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Aflatoxins in Nigerian dry-roasted groundnuts

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Abstract

Samples of dry roasted groundnuts (DRG) purchased from street hawkers, markets and retail shops in southwestern Nigeria were analysed for moisture content, fungal populations and aflatoxin contamination. The moisture content varied from 2.1% to 3.6% while the mould counts, using the dilution plating method, ranged from 2.9×10^2 to 6.3×10^2 colony-forming units per gramme. Aflatoxin B_1 was found in 64.2% of samples with a mean of 25.5 ppb. Aflatoxins B_2, G_1 and G_2 were detected in 26.4%, 11.3% and 2.8% of the samples with mean levels of 10.7, 7.2 and 8 ppb, respectively, in contaminated samples. It is concluded that the regular consumption of DRG by Nigerians might present potential health hazards to consumers.
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1. Introduction

Groundnut (*Arachis hypogaea*) is an important source of food worldwide and constitutes an inexpensive source of protein, fat, minerals and vitamins in the diets of rural populations, especially children. Before large-scale exploration of oil began in Nigeria, the country was the world's leading exporter of groundnut (Hogendorn, 1978), and this was the reason why research into aflatoxin problem in Nigeria started immediately after its discovery in 1960 (McDonald, 1964; McDonald & Harkness, 1965). Groundnut is consumed in the boiled or roasted form, and also as groundnut cake ('kulikuli'). The dry roasted groundnut (DRG) snack is presently the most widely consumed form of groundnut in Nigeria. It can be consumed alone or combined with dry roasted maize (popcorn), 'gari', coconut, bread or plantain.

The traditional method of preparing DRG first involves sorting out the physically damaged and mouldy kernels of raw groundnuts, followed by soaking in water for about 20 min, salting with NaCl to taste, and then roasting by stirring the kernels in hot sand placed in an earthen ware pot on an open fire. On cooling, the roasted groundnuts are separated from the sand by a metal sieve.

Groundnut is frequently infected with fungi that produce mycotoxins during and after harvesting, which affect the quality and safety of human food (Martin, Ba, Dimanche, & Schilling, 1999). The prevalence of human exposure to aflatoxin has been shown to be over 98% in West Africa, including Nigeria (Wild, 1996). In the World Bank Report, Investing in Health (1993), mycotoxin-induced diseases led to a reduced life expectancy in the developing countries (Miller, 1996). Aflatoxin is a very powerful hepatocarcinogen, and naturally-occurring mixtures of aflatoxins have been classified as class1 human carcinogens (IARC, 1993). It has been linked with the high incidence of liver cancer in Africa (Oettle, 1964). Aflatoxin synergises with other agents, such as hepatitis B, in the causation of liver cancer (Henry et al., 1998). Though, the etiology and pathogenesis of kwashiorkor still remain obscure, much higher aflatoxins have been found in the blood, urine and livers of children in Nigeria with the disease than similar age-matched children (Hendrickse, 1983), and the presence

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of the toxin was established in the autopsy brain tissue of some Nigerian children (Oyelami, Maxwell, Adelusola, Aladekoma, & Oyelese, 1996). Aflatoxins have also been proven to be immunotoxic (Turner, Moore, Hall, Prentice, & Wild, 2003), and a recent epidemiological study reveals a striking association between exposure to aflatoxins and growth stunting (Gong et al., 2002). The above facts emphasize the need to closely monitor aflatoxin levels in food products that are widely consumed in the region, particularly those that are highly prone to mycotoxin contamination such as groundnuts.

Literature on the levels of aflatoxins in dry-roasted groundnuts, is sparse and the studies conducted on the raw groundnut and the cakes were carried out mainly in the 1960s. The present study was undertaken to determine the levels of aflatoxins in dry-roasted groundnuts offered for sale in southwestern Nigeria.

2. Materials and methods

A total of 106 samples (approximately 500 g each) of DRG were purchased from street hawkers, markets and retail shops in different locations of southwestern Nigeria. The traders were questioned on whether they sorted the ground nut kernels before using them to prepare DRG and their awareness of the hazards of consuming mouldy foods. Each sample was milled with a Romer mill (Union, MO). The moisture content was determined by oven-drying at 105 °C to constant weight.

The assessment of the level of mould contamination was done by suspending 10 g from each sample in 90 ml of sterile distilled water to obtain 10^{-1} stock. Further 10-fold serial dilutions were made up to 10^{-4}. Duplicate 1 ml volumes were dispensed in Petri dishes (90 mm diameter) containing malt extract agar complemented with 0.5% chloramphenicol (MEAC) and 1 ml of the same suspension was placed in each of another two sets of Petri dishes, and over-laid with MEAC. The plates were incubated at 28 ± 2 °C, and observed daily for 7 days. Counts of fungi obtained from plates having between 15 and 150 colony-forming units (cfu) were used in calculating the total number of cfu/g in each sample. The fungi that developed were purified by repeated sub-cultures. Pure cultures of fungi were examined macroscopically and microscopically and identified by following the methods of Barnett and Hunter (1987), Pitt (1979), Raper and Fennel (1973) and Von Arx (1981).

The DRG samples were analysed for aflatoxin by thin-layer chromatography (TLC), using a standard method (Anon, 1976; Bankole, Eseigbe, & Enikuomehin, 1996). Standard aflatoxins B_1, B_2, G_1 and G_2 were obtained from Sigma (St Louis, MO). 50 g of milled subsample of DRG were extracted with 250 ml of methanol:water, 70:30, and the mixture was shaken for 30 min. The methanol extract was clarified with ammonium sulphate, followed by partitioning with chloroform. The content was filtered through Whatman No. 1 filter paper and evaporated to dryness by rotary evaporation. The suspended extracts were spotted on silica gel (60 G) plates, along with standard aflatoxins, and developed in an isocratic acetonitrile/water/acetic acid (24:76:1, v/v/v) solvent system in a TLC tank. Quantification of the levels of aflatoxin was done by visual comparison of extract fluorescent spots with standard aflatoxin spots under long wave UV light (365 nm). Confirmation of the identity of aflatoxins was achieved by derivatization with trifluoroacetic (Stack & Pohland, 1975).

A series of five aflatoxin B1 spots was applied on each of 10 TLC plates and developed, as described above, to determine the reproducibility of the method used. The mean coefficient of variation of 50 aflatoxin B1 spot measurements was 10.6%, and this represent the errors of application of spots, development of TLC and visual estimation.

3. Results and discussion

The moisture content of the DRG varied from 2.1% to 3.6%. During the survey, it was found that the roasted groundnut was offered for sale by measuring it out in different quantities in small nylons. It was also gathered that the petty traders that process the groundnuts into DRG do not bother themselves to sort out the physically damaged and mouldy kernels from the good ones before roasting, to maximize profit. The consumers, too, do not bother themselves about the quality of the product due to ignorance and also because of the fact that the need to eat outweighs other considerations, such as food safety, among the people in the country, the majority of whom are resource-poor and illiterate. About 92.5% of the DRG sellers claimed that they were not aware of the hazards of consuming mouldy foods.

Table 1 presents the percentage occurrence and mean total count of fungi recovered by the dilution plating method from the DRG samples. Fungi were isolated from all the DRG samples used for the study. The total mould count ranged from 2.9×10^2 to 6.3×10^2 cfu/g in samples. The heat applied to groundnut during roasting is sufficiently high to destroy most of the microbes present, thus giving the low microbial load in the snack samples. The fungi identified in the DRG showed that *Aspergillus flavus* and *A. niger* were the most frequent, as they were recovered from 43.4% and 35.8% of samples. *Penicillium citrinum* and *Cladosporium cladospoioides* ranked next in abundance, with percentage occurrences of 31.1% and 30.2% in samples. Hao and Ann (1999) isolated *A. flavus* from 100% of groundnut cake samples collected from North Vietman. The fungi recovered from the DRG are similar to those contaminating other Nigerian food products in stores (Akano, Atanda, &

Table 1
Fungi isolated from dry-roasted groundnut in southwestern Nigeria

Fungi	NCI (%)	MTC ($\times 10^2$)
A. flavus	46 (43.4)	3.35
A. parasiticus	10 (09.4)	1.38
niger	38 (35.8)	2.86
fumigatus	12 (11.3)	0.66
ochraceus	4 (03.4)	0.21
P. citrinum	33 (31.1)	1.44
P. aurantiogriseum	7 (06.6)	0.47
Cladosporium cladosporioides	32 (30.2)	1.57
C. herbarum	8 (07.5)	0.75
Fusarium spp.	14 (13.2)	0.33
Rhizopus arrhizus	20 (18.9)	2.33
Mucor spp.	7 (06.6)	0.87
Paecilomyces varioti	15 (14.2)	0.58
Syncephalastrum racemosum	8 (07.5)	0.35

NCI, number of cases of isolation out of 106 samples; MTC, mean total count of different fungi per gramme in positive samples.

Ogundipe, 1992) and those displayed for sale in the markets (Atanda, Akano, & Afolabi, 1990; Bankole, 1995). Adebiyi, Adeyemi, and Olorunda (2002) in their study of the effect of different packaging materials on the storability of DRG, also observed the growth of *A. flavus* and *A. parasiticus* on the product. Contamination by these fungi could have occurred during the removal of the skin of the seeds after roasting or when the snack is being distributed into nylons or by cross infection from other products displayed for sale in the markets.

Aflatoxin was detected in 64.2% of the DRG samples (Table 2). Aflatoxin B_1 occurred in all aflatoxin-positive samples, with concentration ranging from 5 to 165 ppb (mean = 25.5 ppb), while 33 samples (31.1%) of the total samples contained aflatoxin B1 above 20 ppb. The percentage of samples positive for aflatoxin $B_2$was 26.4%, with a mean level of 10.7 ppb, while aflatoxins G_1 and G_2 were detected in 11.3% and 2.8% of samples with mean levels of 7.2 and 8.0 ppb, respectively. 38 of the samples that had *A. flavus* contamination were contaminated with aflatoxins, while 30 samples without *A. flavus* contamination contained aflatoxins. Bullerman (1986) has pointed out that the absence of toxigenic fungi does not guarantee the safety of a food product, because the fungi could die as a result of heating or be cleaned off, but the toxin would remain because it diffuses into the food. The linear regression analysis between the measured aflatoxin and the count of *A. flavus* in samples showed no significant positive or negative correlation ($r = 0.08$), and this contrasts with our previous results of a positive correlation on dried yam chips (Bankole & Adebanjo, 2003).

Table 2
Aflatoxin levels (ppb) in dry-roasted groundnut from southwestern Nigeria

Aflatoxin type	AFB_1	AFB_2	AFG_1	AFG_2
Positive samples (%)	68 (64.2)	28 (26.4)	12 (11.3)	3 (2.8)
Mean concentration (ppb)[a]	25.5	10.7	7.2	8.0
Concentration range (ppb)	5–165	6–26	5–20	7–10

[a] Calculated in positive samples.

The total aflatoxin contamination ranged from 10 to 176 ppb in various samples, and was above 20 ppb in 44%, or in 41.5% of total samples. It is established that heating significantly reduces aflatoxins in contaminated samples (Kpodo, 1996). Thus, the level of contamination in the raw groundnuts used for the processing of the DRG would have been considerably higher than the levels obtained in the current work. Few people eat the raw groundnut, though only a small quantity can be consumed in this way. Nigeria was listed as regulating aflatoxin B1 at 20 ppb in all classes of food, while most countries regulate total aflatoxins at 20 ppb (FAO, 1997). For overall sanitary precaution, the European Union has enacted, in 1998, very severe aflatoxin tolerance standards of 2ppb aflatoxin B_1 and 4ppb total aflatoxins in dry groundnuts for human consumption (CEC, 1998), and this has come into effect from January, 2001 (Dimanchie, 2001). Thus, by the Nigerian regulations, the present study indicates that aflatoxin contamination is occurring at unacceptable levels in 31.1% of the DRG being consumed while, by international standards, most of the DRG are not fit for human consumption. Unfortunately, more than half of the Nigerian populace cannot do without DRG in a day, because it is considered as a cheap source of protein, and many of them take the snack with gari for their afternoon meal. This is a major public health concern, and requires investigations into the reasons for these high levels and means of minimizing or eliminating them from the DRG. Since the hazards of aflatoxin contamination are not known to many people, the National Agency for Food and Drug Administration and Control (NAFDAC) and other relevant agencies in Nigeria should take steps to educate the masses on the danger of aflatoxins, so that they could be careful in selecting foods.

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