



Full Length Research Paper

A preliminary survey of aflatoxin in fresh and dried vegetables in Minna, Nigeria

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Aflatoxins are a group of fungal toxins that contaminate foods and feeds with adverse health impact on human beings and animals. A total of sixty vegetable samples comprising of 30 fresh and 30 dried samples were analyzed for the four major aflatoxins B₁, B₂, G₁ and G₂ using High Pressure Liquid Chromatography (HPLC). The vegetable samples were made up of baobab leaves (*Adansonia digitata*) (20), okra (*Abelmoschus esculentus*) (20) and red hot chili pepper (RCP) (*Capsicum annum*) (20) from Minna, Nigeria. The mycotoxins were not found in baobab leaves and okra but detected in 60% of the hot chili pepper samples analyzed at a total aflatoxin concentration of up 19.45µg/kg. Ten of the twenty hot chili samples had aflatoxin concentrations above the European Union maximum tolerance level of 4µg/kg for total aflatoxin which raises public health concern.

Keywords: Aflatoxin, baobab leaves, okra, chili pepper, Nigeria.

INTRODUCTION

Traditional African vegetables are very cheap sources of proteins, vitamins, minerals and essential amino acids as well as antioxidants (Mensah, Okoli, Ohaju-Obodo and Eifediyi, 2008) that are frequently used for dietary and medicinal purposes. Vegetables are cooked in the dried form or as fresh leaves or pods. The bulk is preserved by sundrying and used in the dried form throughout the year. In Nigeria, baobab leaves, okra and hot chili pepper are eaten daily as sauces and soups. Baobab is known as "kuku" in Hausa. Its leaves, fruits and bark are sources of food, fibre and medicine with over three hundred traditional uses in Africa which include being used as immuno-stimulant, anti-inflammatory, analgesic, insect repellent and pesticide, and in the treatment of diarrhoea and dysentery such that they are evaluated as substitutes to western drugs and are currently being commercialized as export crops (Kamatou, Vermaak and Viljoen, 2011). The authors in their review observed that one of the major reasons for the growing interest in baobab fruit pulp is that it has seven to ten times more vitamin C than oranges.

Okra pods are eaten for their fibre, vitamin C and folate contents which help to stabilize blood sugar by

decreasing its rate of absorption into the intestine while the mucilage binds cholesterol with consequent protection against diabetes, atherosclerosis and colorectal cancer (Takano, 2012). The pods also serve as anti-bacterial agents in transfusion, and post-partum haemorrhage in pregnant women in Ghana (Burkill, 1994). Red hot chili pepper on the other hand is one of the most consumed spices in the world and because of its pungency, aroma, colour and taste; RCP is used to modify the taste, colour and flavour of foods and confectionary products. It is also increasingly used for pharmaceutical purposes (Ahmet, 2010).

Aflatoxins are poisonous secondary metabolites produced mainly by *Aspergillus flavus*, *A. parasiticus* and *A. nomius*. The four major aflatoxins, B₁, B₂, G₁ and G₂ are the most important mycotoxins in foods and feeds because of their high prevalence in nature and toxicity (Makun, Dutton, Njobeh, Gbodi and Ogbadu, 2012). Aflatoxins especially AFB₁ created great interest once it was established as a powerful carcinogen (Wogan, 1973). The toxins are also mutagenic and teratogenic (Raisuddin, Singh, Zaidi, Paul and Ray, 1993). It has been estimated that AFB₁ plays a causative role in 4.6-28.2% of all global hepatocellular carcinoma (Liu and Wu, 2010). Since 1982, deaths caused by aflatoxin contaminated maize have repeatedly occurred in the Eastern Province of Kenya (Probst, Njapau and Cotty,

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2007). Similar cases have also previously been recorded at various times in India (Krishnamachari, Bhat, Nagarajan and Tilak, 1975) and Malaysia (Lye, Ghazali, Mohan, Alwin and Nair, 1995). A recent case reported in Kenya is as a result of consuming contaminated maize with 125 deaths out of 317 cases (Azziz-Baumgartner, Lindblade, Gieseke, Rogers, Kieszak, Njapau et al., 2006).

This group of deadly mycotoxins contaminates nuts and oilseeds, cereals, roots and tubers, fruits, vegetables and animal feeds (Makun et al., 2012) particularly in warm, humid regions of the world. Natural occurrence of mycotoxins in vegetables has been demonstrated in many countries (Trucksess and Scott, (2011). However, reports on mycotoxin contamination of African traditional vegetables are exceptionally rare. One of such rare works is that of Hell, Gnonlonfin, Kodjogbe, Lamboni, and Abdourhamane, (2009) who studied fungal and aflatoxin incidence in dried vegetable samples from three West African countries. It is noteworthy to mention here that the work was not conducted in Nigeria neither did it report on aflatoxins in fresh vegetables. Of the few studies (Atanda, Akando, and Afolabi, (1990), Obidoa and Gugnani, (1992), Adebajo and Shopeju, (1993), Adegoke, Allamu, Akingbala, and Akanni, (1996), Muhammad, Shehu and Amusa, (2004), Bankole, Ogunsanwo, Osho and Adewuyi (2006), Jonathan and Esho, (2010) on mycotoxins in vegetables in Nigeria, only three (Atanda et al., 1990, Obidoa and Gugnani, (1992, and Adegoke et al., 1996) are on aflatoxins in dried okra and RCP in the country with no report available on the incidence of the hepatotoxic aflatoxins in baobab leaves and this is despite that these three vegetables are vital, nutritious, daily components of diets in the country and indeed the continent (Hell et al., 2009). In view of the very scarce data on aflatoxins in the highly consumed Nigerian "kuka", okra and RCP that have increasing dietary, industrial and commercial profiles, this study is designed to generate information on the occurrence of aflatoxins in dried and fresh samples of baobab leaves, okra and RCP marketed in Minna, Nigeria.

MATERIALS AND METHODS

Sampling

Fresh and dried samples of the three different vegetables (0.5kg each) were collected from twenty five vegetable vendors in four markets in Minna metropolis, Niger State, Nigeria during 2011. A total of sixty samples; (baobab (20), okra (20) and (chili (20) were collected. For each vegetable, ten fresh and ten dried were sampled. All the fresh and dried ground samples of okra and RCP were obtained from the markets. However, for baobab leaves, only the dried ground form were purchased from the markets while the fresh ones were retrieved randomly

from ten baobab trees in different locations within Minna and its environs. The samples were put in sealed plastic bottles and stored at -20°C in the deep freezer until used for analysis.

Aflatoxin analysis

Extraction

Aflatoxins were extracted as described by Hell et al., (2009) without modifications. Briefly, 10 gm of each fresh or dried ground vegetable was mixed with 50 ml of methanol (BDH Chemicals Ltd Poole, England)/water (85:15 v/v) and blended for 3min, and filtered. The filtrate which was about 40 ml was mixed with an equal volume, 40 ml of 10% sodium chloride (BDH Chemicals Ltd Poole, England). The mixture was poured into a separatory funnel and defatted with 25 ml of n-hexane (BDH Chemicals Ltd Poole, England). The hexane layer was discarded and the aqueous layer partitioned twice with 25 ml of chloroform (May and Baker Ltd Dagenham, England). The chloroform layers were pooled and dried over anhydrous sodium sulphate (Cambrian Chemicals Beddington, England). The chloroform was then evaporated off on a rotary evaporator and the residue transferred to an amber vial with 2×0.5 ml of chloroform which was evaporated off under vacuum to near dryness and stored at -20°C until analyzed. The residue was re-dissolved in 200 μl acetonitrile: water :acetic acid (10:50:40 v/v/v) for high pressure liquid chromatography (HPLC).

Quantitative analysis of aflatoxins

Aflatoxins were analyzed on Agilent Technologies 1200 series HPLC with UV detection as described by Cora, Angre and Ronald, (2005) at wavelength of 365nm. The octadecylsilyl group (ODS) column, 4.6mm x 150mm x 5 μm was used at ambient temperature of 25°C . Acetonitrile : water and acetic acid in ratio 10:50:40 v/v/v respectively was used as mobile phase at flow rate of 0.8ml/min. The injection volume was 20 μl . The analyses were carried out with aflatoxins standards (Sigma Chemical Company, St. Louis, MO, USA) of known concentrations with AFB₁, AFB₂, AFG₁ and AFG₂ eluting at distinct retention time of 1.673, 1.524, 1.387 and 1.248 respectively. Calibration curves with correlation factors of 0.91, 0.99, 0.87 and 0.94 were obtained for AFB₁, AFB₂, AFG₁ and AFG₂ respectively using series of dilutions containing 0.005 $\mu\text{g/ml}$, 0.01 $\mu\text{g/ml}$, 0.015 $\mu\text{g/ml}$, 0.02 $\mu\text{g/ml}$ and 0.025 $\mu\text{g/ml}$ of each of the standard. The detection limit of the machine with regards to the toxins was 0.001 $\mu\text{g/ml}$.

RESULTS AND DISCUSSION

Mycotoxicological data on baobab leaves, okra and RCP

Table 1. Aflatoxin levels ($\mu\text{g}/\text{kg}$) in red hot chili pepper samples from Niger State, Nigeria

Aflatoxin	Type of sample	Frequency of contamination	Range of concentration of positive samples	Mean \pm SD	Mean \pm SD of both fresh and dried samples	Number of samples with aflatoxin exceeding EU limits
AFB ₁	Fresh	6/10	2.00-12.4	3.83 \pm 1.243 ^a	2.212 \pm 3.433	5/10
	Dried	6/10	0.05-2.50	0.59 \pm 1.44 ^a		1/10
AFB ₂	Fresh	6/10	0.55-4.95	1.59 \pm 0.59	0.897 \pm 1.511	
	Dried	6/10	0.05-0.90	0.21 \pm 0.65		
AFG ₁	Fresh	6/10	0.55-10.0	0.95 \pm 0.66	0.580 \pm 1.531	
	Dried	6/10	0.50-4.25	0.64 \pm 0.74		
AFG ₂	Fresh	6/10	0.55-2.70	0.52 \pm 0.38	0.390 \pm 0.984	
	Dried	6/10	0.55-3.20	0.26 \pm 0.19		
Total AF	Fresh	6/10	10.15-19.45	8.347 \pm 7.754	5.858 \pm 6.797	6/10
	Dried	6/10	0.85-14.65	3.369 \pm 4.864		4/10

Note: ^aThe total number of samples analyzed was twenty, ten being fresh and ten dried samples. ^bConcentrations are presented as mean \pm standard deviation and range in $\mu\text{g}/\text{kg}$. ^cValues with similar superscripts are significantly different at $P < 0.05$ level of significance. ^dThe EU maximum tolerable limit for AFB₁ and Total aflatoxin (AF) are $2\mu\text{g}/\text{kg}$ and $4\mu\text{g}/\text{kg}$ respectively.

are rare in Nigeria. Except for the works of Obidoa and Gugnani, (1992) and Adegoke, Allamu, Akingbala, and Akanni, (1996) that detected aflatoxins in dried okra and RCP in Nigeria, all other investigations in the country (Atanda, Akando, and Afolabi, 1990, Adebajo and Shopeju, 1993) provided data on fungi contaminating okra and RCP with virtually nothing on baobab leaves. Thus, this study to the best of our knowledge is the first investigation of aflatoxins in baobab leaves in Nigeria and the second report on the hepatocarcinogenic toxins in okra and red hot chili pepper in the country. The present survey did not find aflatoxins in both fresh and dried "kuka" and okra samples but

demonstrated the mycotoxins as common contaminants of the two studied forms of RCP samples. The four major aflatoxins were detected in 12 of the 20 samples of RCP samples analyzed (Table1) at AFB₁ and total AF concentrations ranges of 0.05 – 12.4 $\mu\text{g}/\text{kg}$ and 0.85 – 19.45 $\mu\text{g}/\text{kg}$ respectively. The fresh samples consistently had higher AFB₁, AFB₂, AFG₁ and AFG₂ levels than the dried samples indicating moisture dependent contamination. Similarly more fresh RCP (6 of 10) samples had aflatoxin concentrations above the action level of European Union than dried samples (4 of 10). The occurrence of aflatoxin is highly pivoted on favourable temperature of between 24°C and 28°C and substrate moisture

content of at least (17.5%) Ominski, Marquardi, Sinha and Abramson, (1994) with higher aflatoxin incidence recorded in poorly stored moist samples than dried ones (CAST, 2003) This might be the reason for the observed moisture dependent aflatoxin contamination trend.

The absolute absence of aflatoxins in baobab leaves in the current work correlates with the findings of Hell et al., (2009) who found no aflatoxins in dried 'kuka' samples that were heavily contaminated with aflatoxigenic fungi. Similarly, PhytoTrade Africa, (2008) did not detect aflatoxins and ochratoxin A in baobab dried fruit pulp. On the other hand the non detection of aflatoxins in the dried and fresh samples of okra in

this study despite its proven susceptibility to aflatoxin producing fungi (Adebanjo and Shopeju, 1993) is in slight variance with the report of Obidoa and Gugnani, (1992) and Hell et al., (2009). The former, using qualitative TLC method demonstrated the presence of aflatoxin in dried okra but did not find the toxin in fresh samples of the vegetable while the latter detected AFB₁ and AFB₂ to the extent of 5.4 µg/kg and 0.6 µg/kg, respectively in 3 out of 30 dried okra samples from Benin, Mali and Togo. The reported presence of aflatoxins in very low frequency of occurrence and concentration in okra in these two surveys and the non detection of the mycotoxins in same crop in the current study imply that okra is an unsuitable substrate for aflatoxin contamination. The phytochemical composition of kuka (Kamatou, Vermaak and Viljoen, 2011) and okra (Singh, Suman, Sharma, Singh, Singh and Maurya, 2008) which includes essential oils especially the fungicidal principles; phenols and tannins that confer inherent resistance to mould infestation and aflatoxin production (Audilakshmi, Stenhouse, Reddy and Prasad, 1999) could be the reason for the absence of the toxins in the crops.

Prior to this study, there were four reports on fungal and aflatoxin contamination of RCP in Nigeria. Atanda et al., (1990) conducted a mycological survey of dry red pepper in south western part of the country and showed aflatoxin producing fungi, *Aspergillus flavus* as a dominant fungal contaminant of RCP but did not find aflatoxin in the samples. Obidoa and Gugnani, (1992) in their survey for aflatoxin in various types of local Nigerian foods using TLC qualitatively detected aflatoxin in dried pepper. Adebanjo and Shopeju, (1993) found *A. flavus* and *A. niger* as the commonest fungi contaminating sundried preserved RCP while Adegoke et al., (1996) isolated *Rhizopus oryza*, *Aspergillus niger*, *A. flavus*, *Geotrichum candidum* and *Saccharomyces* spp as the major fungal contaminants of two pepper varieties- *Capsicum annum* and *Capsicum frutescens*. AFB₁ was not detected in sun dried, matured red *C. frutescens*, but was found in *C. annum* at concentrations of up to 2.2µg/kg. This to the best of our knowledge is the first quantitative report of aflatoxin in RCP in Nigeria while the current study is the second in the country. However, it is noteworthy that the former only reported on AFB₁ while the current work is presenting for the first time, the presence of the four major aflatoxins in Nigerian grown RCP.

The results obtained for RCP are similar to values obtained by Zinedine, Brera, Elakhdari, Catano, Debegnach, Angelini et al., (2006) in red paprika at AFB₁ and total AF maximum level of 5.40µg/kg and 9.68µg/kg in Morocco but higher than the estimated values observed by Hell et al., (2009) in 1 out of 30 samples contaminated with AFB₁ at concentration of 3.2µg/kg. The herein reported concentrations were lower than those determined by Musaiger, Al-jedah and D'souza (2008) (35.9, 52.6 and 69.2µg/kg in three red chili

powder), Jalili and Jinap, (2012) (total AF concentration of 0.2 – 79.7µg/kg in 52 of 80 commercially dried chili) in Bahrain and Malaysia respectively. In their review on mycotoxins in botanicals and dried fruits, Trucksess and Scott, (2011) reported alarming aflatoxin levels in *Capsicum* spp from Ethiopia and India. Eight of the 60 red pepper samples from Ethiopia contained AFB₁ at concentration ranging from 100µg/kg to 500µg/kg. The toxin (AFB₁) was also found in 107 out of the 182 marketed Indian chili pod samples at concentration of up to 969µg/kg. The review also recorded the occurrence of AFB₁ in 18 of 70 ground red pepper samples from Hungary at concentrations of between 6.1µg/kg and 15.7µg/kg. Our results therefore lay credence to the findings that *Capsicum* spp are good substrate for aflatoxigenic fungi growth and aflatoxin production (Ravi Kiran, Narayana and Vijayalakshmi, 2005) and are therefore susceptible to aflatoxin contamination worldwide.

The present aflatoxin contamination of RCP with 50% of the samples having unacceptable toxins levels above the safe limit set by EU could be because these vegetables were sun dried, stored and sold in the open under unwholesome environmental conditions which are suitable for fungal growth and aflatoxin production (Hell et al 2009). Minna, the study area is warm (average annual temperature of 31.7°C) and humid (average annual humidity of 51.6%) most time of the year which are favourable conditions for growth of aflatoxin producing fungi and toxin synthesis (Ominski et al., 1994). Another compelling factor that heightens aflatoxin levels in foods particularly in Sub Saharan Africa which Nigeria is part of, is ignorance of the existence of the toxins (Wagacha and Muthomi, 2008). Since aflatoxins are deadly mycotoxins (Azziz-Baumgartner et al., 2006) implicated in hepatocellular carcinoma ((Liu and Wu, 2010), their presence at unsafe concentrations in the studied Nigerian grown RCP necessitates the inclusion of the crop in the list of foods for mycotoxin control and regulation via good agricultural and manufacturing practices, public enlightenment on the hazards and control of the mycotoxins and strict enforcement of mycotoxin regulatory legislation.

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