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Fungi and mycotoxins associated with food commodities in Cameroon

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Abstract

Objective: Spoiled maize grains and numerous types of snacks that are consumed in the Western Highlands of Cameroon are infected by several mycotoxin producing fungi. The extent of contamination of these food commodities by secondary metabolites of fungal origin has not been well studied. This study aimed to identify the microorganisms that infect maize grains and snacks sold at road side markets, and to sensitize the population on the health risks that are associated with consumption of contaminated commodities.

Methodology and results: Maize and snack samples were collected from various locations in Cameroon. Contaminating microorganisms were isolated and identified using conventional techniques. *Staphylococcus* and *Salmonella* species were the most frequently isolated bacteria while *Fusarium* and *Aspergillus* species were isolated in highest frequency ranging from 20 to 100 % presence in the samples analysed. Chemical analyses revealed the presence of fumonosins (50-26000 ng g^{-1}), Deoxynivalenol (100-1300 ng g^{-1}) and zearalenon (50-180 ng g^{-1}) in the sampled maize.

Conclusion and application of findings: Contamination of agricultural products by microbial toxins is an important but often underestimated risk to public health and can have long-term health implications. Appropriate sanitary measures need to be taken to ensure that conditions for microbial contamination and toxin production are reduced or eliminated during the handling, transportation, packaging and storage of all agricultural products.

Key words: Maize, snacks, fungi, mycotoxins, Cameroon

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Introduction

Mycotoxins are secondary metabolites that are produced by fungi, especially by saprophytic moulds growing on foodstuffs, animal feeds, grains or plants. Thirty years ago, such moulds were considered to affect only the aesthetic quality of products, but not human and animal health. Presently, many adverse effects of mycotoxins are well known, with mycotoxicoses causing major recent epidemics affecting man and animals. Some of the important events include ergotism in Europe in the last millenium, alimentary toxic aleukia (ATA) in the USSR in the 1940s; stachybotryotoxicosis, which killed horses and cattle in the USSR in the 1930s; and death of turkey due to aflatoxicosis in England in 1960.

In 2004 and 2005 aflatoxicosis was reported in Kenya, causing some deaths and leaving many people sick (MMWR Morb Mortal Wkly Rep 2004, 2005).

In the western highlands of Cameroon, most grains are harvested during the rainy season, which creates favorable conditions for infections by fungi and subsequent mycotoxin contamination. The major contaminants of maize grain are *Fusarium* sp., *Aspergillus* sp., *Penicillium* sp., *Acremonium* sp. and *Diplodia* sp. (Marasas, 2001b; Ngoko *et al*., 2001). The contamination of maize by these fungi and their toxic metabolites has been associated with several human and animal diseases including liver and oesophageal cancer, particularly in Africa (Marasas *et al*., 2001a,b; Gong *et al*., 2004). The extent of harmful effects of infected and contaminated grains on human and animal health has not been investigated in Cameroon.

Given the low incomes of many people in both rural and urban areas of Cameroon, infected grains are usually not excluded from food or feed for animals. In addition to grains, a number of snacks are also consumed in the country. Such snacks include tapioca, groundnuts, date palm and cola nuts mostly sold in kiosks and other road-side markets. Due to poor handling, packaging and storage practices, the snacks are exposed to infection by several microorganisms and contamination by mycotoxins. The objectives of this study were to identify microorganisms that infect maize grains and snacks sold at road side markets in Cameroon, and to sensitize the population on the potential risks to health.

Materials and methods

A survey was carried out in the Center, West and the Northwest provinces of Cameroon in 2002 and 2004. Seven hundred and twenty (720) pieces of colanuts *(C. nitida*), 400 groundnut kernels (*A. hypogea*), 400 date palms (*P. dactyfera*), 400 pieces of dried sweet potatoes (*I. batata*), 25 samples (250 g each) of gari, 100 croucrou (mixed maize and groundnuts paste fried in oil) and 1800 maize kernels were collected from 18 locations and analysed. Investigations were carried out on microbial profiles in all samples while mycotoxins content was done only for the maize samples.

Microbial analyses

Bacteriological analysis: 10 g of each commodity were transferred into 90 ml of ¼ Ringers solution. The contents were homogenized before serially diluting up to 10^{-5}. Aliquots of the serial dilution were spread plated on nutrient agar media. Bacterial isolates were selected randomly from plates containing between 30 and 300 colonies, and identified based on their morphological presentation, gram stain and temperature of growth according to Harrigan and McCance (1976).

Mycological analysis: Small pieces of each sampled product were incubated in Petri dishes containing filter paper dampened with sterile distilled water. The Petri dishes were inspected daily for 14 days to observe fungal growth. The identification of fungi was done according to John Webster (1980), Alexopoulos & Mims (1979), Nelson et al. (1983), Marasas & Van der Westhuizen (1979).

Chemical analyses

Aflatoxin was analysed using Thin Layer Chromatography (TLC) as described by Thomas et al. (1975). The fluorescence of the sample spot was compared with the fluorescence of known standards.

Fumonisin B_1, deoxynivalenol and zearalenone content in the maize samples was analysed as described by Sydenham *et al*. (1996) using polyclonal-antibody (PAb)-based competitive direct enzyme-linked immunosorbent assay (Agri-Screening kit catalogue no. 70/8830, Neogen Corp.; Lansing, MI).

Data analysis: Frequencies of appearance of microorganisms were performed using simple statistical analyses.

Results

All the sampled food commodities were found to be contaminated by microorganisms, some of which are capable of producing mycotoxins. Fungi were the most prevalent microorganisms in the different commodities with occurrences varying from 34 to 100 %. Cola nuts, sweet potato, gari, and date palm were the most infected commodities (table 1). All the samples analysed were contaminated with bacteria with isolation frequencies varying between 25-100%. The most commonly isolated bacteria were *Staphylococcus* sp. (8.0 x 10^3 cfu $g^{-1)}$ and *Salmonella* sp. (9 x 10^6 cfu g^{-1}.) Gari, dry sweet potato and cola nuts were the most infected products.

Fusarium sp., *Penicillium* sp. and *Aspergillus* sp. were the fungi of interest because of the health related effects associated with them. *Rhizopus* sp, was also isolated though it has no major effect on health. *Fusarium* and *Aspergillus* were the most prevalent fungi on the sampled commodities with isolation frequency varying from 20 to 100 % (table 2).

Table1: Occurrence of bacterial and fungal microorganisms in various food commodities in Cameroon.

.Commodities	Bacteria (%)	Fungi (%)
Cola nuts	25	90
Ground nuts	35	34
Croucrou	50	50
Dates palm	25	100
Sweet potato	50	100
Tapioca (gari)	90	100

Chemical analyses

Traces of aflatoxin were detected in only a few locations, corresponding to the low incidence of *Aspergillus flavus* that was observed in the maize samples (data not shown). Fumonisins were detected in 16 of the 18 samples analysed, with high levels of contamination in some locations (table 3).

Table 2: Fungal contaminants associated with selected food commodities in Cameroon.

Commodities	Fungi	Frequency of isolation (%)
Cola nuts	*Fusarium* sp	50
	Penicillium sp	5
	Aspergillus sp	20
Ground nuts	*Fusarium* sp	30
	Penicillium sp	5
	Aspergillus sp	20
Croucrou	*Fusarium* sp	80
	Penicillium sp	10
	Aspergillus sp	25
Dates palm	*Fusarium* sp	80
	Penicillium sp	-
	Aspergillus sp	-
Sweet potato	*Fusarium* sp	100
	Penicellium sp	20
	Aspergillus sp	25
	Rhizopus sp	50
Tapioca (gari)	*Fusarium* sp	62.5
	Penicellium sp	38
	Aspergillus sp	30
Maize grains	*Fusarium* sp	25
	Penicellium sp	5
	Aspergillus sp	25

Discussion

Maize is the staple food in the western highlands of Cameroon. Inappropriate post-harvest practices have led to an increase in the chances of harvested produce becoming infected with *Aspergillus and Fusarium sp*., with favourable seasonal temperature and humidity conditions accelerating the infection of grains by fungi (Dawlatana et al., 2002).

This study found that various products sold as snacks including groundnuts, dry sweet potatoes, colanuts, tapioca, croucrou and date palm are often infected by microorganisms that could produce mycotoxins. Similar observations have been made in some other African countries especially on peanut and maize products (Marasas et al., 2001a). The results further showed that more than 25% of the samples had more mycotoxins than the allowed level for human consumption.

Using better storage facilities, proper screening and regular monitoring for fungal contaminants are some of the actions that could be taken to reduce mycotoxin contamination in food and feed (Mphande et al., 2004). However, most developing countries have limited resources and hence are not able to invest in effective surveillance systems. In this study the mycotoxin content in maize was between 50 to 26000 ng/g, which compares well to findings elsewhere (Desjardins, 2000; Dawlatana et al., 2002).

Food items stored for a long time before distribution are more prone to contamination with mycotoxins, though formation of toxins could also start before produce is harvested and put in storage. Therefore, it is necessary to observe strict pre- and post-harvest practices in order to reduce fungal infection and potential mycotoxin contamination. In some countries there are centres to deal with food safety, working to protect human health and the environment by curbing the proliferation of harmful food production technologies, and by promoting organic and other forms of sustainable agriculture. Unfortunately, in Cameroon, regulations on norms for food and feeds either do not exist or are poorly enforced.

Contamination of food and feed is an important but unrecognized risk to public health and can have long-term health implications (Coulter et al. 1986; Bucci et al. 1990; Rheeder et al., 1992; Abdulrazzaq et al., 2004). Appropriate sanitary measures must be taken to ensure that conditions for growth and toxin production are reduced or eliminated during the handling, transportation packaging and storage of food and feeds. The public should be educated and sensitised on the necessity to observe sound food safety measures. Government should reinforce existing legislation, determine and enforce systems for standards, inspection, certification, monitoring and surveillance as well as management structures for quality control of ready-to-eat snacks, foodstuffs and grains. This requires investments in effective quality control capacity in both facilities and personnel.

Table 3: Occurrence of mycotoxins in maize samples in Cameroon.

Sample location	Mycotoxin level (ng/g)		
	Fumonisins (FB1)	Deoxynivalenol	Zearalenon
Ngat 3	1900	100	<50
Nkometou I	26000	200	<50
Etoud II	6800	nd	nd
Bali	1700	200	<50
Njinikom	1900	100	<50
Bamunka	2000	1300	180

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