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RESEARCH ARTICLE

Aflatoxin and fumonisin contamination of cassava products and maize grain from markets in Tanzania and republic of the Congo

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Abstract

Food safety and compliance with international standards is a major challenging to achieve food security in sub-Saharan Africa. The present study evaluated the occurrence of *Aspergillus flavus, Fusarium* spp., and related fungi, and resultant aflatoxins and fumonsins in dried cassava and maize samples from various markets and villages in Tanzania and Congo. The relationship between mycotoxins and length of storage period was also elucidated. The levels of aflatoxin B_1 varied from 0.3 to 4.4 ppb in cassava chips and flour, and from 0.1 to 13.0 ppb in stored cassava samples, with relatively high levels of contamination found in cassava stored for 4 months. Maize kernels showed high aflatoxin concentrations, with means ranging from 0.04 to 120 ppb. On maize, the dominant mycoflora were *Aspergillus* spp. (3.3%–39.5%) and *Fusarium* spp. (42%–70.5%), potentially causing serious health risks to consumers of these products. Low levels of fumonisin ranging from 0 to 0.07 ppm were found in cassava chips and flour with mean values ranging from 0.001 to 0.006 ppm. Maize recorded relatively higher fumonisin levels ranging from 0.02 to 9.4 ppm, indicating that maize is potentially a more serious risk to consumer health than cassava. This needs to be taken into account when developing strategies to reduce toxin contamination and improve health of populations. Aflatoxin in maize is a chronic problem in the two countries surveyed, limiting marketability and income. Nevertheless the collected cassava samples are also prone to aflatoxin contamination, but not fumonisin contamination.

Keywords: *Aflatoxin; fumonisin;* A. flavus*;* Fusarium *spp.; cassava maize*

Introduction

The availability of safe food is a prerequisite for the well-being of people and the development of national economies. The low quality and safety of foods in Africa have a significant impact on human and animal health, and are a major constraint to growers who need access to more remunerating markets. Some of the factors that are a threat to food quality and safety include poor physical quality, chemical contamination, bacterial or mycotoxin contamination (Bankole and Adebanjo, 2003). African countries have a climate that is conducive to the growth of fungi and subsequent toxin production (Bankole and Adebanjo, 2003). It has been reported that regions between the latitudes 40° N and 40° S of the equator in Africa are the conducive zone for aflatoxin infestation of cereal grains (Williams et al., 2004). Aflatoxins, fumonisins, ochratoxin A, zearalenone, and deoxynivalenol are mycotoxins that are detected in cereal crops. Aflatoxins pose the greatest risk to health in tropical Africa due to their widespread prevalence and high toxicity. Mycotoxin exposure contributes to more than 40% of the global disease burden (Williams et al., 2004). In Africa, the reduction of the average human life span has been correlated with exposure to

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mycotoxins (Miller, 1996). Several fatal cases of aflatoxin ingestion have occurred in sub-Saharan Africa, including the recent events in Kenya, where more than 125 deaths were attributed to acute aflatoxin poisoning (Azziz-Baumgartner et al., 2005). The high incidence of aflatoxin contamination of groundnuts and cereal grains in Guinea, Gambia, Nigeria, and Senegal was correlated with an increased incidence of liver cancer (Shephard, 2004). Gong and colleagues (2003) reported that in rural West Africa more than 99% of children have aflatoxin-albumin adducts, the biomarker of aflatoxin exposure, in their blood. Maize and cassava constitute the staple food of inhabitants in tropical Africa. Environmental conditions in these regions are favorable for infection by *A. flavus* and subsequent aflatoxin contamination of these two crops (Williams et al., 2004). *Aspergillus* spp. grow very well when temperatures range from 24 to 35°C under conditions of low water availability (a_w) (Marín et al., 1998).

Fusarium spp. are often endophytic in maize and produce a diverse number of toxins implicated in human and animal health impairments (Miller, 2001). *F. verticillioides* is commonly found in preharvest and stored maize in West Africa (Essien, 2000; Fandohan et al., 2005a). Human consumption of food contaminated with this fungus was associated with higher levels of esophageal cancer in certain regions of South Africa and China (Shephard et al., 1996). High incidence of fumonisins at low levels was reported in surveys of Eastern and Southern Africa. Very little information is available on *Fusarium* infection and fumonisin contamination in maize in Africa outside South Africa (Doko et al., 1995; Doko et al., 1996; Fandohan et al., 2003).

Cassava and maize are a source of dietary energy in sub-Saharan Africa. In Uganda, Tanzania, and the Democratic Republic of Congo, cassava is the primary staple. However, in Burundi and Kenya, diets of indigenous populations are mainly maize-based (EARRNET, 2002). Few studies have reported on mycotoxin contamination in cassava products (Adegoke et al., 1993; Wareing et al., 2001; Gnonlonfin et al., 2008) when compared with the number of studies conducted on cereals, peanuts, dairy products, wheat, and dried chilies (Sibanda et al., 1997; Bankole and Adebanjo, 2003).

The present study investigated the level of mycotoxin contamination of maize samples and dried cassava products collected from village farms and large market traders in Tanzania and the Republic of the Congo. Studies on the mycotoxins contaminating these targeted crops are rare. The general objective was to collect a wide-ranging data set on the risk that mycotoxins pose to the health profile of African populations, so that better targeted control strategies can be developed.

Material and methods

Samples

The incidence of *Aspergillus*, *Fusarium*, and other fungal species and resulting mycotoxins in cassava and maize staples in Tanzania and Congo was determined. Thirty-eight different types of cassava tubers: Makopa fresh, stored, dry, just dry, stored-smoked, non-smoked, and fermented were sampled from 8 different locations in four regions in Tanzania: Mtwara, Mkongi, Zanzibar, and Ugunja Island. Each type of dried cassava sample came from a different storage period. Cassava chips and flour were processed by the traditional method, cassava roots were manually peeled, chipped, and sometimes fermented, and finally manually pounded, sun dried, and stored (Westby et al., 2002). Another 22 samples of cassava chips, flour, and maize grains were collected from six markets in Brazzaville, Republic of the Congo.

All samples were sealed in polythene bags and dried in a desiccator (Niko, Trihei BP-65, Japan) at 35°C for 72 hrs. The samples were ground in a blender (Waring Commercial, Model-HGBTWTG4, Torrington, Connecticut) to a dry, smooth consistency and stored at 4°C.

Toxin analysis

Extraction: Twenty grams of each ground sample were passed through a 20-mesh sieve (U.S. Standard test sieve, ASTM E-11, Newark Wire Cloth Company) and extracted with 100 ml of 70% methanol (B.D.H., England). The samples were filtered through Whatman filter paper No. 1 and 15 ml of the extract collected.

ELISA: The extracts were assayed for aflatoxin B_1 and fumonisin B_1 using a competitive direct enzyme linked immunosorbent assay (ELISA) in a microwell format (EZ-Quant Aflatoxin/Fumonisin kit, Diagnostix Limited, Mississauga, Canada). A 100-μl aliquot of each standard (0, 0.3, 1.5, and 6.0 μg/g) was dispensed separately into four mixing wells, and 100 μl of each sample extract was added to all the remaining mixing wells. To each mixing well, 100 μl of enzyme conjugate (horseradish peroxidase) was dispensed, and after mixing, 100 μl was transferred to the antibody-coated wells and incubated for 10 min at room temperature (27°C). The contents of the antibody wells were removed, and after a wash step, 100 μl of substrate 3,3′,5,5′-tetramethylbenzidine

was added and incubated for 5 min at 28°C. Reactions were stopped by adding 100 μl of stop solution (HCl) to each well, and absorbance was read with an ELISA plate reader at 450 nm optical densities (BioTek Instruments, Inc., Vermont). Aflatoxin, fumonisin concentrations of samples were determined by plotting the optical densities on the standard curve of the known aflatoxin or fumonisin concentrations. Samples with toxin concentration higher than the highest standards were diluted with extraction solvent, and the results obtained were multiplied by the dilution factor. All samples were run in duplicates and the detection limit was 0.001 ppb.

Recovery test: The sensitivity of the quantification method was evaluated by spiking samples with known concentrations of aflatoxins and fumonisin B_1 and determining the percentage recovery of the toxins.

Incidence of fungal infection on maize grains

To determine the fungal infestation, maize ears were shelled, and kernels from each lot were randomly sampled for a total of 100 kernels per sample. The grains were surface sterilized in a sodium hypochlorite solution (1.75%) and then washed three times in sterile distilled water. Ten maize grains were placed in each Petri dish, each of which contained moistened cotton wool covered by an absorbent filter paper according to the method of Waller (2002). The plates were incubated for 7 days at room temperature and the number of grains colonized by fungi was recorded and fungal species were identified. The number of grains free from fungal growth was also recorded. Percent kernel infection per fungal species was calculated on the basis of 100 kernels per sample.

Statistical analysis

Mean values of aflatoxin B_1 were transformed to log (x+1) and all percentages were arcsine-transformed before analysis to normalize the data. Data were analyzed with ANOVA for multiple comparisons among means. Correlation and regression coefficients were calculated using the SAS statistical package version 8 (SAS Institute, 2000).

Results

Aflatoxin and fumonisin in maize

The recovery from maize spiked with aflatoxin B1(AFB1) or fumonisin B_1 exceeded 86.0%, demonstrating a good performance of the method used. Table 1 presents the results of the assessment of the incidence of *Aspergillus* and *Fusarium* spp. on maize grains collected from Brazzaville, Congo. *Aspergillus*, *Fusarium*, and other species of filamentous fungi were observed with varying frequencies on the maize kernels. *Fusarium* was the most frequently observed genus in all the maize samples, infection rates being 2 to 25 times higher than the frequency of *Aspergillus* sp., which ranged from 3% to 40%. Other fungi encountered on maize grains were *Penicillium* sp., and to a lesser extent *Helminthosporium* sp. and *Colletotrichum* sp. In regard to the incidence of kernels with *Aspergillus*, *Fusarium*, other filamentous fungi and kernels with no apparent fungal contamination, no significant differences were observed between the different maize types sold in the market (maize and mixed maize). AFB_1 was detected in all the analyzed samples (Table 2) at levels ranging from 0.4 to 120.9 ppb and the overall mean of AFB_1 incidence was much higher in maize grains than in cassava samples. There were no significant differences in the mean AFB_1 incidence between the different cassava samples. There were, however, highly significant differences ($P < 0.0002$) between the maize types (e.g., maize and mixed maize), with mixed maize showing relatively high aflatoxin and fumonisin levels.

In spite of the high kernel infection with *Fusarium*, low levels of fumonisin B_1 contamination were detected in all the maize samples from Brazzaville (Table 3), with levels ranging from 0 to 9.6 ppm. Fumonisin incidence between the different types of maize samples (maize, mixed maize, and white maize) were significantly different ($P < 0.006$), again with mixed maize having relatively high contamination.

Despite the low percentage of maize grains contaminated with *Aspergillus* (3%–39.5%), a significantly positive correlation was observed between

Table 1. Percentage of Maize Grain Kernels on Market Samples from Brazzaville, Congo, with *Aspergillus* spp, *Fusarium* spp, Other Fungi or No Visible Infection.

		Fungal genera present (%)			
Sample type	Sample size	*Aspergillus*	*Fusarium*	Others	None
Maize	2	12.5a	70.5a	31.0a	6.0a
White maize	4	11.75a	67.5a	30.2a	21.2a
Yellow maize	3	3.33a	73.0a	39.0a	3.3a
Mixed maize	4	39.5a	42.5a	31.5a	10.7ab

Values followed by the same letters are not significantly different.
Least significant difference at $\alpha = 0.05$.

Table 2. Aflatoxins B_1 Content in Cassava and Maize Samples from Markets in Brazzaville, Congo.

			Aflatoxin (ppb)	
Sample type	Sample size	Positive samples >20 ppb (%)	Mean[1]	Range
Cassava chips	6	0	0.35 a	0.4–4.38
Cassava flour	3	0	0.31 a	0.32–1.64
Maize	2	50	1.07 b	1.14–71.67
White maize	4	0	0.55 a	0.4–10.81
Yellow maize	3	0	0.46 a	0.04–10.81
Mixed maize	4	75	1.36 b	1.0–120.09

Values followed by the same letters are not significantly different.
Least significant difference at $\alpha = 0.05$.
[1] Means transformed to the $\log_{10}$, i.e., $y = \log_{10}(x+1)$ previous to analysis.

Table 3. Occurrence of Fumonisin B_1 in Cassava and Maize Samples from Markets in Brazzaville, Congo.

		Fumonisin (ppm)	
Sample type	Sample size	Mean[1]	Range
Cassava chips	6	0.008a	ND
Cassava flour	3	0.009a	ND
Maize	2	0.42b	ND
White maize	4	0.14ab	0.02–1.68
Mixed maize	4	0.34b	0.05–9.62

Values followed by the same letters are not significantly different.
Least significant difference at $\alpha = 0.05$.
[1]Means transformed to the $\log_{10}$, i.e., $y = \log_{10}(x+1)$ previous to analysis

percentage grain infection and aflatoxin levels in maize samples ($P < 0.0003$, $r = 0.84$). The incidence of *Fusarium* spp. was very high, ranging from 42.5% to 73% in the maize grain samples. However, no significant correlation was found between the incidence of *Fusarium* and fumonisin ($P < 0.86$, $r = -0.05$).

Aflatoxin and fumonisin in cassava

All the tested cassava samples collected in Tanzania were aflatoxin-positive with mean content not exceeding 1.2 ppb (Table 4). No statistical differences were observed in the contamination levels between samples of cassava that were fresh, had been stored, or had been smoked. The AFB_1 levels in cassava samples that had been stored for 1 week to 2 months had, however, a higher though not significantly different toxin contamination (0.12–2.08 ppb). In stored cassava, aflatoxin contamination increased significantly with time (Table 5). The mean aflatoxin contamination levels were low (3.7 ppb) in fresh samples (3–4 days of storage), but increased sixfold (12.9 ppb) when stored for 4 months. It seems that cassava is a good substrate for aflatoxin biosynthesis. Fumonisin

Table 4. Aflatoxin B_1 Contamination in Cassava Chip Samples from Villages in Tanzania.

		Aflatoxin (ppb)	
Sample type	Sample size	Mean[1]	Range
Fresh	7	0.25 a	0–1.58
Stored	13	0.28 a	0.12–2.08
Smoked	5	0.35 a	0.9–1.44

Sample type fresh—cassava tubers not processed, Stored—cassava tubers stored for a week, after solar drying, non-smoked, Smoked—cassava tubers smoked after solar drying.
Values followed by the same letters are not significantly different.
Least significant difference at $\alpha = 0.05$.
[1] Means transformed to the $\log_{10}$, i.e., $y = \log_{10}(x+1)$ previous to analysis.

Table 5. Aflatoxin B_1 Content in Cassava Chips Stored Fresh for 4 to 7 Days, for 1 to 4 Months in Tanzania.

		Aflatoxin (ppb)	
Storage period	Sample size	Mean[1]	Range
Fresh samples	6	0.42 a	0.86–6.98
Stored (3–7 days)	24	0.53 ab	0.1–17.11
Stored (1–4 months)	9	0.89 b	2.3–33.8

Values followed by the same letters are not significantly different.
Least significant difference at $\alpha = 0.05$.
[1] Means transformed to the $\log_{10}$, i.e., $y = \log_{10}(x+1)$.

was detected in traces in the tested cassava samples, only one of the 24 samples being fumonisin positive (0.32 ppm).

Discussion

The present study shows that mycotoxin contamination of cassava is a concern and regular quality control is necessary, so that populations are not at risk for toxin contamination. The cassava samples from markets in Congo and Tanzania showed AFB_1 levels in the range of 0.4 to 4.38 ppb and 0.86 to 33.8 ppb, respectively. Wareing and colleagues (2001) isolated predominantly *Fusarium* spp. on Ghanaian cassava chips and to a lesser extent *Aspergillus* and *Penicillium* spp. Similarly, McFarlane (1982) reported that cassava chips were infested with fungi of the genera *Aspergillus* spp., *Penicillium* spp. and *Rhizopus* spp., and contamination with mycotoxin was observed on processed cassava chips.

Aflatoxin contamination seems to increase with storage, with cassava tubers stored for 6 months having the highest AFB_1 levels (Table 5). Poor storage and handling methods contribute to the accumulation of mycotoxins (Setamou et al., 1997). Aflatoxin contamination in maize increased with storage time (Hell et al., 2003), with maize that was stored for 2 years having significantly higher levels of toxin than

that stored for 1 year (Liu et al., 2006). Adequate drying to less than 14% moisture content is necessary to prevent biological activity and to preserve quality in storage for maize (Williams et al., 2004), with cassava products requiring drying below 12% (Christensen et al., 1986).

Fusarium was the predominant fungal species isolated on maize samples from the Congo. In West Africa, *Fusarium* spp. have been found as the causal agent of fumonisin contamination of maize (Schulthess et al., 2002; Fandohan et al., 2005b). Nikiema and colleagues (2004) reported that maize samples from markets in Burkina Faso were fumonisin positive (mean 2,900 ppb), with a high likelihood of a fatal exposure, as maize is a staple crop. The collected maize samples were contaminated with AFB_1, ranging from 0.04 to 120.09 ppb (Table 2). Fumonisin contamination was found in traces in cassava samples (0–0.07 ppm), and was detected in the range of 0.02 to 9.62 ppm in the maize grains (Table 3). Although there was a high percentage of *Fusarium* infection in the collected maize grain samples from Tanzania and Congo, no significant contamination with fumonisin was observed. Nevertheless the presence of fungal contamination indicates the potential for fumonisin production. The high infestation of the grains with *Fusarium* and the observed low fumonisin production may be due to inhibitory or synergistic effects (Stoev et al., 2001) of co-occurring fungi. Fumonisin contamination is also reported to be strongly influenced by several environmental factors including temperature, humidity, drought stress, and rainfall during preharvest and harvest periods (Fandohan et al., 2005a). Favorable conditions need to be met before the production of this toxin, despite high *Fusarium* infection.

High aflatoxin contamination of stored maize grains has previously been reported from Benin by Hell and colleagues (2000) and in Ghana ranging from 20 to 355 ppb by Kpodo and colleagues (1996). In Benin 99% of the children had aflatoxin in their blood, with maize as one of the main sources of contamination (Egal et al., 2005). One of the effective ways for reducing mycotoxin contamination is sorting out damaged and discolored grains (Afolabi et al., 2006; Fandohan et al., 2005b). During the survey, when maize samples were collected in Brazzaville markets, it was observed that market women were sorting corn manually or using sieves (K. Hell, personal observation). No differences were observed between the contamination levels in the different types of maize, though there seems to be a trend whereby mixed maize was of a lower quality since a higher percentage of fungal growth was observed on it. Our conclusion is that this maize was destined for the feed market. Kedera and colleagues (1999) demonstrated relatively high levels of fumonisins (> 1000 ppm) in 5% of poor quality maize grains and low levels (100 ppm) among 47% samples from good quality maize, indicating that low quality maize could be a source of higher contamination.

The storage time in the cassava samples was 4 months, and aflatoxin accumulated threefold relative to freshly harvested samples. Similarly, *A. flavus* contamination of garri, a cassava-based product, increased with storage time (Ogiehor and Ikenebomeh, 2004). Previously it has been reported that a higher incidence of aflatoxin was found in maize samples stored for 6 months (15.0%–32.2%), as compared with freshly harvested samples (9.9%–32.0%) (Hell et al., 2003). Egal and colleagues (2005) reported that consumption of maize was an important source of aflatoxin exposure for the survey population.

Environmental factors, genotype, management, and cultural practices have an impact on the growth of fungi and mycotoxin accumulation (Setamou et al., 1997). Smoking of cassava chips will probably reduce insect infestation, fungal infection, and aflatoxin accumulation by lowering the moisture content, and in our data (Table 4) the incidence of aflatoxin was indeed quite low (1.27 ppb) on smoked cassava chips. The effectiveness of smoke drying in reducing insect and fungal infection was confirmed by Udoh and colleagues (2000). Temperature and water availability directly influence mycotoxin development and *A. flavus* shows optimal development at temperatures around 30°C and ± 96 a_w (Marín et al., 1998). The exact genotypes of the maize samples of the present study are not known, but in a Texan study the white or yellow grain characteristics did not influence aflatoxin accumulation (Betran et al., 2004).

Cassava and maize occupy an important position in Africa's agricultural food consumption and economy. Lack of genotypes that are resistant to mycotoxin contamination emphasizes the need of adapting aflatoxin-minimizing strategies like the use of varieties with less risk of contamination, use of biological control, model-based risk-forecasting systems, and improved pre- and postharvest management strategies to minimize qualitative and quantitative losses of these dietary staples. Deployment of such control strategies in Africa could limit the levels of food-borne hazards while enhancing export possibilities for maize and cassava.

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