Fungal and aflatoxin detection in fresh and stored 'garri ijebu' (locally processed food)

Manihot esculenta

Jonathan SG [1] , Abdul-Lateef MB [1] and Ayansina ADV[2]
Mycology & Biotechnology unit,[1,] Department of Botany& Microbiology, University of Ibadan, Ibadan. Nigeria
[2] Department of Biological Sciences, BOWEN University. Iwo. Nigeria
gbolagadejonathan@gmail.com

Abstract: Eight samples of both fresh and stored 'garri ijebu'(GI) which is usually being processed in Ijebu land and consumed by the Ijebus and other Yoruba people of southwest Nigeria were analyzed for proximate composition, fungi detection and the level of aflatoxin B_1 B_2, G_1 and G_2 contamination. Moisture content of 66.1% and 42.0 were recorded for fresh and 18- month old GI samples respectively. Results of carbohydrate analysis showed that fresh GI sample had 41.1% starch which reduced significantly to 20.6% in 18- month stored GI. It was also observed that the crude protein reduced significantly ($P<0.05$) from 4.9 to 4.4% respectively. The fat contents also reduced as the storage time increases. Fungi that were found in biodereriorating GI samples included *Aspergillus tamari, Aspergillus niger, Fusarium oxysporum, Aspergillus flavus, Penicillium chrysogenum, Fusarium compacticum and Saccharomyces* spp**.** The study also presented level of mycotoxin contamination in fresh and stored 'garri ijebu'. In the samples, Aflatoxin B_2 (0.0085ug/kg) was detected in 18-month stored sample, representing 8.8% of the total aflatoxin detected. The nutritional indices and health implications of both fresh and stored GI was discussed.

[Jonathan SG , Abdul-Lateef MB and Ayansina ADV. **Fungal and aflatoxin detection in fresh and stored 'garri ijebu' (locally processed food).** *Rep Opinion* 2013;5(2):13-19]. (ISSN: 1553-9873). http://www.sciencepub.net/report. 4

Keywords: biodeterioration, fungi, aflatoxins, '*garri* ijebu'

1. Introduction.

Nigeria is expressing increasing population growth; National Population Commission estimated Nigerian population to be 140,003,542 (NPC, 2006). The high population without equivalent increase in food production and availability to the citizens could result to malnutrition, disease outbreak and death. This is an important factor which will need attention by policy makers in Nigeria.Children and lactating mothers need adequate protein and necessary nutrients or balance diet. The resultant effect poor food is rampant malnutrition, low productivity level among the mothers and chidren(Jonathan *et al.,*2011). Balagopalan and Diop.(1988) suggested that malnutrition is prevalent in Nigeria with about 76% children malnourished. Their findings further revealed that 42% of children are stunted, 25% underweight and 9% wasted. It was also pointed out that 16.4% of the women malnourished were from dry savannah, 9.9% moist savannah and 9% from humid forest. Among the factors affecting inadequate food suffered by the citizens is low socio-economic level of the people which is serious problem. The above problems prompted the campaign for increase production, utilization and consumption of traditional foods (such as fresh and processed cassava) among the citizens (FAO, 1998).

Cassava (*Manihot esculentus*) (Fam. Euphorbiaceae) is a perennial plant growing to a height ranging from 1 to 5 m with three-core single or multitude branching stems. The leaves are deeply, palmately lobed and the roots are enlarged by deposition of starch cells which constitute the principal source of nutrients . It is also fairly drought tolerant and has the ability to growithstand marginal lands in low-nutrient soils where othother crops do not grow well. Cassava is adapted to the land within latitudes 30° north and south of the equator, at elevations of not more than two thousand meters above sea level, in temperatures ranging from 18 - 25°C to raingrowth and development of the children and low

It requires rainfall of 50-55mm annually and poor soils with a pH from 4 to 9. Roots' bulking occurs usually between the 45^{th} and 60^{th} day after planting and storage roots building is a continuous process. An average storage root yield of 5 - 12 tonnes/ha had been reported by traditional methods of cultivation; but by cultivating high yielding varieties and following better production practices, yield can increase to 40 - 60 tonnes/ha. Cassava's productivity in terms of calories per unit land area per unit of time is significantly higher than other staple crops as cassava can produce 250 x 10^3 cal/ha/day compared with 176 x 10^3 for rice, 110 x 10^3 for wheat, 200 x 10^3 for maize and 114 x 10^3 for sorghum (Balagopalan and Diop ,1988).

The traditional method used in West Africa is to peel the roots and put them into water for three days to ferment. The roots then are dried or cooked. In

Nigeria and several other West African countries, including Ghana, Benin, Togo, Ivory Coast, and Burkina Faso. They are usually grated and lightly fried in palm oil to preserve them. The result is a foodstuff called *garri*. Fermentation is also used in other places such as Indonesia. The fermentation process also reduces the level of antinutrients, making the cassava a more nutritious food (Oboh and Oladunmoye, 2007). Garri is a fermented, gritty, starchy food or free flowing dry granular product produced from cassava (IITA, 1990). It is processed by fermenting peeled and grated roots followed by dewatering, sieving and frying. . Garri is consumed principally as a main meal (eba) or taken as a snack when soaked in cold water, sweetened with sugar and consumed with roasted groundnut, coconut and sometimes dry fish or . Garri features more frequentl'kulikuli' up to 3 times in the daily diet of most households in the producing areas (Adindu and Aprioku, 2006). However, as important as' garri ijebu' is to the natives, the problem of its contamination with aflatoxigenic fungi and elaboration of toxins has received a great deal of attention.

Aflatoxins are potent, toxic, carcinogenic, mutagenic, immunosuppressive agents, produced as secondary metabolites by the fungus *Aspergillus flavus* and *A. parasticus* on variety of food products. Among 18 different types of aflatoxins identified, major members are aflatoxin B_1, B_2, G_1, and G_2 (FAO, 2002). Aflatoxin B_1 (AFB_1) is normally predominant in cultures as well as in food products. Pure AFB_1 is pale-white to yellow crystalline, odourless solid (Adebayo -Tayo *et al.*2006,)

Aflatoxins have been associated with various diseases, such as aflatoxicosis, in livestock, domestic animals and humans throughout the world. The occurrence of aflatoxins is influenced by certain environmental factors; hence the extent of contamination will vary with geographic location, agricultural and agronomic practices and the susceptibility of commodities to fungal invasion during pre-harvest, storage, and processing periods (onathan and Esho,2010).

Food products contaminated with aflatoxins include cereal (maize, sorghum, pearl millet, rice, and wheat), oilseeds (groundnut, soyabean, sunflower, and cotton), spices (chillies, black pepper, coriander, turmeric, and ginger), tree nuts (almonds, pistachio, walnuts, and coconut) and milk. Aflatoxins have also been reported in yam and cassava products such as chips and flours (Adebayo-Tayo *et al*., 2006).

In view of the above, the study aimed at determining the proximate composition, detection of fungi and aflatoxin in fresh and stored garri (stored under market condition(s)

2.Materials and Methods

2.1Preparation of Garri samples

Cassava (*Manihot esculentus*) flakes (Garri) were purchased from Oke-Aje market and Ita-Osu market in Ijebu-ode, Ogun state, Nigeria. Eight different samples were purchased for Garri; ranging from freshly prepared flour to one month, three months, six months, nine months, twelve months, fifteen months and eighteen months. The samples were stored in dry containers at ambient temperature for laboratory analyses proximate composition, fungi isolation, and aflatoxin determination. All experiments were carried out in triplicates.

2.1. Determination of proximate composition

Samples were analyzed chemically following the official procedure of proximate composition analysis described by the Association of Official Chemist (A.O.A.C 1992)

2.2. Isolation of fungi:

All glass wares were washed dried and the surfaces were sterilized with 95% ethanol. The culture medium used was potato Dextrose Agar (PDA). A small quantity of flour sample was poured onto the PDA agar plates and inoculated for seven days at 30 + 2^{o}C in a Gallenkhamp incubator. After seven days, sub culturing was done to get pure culture from the mixed culture(Kighigha,and Jonathan,2012).

One gram each of the samples was weighed into 9ml of sterilized distilled water, it was vigorously shaken for 15 minutes to dislodge all the mycelia and spores from the flour sample, and then kept in suspension to 9ml of sterilized water in test-tuber, serial dilution was done using media preparation of 6 fold. It was then vigorously shaken; after which selected dilution was plated on PDA using pour plate method. The plates were then inoculated in the dark for 3 days and then sub-culturing was done to get pure culture from the mixed culture. Pure culture of fungi species isolated from garri samples were preserved on slant PDA contained in sterile super bottle and kept in the refrigerator at 4^{o}C as stock culture (Jonathan *et al.,*2012).

The fungi isolated were subjected to both macro and microscopic tests. Cotton blue-in-lactophenol was used for fungi microscopic test. A sterile inoculating needle was used to pick a part of the fungi mycelium from plates containing the fungi colonies of 24 hours old. Cotton-in-lactophenol was placed on a clean slide and a small piece of mycelium was carefully transferred into it with the aid of inoculating needle. It was then gently teased and the slide covered with a clean slip, was subsequently examined under the high-power magnification (x 40) objective lens of the microscope. The cultures that

appeared were primarily identified using cultural and morphological features (Banrnet and Hunter, 1972), and identification was done using laboratory manual of microbiology.

2.3.Quantification of aflatoxin:

Five grammes of each sample were filled into 250ml conical flask. 25ml of methanol -water mixture were added and shaken thoroughly for approximately 30minutes in iin a mechanical shaker. The solution was allowed to sediment and filtered through Whatman filler paper No 1. The filtrate was transfer into a 250ml separating funnel; 30ml of saturated sodium chloride (NaCl) and 50ml hexane were added and shaken vigorously for 20minutes. The solutions were allowed to separate. The lower methanol -water layer was collected into another clean dry 250ml separating funnel. 50ml of chloroform was added and shaken vigorously. The chloroform layer was drained into a 250ml conical containing 5g of cupric carbonate, shaken and allowed the cupric carbonate to settle. The mixture was filtered using Whatman No. 4 filter paper having a bed of anhydrous sodium sulphate into a 250ml beaker. Carbonate was washed off again with 25ml chloroform extracts in the 250ml beaker. The chloroform extract was evaporated in a water bath to dryness.

The residue was dissolved in 2ml chloroform and transferred into a screw cap tube for the quantitative estimate. ATLC plates of 20 x 20 x 0.25cm were prepared using glass plates that have been cleaned with acetone to remove all grease marks and finger prints. The glass plates were coated with kieselged G-HR, dried in a dust face chamber 15m minutes and made ready for boiling IIAR&T, 2009).

Aflatoxin standard for B_1 and $G_1$5µl, 10µl and 15µl of each chloroform extracts were spotted on the base line of TLC plate (1cm). 5µl of the aflatoxin (B_1 or G_1) standard of concentration 0.5ug/ncl was spotted on either side of the above spots. The spotted plates were developed in a 100ml chloroform/ acetone mixture ratio ($96:4^{v}/_{v}$) solvent system F for approximately 20minutes so that the solvent front moves about 10cm. Once the area containing the toxin of interest is located under UV light examination, it is scrapped off, elute with chloroform and filter through whatman No 42 filter paper. This was evaporated again to dryness over a hot water bath and reconstitute with 3ml of chloroform. The Absorbance of chloroform extracts for each sample and standard Aflatoxin B_1 or G_1 of 0.5ug/ml were read on UV (Ultra Violet) spectrophotometer at a wave length of 365nm. Aflatoxin concentration in ug/kg was calculated using the formula:

Absorbance Sample x Standard Concentration x Dilution Factor x 1000 Weight of Sample

Colour of aflatoxin under ultraviolet light:

B_1 = Bright green yellow; B_2 = Bright green; G_1 = Violet – brown (dark); G_2 = Violet – light brown

3.0 Results:

Table 1: Proximate composition of stored cassava (garri)

Sample	Starch (%)	Crude Protein (%)	Fat (%)	Moisture (%)	Crude fibre	Ash (%)
G_0	41.1a	4.9^{b}	3.7^{b}	12.8g	4.6^{cd}	3.7^{a}
G_1	39.1^{b}	5.3^{a}	4.0^{a}	13.3f	4.9^{a}	3.7^{a}
G_2	34.6^{c}	4.9^{b}	3.3^{c}	13.9e	4.8^{ab}	3.2^{bc}
G_3	33.4^{d}	4.8^{b}	3.1^{cd}	14.5^{d}	4.7^{bc}	2.9^{c}
G_4	32.0^{e}	4.7^{b}	3.0^{d}	15.9^{bc}	4.5^{d}	2.6^{de}
G_5	25.9^{f}	4.9^{b}	3.0^{e}	14.2^{b}	4.7^{bc}	2.5^{e}
G_6	23.8^{g}	4.9^{b}	3.0^{e}	14.7^{b}	4.7^{bc}	2.3^{f}
G_7	20.6^{h}	4.4^{c}	2.6^{f}	15.1^{a}	4.3^{e}	2.0^{g}

*Values followed by the same letter(s) along each vertical column are not significantly different by Duncan's multiple range test ($p < 0.05$). Each value is a mean of triplicates of samples.

Key

G_0 – Fresh Garri flour; G_1 – One-month stored Garri; G_2 – Three-month stored Garri;
G_3 - Six-month stored Garri; G_4 – Nine-month stored Garri; G_5 – Twelve-month stored Gari;
G_6 - fifteen-month stored Garri; G_7 – eighteen-month stored Garri

3.1.Proximate analyses:

As presented in Table 1 above, the analysis of carbohydrate showed that percentage starch in the samples ranged from 20.6 - 41.1%, with fresh sample having the highest carbohydrate content of 41.1% and eighteen-month stored sample having the lowest (20.6%). Crude protein analysis revealed that percentage protein in the garri samples analyzed ranged from 4.4 - 5.3%; one-month stored sample (G_1) had the highest protein content of 5.3%, while eighteen-month stored sample had the lowest protein content of 4.4%. However, fresh, three-month stored,

twelve-month stored sample and fifteen-month stored samples each have 4.9% protein content, while six-month stored and nine-month stored samples have 4.8% and 4.7% crude protein respectively. Percentage fat, crude fibre and ash content in the analyzed garri ijebu samples showed similar trend as observed in the protein content analysis. Fat content ranged from 2.6 – 4.0%; crude fibre, 4.3 – 4.9% and Ash content 2.0 – 3.7%, with one-month stored garri sample having the highest amount of these substances while eighteen-month stored sample had the lowest

3,2.Mycoorganisms

The result of the microscopic and macroscopic tests carried out on the samples showed the presence of *Aspergillus tamari, Aspergillus niger, Fusarium oxysporum, Aspergillus flavus, Penicillium chrysogenum, Fusarium compacticum* and *Saccharomyces* spp in the contaminated samples. These isolates were characterized and identified based on their appearance on gowth on media and microscopic examinations. The growth of *Aspergillus tamari* was deep yellow-green in colour and covered all the plate after 48hours. It later matured and turned brownish green. Simple chain of conidia was visible having dark conidia head with thin walled vesicles. *Aspergillus niger* being a very fast growing fungus, appeared dark known at first and later turned black. The conidia head were globose and later spilled to conidia chain which were brownish, smooth and crushed like pieces of cane(Alexopolous *et al*.,1996).

However, the growth of *Aspergillus flavus* showed yellowish-green colour. The conidia are globose to subglobose in shape. It occurs in chain of two or more they produce toxin. The growth of *Penicillum chrysogenum* was greenish in colour. It had a velvety surface and growth was much after 48hours(Jonathan *et al*.,2011a). It later turned powdery after few days, microscopically, the hyphae were septate. The conidia were born on the conidiospore and they resemble a painting brush. *Fusarium oxysporum* was whitish and fluffy; *Fusarium compacticum* produced white dry surface spores and mycelium and *Saccharomyces* grew like bacteria with creamy colour. No mycelium or hyphae found. No toxin but gave a saccharomyces odour.

Table 2: Aflatoxin levels in cassava flour (garri) samples

Sample	Aflatoxin B_1 (µg/Kg)	Aflatoxin B_2 (µg/Kg)	Aflatoxin G_1 (µg/Kg)	Aflatoxin G_2 (µg/Kg)
G_0	0.0000	0.0000	0.0000	0.0000
G_1	0.0012^{s}	0.0010^{de}	0.0010^{f}	0.0010^{c}
G_2	0.0016^{f}	0.0013^{dc}	0.0030^{d}	0.0010^{c}
G_3	0.0018^{e}	0.0015^{dc}	0.0040^{c}	0.0030^{abc}
G_4	0.0023^{d}	0.0022^{dc}	0.0050^{d}	0.0040^{abc}
G_5	0.0027^{c}	0.0025^{c}	0.0050^{d}	0.0050^{ab}
G_6	0.0031^{d}	0.0050^{b}	0.0070^{a}	0.0050^{abc}
G_7	0.0033^{a}	0.0085^{a}	0.0080^{c}	0.0065^{a}

*Values followed by the same letter(s) along each vertical column are not significantly different by Duncan's multiple range test ($p < 0.05$). Each value is a mean of triplicates of samples.

3.3Aflatoxins:

Table 2 above represents aflatoxin levels in the analyzed Garri samples. It revealed that generally, aflatoxin levels increased with the period of storage. Eighteen-month stored flour sample had the highest concentration of Aflatoxin B_1, B_2, G_1 and G_2 with 0.0033µg/kg, 0.0085µg/kg, 0.0080µg/kg and 0.0065µg/kg respectively. Fresh flour samples had no aflatoxins detected (i.e. 0.0000µg/kg aflatoxin levels). However, Aflatoxins B_2 and G_1 are more in concentrations than Aflatoxins G_1 and G_2 in the stored samples.

4.0.Discussion

The results of the proximate analysis of garri ijebu samples revealed that percentage moisture content increased with storage.Similar observation were made by Jonathan and Esho(2010), and Jonathan *et al*., (2011a), for stored mushrooms and yam snacks respectively. This increasing moisture content observed in our samples is probably due to exposure to humid environment during storage and market sales. Kaaya *et al.* (2009) reported that storage structures commonly used by farmers in Nigeria are traditional and may not maintain an even, cool and dry internal atmosphere. This finding further indicates the tendency of the flours to grow mould because higher moisture content encourages the growth of micro-organisms and therefore might be favourable for prolong storage of flour (Gbolagade,2005;Abulude and Ojediran, 2006; Jonathan *et al*., 2011a).

Furthermore, it was observed that percentage sugar, protein, fat, crude fibre and ash (dry matter) contents decreased with increasing storage period. This

basically might be as a result of microbial degradation of the nutritive substances in the flour samples by certain fungi making use of these foods as substrates for their growth and development. In addition to this, Aina *et al*.,(2012), and Jonathan *et al*.,(2012a) reported that traditional storage conditions for most vegetables and fruits including their processed forms in most parts of West Africa are far from being ideal. This in turn can cause changes in chemical composition induced by biochemical reactions which take place in the stored food, favoring bacterial and fungal growth, particularly in grains, and reducing the nutrient content (Gbolagade,2006;Gbolagade *et al*.,2006). Storage may affect nutrient content indirectly by inducing physical changes in the grain structure that will influence milling losses and require higher heat processing conditions.

Jonathan and Esho (2010) stressed that storage of processed products deserves attention. Processing is carried out to make the food edible and to stabilize it before consumption. Storage under cold conditions or in sealed containers with no or minimum gaseous exchange and protection from light may induce little change (Jonathan *et al*.,2012b). However, due to the cost of packaging materials in developing countries, plastic or cellophane materials are used, particularly for grain flour and other processed products. An additional point is that often processing of foods may favour their deterioration in storage if conditions are not appropriate and controlled because of the chemical changes that took place during processing.

Furthermore, microscopic and macroscopic examinations on stored bio deteriorating garri ijebu samples revealed the presence of *Aspergillus tamari*, *Aspergillus niger*, *Fusarium oxysporum*, *Aspergillus flavus*, *Penicillum chrysogenum*, *Fusarium compacticum* and *Saccharomyces* spp . The fungal species that colonized the stored garri ijebu samples must have been present in the atmosphere in the form of spores during the cassava chip processing or gained their entrance during storage period as a result of inadequate storage facilities as well in the market.

From the results presented, all the garri samples tested for aflatoxins, with the exception of the fresh sample, were positive for all the four aflatoxins specifically with high aflatoxins G_1 and G_2 than aflatoxins B_1 and B_2. This however does not presuppose that this observation is general as Akingbala *et al.* (1989), Kenji *et al.* (2000), and Akingbala *et al.* (2005) reported that the amount of varies from country to country.

Furthermore, total aflatoxin AFB_1 + AFB_2 + AFG_1 + AFG_2 detected in all the stored samples exceeded 0.001. Most countries limit aflatoxin in food to 20µg/kg (Bankole and Adebanjo, 2003). This according to Okello *et al.* (2010) could have direct negative health implication resulting in loss lives from man and animals that consume aflatoxins contaminated cassava based products . Jonathan *et al.,*2011b) found that the presence of high levels of aflatoxins in stored foods may made it unacceptable for marketing, causing financial loss to the farmers or retailers. Depending on the market, economic losses may reach 100%, when the entire produce/product is rejected by the market if aflatoxigenic fungi are physically observed. It was estimated that Africa loses over US dollars 670 million annually due to lack of adequate storage facilities (Otsuki *et al*., 2001; Guo *et al*., 2009). It is therefore, very essential that all parties involved in the process of producing and marketing garri should ensure that contamination from fungi and mycotoxins is minimized as much as possible

5.0Conclusion

Aflatoxins are the most dangerous toxic substances produced by fungi and could be classified according to their fungal origin, chemical structure and biological activity. Occurrence of these toxins in stored garri ijebu have been observed in this study.. These fungal contaminations not only pose serious health risk to consumers but in addition diminish the nutritional value and economic benefits of this local food .

In view of this, it is expedient that raw materials which are easily susceptible to mould growth and aflatoxin formation be very closely kept in ideal storage conditions. The food industry and farmers should observe all conditions attached to the food processing hygiene. This includes sanitation of raw materials and their manufacturing. Moldy foodstuff should neither be used for the production of garri for human consumption nor for feeding animals.

Technologies that match location-specific needs with the socio-economic profiles of farmers in developing countries. Regulations for monitoring susceptible produce from farm level through buying points to retail markets should be put in place and strict measures on food quality at both household and market levels should be embraced and enforced by all policy makers.

.

Correspondence to:
Dr Segun Gbolagade Jonathan
Mycology & Biotechnology unit
Department of Botany & Microbiology
University of Ibadan
Ibadan. Nigeria
Email: gbolagadejonathan@gmail.com
Tel: +2348164746758

References

1. Abulude, F.O. and Ojediran, V.A. 2006. Development and quality evaluation of fortified 'Amala'. *ACTA Scientiarum Polonorum Technology Alimentaria* 5(2): 127 – 134.
2. Adebayo-Tayo, B.C., Onilude, A.A., Ogunjobi, A.A. Gbolagade, J.S. and Oladapo, M.O. 2006. Detection of fungi and aflatoxin in shelved bush mango seeds (*Irvingia* spp) stored for sale in Uyo, Nigeria. *Electronic Journal of Environmental, Agricultural and Food Chemistry* (EJEAFche) 5(5): 1569 – 1574.
3. Adindu, P.D. and Aprioku, J.K. 2006. Production and characterization of unripe Cassava. *Interciencui* 33: 290-296.
4. Akingbala, J.O., B.O. Oyewole, I. Uzo-Peters, O.R. Karim and G.S.H. Baccus-Taylor, (2005). Evaluating stored cassava quality in Gari Production. *J. Agric and Environ.,* 3: 75- 80.
5. Akingbala, J.O., Osunsami, A.T. and Oguntimein, G.B. 1989. Effect of storage on starch content and modification of cassava. *Starch/Stärke*, 41: 54-57.
6. Alexopoulus, C.J. 1996. Introductory Mycology, 4th Edn., John Wiley, New York.
7. Association of Official Analytical Chemist (AOAC) 1992. Official methods of analysis (15th Edn). Association of Official Analytical Chemist, Washington D.C. *Biometrics*,
8. Aina, DA.Jonathan SG Olawuyi, OJ. Ojelabi, DO and Durowoju, BM (2012).Antioxidant, antimicrobial and phytochemical properties of alcoholic extracts of *Cantharellus cibarius* – Nigerian mushroom. *New York Science Journal* 5(10):114-120
9. Atanda, O.O., Akpan, I. and Enikuomehin, O.A. 2006. Palm kernel agar: An alternative culture medium for rapid detection of aflatoxins in agricultural commodities, *African Journal of Biotechnology* 5 (10): 1029 -1033.
10. Balagopalan, K., and Diop, A. 1988. Storage and Processing of Roots and Tubers in the Tropics. Food and Agriculture Organization of the United Nations (LAO) Ld. D..I.B. Calverley.
11. Bankole, S. A and Adebanjo, A. 2003. Aflatoxin contamination of dried yam chips marketed in Nigeria. *Tropical Science* 43: 201 - 203.
12. Banrnet, H. H and Hunter, B. B. 1972. *Illustrated Genera of Imperfect fungi.* Minneapolis Burgress Publishing Company. Minneapolis, MN, p24 1.
13. FAO Food and Agriculture Organisation, 2004 *Statistics series* No. 95. Food and Agriculture Organisation of the Uniled Nation, Rome.
14. FAO. 1998. Mycotoxin Prevention and Control in Food Grain. Food and Agricultural Organization of the United Nations. Rome.
15. FAO. 2002. Evaluation of Certain Mycotoxins in Food. Fifty-sixth Report of the Joint FAO/WHO Expert Committee on Food Additives. World Health Organization. Geneva.
16. Gbolagade J.S 2005. Bacteria associated with cultures of *Psathyrella atrombonata* (Pegler), *and Schizophyllum commune* (Fr.Ex.Fr), Nigerian edible mushrooms *Acta Phytopathologica. Et Entomologica Hungarica*, 40 :(2-3), 333-340
17. Gbolagade J.S, 2006. Bacteria associated with compost used for cultivation of Nigerian edible Mushrooms: *Pleurotus tuber-regium* (Fr.) Singer, and *Lentinus squarrosulus* (Berk.) *African Journal of Biotechnology*.5 (4) :338-342
18. Gbolagade J.S, Fasidi,I.O , Ajayi E.J and Sobowale A.A 2006. Effect of physico-chemical factors and semi-synthetic media on vegetative growth of *Lentinus subnudus* (Berk.), an edible mushroom from Nigeria. *Food Chemistry*. 99:742-747
19. Guo, B., Yu, J., Holbrook, C.C., Cleveland, T.E., Nierman, W.C., and Scully, B.T. 2009. Strategies in Prevention of Preharvest Aflatoxin Contamination in Peanuts: Aflatoxin Biosynthesis, Genetics and Genomics. *Peanut Science* 36:11-20.
20. IAR&T (2009). Method for aflatoxin analysis, Apata Ibadan
21. Jonathan, S.G. and Esho, E.O. 2010. Fungi and Aflatoxin detection in two oyster mushrooms *Pleurotus ostreatus* and *Pleurotus pulmonarius* from Nigeria. *Electronic Journal of Environmental, Agricultural and Food Chemistry* (EJEAFche) 9(11): 1722 – 1730.
22. Jonathan, G., Ajayi, L. and Y. Omitade. 2011a. Nutritional compositions, fungi and aflatoxins detection in sotred 'gbodo' fermented (*Dioscorea rotundata*) and 'elubo ogede' fermented (*Musa parasidiaca*) from South Western Nigeria. *African Journal of Food Science* 5(2) : 105 – 110
23. Jonathan, S.G., Amos Tautau, B.M.W. and Olawuyi, O.J. 2011b. Food values, heavy metal accumulation, aflatoxin contamination and detection of exo-polysacharrides in *Lentinus Squar-rosulus Berk,* a nigerian mushroom. *African Journal of Agricultural Research*. 6(13):3007-3012
24. Kaaya, A. N., Kyamuhangire, W., and Kyamanywa, S. 2009. Factors Affecting Aflatoxin Contamination of Harvested Maize in the Three Agroecological Zones of Uganda. *Journal of Applied Sciences* 6:2401 - 2407.
25. Kenji, G.M., Mvula, A. M., Koaze, If, Baba, N. 2000. Aflatoxin contamination of Kenyan maize Hour and malted Kenyan and Malawian

grain. *Scientific Reports of the Faculty of Agriculture, Okayama University*. 89: 5-7.
26. Jonathan SG, Ajayi I and Omitade Y(2011). Nutritional compositions, fungi and aflatoxins detection in stored 'gbodo' fermented (*Dioscorea rotundata*) and 'elubo ogede' fermented (*Musa parasidiaca*) from south western Nigeria. *African Journal of Food Science* 5(2):105-110
27. Kigigha LT and Jonathan SG. 2012. Microbiological assessment of opened soft drink bottles for pathogenic bacteria associated with drinking directly from the orifice. *Continental J. Microbiology 6 (1)*: 26 - 32,
28. Jonathan SG, Olawuyi OJ , Aina DA , Dada HG and Oyelakin AO 2012. A Influence of storage time on bio deterioration, aflatoxin contamination and food values of onion (*Allium cepa*) *Nature and Science* 10(11):26-33 .
29. Jonathan SG, Abdul-Lateef MB, Olawuyi OJ and Oyelakin A O 2012. Studies on bio-deterioration, aflatoxin contamination and food values of fermented, dried and stored *ipomoea batatas* chips. *Nature and Science* 10(11):123-128
30. Oboh G. and Oladunmoye M.K. 2007. "Biochemical changes in micro-fungi fermented cassava flour produced from low- and medium-cyanide variety of cassava tubers". *Nutr Health* **18** (4): 355–67. PMID 18087867.
31. Okello, K.D., Biruma, M., and Deom, C.M. 2010. Overview of groundnuts research in Uganda: Past, present and future. *African Journal of Biotechnology* 9:6448-6459.
32. Otsuki, T., Wilson, J.S., and Sewadeh, M. 2001. What Price Precaution? European Harmonisation of Aflatoxin Regulations and African Groundnut Exports. *European Review of Agricultural Economics* 28:263-284.

12/12/2012