the post-processing of this product.

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 (Basilico et al., 2001; Magnoli et al., 2006). This microbial contaminant may be a source of food borne diseases (Islam et al., 1993; Maria et al., 2001; Venugopal et al., 2001; Ellin, 2002; Oyarzabal et al., 2003).

Previous reports have revealed high bio-load and a vast array of microorganisms in market sample of garri (Amadi and Adebola, 2008; Ijabadeniyi, 2007; Ogiehor et al., 2007). The microorganisms isolated from this market sample include *Bacillus* spp., *Pseudomonas* spp., *Clostridium* spp., *Salmonella* spp., *Klebseiella* spp., *Fusarium* spp., *Cladosporium* spp., etc. These microorganisms can cause deterioration in food quality and spoilage, which may lead to economy losses, serious food borne illness and may pose a threat to public health.

Cassava is fermented to remove cyanide and to produce the desirable flavors it must be properly controlled, as too short a period will result in an incomplete bland product. Too long a period will give the product a strong sour taste. Under-fermentation also badly affect texture of the final garri (ICP, 2005). Roasting of the cassava is done to destroy enzymes and microorganisms, gas, and to dry the product. Preservation is achieved by heating during roasting. Low moisture content inhibits recontamination by bacteria. High humidity is needed for packing to retain the low moisture content (ICP, 2005).

However, the source of these microbial contaminants may also be a portal for contamination by more potent pathogenic microbes, which may cause an epidemic considering the popularity of the food products. This study aimed at investigating the fungi or microorganisms associated with deterioration of garri samples in some selected places within Ogun State.

MATERIALS AND METHODS

Study area

Ogun State is the study site which constitutes one of the largest populations in western Nigeria involved in the production of garri. This state is made up of twenty local government areas in Nigeria with over four million people.

Sample collection

A total of 100 samples, each of white and yellow garri were aseptically collected randomly from 16 different markets in three different zone of Ogun State namely: Abeokuta, Remo and Ijebu. These samples were collected at regular interval and are evenly spread during the study period.

Microbiological analysis

Enumeration of the microbial count in garri was carried out using the surface spread plate method. Ten grams of packed samples were added in 90 ml of 0.1% of sterile peptone water in a sterile 500 ml beaker and allowed to stand with occasional stirring using method described by Ogiehor and Ikenebomeh (2005). Subsequently, tenfold dilution of the samples was prepared and 0.1 ml aliquots were spread plated on Sabouraud Dextrose Agar for total fungal plate counts. These were incubated for 3 to 5 days at 25°C.

The fungal isolates were identified by wet mounting the fungal spores in lacto phenol cotton blue and then observed under x40 objectives. Features such as vesicle shape, the length and colour of the stipe, the foot cell, the type of conidia and the serration were some of the characteristics observed under the microscope, while macroscopic features such as colour of the organisms on plate, the diameter of the mould and the reverse colour of the plates were also considered as described previously.

Biochemical analysis

The pH of the garri sample was obtained according to the method described by Ogiehor and Ikenebomeh (2005). Ten gram of each sample was homogenized in 10 ml of distilled water and then the pH measured using pH meter (Mettler Delta 340, mettle-toledo LTD. UK). The moisture content of garri was determined by drying the samples in an oven at 105°C until a constant weight was obtained (ADAC, 1990).

Sensory evaluation

The sensory quality (general acceptability) was assessed based on parameters such as test, colour, flavour, mouth feel, swelling index, and draw ability. Using a nine-point hedonic scale a ten member panel who consumes garri on a regular basis was used to score the various qualities attributes for overall acceptability.

Statistical analysis

Data was subjected to statistical analysis to determined means and standard deviations of means. Significance differences between means of experiment were determined by analysis of variance (ANOVA). A significant level of 0.05 was chosen.

RESULTS

Table 1 represents cultural and microscopic identification of fungal contaminant of dried cassava powder garri circulating in Ogun State, Nigeria.

From Table 2, *Aspergillus niger* is the most predominant fungi isolates in yellow garri while *Penicillium* spp. was the most frequently isolated fungi isolate in white garri powder. Some other mycotoxigenic or xerophilic mould such as *Aspergillus flavus* and *Aspergillus ochraceous* were also recovered from the investigated garri powder.

The mycological quality of the garri powder was also compared using two parameters via yeast and mould count (Table 3). The total yeast count of 4.55 ± 0.55 in yellow garri was found to be significantly greater than the yeast count of 3.01 ± 0.29 in white garri ($T = 2.48$, $p < 0.05$). However, mould count of 7.46 ± 0.30 in yellow garri was also found to be higher than 6.26 ± 0.61 in white garri but this was not statistically significant ($t = 1.78$, $P > 0.05$).

Some chemical parameter of yellow and white garri were statistically compared and they included moisture content, pH, protein content, fat content, ash content and carbohydrate ($C_6H_{12}O_6$) content (Table 4).

In Table 5, it depict the regression coefficient for predicting the total viable fungal count from the intrinsic factor of garri which is given by the model, $A = \alpha + B_1X_1 + B_2X_2$. Where A = Total viable fungal count from the intrinsic factor, B_1 and B_2 = coefficient (constant), X_1 = pH and X_2 = moisture content; hence, if the pH and the moisture content of garri sample circulating in Ogun state is known, then the total fungal content in garri can be statistically determined using the form model, that is, $A = \alpha + B_1X_1 + B_2X_2$.

The Outer Membrane Protein Bound (OMPB) of the fungi contaminant is tabulated in Table 6. Except for *Aspergillus niger* that revealed three (3) protein bands, All other species of the isolated mould showed two (2) different bands. The molecular weight of the isolated fungal ranged from 10 to 15, 11 to 13, 13 to 14, 11 to 13 for *A. niger, A. flavus, Penicillium* spp. and *A. ochraceus*, respectively.

Table 1. Cultural and microscopic identification of fungi.

Fungi	Colonial morphology appearance	Microscopic
Aspergillus niger	White colonies become brown	Single celled spores (conidia), black as culture matures chains developing end of the sterigma arising from the terminal conidiospores, the vesicle, long conidiophores arise from a septate mycelium
Aspergillus flavus	White colonies becomes greenish Yellow as culture matures	Same as above
Penicillium species	Matures culture usually greenish	Singlecelled spores (conidia) or blue green inchains develop at the end of the sterigma arising from the medulla of the conidiophores arise from a septate mycelium.
Aspergillus ochraceus	Yellow-buff coloured colonies, Pink to purple sclerotia. Uncoloured, yellow or dull red exudates sometimes formed	Single cell spores as in other small and nearly smooth conidia.

Table 2. Distribution of fungal contaminants of garri circulating in Ogun State, Nigeria.

Fungal isolates	N	Garri sample			
		White garri		Yellow garri	
		n	%	n	%
Aspergillus niger	80	21	26	24	30
Aspergillus flavus	80	22	28	12	15
Penicillium species	80	31	39	22	28
Aspergillus ochraceus	80	6	7	22	27

N = Number of samples collected; n = frequency of organisms isolated; % = Percentage of isolated organism.

Table 3. Mycological quality of garri circulating in Ogun State, Nigeria.

Parameters	Types of garri (means ± SEM)		T-value	P-value
	Yellow	White		
Yeast counts	4.55 ± 0.55	3.01± 0.29	2.48	<0.05
Mould counts	7.46 ± 0.30	6.26 ± 0.61	1.78	>0.05

Table 4. Chemical parameters of garri circulating in Ogun State, Nigeria.

Parameters	Types of garri (Mean ± SEM)		t-value	p-value
	Yellow	White		
Moisture content	8.98 ± 0.00	9.62 ± 0.64	1.61	p > 0.05
pH	5.97 ± 0.33	5.50 ± 0.45	1.40	p > 0.05
Protein content (%)	5.63 ± 0.00	5.63 ± 0.01	1.38	p > 0.05
Fat content (%)	6.20 ± 0.00	4.20 ± 0.02	2.40	p < 0.05
Ash content (%)	1.15 ± 0.05	1.20 ± 0.00	1.41	p > 0.05
Carbohydrate content (%)	78.04 ± 0.10	79.35 ± 0.00	1.62	p > 0.05

Table 5. Regression coefficient for predicting total viable fungi count from the intrinsic factors of garri.

Intrinsic factors of garri	Coefficient (β)	Standard error	t-value	p-value
Constant (α)	5.6×10^{-8}	1×10^{-8}	-5.59	0.31*
pH	4.5×10^{-7}	7449511	6.059	0.026*
Moisture content	3115648	1403683	2.20	0.157

Model, $A = \alpha + \beta_1 X_1 + \beta_2 X_2$.

Table 6. Protein bands of fungal contaminant of garri as revealed by Sabouraud Dextrose Agar (SDA) PAGE.

Fungal isolates	Characteristics	
	Number of protein bands	Molecular weight range (kDa)
Aspergillus niger	+++ (3)	10-15
Aspergillus flavus	++ (2)	11-13
Penicillium species	++ (2)	13-14
Aspergillus ochraceus	++ (2)	11-13

DISCUSSION

The results of this study clearly show that yellow and white garri harbors arrays of fungal contaminant. Some of this fungi contaminant had been reported in other studies (Adeniji, 1976; Alkano et al., 1984; Oyeniran, 1989; Edward and Oyedeji, 1992; Ogiehor and Ikenebomeh, 2005).

Penicillium spp. was the most predominant organism isolated from white garri sample. This finding is in parallel with that of Achar et al. (2009), who reported similar findings. The organism is uncommon pathogens in food sample; the presence of *Penicillium* spp. in food put the consumers at risk of ingesting citrinin which had been reported to be nephrotoxin at 0.22 ppm in pigs and 4 ppm in broilers. They may also cause tremors, coagulopathy and enteritis (Ojo, 2003).

A. niger was the most predominant in yellow garri and has long been implicated as one of the most important fungi that causes black mold disease in fruits (Chang et al., 2000), ear infections in human and food poisoning (Adeniyi, 1976; Edward and Oyedeji, 1992). The presence of *Aspergillus* spp. might also be due to be ability of the fungi to produce many spores known as conidia, which are abundantly disseminated into the air. It was reported that the fungi disseminated its conidia into the air by simple disturbance of the environment (Bhattacharya and Raha, 2002).

The mould infestation observed in this study may be due to contamination as a result of local method of processing and also the way it is displayed at the market without any covering (Amadi and Adebola, 2008). The presence of isolated fungi in food suggest an imminent public health danger since there are metabolite (mycotoxins) in food (Jayeola and Oluwadun, 2010) like garri may lead to serious and devastating clinical conditions in the consumers. In conclusion, this study shown that fungal involved in contamination of garri are xerophilic mould such as *A. niger, A. flavus, A. ochraceus and Penicillium* species.

Conclusion

It is therefore important to develop a strategy to antagonize the growth and survival in this commodity, in order to prevent the spread of foodborne disease caused by these xerophilic moulds in our markets and regions where garri is taken as food.

REFERENCES

Achar PN, Hermetz SR, Apkarian R, Taylor J, **2009**. Microscopic Studies on the *Aspergillus flavus* infected kernels of commercial peanuts in Georgia. Ecotoxicol Environ Safety, 72(8):2115-2120.

Adeniyi O, **1976**. Fungi associated with deterioration of garri. Nig J Plant Protection. 2: 74-77.

Akindahunsi AA, Oboh G, Oshodi AA, **1999**. Effect of fermenting cassava with *Rhizopus oryzae* on the chemical composition of its flour and garri products. Riv Ital Sostanze Gr, 76:437-440.

Amadi JE, **Adebola** MO, **2008**. Effect of moisture content and storage condition on the storability of garri. Afr J Biotechnol, 7(24):4591-4594.

Asegbeloyin JN, **Onyimonyi** AE, **2007**. The effect of cassava with *Rhizopus oryzae* on the chemical composition of its different processing methods on the residual cyanide of 'garri'. Pak J Food Nutr, 6(2):163-166.

Basilico JC, Debasilico MZ, Chiericatti, C and Vinderola, CG, **2001**. Characterization and control of threat mould in cheese. Lett Appl Microbiol, 32:419-423.

Bhattacharya K, **Raha** S, **2002**. Deteriorative change of maize, groundnut and soybeans seeds by fungi storage. J Mycopathologia, 155:135-140.

Chang PK, Yu J, Bhatnagar D, Cleveland TE, **2000**. Characterization of *Aspergillus parasiticus* major nitrogen regulatory gene, areA. Biochimi Biophys Acta, 1491:263-266.

Edem DO, Ayatse JO, Itam EH, **2001**. Effect of soy protein supplementation on the nutritive value of garri (Farina) from *Manihot esculenta.* Food Chem, 75:57-62.

Edward AW, **Oyedeji** NO, **1992**. Variations in population dynamic of micro flora of garri in storage. Nig J Pure Appl Sci, 7:199-209.

Ellin M, **2002**. Survival and growth of bacterial pathogens on raw meat during chilling. Food Research Institute. 8: 1-4.

FAO, **2010**. Agriculture statistic. Food and Agriculture Organization of the United Nation. Nation. Rome, www.fao.org.

Harbor CI, **Ogundu** EC, **2009**. Effects of processing on cyanide reduction in different cassava product. Nig J Biochem Mol Biol, 24(1): 35-37.

Huch NKM, Hanak A, Spedit I, Dorty CM, Thonart P, Mbugua S, Holzapfel WH, Hertel C, Franz CMAP, **2008**. Use of Lactobacillus strains to start cassava fermentations of garri production. Int J Food Microbiol, 128:258-267

ICP, **2005**. Integrate cassava project. Nigeria. IITA-ICP (9*)* cigar.org.

Ijabadeniyi AO, **2007**. Microbiology safety of gari, lafun, and ogiri in Akure Metropolis, Nigeria. Afr J Biotechnol, 6(22): 2633-2635.

Islam MS, Hassan MK, Khan SI, **1993**. Growth and survival of *Shigella flexneri* in common Bangladeshi food under various conditions of time and temperature. Appl Environ Microbiol, 59(2): 652-654.

Jayeola CO, **Oluwadun** AO, **2010**. Mycoflora and nutritional components of cocoa powder samples in South West Nigeria. Afr J Agric Res, 5(19): 2694-2698.

Jekayinfa SO, **Olajide** JO, **2007**. Analysis of energy usage in the production of three selected cassava-based foods in Nigeria. J Food Eng, 82:217-226.

Magnoli C, Hallak C, Astoreca A, Pansne, L, Chiacchiera S, and Dalcero, AM, **2006**. Occurrence of ochratoxin A- Producing fungi in commercial corn kernels in Argentina. Mycopathologia, 161:53-58.

Maria LA, Rafael MR, Carolina C, Florencia A, **2001**. Effect of storage temperatures on growth and survival of Escherichia coli 0157:H7 inoculated in foods from a neotropical environment. Rev Biol Trop, 49(2): 517-524.

Oduro I, Ellis WO, Dziedzoave NT, Nimako-Yeboah K, **2000**. Quality of gari selected processing zones in Ghana. Food Control, 11:297-303.

Ogiehor IS, Ikenebomeh, MJ, Ekundayo, AO, **2007**. The bioload and aflatoxin content of market gari from some selected states in southern Nigeria: public health significance. Afr Health Sci, 7(4):223-227.

Ogiehor IS, **Ikeneborneh** MJ, **2005**. Extension of shelf life garri by hygienic handling and sodium benzoate treatment. Afr J Biotechnol, 4(7):744-748.

Ojo MO, **2003**. Food and Animals-borne Diseases, Microbes, Human and Animals Vol 1. Dart ventures, Ibadan, Nigeria: 64:77

Osho SM, **Dashiell** KE, **2002**. The processing and acceptability of fortified cassava based product (gari) with soybean. Discov Innov, 14:186-191.

Oyarzabal OA, Nogueira MC, Gombas DE, **2003**. Survival of *Escherichia coli* 0157:H7, *Listeria monocytogenes* and Salmonella in juice concentrates. J Food Protect, 66(9):1595-1598.

Ray RC, **Sivakumar** PS, **2009**. Traditional and novel fermented foods and beverages from tropical root and tuber crops: Review. J Food Sci Technol, 4: 1073-1078.

Steinkraus KH, **1997**. Classification of fermented foods: worldwide review of household fermentation techniques. Food Control, 8(5):311-317.

Venugopal MN, Karunasagar I, Karunasagar I, Varadaraj MC, **2001**. Growth and survival of kanagawa positive*Vibrio parahaemolyticus* in fish and prawn preparations held at ambient and elevated temperature. Asian Fish Sci, 14(1): 83-88.

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APPENDIX

Steps in production of garri

The procedure for the production of garri from cassava root is represented by the following steps:

i) Harvesting / sorting of cassava: selected fresh, mature cassava, roots without rot.
ii) Peeling: peel by hands or remove woody tops.
iii) Washing: wash in clean water to remove pieces of peel, sand, etc.
iv) Sifting: using a wooden sieve, separate fibrous materials to control size of particles
v) Garri frying: roast in a large, shallow casting pan over a fire, with constant stirring, usually with a piece of broken calabash (gourd) or a wooden paddle for 20-30 min use rotary.
vi) Cooling
vii) Sieving (optional): sieve to obtain granule of uniform size, larger particles of garri that are separated may be sold at a cheaper grade.
viii) Packing
ix) Storing (ICP, 2005: 6)