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Ochratoxin A producing filamentous fungi in garri circulating in Ogun State, Nigeria

Benjamin Thoha Thomas and Liasu Adebayo Ogunkanmi

Department of Cell Biology and Genetics, University of Lagos, Lagos State, Nigeria.

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ABSTRACT

This study evaluated the presence of ochratoxin producing fungi and levels of ochratoxin A contamination in the most popular cassava food product (garri) in West Africa circulating in Ogun State, Nigeria. Results obtained revealed that the rate of total filamentous fungal contamination in all the sampled zones of Ogun State, Nigeria were not statistically significant (F= 0.327, P>0.05). For the black *Aspergilli*, the maximum contamination rate was 4.2 x 10^7 $CFug^{-1}$ for a sample in Egba zone while the minimum concentration of 2.5x10^2 $CFug^{-1}$ was obtained in a particular market in Remo zone. It was noted, that *Aspergillus niger* and *Aspergillus carbonarius* have the highest isolation rate of 21(24%) among the isolated filamentous fungi (F= 88.167, p< 0.05). 918 (92%) of the total samples of 1000 examined, contained detectable levels of OTA while samples from egba region have highest contamination rate (Fvalue = 3.504, P<0.005). These detectable levels found in our study satisfies the 0-5.0ppb recommended by the Codex Alimentarius Commission (1997). It can be concluded, that garri found in Ogun State are variously contaminated by different filamentous fungi, however black aspergilli remain the main ochratoxigenic moulds present in this staple food.

Introduction

Ochratoxigenic molds are ubiquitous contaminants of pre and post harvest commodities (Sanchis and Magan, 2004), including the ready to eat foods (Palli *et al*; 1991; Sayed *et al.*, 2001; Takahashi *et al*, 2004; Cavaliere *et al.* 2006; Trucksess *et al*, 2006). These organisms attracts particular attention through the damage it does to humans and animals (Abarca *et al*, 1998), as a result of their toxic secondary metabolites known as ochratoxins. Of these ochratoxins, Ochratoxin A seems to be the most popular and has been classified as Group 2B human carcinogen following experiments animals (IARC, 1993). The Mycotoxin "ochratoxin A" is principally produced by certain *Penicillium* and *Aspergillus* species of storage fungi (Pitt *et al*., 2000; Abrunhosa *et al*.,2001;O' Callaghan *et al*, 2003). These storage fungi are known as common saprophytes and are present in different environments, fields and warehouses (Battilani and Pietri, 2000).

The presence of ochratoxin A in several foods (Ogunledun, 2007, Jayeola and Oluwadun, 2010; Dongo *et al*., 2008; Thirumala – Devi *et al*.,2001; Pittet *et al*.,1996;WHO, 2001; Gareis and Schever, 2000) and its accumulated effect such as immunotoxicity, neurotoxicity, genotoxity and possibly carcinogenicity has been documented (Council and Mycotoxins, 2003). In another vein, ochratoxin A has been suspected to be a risk factor for testicular cancer (Jonsyn-Ellin, 2000). According to Marquardt and Frohlich (1992), ochratoxin A disturb cellular physiology in multiple ways, but it seems that the primary effects are associated with enzymes involved in phenylalanine metabolism, mostly with the enzymes involved in the synthesis of the phenylalanine tRNA complex. In addition, it inhibit mitochondrial ATP production (Meisner *et al*.,1981) and stimulates lipid peroxidation (Rahimtula *et al*, 1998).

Despite the ubiquitous nature of ochratoxin A in several foods and even drinks, there is still dearth of information regarding the extent of ochratoxin A contamination in garri samples circulating in Ogun State, Nigeria. Garri is the most popular form in which cassava is consumed in West Africa (Ikediobi *et al*.,1980) and indeed in Africa (Oluwole *et al*.,2004). This food is a roasted granule of cassava that is widely consumed in both rural and urban areas (FAO, 2010). To prepare this food, fresh cassava roots are peeled, washed and grated. The resultant pulp is pulverized, sifted and the resulting semi dried mash is toasted in a pan (Nweke *et al*.,2002). The product obtained after the toasting process is called garri and is highly preferred because it is either consumed dry or with cold water and/or reconstituted with hot water to form dough which can be eaten with different types of soup (Oluwole *et al*.,2004).

However, the practices associated with its production, processing and post processing handling such as spreading on the floor, mats, displays in open bowls in the markets and use of various packaging materials to haul finished products from rural to urban areas may exacerbate microbial contamination (Ogiehor and Ikenebomeh, 2006). These microbial contaminants may serves as vehicle of food borne diseases (Maria *et al*.,2001; Omar *et al*.,2003), while the mycotoxigenic fungi may be responsible for substantial effects in stored food stuffs including discolouration, losses in nutritional value, production of off odours, deterioration in technological quality and contamination with mycotoxins (Basilico *et al*.,2001; Magnoli *et al*.,2006). This research is aimed at identifying filamentous fungi contaminating garri in Ogun State, Nigeria and to determine the presence of their secondary metabolite (ochratoxin A) in the studied garri samples.

Materials and Methods

Collection of Garri Samples

A total of 1000 garri samples (250 each from 10 different markets in each zone) were collected in pre sterilized aluminum pan from the four geopolitical zones of Ogun State, Nigeria

Tele:

E-mail addresses: benthoa2013@gmail.com

between March, 2013 to February, 2014. The samples were collected at interval and spreaded over the study period. The collection of samples was done as described by ICMSF(2002).

Microbiological Analyses

The filamentous fungi population was enumerated by inoculation on the surface of PDA medium (Biotech, United Kingdom). The inoculums were obtained by weighing 10g of the garri powder into 90ml of a peptone water solution (0.1%w/v; Biotech, United Kingdom) for 10 minutes (Hocking and Pitt, 1980). Dishes were incubated at 25^0C for 5 to 7 days and the result was expressed in $CFUg^{-1}$ for the total filamentous fungi and black *Aspergilli*. The filamentous fungi isolates were selected randomly according to phenotypic criteria (2 Isolates for each phenotypic group). Isolates were identified using Morphological attributes (Samson *et al.*,1995) and ITS sequencing (Spadaro *et al.*,2009). The ITS sequence obtained were blasted for using *MEGA* version 4 from the National Centre for Biotechnology Information (NCBI) GenBank, New York, NY, USA (Tamura *et al.*,2007).

Determination of OTA in Garri Samples

To determine the total ochratoxin A level in each of the analyzed garri samples, five grammes of each of the garri samples was weighed into a weighing bottle containing 25ml of 50% methanol. The content was shaken vigorously for 3 minutes on the horizontal shaker. A 5ml aliquot of the resulting solution was filtered through whatman no 1 filter paper. In all cases, OTA was quantified from the extracts by competitive direct ELISA in a microwell format using Neogen Veratox kit (Sigma, USA). The operating conditions were as follows: 100µl each of the extracted garri sample and ochratoxin A control were mixed, followed by the addition of 100µl conjugate enzyme solution. The resulting 300µl solution was mixed thoroughly using a multi channel pipettor. A 100µl aliquot was then transferred to an anti ochratoxin antibody (1:5000 Rabbit IgG in 0.5M potassium phosphate buffer pH 7.0) (Sigma, USA) coated microwell and incubated at 25^0 C for 10minutes. The content of the microwell was washed off and rinsed 3 times with sterile distilled water. Substrate solution (100µl) was then added to the microwell followed by incubation at 25^0C for 10 minutes. The reaction was stopped by the addition of the stop solution (tetraoxosulphate (vi) oxide). The microwell was wiped with a towel and the optical density (OD) was read on a ELISA reader (Bioline Technologies, India) at 650nm.

Results

Quantification and prevalence of filamentous fungi in garri

Table 1 gives the identification and the enumeration of filamentous fungi present in garri samples examined from the four geopolitical zones of Ogun State, Nigeria. Filamentous fungi were found in all the samples from Egba and Remo zones of Ogun State while the samples from Yewa and Ijebu have samples without fungal contamination. However, the rate of total filamentous fungal contamination in all the sampled zones of Ogun State, Nigeria were found not to be statistically significant (F= 0.327, P>0.05). For the black *Aspergilli*, the maximum contamination rate was 4.2×10^7 $CFug^{-1}$ for one of the sample from a market in Egba zone while the minimum concentration of $2.5x10^2$ $CFug^{-1}$ was obtained in a particular sample from Remo market. It was noted, that the number of black Aspergilli (*A. niger* and *A.carbonarius*) were not significantly different statistically in the four geopolitical zones of Ogun State, Nigeria (F=0.863, P>0.05).

Table 2 depicts the prevalence rate of filamentous fungi in the four geopolitical zones of Ogun State, Nigeria. As shown in the table, *Aspergillus niger* and *Aspergillus carbonarius* have the highest isolation rate of 21(24%) among the isolated filamentous fungi. The occurrence of these Ochratoxigenic molds were statistically found to be significantly higher than any other isolated fungi (F= 88.167, p< 0.05).

Recovery and levels of OTA in garri

The recovery of OTA averaged 2.24±0.40, 3.22±1.41, 2.62±0.25 and 2.55±1.46 was observed in ready for sale garri samples spiked with 2.5, 5, 3.13 and 3.80ppb from Yewa, Egba, Remo and Ijebu zones of Ogun State respectively . The mean recovery rate for the different geopolitical zones were not statistically significant (p >0.05) (table 3).

Out of the 1000 garri samples collected from the four geopolitical zones of Ogun State, Nigeria, tested for the presence of ochratoxin A, 918 samples (92%) contained detectable levels of OTA (Table 4).

OTA was detected in all the samples from Egba zone while 212, 243 and 213 samples from Yewa, Remo and Ijebu zones harbor OTA respectively. The most contaminated samples were from the Egba region of the State and this observation was also found to be statistically significant(Fvalue= 3.504, P<0.005). Among the samples from Yewa zone, approximately 85% were positive with an average OTA content of (2.24±0.40) ppb. 243 out of 250 samples from Remo Zone were positive with mean ochratoxin A level found to be (2.24±0.40) ppb while the mean ochratoxin A content for Egba and Ijebu were (3.22±1.41)ppb and (2.55±1.46)ppb respectively. Table 5 shows the level of ochratoxin A contamination of the 1000 samples of garri analyzed. The obtained ochratoxin A level of each garri sample was compared with the regulated acceptable level of 0-5.0ppb recommended by the Codex Alimentarius Commission (1997). It was however found that 92.2% of the garri samples circulating in Ogun State, Nigeria satisfied the codex regulated limit while only 7.8% had value above the regulated limit of 5.0ppb (table 6)

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Discussion

The role of filamentous fungi in food deterioration has been well documented (Schawn and Wheals, 2004; Ogiehor and Ikenebomeh, 2005). In this study, the main filamentous fungi isolated were *Aspergillus niger, Aspergillus flavus, Penicillium verrucosum, Fusarium moniliforme, Trichoderma* species, *Absidia Corymbifera* and *Rhizopus* spp. Our result differed from those quoted in the literature for the filamentous fungi associated with ready for sale garri samples (Ogiehor and Ikenebomeh, 2005; Thomas *et al.*, 2012). In those studies, *Absidia corymbifera*, *Trichoderma* spp and *Penicillium verrucosum* were not isolated. However, Thomas *et al.* (2012) recovered only *Penicillium* species, *Aspergillus nige*r and *Aspegillius flavus*. The variation observed in the two studies may not be unconnected to the fact that a larger sample sizes and markets were sampled in our recent study. Irrespective of the types of xerophilic mould isolated, their presence in foods result in changes in the organoleptic, microbiological as well as the nutritional quality and subsequently in food spoilage (Ogiehor and Ikenebomeh,2005;Magnolia *et al.*,2006). From the public health perspective, the growth of these xerophilic organisms in garri suggest an imminent danger to the consumer especially when their secondary metabolites has been produced in the food already (Zimmerli and Dick, 1996; Otteneder and Majerus, 2000).

Table 1. Quantification of black *Aspergilli* and filamentous fungi in garri circulating in Ogun State, Nigeria

GEOPOLITICAL ZONES	MARKETS	ROCFF	ROCFBA	FILAMENTOUS FUNGI
YEWA	1	4×10^{7}	nd	ac,af,afu
	2	5.2×10^{6}	3.8×10^{5}	rs,an,aca
	3	Nd	nd	nd
	4	4.5×10^{4}	2.1×10^{3}	an.aca,ac
	5	4×10^{5}	4×10^{5}	an,ac
	6	2.8×10^{5}	2.6×10^{5}	ts,pv, aca
	7	5×10^{5}	3×10^{5}	an,aca, pv
	8	3.8×10^{6}	2.8×10^{6}	pv, ts, an
	9	3.5×10^{4}	3.5×10^{4}	an,ac
	10	1.6×10^{6}	nd	at, afl,afu
Egba	1	5.2×10^{7}	4.2×10^{7}	an, aca, ac
	2	1.4×10^{4}	1.4×10^{4}	an, aca
	3	7.8×10^{3}	6.2×10^{3}	an, aw, pv
	4	5.4×10^{4}	2.8×10^{4}	at, afu, an
	5	2.3×10^{6}	1.9×10^{5}	pv,ac, aca
	6	2.3×10^{5}	2.8×10^{4}	aw, ts, an
	7	4.2×10^{4}	1.2×10^{4}	afl,afu,an
	8	3.2×10^{6}	1.4×10^{5}	aca,an
	9	2.0×10^{4}	3.5×10^{3}	aca, pv
	10	6.4×10^{6}	3.5×10^{6}	at, aca

Keys : an = *Aspergillus niger*, af = *Aspergillus flavus*, afu = *Aspergillus fumigatus*, aca =*Aspergillus carbonarius*, at = *Aspergillus terreus*, aw = *Aspergillus wenti*, pv = *Penicilliun verrucosum*, fm = *Fusarium moniliforme*, ts = *Trichoderma* species, ac = *Absidia corymbifera*, rs = *Rhizopus* species, ROCFF = rate of contamination for filamentous fungi, ROCFBA = rate of contamination for black aspergilli

Table 1 contd . Quantification of black *Aspergilli* and filamentous fungi in garri circulating in Ogun State, Nigeria

REMO	1	7.8×10^{6}	nd	an, aca
	2	4.2×10^{5}	7.8×10^{6}	afu, aca
	3	1.8×10^{5}	3.8×10^{4}	an, fs
	4	5.4×10^{7}	9.0×10^{4}	an, afu,at
	5	2×10^{4}	7.2×10^{6}	ac, aca pv
	6	2.5×10^{3}	2.0×10^{4}	aw,ts an
	7	2.5×10^{4}	2.5×10^{3}	afl, afu, an
	8	4.0×10^{6}	3.9×10	an, aca
	9	3.0×10^{5}	2.6×10^{5}	pv aca
	10	3.9×10^{6}	2.8×10^{5}	at, aca
Ijebu	1	5.5×10^{6}	nd	fs, ts
	2	4.9×10^{6}	nd	aw,pv, ac
	3	1.4×10^{6}	nd	nd
	4	4.6×10^{4}	4.2×10^{4}	aca,afu
	5	nd	nd	nd
	6	nd	nd	nd
	7	2.0×10^{4}	2.0×10^{4}	aca
	8	3.0×10^{6}	3.0×10^{5}	an, aca
	9	2.5×10^{5}	2.8×10^{4}	an, pv
	10	Nd	nd	nd

Table 2. Prevalence of fungal contaminats in garri samples circulating in Ogun State, Nigeria

Organisms	YEWA		EGBA		REMO		IJEBU		TOTAL	
	n	%	n	%	n	%	n	%	N	%
A. niger	6	24	7	26.9	6	25	2	16.7	21	24
A. flavus	2	8	1	3.8	1	4.2	-	-	4	4.6
A.fumigatus	2	8	2	7.6	3	12.5	1	8.3	8	9.2
A. carbonarius	6	24	6	23.1	6	25	3	25	21	24
A. terreus	1	4	2	7.6	2	8.3	-	-	5	5.7
A. wenti	-	-	2	7.6	1	4.2	1	8.3	4	4.6
P. verrucosum	3	12	3	11.6	2	8.3	2	16.7	10	11.4
F. moniliforme	-	-	-	-	1	4.2	1	8.3	2	2.3
T. spp	2	8	1	3.8	1	4.2	1	8.3	5	5.7
A. corymbifera	2	8	2	7.6	1	4.2	1	8.3	6	6.9
R. spp	1	4	-	-	-	-	-	-	1	1.15
Total	25	(100)	26	(100)	24	(100)	12	(100)	87	(100)

Table 3: Recovery of OTA from spiked Garri Samples

Added OTA (ppb)	N	Garri Samples	Detected (%)[e]
2.5	3	2.24±0.40	87.9±7.81*
5	3	3.22±1.41	67.5±13.65
3.13	3	2.62±0.25	83.7±4.61
3.80	3	2.55±1.46	67.1 ±22.2

Each Sample was replicated thrice, Values are mean ± SEM, [e]Detected = OTA (ppb)/ added OTA (ppb) x 100, *P>0.05

Table 4. Occurrence of (OTA) in ready for sale garri samples in Ogun State, Nigeria

ZONES	N	Number Analyzed	OTA Positive Samples	Mean of Positive (ppb)	Range (ppb)
YEWA	10	250	212	2.24±0.40	0.41-4.90
EGBA	10	250	250	3.22±1.41[f]	2.61-3.87
REMO	10	250	243	2.62±0.25	1.57-3.73
IJEBU	10	250	213	2.55±1.46	1.2-3.73

[f]p <0.05, Fvalue = 3.504, N = number of market sampled in a zone

Table 5. Mean ochratoxin A content in each of the sampled market

Markets /Zone	No	Range ppb	(Mean± SEM) ppb	Median
YEWA				
1	25	0.5-7.8	1.43 ± 0.46	0.5
2	25	2.2 – 5.5	2.30 ± 0.18	2.6
3	25	0.8 – 23	0.41 ± 0.14	0.8
4	25	1.8 – 12.4	4.90 ± 0.63[a]	11.4
5	25	0.5 – 2.0	1.20 ± 0.10	0.5
6	25	0.5-6.2	2.91 ± 0.30	3.3
7	25	0.8-5.2	2.58±0.29	4.2
8	25	0.4-4.8	2.94±0.24	2.1
9	25	0.7-4.8	2.42±0.23	2.4
10	25	0.5-44	1.35±0.12	1.9
EGBA				
1	25	0.9-46	3.57±0.16	4.1
2	25	1.4 -14.0	3.72±0.56	1.4

3	25	1.5-3.8	3.30±0.19	3.1
4	25	1.4-7.3	3.51±0.33	3.6
5	25	1.5-7.8	3.87±0.49[b]	7.8
6	25	1.6-5.3	3.24±0.17	2.6
7	25	0.7-2.9	2.60±0.23	1.7
8	25	1.4-3.9	2.65±0.13	2.7
10	25	1.8-4.8	2.89±0.15	2.5
REMO				
1	25	0.1-9.8	3.03±0.42	5.4
2	25	0.4-8.6	3.73±0.54[c]	5.1
3	25	0.3-3.8	2.12±0.23	1.8
4	25	0.9-6.6	3.27±.29	3.4
5	25	0.1-4.1	1.57±0.93	1.6
6	25	1.2-4.9	2.49±0.23	2.7
7	25	0.3-6.1	2.79±0.29	5.2
8	25	1.2-8.2	3.24±0.28	3.7
9	25	0.3-3.9	2.05±0.23	0.8
10	25	0.2-4.4	1.94±0.27	2.0
1	25	1.0-9.1	2.30±0.36	1.1
2	25	0.2-5.0	1.30±0.29	1.4
3.	25	1.1-4.8	3.73±0.20[d]	4.5
4	25	0.4-3.8	1.34±0.20	1.9
5	25	0.1-84	1.89±0.41	1.4
6	25	0.4-4.8	1.99±0.27	1.4
7	25	0.1-6.9	2.37±0.35	5.8
8	25	1.0-6.1	2.94±0.34	2.9
9	25	1.3-4.7	1.93±0.26	0.6
10	25	0.8-2.9	1.2±0.22	0.8

Table 6: Ochratoxin A level in Garri relative to Codex Alimentarius Commission regulated limits ochratoxin A level (ppb)

N	Acceptable	Unacceptable	
Yewa Zone	250	222(88.8%)	28(11.2%)
Egba Zone	250	236 (34.4%)	14(5.6%)
Remo Zone	250	230(92%)	20(8%)
Ijebu Zone	250	234(94%)	15 (6%)
N	1000	922(92.2%)	78(7.8%)

[@]Accepatablity was based on human ochratoxin safety level of 0-5 -0ppb (Codex Alimentarius, 1997)

Aspergilli which were the most predominant in this study, are among the most abundant organisms that live on the earth without causing diseases (Samson *et al.,* 2000; Bennett and Klich, 2003; Chessborough, 2005). Among the isolated *Aspergilli, Aspergillius niger and Aspergillus carbonarius* have the highest prevelant rate of 21 (24%), confirming them as the major contaminants of garri in the studied regions. This high contamination rate may be due to the practices associated with the production, processing and post processing handling of garri which includes spreading on the floor, mats, displaying in open bowl in the markets as well as use of various packaging materials to haul finished products from rural to urban areas (Ogiehor and Ikenebomeh, 2005). The study of the presence of ochratoxin A in garri samples disclosed varying degree of contamination level in the four geopolitical zones. When the decision criteria was based on the codex alimentarius commission regulated limits used for beverages, 922 (92.2%) of the examined garri samples satisfied the codex regulated limits while 78(7.8%) had limit above the recommended standard. This findings is not unexpected as OTA has been reported in several foods globally. In Italy, 57% of 6476 marketed food samples were reported to be OTA contaminated (MAFF, 1997; Wolff *et al*.,2000), while 22% of their cocoa products were contaminated above detectable limits (Tafuri *et al.*,2004). In this study, significant statistical variation occur in the mean ochratoxin A level of the garri sampled from the four geopolitical zones of Ogun State while samples from Egba region had the highest contamination rate. The reason for the highest contamination rate of Egba in term of ochratoxin A content may be correlated to the presence of higher number of black Aspergilli (Kapetanakou *et al.*,2009; Bellí *et al.*,2005). Results of this study have shown that garri samples circulating in Ogun State, Nigeria are variously contaminated with different xerophilic moulds while 92.2% of these samples harbour less than 5.0ppb of Ochratioxin A . We however, planned to use molecular based techniques to study the most prevalent filamentous fungi (black Aspergilli) genetic biodiversity in relation to their toxigencity.

References

Abarca, M. L., M. R.Bragulat, M. T.Bruguera and F. J.Cabanes, 1988. Comparison of some screening methods for aflatoxigenic moulds. *Mycopathologia*, **104**:75-79.

Abrunhosa, L., R.R.M. Paterson, Z.Kozakiewicz, N.Lima and A.Venancio, 2001. Mycotoxin production from fungi isolated from grapes. *Letters in Applied Microbiology*, **32**: 240–242.

Basilico, J.C., M.Z.De Basilica, C.Chierizatti, and C.G.Vinderola, 2001. Characterization and Control of thread mould in cheese. *Letters in Applied Microbiology*, **32**: 419-423.

Battilani P. and A. Pietri, 2002. Ochratoxin A in grapes and wine. *European Journal of Plant Pathology*, **108**: 639–643.

Bellí N., A.J. Ramos, I. Coronas, V. Sanchis, and S. Marín, 2005. *Aspergillus carbonarius* growth and ochratoxin A production on a synthetic grape medium in relation to environmental factors. *Journal of Applied Microbiology*, **98**: 839–844.

Bennett, J.W and M.Klich, 2003. Mycotoxins. Clinical microbiology reviews 16(3):497.

Cavaliere,G., P.Fogia, E.Pastorini, R.Samperi and A.Lagana, 2006. A liquid chromatography/tandem mass spectrometric confirmatory method for determining aflatoxin m1 in cow milk comparison between electrospray and atmospheric pressure photoionization sources. *Journal of Chromatography*,**1101**: 69-78

Cheesebrough, L.M.,2005. Medical Laboratory manual for tropical countries. Vol II. Butterworth & Co. Publishers, London, UK.

Council for Agricultural Science and Technology, 2003.Mycotoxins: Risks in plant, animal and human systems. *Task Force Report*, pp:139.

Dongo, L., R.Bangyopadhyay, M.Kumar, and P.S.Ojiambo, 2008. Occurrence of ochratoxin A in Nigerian ready for sale cocoa beans. *Agricultural Journal*, **3(1):** 4-9.

Gareis, M and R.Scheuer, 2000. Ochratoxin A in meat and meat products. *Archive for Lebensmittel Hygiene*,**51**:102–104.

IARC (International Agency for Research on Cancer),1993. Some naturally occurring substances: food items and constituents heterocyclic aromatic amines and mycotoxins France: Lyon, **56**: 245-395.

ICMSF, 2002. Microorganisms in foods. Vol 7. USA Kluwer academic/plenum. Pp 123-142.

Ikediobi, C.O., G.O.C.Onfia and C.E.Eluwah,1980. A rapid and inexpensive enzymatic assay for total cyanide in cassava (*Manihot esculenta* crantz) cassava products. *Agri. Bio. Chem.*, **44**: 2803-2809.

Jayeola,C.O. and A.Oluwadun, 2000. Mycoflora and nutritional components of cocoa powder samples in South West Nigeria. *Africa Journal of Agri.Research*, **5:**2694-2698

Jonsyn-Ellins, F.E.2000. Seasonal variation in exposure frequency and concentration levels of aflatoxins and ochratoxins in urine samples of boys and girls. *Mycopathologia*, **15**: 216-219

Kapetanakou A.E., E.Z. Panagou, M. Gialitaki, E.H. Drosinos and P.N. Skandamis, 2009. Evaluating the combined effect of water activity, pH and temperature on ochratoxin A production by *Aspergillus ochraceus* and *Aspergillus carbonarius* on culture medium and Corinth raisins. *Food Control*, **20:** 725–732.

MAFF (Ministry of Agriculture, Fisheries and Foods),Survey of aflatoxins and OTA in cereals and retail products. Food surveillance information sheet no 130; nov.1997. available:http://www.maff.gov.uk/food/infsheet/1999/no185/185ochra.htm.

Magnolia, C., C.Hallak, A.Astoreeca, L.Ponsone, S.Chiazchiera and Dalcero,A.M.2006., Occurrence of ochratoxin A producing fungi in commercial kernels in Argentina. *Mycopathologia*, **161**:53-58

Marquardt, R.R and A.A. Frohlich, 1992. A review of recent advances in understanding ochratoxicosis. *Journal of Animal Sciences*,**70**: 3968-3988.

Misner,H. and P.Meisner,1981. Ochratoxin A- an inhibitor of renal phosphoenol pyruvate carboxylase. *Archives Biochemical Biophys*,**208**:146-151.

Nweke, F.I., D.S.C.Spencer and J. K. Iynan, 2006. The Cassava Press East Lansing USA, 7-206.

O'Callaghan J., P.C. Stapleton and A.D.W. Dobson, 2006. Ochratoxin A biosynthetic genes in *Aspergillus ochraceus* are differentially regulated by pH and nutritional stimuli. *Fungal Genetics and Biology*, **43**, 213–221.

Ogiehor, I.S. and M.J.Ikenebomeh, 2005. The effect of different packaging materials on the shelf stability of garri. *African Journal of Biotechnology*, **523**:2412-2416.

Ogiehor, I.S., and M.J. Ikenebomeh, 2006. The effect of different packaging materials on the shelf stability of garri. *African Journal of Biotechnology*, **523**:2412-2416.

Ogunledun, A. 2007.Incidence of microbial contaminants and nutrient composition of selected cocoa based beverages in Ibadan, Nigeria. PhD thesis, University of Ibadan.

Oluwole, O.B., O.O.Olatunji and S.A. Odunfa, 2004. A process technology for conservation of dried Omar, A.O., C.Mara, L.Nogueira and E.G. David, 2003. Survival of *E.coli* 0157:H7,

Listeria monocytogenes and *Salmonella* in Juice Concentrates. *Journal of Food Protection*, **66**(9): 1595-1598.

Otteneder,H. and P. Majerus, 2000. Occurrence of ochratoxin A (OTA) in wines: influence of the type of wine and its geographical origin. *Food Additives and Contaminations*, **17**: 793–798.

Palli, D., M.Miraglia, C.Saeieva, G.Masala, N.Colatostic, A.M.Corisi, A.Russon and C.Brera, 1999. Serum levels of ochratoxin A in healthy adults in tucany: correlation with individuals characteristics and between measurements. *Cancer Epidemiology Biomarkers*, **8** : 265- 269

Pitt, J. I., 2000. Toxigenic fungi: which are important? *Medical Mycology*,**38**:17-22.

Pittet, A., D.Tounare, A.Huggett and R.Viani, 1996. Liquid chromatographic determination of ochratoxin A in pure and adulterated soluble coffee using an immunoaffinity column cleanup procedure. *Journal of Agricultural and Food Chemistry*, **44**: 3564–3569.

Rahimtula,A.D., J.C.Bereziat, V.Bussacchini-Griat and H.Bartsch,1988. Lipid peroxidation as a possible cause of ochratoxin A toxicity. *Biochem. Pharmacy*.**37**: 4469-4477.

Samson, R. A.,2001. Current fungal taxonomy and mycotoxins. Mycotoxins and phycotoxins in perspective at the turn of the millennium. In: Proceedings of the Xth International IUPAC Symposium on Mycotoxins and Phycotoxins, 21-25 May, 2000 Guarujá (Brazil). Ponsen & Looyen, Wageningen, The Netherlands.

Samson, R.A., E.S. Hoekstra, J.C.Frisval and O. Filtenborg, 1995. Introduction to Food-borne Fungi. Centraal Bureau Voor Schimmel Cultures, Baarn, Delft, Netherlands.

Sanchis,V.,2000.Environmental conditions affecting mycotoxins. In: Magan, N. and Olsen,M.(eds),Mycotoxin in food detection and control. Wood Head Publishing Limited, England : CRC Press, pp 175-180.

Sayed, M.A., E.A.Mohamoud and A.A .Abouelalla, 2000. Mycoflora and natural occurrence of mycotoxin in meat of imported bulls, poultry and some meat product. *Assaults Vet Med. J.*, **43**(86):188-200

Schwan, R.F. and A.E. Wheals, 2004. The microbiology of cocoa fermentation and its role in chocolate quality. *Critical Reviews in Food Science Nutrition*, **44**:205–221.

Spadaro, D., S.Patharajan, M., A.Karthikeyan, A. Lorè Garibaldi and M.L.Gullino,2009. Molecular strategies for the identification of *Aspergillus* species in vineyard. *Journal of Plant Pathology*,**91**:S4- 41.

Tafuri, A., R. Ferracane and A. Ritieni, 2004. Ochratoxin A in Italian cocoa products. *Food Chemistry* **88,** 487–494.

Takahashi-Ando,M., S.Ohsata, T.Shibata, H.Hamamaoto, I.Yamagunich and M.Kimura, 2004. Metabolism of zearaleone genetically modified organism expressing the detoxification gene from *Clonastachys tosea*, *Appl.Environ.Microbiol*, **70** (6):3239-3245.

Tamura,K., J.Dudley, M.Nei and S.Kumar, *2007*. *MEGA4*: Molecular evolutionary genetics analysis (MEGA) software version 4.0. Molecular Biology and Evolution 24:1596-1599. (Publication PDF at http://www.kumarlab.net/publications)

Thirumala-Devi, K., M.A.Mayo, K.S.V Reddy and D.V. Reddy, 2000. Production of polyclonal antibodies against OTA and its detection in Chiles by ELISA. *J. Agric Food Chem*., **48**: 5079-5082

Thomas, B.T., H.I. Effedua, G.Agu, O.S.Musa, M.T.Adeyemi, O.D.Odunsi, K.O.Adesoga,O. Ogundero A.Oluwadun,2012. Fungi associated with the deterioration of garri in Ogun State, Nigeria. *Reseacher*, **4**(2):8-12.

Trucksess, M., C.Weaver, C.Oles, K.K.Dovido and J. Rader,2006. Determination of aflatoxins and ochratoxins A in gingseg and other botanical roots by immune affinity column clean up and liquid chromatography with fluorescence detection. *J.AOAC.*, **89**(3): 624-630

WHO (World Health Organization), 2001. Report of the 56th meeting of the joint FAO/WHO expert committee on food additives. February 6–15, 2001. Geneva, Switzerland.

Wolff, J., H.Bresch, C.Cholmakov-Bodechtel, G.Engel, M.Garais, P.Majerus, H.Rosner and R.Scheuer, 2000. Contamination of foods and consumer exposure. *Archive for Lebensmittel Hygiene*,**51**: 81-128.

Zimmerli ,B. and R. Dick, 1996. Ochratoxin A in table wine and grape-juice: occurrence and risk wine and its geographical origin. *Food Additives and Contaminations*,**17**:793–798.