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ABILITY OF CASSAVA PRODUCTS TO SUPPORT MYCOTOXIN FORMATION

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Abstract

Fungi were isolated from several cassava products from Côte d'Ivoire, Ghana, Uganda, and Zaire. Of the potentially toxigenic fungi isolated, *Penicillium*, *Fusarium*, and *Aspergillus* were the commonest. Isolates were tested for their ability to produce mycotoxins on sterile rice (a known good substrate) and sterile cassava. Toxins were quantified by high-performance thin-layer chromatography (HPTLC). Most isolates were able to produce at least one toxin. Also determined were mycological and mycotoxin profiles of 26 samples of cassava chips and flours from Côte d'Ivoire and Uganda. Again, *Penicillium* and *Fusarium* were the commonest, and a wide range of mycotoxins were detected, including neosolaniol (8 samples, 0.18-3.11 mg/kg), patulin (7 samples, 0.02-4.20 mg/kg), cyclopiazonic acid (7 samples, 0.11-1.61 mg/kg), penicillic acid (4 samples, 0.07-3.60 mg/kg), and diacetoxyscirpenol (4 samples, 0.45-7.75 mg/kg). Aflatoxin was not detected in any sample.

Introduction

Dried cassava chips and flour are important processed products in sub-Saharan Africa (NRI 1992). Mould growth during the production of such products is common (Clerk and Caurie 1968; Essers and Nout 1989; Jonsyn 1989). The ability of cassava products to support mycotoxin formation as a result of mould growth during poor drying or poor storage is largely unknown.

In this paper, we summarize work undertaken at the Natural Resources Institute (NRI) to determine the ability of cassava to support mycotoxin formation. First, potentially toxigenic fungi were isolated from a variety of African cassava products, and the fungi's ability to produce a range of toxins on rice (a known good substrate), with added nutrients, and on sterile cassava was determined. Several cassava products, collected as part of the Collaborative Study of Cassava in Africa (COSCA), were also analysed for mycotoxins.

Materials and Methods

Isolating and identifying cultivars

The following cassava products were examined: *kokonte* from Volta Region, Ghana (1 sample) and Abidjan, Côte d'Ivoire (2 samples); *makopa* from Bamdundu Region, Zaire (2 samples) and Bas-Zaire (2 samples); *cossette presse* from Zaire (1 sample); *miette presse* from Zaire (1 sample); *miette normale* from Zaire (2 samples); and dried cassava chips from Kampala, Uganda.

Fungi were counted after incubation for 7 days at 25 ^{o}C on Dichloran rose bengal chloramphenicol agar (Unipath), Dichloran 18% glycerol agar (DG18; Unipath), and malt extract agar (Unipath), using standard dilution plating techniques. Each medium contained 200 mg/kg of chloramphenicol to inhibit bacterial growth.

Penicillium spp. were identified according to the Pitt schemes; *Fusarium* spp. to the Brayford scheme; *Aspergillus* spp. to their morphological characteristics.

Growing cultures to determine toxigenicity

Sterile white rice (40% moisture content [MC]), with added nutrients, and irradiated cassava (25 kGy) were prepared according to the method of Westby et al. (1994). Samples were hydrated to 40% MC with sterile distilled water. Substrates were inoculated with spore suspensions (Westby et al. 1994) of the relevant cultures and incubated at 28-30 ^{o}C for 5-7 days (*Aspergillus* spp. and *Penicillium* spp.) in an orbital incubator (120 rpm). Cultures of *Fusarium* spp. were incubated at 25 ^{o}C at constant room temperature for 14 days without agitation.

Examining cassava products for mycotoxin content

Twenty-six cassava products were collected in Côte d'Ivoire and Uganda as part of COSCA, which studies all aspects of cassava production, processing, marketing, and consumption in six African countries (Nweke 1988). The mycological profiles of the samples were determined by using the microbiological methods described above. Fungi were identified to genus level or better according to their morphological characteristics. Toxin analyses of the samples were then carried out on a particular sample when the fungal count for the relevant species was $>10^4$ colony-forming colonies (cfu) per gram (10^3 cfu/g for *A. flavus*).

Extracting and screening cultures for mycotoxins

Aflatoxins were extracted and determined by the method of Westby et al. (1994). Other mycotoxins were extracted with chloroform or acetone and partially cleaned by partition among various solvents and then concentrated. The toxins were then determined semi-quantitatively for screening experiments or quantitatively for examining products, using high-performance thin-layer chromatography (HPTLC) and densitometric measurements. All analyses of mycotoxins in processed products were confirmed. The methods are detailed in Westby et al. (nd).

Results and Discussion

Mycoflora of cassava products

Total mycological counts of cassava samples used to isolate fungi varied from 5.0×10^3 to 4.9×10^8 cfu/g. All the products contained at least one potentially toxigenic species (data not shown). *Fusarium* and *Penicillium* spp. were the most widely distributed genera, but other potentially mycotoxigenic fungi such as *Aspergillus flavus, Aspergillus parasiticus, Aspergillus clavatus*, *Alternaria alternata*, *Cladosporium* spp., and *Wallemia sebi* were also identified. Fourteen isolates of *Penicillium*, 25 isolates of *Fusarium*, and two isolates of *A. clavatus* were identified and screened for their ability to produce mycotoxins. Analyses for toxins were carried out, based on reported abilities (Cole and Cox 1981) of species to produce specific toxins (Table 1).

Analyses of cassava products for mycotoxins

Only two (CI3 and CI12) of the 26 samples of cassava flour or chips did not contain fungi that were potentially mycotoxigenic (i.e., species of *Aspergillus*, *Penicillium*, *Phoma*, or *Alternaria* genera). Of the mycotoxigenic species, the commonest belonged to the genera *Fusarium* (detected in 21 samples, of which 13 had $>10^4$ cfu/g) and *Penicillium* (detected in 13 samples, of which 10 contained $>10^4$ cfu/g) (Table 2). *Aspergillus flavus* was present in six samples, but only three contained $>10^4$ cfu/g. *Aspergillus ochraceous* was present at low levels in two samples, *Alternaria* spp. in one sample (CI2, 5×10^4 cfu/g), and *Phoma sorghina* in one sample (CI18, 5×10^4 cfu/g). No specific toxin analyses were carried out for *A. ochraceous*.

Mycotoxin analyses

Fusarium spp. toxins were detected in 12 of the 13 samples that had counts of $>10^4$ cfu/g (Table 3). Neosolaniol was the commonest toxin present in eight samples at concentrations ranging from 0.20 to 3.11 mg/kg. The following were also detected: diacetoxyscirpenol (four samples, 0.45-7.75 mg/kg), T-2 toxin (1 sample, 1.39 mg/kg), moniliformin (1 sample, 0.11 mg/kg) and fusarenon-X (1 sample, 0.27 mg/kg).

The effects of other *Fusarium* toxins on humans are largely unknown (Hocking 1991), except for the well-documented case of T-2 toxin, the agent for alimentary toxic aleukia (ATA), which caused an estimated 100,000 deaths in the Soviet Union during 1942-1948 (Joffe 1978). Diacetoxyscirpenol, at concentrations ranging from 0.38 to 0.50 mg/kg, has caused hemorrhagic bowel syndrome in swine. Deoxynivalenol, at concentrations between 0.0005 and 7.0 mg/kg of feed, has caused vomiting, feed refusal, and infertility in swine and dogs (Joffe 1986). Moniliformin can be extremely toxic to rats, ducklings, mice, and chicks (Joffe 1986).

Toxins were detectable in 12 of the 13 samples containing *Penicillium* spp. (Table 4). The commonest were patulin (7 samples, 0.02-4.70 mg/kg), cyclopiazonic acid (7 samples, 0.11-1.61 mg/kg), and penicillic acid (4 samples, 0.07-3.60 mg/kg). Other toxins detected were citrinin (2 samples, 0.03 and 0.04 mg/kg), PR toxin (1 sample, 0.21 mg/kg), and secalonic acid D (1 sample, 0.06 mg/kg). In humans, patulin may produce nausea and stomach irritation when administered orally (Scott 1977). Penicillic acid is moderately toxic to mice and guinea pigs (Scott 1977), but its effect on humans has not yet been reported. CPA produces diarrhoea and convulsions in ducks, rats, and chickens (Moreau 1979), and secalonic acid D causes kidney and liver damage in mice. Citrinin is also thought to cause kidney damage after prolonged ingestion (Pitt 1991).

Samples UG1, UG2, and CI5, containing *A. flavus* at levels of $>10^4$ cfu/g, did not contain aflatoxin (limit of detection is 0.01 mg/kg). This supports observations made of pure cultures that few *Aspergillus* isolates can produce toxins on cassava.

Implications of the Data Obtained

Our data demonstrate that a wide range of potentially toxigenic fungi can be isolated from cassava, particularly those of *Fusarium* and *Penicillium* genera. Although the mycotoxins tend to have lower toxicity levels than does aflatoxin B1, some cause for concern still exists.

Because only a small number of samples were examined in this preliminary study, we cannot readily generalize to the probable overall levels of mycotoxin consumption by

communities who eat dried cassava products. Nor can we generalize about the level of risk this consumption may pose. More detailed surveys are needed of the levels of mycotoxins in dried cassava products, together with epidemiological studies to assess the impact on people's health.

Control measures need field testing before being implemented in rural populations. Field infection of roots is perhaps the most difficult to deal with, requiring breeding for resistance to infection or production of mycotoxins. The promotion of improved techniques and practices (e.g., smaller sized chips, fire-assisted drying, or dry-season processing) or the introduction of alternative products that prevent mould growth (e.g., roasted granules or fermented pastes) is needed to combat mould growth associated with poor drying techniques. The adoption of improved storage practices that prevent moisture uptake by dried products and insect infestation should help with problems of poor storage.

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Table 1. Summary of ability of isolates from cassava products to produce various mycotoxins on sterile rice with added nutrients and sterile cassava in pure culture.

Isolate	Isolates examined (no.)	Toxin	No. of isolates producing mycotoxins on:	
			Rice	Cassava
Fusarium spp.				
tricinctum	2	T-2 toxin	1	0
		Diacetoxyscirpenol	1	1
		Zearalenone	1	0
sambucinum	3	Diacetoxyscirpenol	0	0
		Zearalenone	2	1
solani	9	Neosolaniol	5	3
		T-2 toxin	6	3
		Fusarenon X	2	2
oxysporum	5	Zearalenone	4	3
lateritium	2	Diacetoxyscirpenol	0	0
		Neosolaniol	2	2
		Fusarenon X	1	0
		Zearalenone	1	1
moniliforme	1	Moniliformin	1	1
Penicillium spp.				
citrinum	3	Citrinin	3	3
chrysogenum	1	Penicillic acid	0	0
citreonigrum	11	Citrinin	0	0
		Citreoviridin		
oxalicum	2	Secalonic acid D	1	2
griseroseum	3	PR toxin	2	1
		Penicillic acid	2	3
		Cyclopiazonic acid	2	1
bilai	3	PR toxin	3	1
		Penicillic acid	2	1
		Cyclopiazonic acid	2	2

Paecilomyces spp.				
variottii	1	Patulin	0	0
Aspergillus spp.				
clavatus	2	Patulin	2	2
flavus	7	Aflatoxin B1	5	0
		Aflatoxin G1	2	0
parasiticus	1	Aflatoxin B1	1	0
		Aflatoxin G1	1	0

Table 2. Mycological composition of samples of cassava chips and flours from Uganda and Côte d'Ivoire; all counts are log 10 cfu/g.

Sample code	*Fusarium* spp.	*Penicillium*spp.	*A. flavus; A. parasiticus*	*Aspergillusochraceous*	*Alternaria* spp.	*Phoma sorghina*
UG1	4.30	-	4.85	-	-	-
UG2	5.30	-	5.00	-	-	-
UG3	5.40	5.00	-	-	-	-
UG4	3.18	-	-	-	-	-
UG5	2.70	-	-	-	-	-
						-
CII	-	-	3.18	-	-	-
CI2	4.40	-	-	-	4.70	-
CI3	-	-	-	-	-	-
CI4	4.18	6.00	3.30	-	-	-
CI5	5.40	5.40	4.40	2.70	-	-
CI6	5.70	-	-	-	-	-
CI7	3.70	4.30	3.18	-	-	-
CI8	5.48	4.10	-	-	-	5.00
CI9	3.70	-	-	-	-	-
CI10	4.18	4.70	-	-	-	-
CI11	3.40	4.54	-	-	-	-
CI12	-	-	-	-	-	-
CI13	4.00	5.30	-	3.40	-	-
CI14	3.18	-	-	-	-	-
CI15	3.40	-	-	-	-	-
CI16	5.00	4.90	-	-	-	-
CI17	5.00	-	-	-	-	-
CI18	3.70	3.70	-	-	-	-
CI19	-	3.70	-	-	-	-
CI20	4.18	3.40	-	-	-	-
CI21	-	5.00	-	-	-	-

Table 3. Toxins of *Fusarium* species in samples of cassava pieces and flours from Uganda and Côte d'Ivoire; all concentrations are mg/kg, wet wt basis.

Sample code	T-2	DAS[a]	Neosolaniol	Fusarenon-X	Moniliformin
UG1	-[b]	-	-	-	-
UG2	-	-	1.50	-	-
UG3	-	-	2.55	-	-
CI2[c]	-	7.75	3.11	-	-
CI4	-	-	2.80	-	-
CI5	-	-	-	-	-
CI6	1.39	-	1.40	-	-
CI8[d]	-	1.96	0.65	-	-
CI10	-	-	-	-	-
CI13	-	1.10	0.20	-	-
CI16	-	0.45	-	-	-
CI17	-	-	1.87	-	0.11
CI20	-	-	-	0.27	-

a. DAS = diacetexyscirpenol.

b. Lower than limit of detection.

c. Sample CI2 was also analysed for tenuazonic acid and alternariol monomethyl ether; neither toxin was detected.

d. Sample CI8 also contained tenuazonic acid at a concentration of 1.45 mg/kg.

Table 4. *Penicillium* spp. toxins in samples of cassava chips and flours from Uganda and Côte d'Ivoire; all concentrations are in mg/kg on a wet wt basis.

Sample code	Patulin	CPA[a]	Penicillic acid	Citrinin	Secalonic acid D	PR toxin
UG3	4.70	1.61	3.60	-	-	-
CI4	-[b]	0.11	-	-	0.06	-
CI5	0.54	-	0.46	-	-	-
CI7	0.67	0.51	-	0.03	-	-
CI8	0.30	0.35	-	0.04	-	-
CI10	-	-	-	-	-	-
CI11	0.09	-	0.07	-	-	-
CI13	-	-	-	-	-	-
CI16	-	-	0.40	-	-	-
CI18[c]	0.02	0.28	-	-	-	-
CI19	-	0.17	-	-	-	-
CI20	0.11	-	-	-	-	-
CI21	-	0.15	-	-	-	0.21

a. Cyclopiazonic acid.

b. Less than limit of detection.

c. Sample CI18 also contained sterigmatocystin at a concentration of 0.10 mg/kg.