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Mycoflora and Aflatoxin Production in Market Samples of Some Selected Nigerian Foodstuffs

[1]K.O. Jimoh and [2]A.L. Kolapo
[1]Department of Food Science and Technology, The Polytechnic, Ibadan, Saki Campus, Nigeria
[2]Department of Biology, The Polytechnic, Ibadan, Nigeria

Abstract: Study on the fungi and aflatoxin production in some selected Nigerian foodstuffs was conducted in Ibadan, Nigeria. Foodstuffs studied include dry tatase pepper (*Capsium annum*), cassava chips, yam chips, groundnut and maize. The investigated foodstuffs sold at 4 major markets in Ibadan were contaminated with *Rhizopus nigricans*, *Fusarium oxysporum*, *Aspergillus flavus* and *A. niger*. The rate of occurrence of aflatoxigenic fungi was highest in groundnut while non-aflatoxigenic fungi dominated dry tatase pepper. Aflatoxins B_1 and G_1 were detected only in groundnut and yam chips with their concentrations ranging from 7-24 and 5-27 μg kg^{-1}, respectively. There was a significant difference ($p<0.05$) between the aflatoxin contents of groundnut samples from different market and this was possibly due to the wide variations in the moisture contents of groundnut samples. Result from this study is suggesting that aflatoxin intake in this part of the world may be consequent upon the consumption of staples like groundnut and yam chips. Therefore, resources and efforts should be directed at reducing aflatoxin contents of these culprit foodstuffs so as to produce a more healthy and productive populace.

Key words: Aflatoxin, *Capsium annum*, yam chips, cassava chips, groundnut, maize, Nigeria and market

INTRODUCTION

Most microbes infecting plant tissues often produce secondary metabolites in their hosts, which are known to be hazardous to animals including man (Krogh, 1992). Some of these metabolites include the ergot alkaloids on cereals by *Clavisep* sp., fumonisin on maize by *Fusarium* sp., aflatoxins and ochratoxins on several plants produced by *Aspergillus* sp. (Prasad, 1992). Aflatoxins, which are a group of highly toxic, mutagenic and carcinogenic polyketide compounds were first reported in groundnut seed that poisoned thousands of poultry and pigs (Goldbatt, 1983). Ciegler (1977) stated that the production of aflatoxin is affected by several factors, which influence the mould growth such as moisture contents, relative humidity, temperature, substrate composition and the presence of competing microorganisms.

Aflatoxins have been reportedly detected in grapes and musts in France (Sage *et al*., 2002), edible nuts and nut products, milk and milk products (Prasad, 1992; Taveira and Midio, 2001). Aflatoxin B_1 was detected in 22% of yam chips investigated in Ogun and Oyo states of Nigeria (Bankole and Mabekoje, 2003). Also, in Nigeria Adebajo (1993) reported the presence of aflatoxins in tiger nut (*Cyperus esculentus*) while the occurrence of aflatoxins has been established in rice and millet (Makun *et al*., 2007a, b), shelved bush mango seeds (Adebayo-Tayo *et al*., 2006), marketed pawpaw fruit (Baiyewu *et al*., 2007), groundnut and groundnut products (Hudson *et al*., 1992; Dawlatana *et al*., 2002; Williams *et al*., 2004) and herbal drug plants stored for sale (Efuntoye, 1996).

Corresponding Author: A.L. Kolapo, Department of Biology, The Polytechnic, Ibadan, Nigeria
Tel: +234 805 488 2370

The prominent Nigerian staples include; cereals, tubers, legumes and their products. It is a common practice for these staples to be eaten with sauce of which tatase-a dry pepper (*Capsium annum*) is a major component. There are ample documented reports on the aflatoxin status of most Nigerian staples. However there is dearth of literature on the aflatoxin status of tatase (a normal accompaniment of these staples) though the associated mycoflora has been documented by Atanda *et al.* (1990). In this regard, the present study is aimed at reporting the mycoflora and aflatoxin status of market samples of tatashe and few Nigerian staples.

MATERIALS AND METHODS

Collection of Samples

Ibadan is the largest city in the Sub-Saharan Africa and a significant amount of foodstuff produced in other parts of Nigeria found their way to this city for sale. The vegetation is typically tropic. The climate is characterized by dry November to April and wet May to October seasons. The mean annual rainfall of 1150-1500 mm occurs mainly between April and October with major peak in June.

Samples of dry pepper (tatase), dry maize, dry yam chips, dry cassava chips and groundnut were randomly collected from 4 major foodstuff markets in Ibadan, Nigeria; Oritamerin, Bodija, Sango and Shasha, between March and April 2006. The collected materials were representative of stored samples from the previous year's farming season. A total of twenty samples of each item were collected (5 samples from each market). The samples collected were placed in clean, sterile polythene bags, securely tied, labeled and transported to the laboratory. One kilogram of each sample was taken and divided into two. One half was stored in the freezer at sub zero degree for aflatoxin analysis and the other half was used immediately for isolation of fungi. The relative humidity at the time of sampling was between 75 and 79% while the temperature ranged between 28 and 30°C.

Isolation and Identification of Fungi

All the samples were surface sterilized by dipping them in 90% ethyl alcohol for 1 min and then rinsed in several changes of sterile distilled water. About two grams of a sample taken at random were placed in each of the petri-dishes containing Potato Dextrose Agar (PDA) and chloramphenicol (500 mg L^{-1}). The dishes were incubated at 27±2°C for 3-5 days. Fungal cultures obtained were subsequently purified and were later transferred to PDA slant for subsequent characterization tests. The purified cultures were primarily identified using cultural and morphological features (Barnett and Hunter, 1972) and by comparison with reference cultures from plant pathology laboratory of the Institute of Agricultural Research and Training, Moor Plantation, Ibadan, Nigeria. The moisture content of all the foodstuffs was determined by oven drying at 105°C for 4-5 h.

Detection of Aflatoxins Using Thin Layer Chromatography (TLC)

The method described by Seitz and Mohr (1977) was used for detecting aflatoxins in the pulverized samples. Aflatoxins were identified on the basis of co-migration with aflatoxin standards (Fluka) and their characteristic fluorescent colour under long Ultra Violet (UV) light at a distance of 360 mm. The concentration of aflatoxins (B_1 and G_1) in the extract was determined by measuring its absorbance at 360 nm and then calculated according to the method of Masri *et al.* (1969).

RESULTS AND DISCUSSION

Results obtained in this study showed that the mycoflora found associated with marketed foodstuffs include *Rhizopus nigricans*, *Fusarium oxysporum*, *Aspergillus flavus* and *A. niger* (Fig. 1). From the results, it is evident that groundnut and yam chips supported the growth of wide varieties

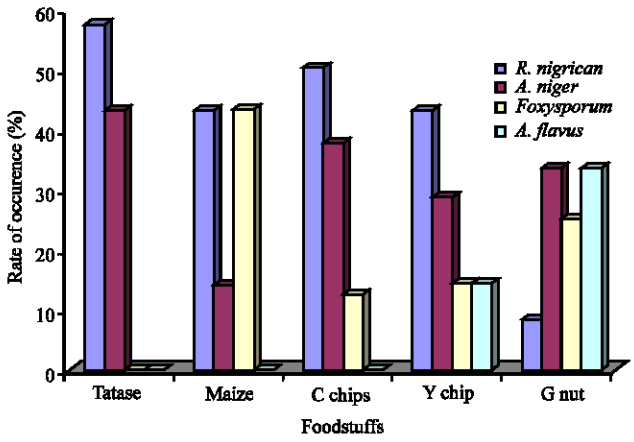

Fig. 1: Rate of occurrence of fungi associated with marketed foodstuffs in Ibadan, Nigeria

of fungi. This was closely followed by maize and cassava chips, while tatase supported the least kinds of fungi. However, between the foodstuffs that supported wide varieties of fungi, the rate of occurrence of aflatoxigenic fungi (*Aspergilus* sp.) was higher in groundnut samples. In a contrasting situation, the growth of non-aflatoxigenic fungus dominated the marketed tatase samples (Fig. 1).

The mycoflora of the market samples of the investigated foodstuffs in the present study is similar to the previous reports. Oboh *et al*. (2000) reported on the existence of non-aflatoxin producing strain of *Aspergillus* in cassava flour. Prasad (1992) stated that fumonisin (a mycotoxin) is often produced in maize by *Fusarium* sp. while *A. flavus* have been implicated as the chief producers of aflatoxin in groundnuts and its products (McDonald, 1964; Akano and Atanda, 1990). In addition, Atanda *et al.* (1990) reported the occurrence of *Aspergillus niger*, *A. flavus* and *Geotrichum candidum* as the major component of tatase mycoflora.

Tatase had moisture content of between 5 and 12%; maize, 3-8%; cassava chips, 6-9%; yam chips, 6-7% and groundnut 2-9% (Fig. 2). These values compare favourably with the value of 2-21% reported for shelved bush mango seeds (*Irvingia* sp.) in Uyo, Nigeria (Adebayo-Tayo *et al*., 2006), a town that share a similar climate with Ibadan, Nigeria. Bankole and Adebanjo (2003) stated that storage fungi usually grow in grain with moisture content in equilibrium with 70-90% relative humidity, which corresponds to less than 18% moisture content in cereals. Results from the present study have shown that at a moisture content level that is much lesser than 18%, fungi can still grow and produce aflatoxin in some marketed foodstuff.

Both Aflatoxins B_1 and G_1 were detected and quantified only in groundnut samples from all the markets and yam chips in one of the market (Sango). Out of the samples that were found to contain aflatoxins, groundnut samples from two markets (Oritamerin and Bodija) and yam chips from Sango market contained aflatoxins B_1 and G_1 at levels that are higher than 20 μg kg^{-1} total aflatoxin level recommended by WHO and FDA (Food and Drug Administration, of United States) (ICRISAT, 2000).

The present report is in agreement with some documented reports. Dawlatana *et al.* (2002) reported aflatoxin contamination rate of 65 μg kg^{-1} in groundnut samples from Bangladesh; an aflatoxin level of 162 μg kg^{-1} was reported in Gambian groundnut samples (Hudson *et al*., 1992; Williams *et al*., 2004). Bankole and Mabekoje (2003) reported that Yam chips had an aflatoxin contamination level of 4-186 μg kg^{-1}. Hell *et al.* (2000) found that no aflatoxin was detected in maize that was free of insect damage, whereas in maize with more than 70% of cobs damaged by insects, 30.3% were aflatoxin positive. Erdogan (2004) reported aflatoxin contamination rate of 1.1-97.5 μg kg^{-1} in some Turkish red pepper.

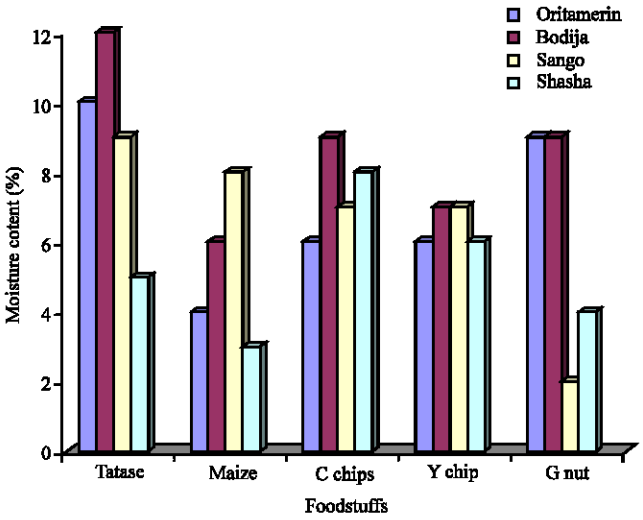

Fig. 2: Moisture content of marketed foodstuffs in Ibadan, Nigeria

Table 1: Aflatoxin content (μg kg^{-1}) of selected foodstuffs obtained from four markets in Ibadan, Nigeria

	Oritamerin		Bodija		Sango		Shasha	
Food sample	AFB_1	AFG_1	AFB_1	AFG_1	AFB_1	AFG_1	AFB_1	AFG_1
Tatase	-	-	-	-	-	-	-	-
Maize	-	-	-	-	-	-	-	-
Cassava chips	-	-	-	-	-	-	-	-
Yam chips	-	-	-	-	14	17	-	-
Ground nut	24	27	23	25	7	5	9	6

Values are means of five replicates, - : Not detected

There was a significant difference ($p<0.05$) between the aflatoxin contents of groundnut samples from the different markets. This may be strongly related to the variability in the moisture contents of these various samples. Groundnuts samples from Sango and Shasha markets had a significantly lower moisture contents and this might have been responsible for the low level of Aflatoxins B_1 and G_1 found in them (Table 1). The present report is in keeping with the earlier submission that the production of aflatoxins is affected by several factors, which influence the mold growth such as moisture contents (Ciegler, 1977) and that poor storage practices resulted in spontaneous heating and aflatoxin accumulation in some Thailand maize (Jones *et al*., 1986).

In the present study, all the investigated marketed samples supported the growth of aflatoxigenic fungus (*Aspergillus*), yet not all contained aflatoxins B_1 and G_1. This may not be particularly strange as Orum *et al*. (1998) and Oboh *et al*. (2000), have earlier stated that not all strains of *Aspergillus flavus* (a renowned aflatoxin producer) produce aflatoxin.

A recent study in Nigeria found that blood and semen aflatoxin levels ranged from 700 to 1393 and 60 to 148 ng mL^{-1}, respectively in infertile men and were significantly higher than that in fertile men (Uriah *et al*., 2001). Results from the present study have shown that though, molds like *Aspergillus niger* and *Rhizopus nigricans* may be associated with tatase, yet these associated fungi may not be producing aflatoxin in this most often used food accompaniment in Nigeria. The dietary intake of aflatoxin in this part of the world may be consequent upon the consumption of staples like groundnut and yam chips/flour. Therefore, more resources and efforts should be directed at reduction of aflatoxin contents of these culprit foodstuffs to a bearest minimum in other to guarantee a more healthy and a productive populace.

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