Nutritional value, Fungi bio-deterioration and Aflatoxin Contaminations of aadun (Maize Snacks) a Novel Nigerian Indigenous Snacks

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Abstract: This study on Aadun (a popular staple food in Nigeria, prepared as snack from maize) was carried out on samples collected from different areas in South Western Nigeria and compared with a laboratory prepared ones. Proximate and aflatoxin contents of the food were detected and fungi responsible for its bio-deterioration were isolated and studied. The prominent fungi isolated are *Rhizopus stolonifer*, *Aspergillus flavus*, *Aspergillus niger* and *Trichoderma koningi*. Proximate composition of the samples were significantly different ($P<0.05$) with moisture (9.80% to 11.03%), ash (5.53% to 6.90%) and carbohydrate (47.40% to 52.57%) contents but low in fibre (3.43% to 3.93%) and fat (16.63% to 21.13%) contents. Most of the vented samples are confirmed to contain certain amount of aflatoxins concentrations but vary generally based on the sample locations, Aflatoxin G_1 and G_2 were found in all the samples except the control (laboratory prepared) and high level of Aflatoxin G_1 of 12.8 (µ/kg) and 12.3 (µ/kg) were detected on Oja Oba and Ibode samples while the control gavethe least (0.13 µ/kg). Aflatoxins B_2, G_1 and G_2 are below the tolerable limits for human consumption (FAO standard). Aflatoxin B_1 was generally higher on the samples and this can be attributed to the fact that when aflatoxin is produced by either *A*spergillus *flavus* or *Aspergillus parasiticus*, aflatoxin B_1 is the first metabolite released before others (aflatoxins B_2, G_1 and G_2) depending on the production rate which takes time, therefore the level of carcinogenicity can be put as $B_1>B_2>G_1>G_2$.

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1. Introduction:

Aadun is an indigenous staple food among the Yoruba people of Nigeria, it is red in color and usually molded into balls. It is a snack prepared from maize (*Zea mays*) which constitutes about 80% of the total composition. It is used traditionally for marriage, naming and twin's festival, and so on. It is also used by past warriors, children, women and people of all ages (Adedokun, 2006). Aadun is a street vended food and described by FAO (1998), it is a ready to eat food prepared eaten or sold by vendors and hawkers especially in streets around markets and public places.

However, the source of production, hygiene, methods of production and the integrity of the raw materials used for most street vended food are usually not known and people who consume them may stand a risk of food borne illnesses because when a food with unsafe level of pathogens is consumed, it poses a risk to those who eat it and put a terrible economic burden on persons, society or country (WHO. 2000). Most foods that are being sold may become compromised hygienically if safety regulations are not taken seriously, hence exposing them to microbial contamination which could come from fungi, bacteria, viruses, nematodes and so on (Fakoor *et al*., 2012).

Fungi are cosmopolitan in nature as they can be found everywhere including foods and this is easy because they are either parasitic or saprophytic in nature. During their association with these materials they carry out many activities which are propelled by the presence of extracellular enzymes and their mycelia.

Biodeterioration can be used to describe the activities of fungi on particular substrates which results in the depletion of a particular amount or volume of substance, thereby reducing its nutrient value and this is usually aided by the presence of high moisture in the substrate (Jonathan et al., 2012a and b). Some metabolites are produced during the activities of fungi with the substrates they grow on, an example of such is mycotoxin which literally means 'fungal poison' and of all the existing mycotoxins, aflatoxins are the most deadly and the number one carcinogen known due to the detrimental effects they exert on their consumers.

Mycotoxins are secondary metabolites of fungal origin which produce toxic responses when ingested by animals or humans (Jonathan *et al*., 2011a). The word 'mycotoxin' is a combination of a Greek word, 'mykes' meaning 'fungus' and a Latin word 'toxicum' meaning 'by poison' (Olayiwola *et al*., 2013) and Mycotoxicosis is a term used to denote the diseases that result from the ingestion of mycotoxin by animals and humans (Kumar *et al*., 2008). The severity

of mycotoxicosis may vary in different victims based on the quantity, type, level of toxicity, dosage, age as well as the nutritional and immunity status of the victim (Peraica *et al*., 1999).

There are five different types of aflatoxins that exist in nature; aflatoxin B_1, aflatoxin B_2, aflatoxin G_1, aflatoxin G_2 and aflatoxin M_1 respectively. They are toxins produced mainly by *Aspergillus flavus* and *Aspergillus parasiticus* and the categories of foods they contaminate are cereals and cereals' products; herbs and spices; nuts and oil seeds; meat and poultry products; animal feeds and milk and milk products. This contamination is highly pronounced in maize (Shepherd, 2008), hence serves as the main gateway of human exposure to aflatoxin consumption because it is highly eaten by the world's population and sadly is also the foremost susceptible crop to aflatoxin contamination (Wu and Klangwiset, 2010).

Aadun falls into the category of foods targeted by aflatoxins therefore the objective of this study was to isolate the fungi associated with bio-deterioration of Aadun samples; check whether the food value has been reduced or affected due to the activities of fungal organisms; ascertain the presence of aflatoxins (fungi metabolites) and the types of aflatoxin in the samples of Aadun and to quantify the amount of aflatoxins present in samples of Aadun collected from five different locations in Ibadan, Oyo state of Nigeria.

2. MATERIALS AND METHODS

2.1 Sources of Materials: For Aadun, white variety of maize (*zea mays*), pepper, palm oil and salt were purchased from Bodija market, Ibadan, Oyo state, Nigeria

2.2. Collection of Samples: Five samples of Aadun were purchased from different locations in Ibadan and each of the samples was packaged in sterile polythene bags before they were taken to Botany department, University of Ibadan. A laboratory prepared sample was used as control. The location of collected aadun sample are:

i. Oja Oba
ii. Ojoo
iii. Oje
iv. Ibode
v. Bodija

2.3 Preparation of Laboratory Sample: The laboratory prepared sample was produced using the recipe and method of Idowu and Adedokun, (2011). Maize grains were first cleaned, roasted with the aid of an electric hot plate for 15 minutes and was later milled. The maize powder and milled powder pepper were mixed together thoroughly and the mixture was later sieved (<0.4mm) to obtain a fine powder. The sieved powdered mixture was later mixed with palm oil and salt to make a paste. The paste was molded into balls before they were put inside sterile polythene bags and was put inside the refrigerator.

2.4 Isolation of Fungal Biota from the Mixture: Fungi isolation from the samples were carried out using ten-fold serial dilution plating technique as explained by Chambert *et al*. (1999). 10g of each of the samples was soaked in 500ml beaker containing 500 ml distilled water and then covered with aluminum foil paper for 24hrs. 1.0 ml of the suspension was poured into 9.0 ml of sterile distilled water in a test-tube and serially diluted from 10^{-2} to 10^{-10} and two drops of 10^{-10} dilution was aseptically inoculated in potato dextrose agar and incubated at 28± 2 °C for 7 days for culture development, after which sub-culturing was done in a fresh potato dextrose agar plates in order to obtain a pure culture of the fungal isolates.

2.4 Characterization and Identification: Pure cultures of fungi isolated from samples of Aadun were characterized based on their macroscopic appearance on the culture medium (PDA), microscopic morphology and type of asexual spores produced through use of photomicrograph and identified by reference to the compendium of soil fungi (Domsc and Gams, 1980).

2.5 Proximate Analysis: Samples of Aadun were taken for proximate analysis and the determination of various parameters were carried out at KAPPA laboratories, Ibadan. The moisture, crude protein, crude fat, crude fibre and total ash were determined using the standard methods of AOAC. (2008) while the carbohydrate was determined by difference.

2.6 Aflatoxin Analysis: The aflatoxin analysis was carried out in pathology laboratory of International Institute of Tropical Agriculture (IITA), Ibadan using the High Perfomance Liquid Cromatographic (HPLC) methods. The detected aflatoxins are:

1. Aflatoxin B1 (AFB1)
2. Aflatoxin B2 (AFB2)
3. Aflatoxin G1 (AFG1) and
4. Aflatoxin G2 (AFG2)

3. RESULTS AND DISCUSSION

3.1 Fungal Isolates: In this study, *Aspergillus niger*, *Rhizopus stolonifer*, *Aspergillus flavus* and *Trichoderma sp* were found to be the multiple pathogens responsible for biodeterioration of Aadun samples collected from five different locations in Ibadan (Plates 1 to 4) and this resemble the reports of Jonathan *et al.* (2011a and b, 2012a and b, 2013); Jonathan and Esho, (2010) and Fapounda *et al*. (2012) who reported production of similar mycotoxins from

many of the fungi. This can be related to the high moisture content in the samples hence predisposing them to microbial infection due to the high water activity. The Aadun samples started showing symptoms of discoloration after six weeks on the outside but deterioration occurred from the inside after a week.

The activities of these fungal isolates were found to have depreciated the food value of the snack especially the moisture, protein and the carbohydrate content while the control maintained higher values of the parameter. Of all the Aadun samples, Oja oba sample was found to be heavily invaded with fungal organisms; *Aspergillus niger*, *Aspergillus flavus*, *Rhizopus stolonifer* and *Trichoderma sp* and was followed by Ojoo sample.

3.2 Result of Proximate Analysis: The values of moisture content, carbohydrate and ash content in samples of Aadun collected from five locations in Ibadan were higher than the values reported by Idowu *et al*. (2012) and Fapounda *et al.* (2012), the values of fibre and fat contents are lower but the values of protein content falls within the same range especially the values of Oja Oba, Ojoo, Oje, Ibade, and Bodija respectively. Aadun samples were significantly different ($P<0.05$) in their proximate composition (Table 1). It was generally high in moisture (9.80% to 11.03%), ash (5.53% to 6.90%) and carbohydrate (47.40% to 52.57%) contents but low in fibre (3.43% to 3.93%) and fat (16.63% to 21.13%) contents.

Table 1: The proximate analysis of Aadun

Location	Moisture content (%)	Protein content (%)	Fat content (%)	Ash content (%)	Fiber content (%)	Carbohydrate content (%)
Control	10.67^{b}	10.40^{b}	16.63^{f}	6.90^{a}	3.60^{bc}	51.80^{b}
Ibode	10.37^{c}	8.43^{e}	18.73^{e}	6.10^{b}	3.80^{ab}	52.57^{a}
Oja oba	10.53^{bc}	11.00^{a}	21.13^{a}	6.43^{b}	3.50^{c}	47.40^{e}
Ojo	10.77^{b}	9.20^{d}	20.23^{c}	5.53^{c}	3.93^{a}	50.33^{d}
Bodija	11.03^{a}	9.13^{d}	19.73^{d}	5.57^{c}	3.77^{ab}	50.97^{c}
Oje	9.80^{d}	10.03^{c}	20.77^{b}	5.63^{c}	3.43^{c}	50.33^{d}

Each value is a mean of three replicate. Values in the same column with different letters as superscripts are significantly different by Duncan multiple range test ($P\leq0.05$)

Table 2: Mean Square effects of aflatoxin concentration on Aadun samples in five locations in Ibadan

	AFB1 (µ/kg)	AFB2 (µ/kg)	AFG1 (µ/kg)	AFG2 (µ/kg)
Control	0.1	0.6	0	0
Oja Oba	12.8	3.2	3	3.2
Ojoo	3.6	2.8	2.6	3.1
Oje	3.9	2.9	2.3	1.6
Ibode	12.3	3.1	2.3	2.6
Bodija	3.4	2.8	1.7	1.8

	AFB1		AFB2		AFG1		AFG2	
	MS	SE	MS	SE	MS	SE	MS	SE
Aadun	45.56**	2.96	2.99**	0.45	1.68**	1.95	0.35**	0.30

** means $P<0.05$-significant*
*** means $P<0.01$-highly significant*

3.3 Results on Aflatoxin Analysis

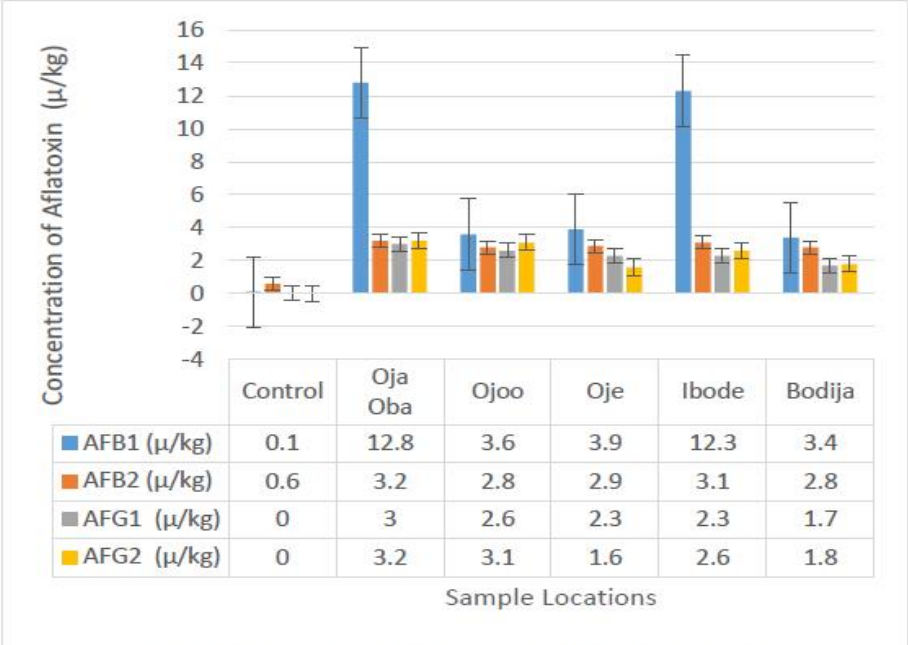
Detailed quantification of aflatoxin contents of the food samples is presented in Fig. 1. All the samples of Aadun collected from five locations in Ibadan were confirmed to contain certain amount of aflatoxins though the concentrations vary but generally.

Aflatoxins content of the laboratory prepared sample was seen to be very low AFB1 (0.13 µ/kg), (AFB2 0.6 µ/kg), AFG1 (0.0µ/kg) and AFG2 (0.0µ/kg) compared to the street vended samples (Fig. 1). Also, Aflatoxin G_1 are generally higher in all the samples compared to other aflatoxins and Oja Oba samples has higher Aflatoxin contents compare to other locations.

Aflatoxin B1 (Fig. 1.) was found in all the samples except the control, but at various concentrations, but aflatoxins B_2, G_1 and G_2 are below the tolerable limits for human consumption (FAO standard) but aflatoxin B1 concentration of 12.8 and 12.3µ/kg were detected in both Oja Oba and Ibode samples respectively.

Surprisingly control sample was confirmed to be free from aflatoxins (G_1 and G_2) and this implies that the fact that fungus/fungi is/are present in a material

does not necessarily mean toxins will be produced, therefore bio-deterioration and toxin production are two different activities of fungi though the former may lead to the latter.



	Control	Oja Oba	Ojoo	Oje	Ibode	Bodija
AFB1 (µ/kg)	0.1	12.8	3.6	3.9	12.3	3.4
AFB2 (µ/kg)	0.6	3.2	2.8	2.9	3.1	2.8
AFG1 (µ/kg)	0	3	2.6	2.3	2.3	1.7
AFG2 (µ/kg)	0	3.2	3.1	1.6	2.6	1.8

Fig. 1. Aflatoxin quantification of Aadun samples

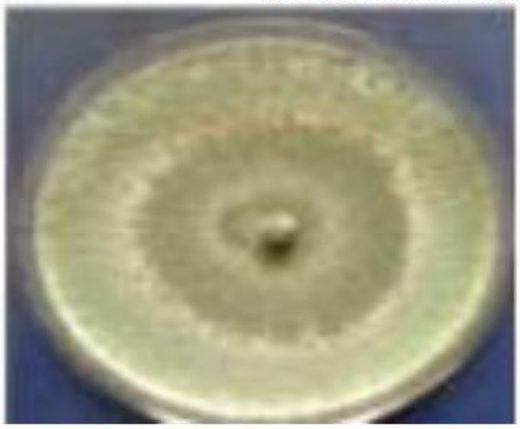


Plate 1a: *Trichoderma koningii* on plate

Plate 2a: showing *Aspergillus niger*

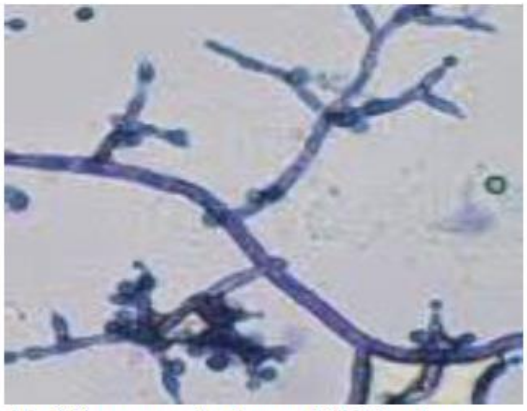


Plate 1b. Micromorphology of *Trichoderma koningii*

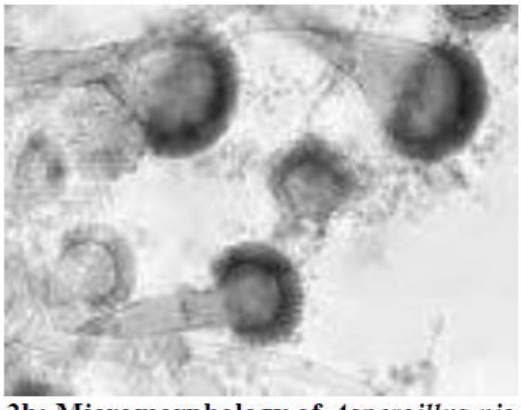


Plate 2b: Micromorphology of *Aspergillus niger*

These report with some other reports have shown that maize happens to be one of the major crops that are affected by aflatoxin producing organisms most especially *Aspergillus flavus* and Aadun is its product but samples of Aadun analysed showed that they contained low levels of some aflatoxins; this could be due the following reasons:

a. Access to aflasafe corns due to research breakthroughs. For example, IITA (Institute of Tropical Agriculture) a research institute situated in Ibadan for some years has involved in a research centered on production of aflasafe corns and as a result of this, farmers in Ibadan now have access to these corns.
b. Exposure of the corn grains to heat during production of Aadun.
c. Proper process that reduces the risk of contamination prior to storage.

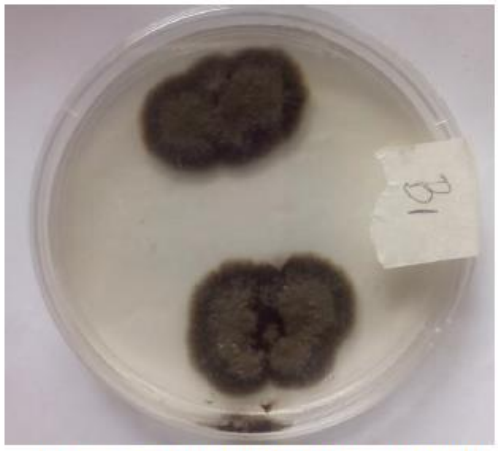


Plate 3a: Showing *Rhizopus stolonifer*

Plate 3b: Micromorphology of *Rhizopus stolonifer*

Plate 4a: Showing *Aspergillus flavus*

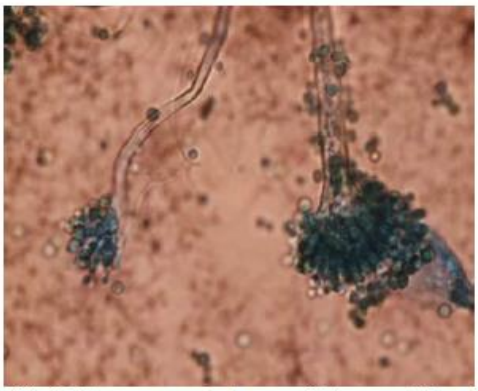


Plate 4b: Micromorphology of *Aspergillus flavus*

4. CONCLUSION

Campaign against aflatoxins in foods should be taken with all seriousness because some of them have been described as hepatotoxic, mutagenic, cancinogenic substance at higher doses, even some may causes liver damage.

The detected aflatoxin contents differs across the location based on different levels of hygienic measures implemented. The laboratory prepared samples were freer of mycotoxins because it was prepared under an aseptic condition. Therefore, there should be a good safe handling of vended food by farmers storing the corn or those in charge of storing the food product (aadun) control the level of contamination by fungi.

COMPETING INTERESTS

The authors declare that there is no conflict of interest regarding the publication of this manuscript.

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