Internet Journal of Food Safety, Vol.14, 2012, p.83-88

Aflatoxigenic moulds and aflatoxins in street-vended snacks in Lagos, Nigeria

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Abstract

Twenty-five snack samples made separately from corn, groundnut, nuts (coconut, tiger nut and walnut) and wheat were evaluated for the incidence of aflatoxigenic moulds and aflatoxins. The snacks were collected from street vendors in five locations within Lagos, Nigeria. Only 9 out of the 25 snacks belonging to the corn- and groundnut-based categories yielded isolates of Aspergillus flavus and A. tamarii. A. flavus was significantly ($p<0.05$) predominant (>90%) than A. tamarii in both snack groups. About 36% of the Aspergillus isolates produced aflatoxin in neutral red desiccated coconut agar medium. Approximately 81% of the toxigenic A. flavus from groundnut-based snacks produced more aflatoxin than those from corn-based snacks. Aflatoxins were detected in 68% (17/25) of the snacks at total aflatoxin (TA) concentrations of 0–50µg/kg. Only aflatoxin B1 (AFB1), AFB2 and AFG1 were detected in the snacks. AFB1 was the most prevalent aflatoxin in all snacks. With exception of nut-based snacks, all other snacks were contaminated with TA concentrations at levels exceeding the maximum allowable limits (MAL) for TA in foods (15µg/kg) as recommended by the National Agency for Food and Drug Administration and Control (NAFDAC).

Key words: ***Aspergillus*, Corn, Groundnut, Nuts, Snack, Wheat**

Introduction

Snacks are quick foods usually derived from one or more basic food items, and are eaten between meals. They can be processed from food group origin such as cereals, pulses, starchy fruits, roots and tubers, beef, dairy and poultry (Aworh and Muller 1987; Okoruwa 1997; Alabi 2007). Processed snacks are designed to be less perishable, more durable and more portable than prepared foods. They often contain substantial amounts of sweeteners, preservatives and appealing ingredients such as chocolates, peanuts and specially designed flavors (such as flavored potato chips) (Street Foods 2006). Snacks are consumed by people in both the developed and developing world, and across all age groups. In Nigeria and other Sub-Saharan African countries, street snack foods constitute a major meal for a handful of indigenes due to its availability, affordability and accessibility (Draper 1996). Commonly consumed snacks in Nigeria include baked products (biscuits, cake, hamburger, meat pie and sausage rolls), fried and roasted snacks (cashew nut, doughnuts, peanuts and popcorn), and nuts (walnut, tiger nut).

Snack food prepared outside the home setting, either on the street, shops, kiosks, factories or fast food joint, and vended on the street, supermarket, shops or motor parks and even in schools, are patronized in leaps and bounds by urban dwellers to satisfy their hunger pangs or as refreshments without giving cognizance to the preparation and packaging methods or nutritional contents in most cases. Therefore, there is the need to ascertain its safety in terms of the presence, diversity and quantity of aflatoxigenic moulds and aflatoxins.

Aflatoxins are mycotoxins produced by *Aspergillus* species that grow in many cereals and oilseeds, and are known to be hepatotoxic, carcinogenic and teratogenic. Hepatocellular carcinoma resulting from chronic aflatoxin exposure is well documented (IARC 2006). High levels of aflatoxin B1, B2,

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G1, G2 and citrinin were discovered in dough samples of kenkey, a Ghanaian maize dietary staple (Kpodo 1996). A positive correlation has also been established between the consumption of aflatoxin contaminated foods and increased incidence of liver cancer in several Southeast Asian and African populations (Foli and Christian 1976; Kpodo et al. 1996; Caldas et al. 2011). Since snacks form a major part of the diet for residents of West African states, and there is paucity of data regarding the incidence of aflatoxigenic moulds and aflatoxins in a wide variety of processed snacks from Nigeria, this research was designed. The study aimed at the investigation of some snacks made from different ingredients for the contamination level of aflatoxigenic *Aspergillus* and total aflatoxin, so as to obtain relevant data for food and public health safety.

Materials and Methods

Samples. A total of 25 snack samples were purchased from vendors who hawked the snacks in five markets and street corners within Lagos metropolis (Surulere, Ilupeju, Oshodi, Mushin and Shomolu). The samples comprised of coconut chips, corn cake, groundnut cake, gurundi, kokoro, peanut candy, sausage roll, tiger nut, roasted and boiled groundnut, and walnut. All samples were grouped into four based on the main ingredient: corn-based (corn cake and kokoro), groundnut-based (groundnut cake, peanut candy, roasted and boiled groundnut), nut-based (tiger nut, gurundi, coconut chips and walnut) and wheat-based (sausage).

Each sample was collected as 400–500g bulk sample obtained by combining three to four parts of the same snack from two to three vendors' trays. Each snack sample size was collected into a sterile *Zip-lock* bag, labeled accurately and transported to the Microbiology laboratory, Biosciences and Biotechnology department, Babcock University, Nigeria. The samples were comminuted immediately and stored at 4^{o}C prior to further analysis which was carried out within 72 hours.

Isolation and characterization of *Aspergillus* section *Flavi*. The isolation of *Aspergillus* from the snacks was performed on modified Rose Bengal Agar (mRBA) according to the dilution plating technique described by Samson et al. (1995). One gram of each powdered sample was diluted in 10ml of sterile water containing 0.1% peptone, and vortexed for 1 min. Appropriate dilutions of the mixture were inoculated on a set of triplicate mRBA plate and the plates were incubated without illumination at 31°C for 3 days.

The isolates obtained from the snacks were characterized by combining distinctive morphological features with aflatoxin profile as described by Diedhiou et al. (2011). Ten random colonies that resembled *Aspergillus* section *Flavi* were transferred from mRBA plates for each sample to the central point of 5/2 agar plates (5% V8 juice and 2% agar, pH 5.2). The 5/2 plates were incubated in the dark at 31°C for 5days after which the tentative identity of each isolate was determined. To determine the aflatoxin profile of the fungi, each isolate was placed at the central point of Petri dishes containing neutral red desiccated coconut agar medium (NRDCA) (Atanda et al. 2011). The plates were incubated in the dark at 31°C for 3 to 5 days, and then visualized under 365nm UV for characteristic fluorescence of aflatoxin.

S**creening of *Aspergillus* isolates for aflatoxin-producing potential.** The ability of 90 isolates belonging to the section *Flavi* to produce aflatoxin was tested using NRDCA as described above. The *A. flavus* isolates that produced aflatoxins as characterized by the fluorescence in the medium, were regarded as toxigenic *A. flavus* strains. The proportion of the toxigenic strains to atoxigenic strains was calculated. The capacity of the toxigenic *A. flavus* strains to liberate aflatoxin was estimated by visual scoring of the intensities under UV at 365nm immediately after the fifth day of incubating the plates.

Aflatoxin estimation in the snacks. The total aflatoxin (TA) contents in the 25 snack samples were determined by Thin-layer chromatography with fluorescent detection. Aflatoxin extraction was carried out as reported by Udom et al. (2012) but with slight modification. The modification was in the use of different concentrations of the extraction solvent. Due to the differences in ingredients of the snacks, we varied the concentration of the extraction solvent such that all other snack types except the groundnut-based were extracted with 70% methanol. We used 85% methanol for the groundnut-based snacks since this was the best concentration that gave optimal recovery from a pre-analyzed sample.

Twenty-five grams of each snack sample was weighed into a Warring blender (Marlex Emerald UNIT III, Daman) and extracted with 125 ml of the appropriate concentration of aqueous methanol (v/v) and 1g of NaCl for 3 min. The mixture was shaken for 30 min in an orbital shaker and partitioned with n-hexane and chloroform (20 ml each). The lower layer was passed through a bed of anhydrous sodium sulphate into a polypropylene cup to remove residual water. The extract was concentrated on a hot plate and re-constituted in 500 µl chloroform. To analyze the extracts for aflatoxin concentration, 40µl extract and 50µl aliquots of 0.50 µg/ml total aflatoxin (TA) standards were spotted and separated on pre-coated TLC plates (silica gel 60 F_{254}; 20 × 10cm; Merck, Germany) in chloroform-acetone-water (88:12:1.5). The plates were dried and visualized under UV light at 365nm. The aflatoxin bands for each sample spot were identified on the basis of characteristic fluorescence and co-migration with aflatoxin standard. Aflatoxin concentrations (µg/kg) in the samples were estimated as described by Atanda et al. (2011).

Data analysis. The data obtained were analyzed using SPSS® version 14.0 (Windows SPSS, IL, USA). Means were calculated by One-way ANOVA, separated and tested

for significance (α = 0.05) using the Duncan's multiple Range test.

Results

Aspergillus* section *Flavi in the snacks. A total of 90 isolates of *Aspergillus* section *Flavi* were obtained only from the corn- and groundnut-based snacks. The other snack types (nut- and wheat-based) did not yield any isolate of *Aspergillus* section *Flavi* after repeated isolations. Only 36% (9 out of 25) of the snacks yielded the isolates obtained in this study. The isolates obtained from the snacks belonged to *A. flavus* and *A. tamarii*. The incidence of *A. flavus* was significant (p<0.05) and higher (>90%) than *A. tamarii* (<10%) in the snacks (Table 1).

Table 1. Incidence (%) of *Aspergillus* section *Flavi* in two snack types vended within five locations Lagos, Nigeria

	Snack type		
Aspergillus species	Corn	Groundnut	Mean
A. flavus L strain	93.7[a]	97.3[a]	95.6
A. tamarii	6.3[b]	2.7[b]	4.4

*Means with different superscript alphabets in a column are significantly different (p<0.05) by the Duncan's multiple Range test.

Aflatoxigenicity of *Aspergillus* section *Flavi* in the snacks. Only 35.6% of the 90 *Aspergillus* section *Flavi* isolates produced aflatoxin in NRDCA. All the *A. tamarii* isolates did not produce aflatoxin in the medium. The proportion of aflatoxigenic isolates were significantly (p<0.05) lower as compared to that of the atoxigenics in both snack types (Fig. 1). We observed that the toxigenic *A. flavus* isolates produced aflatoxin in varying quantities as visualized from the intensities of fluorescence in NRDCA medium (Table 2). About 81% of the toxigenic *A. flavus* from groundnut-based snacks produced more aflatoxin in NRDCA than those from corn-based snacks.

Table 2. Aflatoxigenic capacity of *A. flavus* isolated from two snack types on neutral red desiccated coconut agar (NRDCA)

Snack type	Incidence (%) of toxigenic strains based on intensity[a] of fluorescence of aflatoxin produced in NRDCA			
	±	+	++	+++
Corn-based (N= 11)	27.2	36.4	36.4	0.0
Groundnut-based (N^b= 21)	4.8	4.8	9.5	80.9

[a]Intensity of aflatoxin fluorescence in NRDCA is expressed as ± (very weak intensity), + (weak intensity), ++ (high intensity) and +++ (very high intensity).

[b]Number of toxigenic *A. flavus* isolated from each snack type.

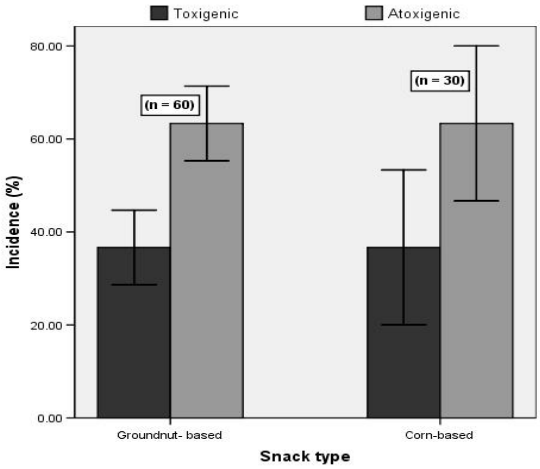

Figure 1. Incidence of toxigenic and atoxigenic *Aspergillus* section *Flavi* in two snack types vended within five locations in Lagos, Nigeria.

Aflatoxin contamination in the snack samples. A summary of the data on aflatoxin concentrations in the snacks (Table 3) show that 68% of the snacks were contaminated with aflatoxin. Specifically, aflatoxins contaminated 75% of groundnut-based, 80% of wheat-based and 100% of corn-based snacks. Only aflatoxin B1 (AFB1), AFB2 and AFG1 were detected in the snacks, AFB1 being the most prevalent in all snacks types. Of the five nut-based snack samples, only one (20%) sample (coconut chips) was positive for aflatoxin (AFB1). The mean concentration of AFB1 was highest in the wheat-based snacks (17.8µg/kg). The TA concentrations in the snacks ranged from 0–50µg/kg. With the exception of the nut-based snacks, all other snacks had TA concentrations at levels exceeding the NAFDAC's MAL for TA in foods (15µg/kg).

Table 3. Estimated total aflatoxin concentrations in snacks obtained from five locations in Lagos, Nigeria

Snack type	N^a	N^b (%)		Mean aflatoxins (µg/kg)			
				AFB1	AFB2	AFG1	Total
Corn-based	3	3 (100)	Mean	14.0	8.0	6.0	18.7
			Range	6.0–30.0	-	-	12.0–30.0
Groundnut-based	12	9 (75)	Mean	8.5	9.0	15.8	20.1
			Range	0.0–12.5	0.0–9.0	0.0–31.3	0.0–43.8
Nut-based	5	1 (20)	Mean	6.0	[c]	[c]	6.0
			Range	-	-	-	0.0–6.0
Wheat-based	5	4 (80)	Mean	17.8	[c]	13.0	21.0
			Range	0.0–50.0	-	-	0.0–50.0

[a]Number of snacks analyzed for total aflatoxin within each snack category.
[b]Number of snacks positive for total aflatoxin within each snack category.
[c]Aflatoxin concentration was not detected.

Discussion

Microbial contamination of street-vended foods has been well documented and several outbreaks of disease, including cholera outbreaks, have been traced to its consumption (Akinyele 1992; Rath and Patra 2012). However, little is known of the implications of food borne toxigenic moulds in daily consumed snacks. We are aware that food borne illness of microbial origin is a major international health problem associated to food safety in developing countries (WHO 2002). Contamination of street-vended food has been attributed to exposure to polluted environment, poor sanitation and poor hygienic practices by the vendors (Mensah et al. 2002; Oladimeji and Kolapo 2006; Ackah et al. 2011; Ezekiel et al. 2011).

Microbiological load of snacks in developing world is highly dependent on the raw materials, traditional methods of processing and packaging, holding temperature which can determine the rate of deterioration of the ready-to-eat food products and snack foods. In this study, the presence of *Aspergillus* species in only corn- and groundnut-based snacks is a reflection of the susceptible nature of the raw grains to infestation by this fungus unlike the raw materials used for other snack types especially the coconut-based snacks. This is in line with previous reports which placed corn and groundnut as the prime crops susceptible to infestation by aflatoxigenic fungi (Vaamonde et al. 2003; Bandyopadhyay et al. 2007). We observed that about one-third of the entire *Aspergillus* isolated from the snacks had potentials to biosynthesize aflatoxins in culture. This poses a threat to the consumers of the snacks and especially in the groundnut-based type where the aflatoxigenic isolates were prolific producers of aflatoxin.

From the results of aflatoxin estimation in the snacks, the prevalence of aflatoxin contamination in corn-based products was 100% and concentrations ranged from 6–30µg/kg for AFB1 and 12–30µg/kg for TA. This indicates that corn can be highly susceptible to aflatoxin contamination; a fact that corroborates the study carried out in Accra (Kpodo et al. 1996) which showed that Kenkey, fermented ready-to-eat maize dough, samples were highly contaminated with total aflatoxin and citrinin at concentration levels up to 289 µg/kg. Bandyopadhyay et al. (2007) also stated that maize contamination with aflatoxins is very high and usually higher than other crops. The groundnut-based snacks also contained aflatoxins, and TA concentrations (mean = 20.1µg/kg) in this snack type exceeded the MAL as recommended by NAFDAC. This supports the previous studies carried out on peanut cake from markets within five states in Nigeria, which revealed high load of aflatoxigenic fungi and very high concentrations AFB1 in all the peanut cake samples (Ezekiel et al. 2012: manuscript under review).

Of the nut-based snacks, only one coconut chips sample was contaminated with AFB1 and at a very low concentration of 6µg/kg. This placed the nut-based snacks as the least contaminated of all snack types evaluated in this study. There are no reports on aflatoxin contamination of coconut products but reports are available for aflatoxins in other nuts including chestnut and tiger nuts (Pietri et al. 2012; Rubert et al. 2012). The low incidence and concentration of aflatoxin in the nut-based samples supports the report of Rubert et al. (2012) where very low aflatoxin concentrations (<2 µg/kg) were detected in Spanish tiger nuts. It may therefore be deduced that coconut is resistant to *Aspergillus* infestation and consequent aflatoxin biosynthesis. Since the aflatoxin concentration (6µg/kg) was below NAFDAC's MAL consumption of this class of product may pose no risk to the consumers if there are no other microbial and toxin contaminants. The wheat-based snack evaluated in this study was produced at the cottage

level; the high aflatoxin levels (mean AFB1 = 17.8µg/kg; mean TA = 21µg/kg) could have been as a result of poor handling or storage. This calls for caution in the selection of snacks that are purchased for consumption.

Considering the data reported in this study, we present the several suggested interventions on how to improve the hygiene of street foods: (1) education and training programs for vendors, (2) the improvement of vendors' equipment for preparation and storage, (3) the provision of adequate sanitation and refuse disposal facilities, and (4) the provision of special food centers (WHO 1996). WHO (1996) also proposed the adoption of HACCP system in the street food control in order to improve the efficiency of the surveillance system by detecting the hazards and focusing on the critical control points. This approach can be applied at any step of the food chain to identify and characterize the critical points where risk occurs and to establish priorities for intervention and control. This information can thus be used to set priorities, formulate interventions, and identify the needs of vendors and customers for education and training in the bid to ensure food safety.

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