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Formation of aflatoxins by *Aspergillus flavus* and *A. parasiticus* isolates from cassava products

Formation d'aflatoxines par Aspergillus flavus et A. parasiticus *isolés de produits dérivés du manioc*

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- Abstract -

Isolates of *Aspergillus flavus* and *parasiticus* from dried cassava products were tested for their ability to produce aflatoxins in sterile rice with additional nutrients and in sterile cassava chips at 40% moisture content. In rice, 5 out of 7 isolates of *A. flavus* produced significant amounts of aflatoxin B_1 and one isolate of *A. parasiticus* produced large amounts of aflatoxins B_1 and G_1. In sterile cassava, all isolates of *A. flavus* and *A. parasiticus* grew, but none produced aflatoxins. Aflatoxin formation by *A. parasiticus* could be stimulated by the addition of extra nutrients. *A. parasiticus* also produced large amounts of aflatoxins on processed cassava products at 40% moisture content.

- Résumé -

L'aptitude à produire des aflatoxines d'isolats d'*Aspergillus flavus* et d'*A. parasiticus* provenant de produits séchés à base de manioc a été testée sur deux milieux stérilisés constitués, pour le premier, de riz additionné de nutriments et, pour le second, de cossettes de manioc contenant 40% d'eau.

Avec le riz, 5 des 7 isolats d'*A. flavus* ont produit des quantités significatives d'Aflatoxine B_1 et un isolat d'*A. parasiticus* a produit de grandes quantités d'aflatoxines B_1 et G_1. Sur le manioc stérile, tous les isolats *d'A. flavus et d'A. parasiticus* se sont développés mais aucun n'a produit d'aflatoxine. La production d'aflatoxines par *A. parasiticus* a pu être stimulée par l'addition de nutriments. *A. parasiticus* a produit également de grande quantité d'aflatoxines sur des produits transformés dérivés du manioc contenant 40% d'eau.

Introduction

Sun dried pieces and flours processed from cassava are one of the most important classes of processed products in Sub-Saharan Africa. Recent data from the Collaborative Study of Cassava in Africa (COSCA) (Natural Resources Institute, 1992) has shown that, in the six study countries, flours and dry pieces accounted for 45% of the three most important products in the 233 villages surveyed. Drying times are usually long; in COSCA 55% of the flours/dry pieces were dried for between 6 and 10 days (Natural Resources Institute 1992). Mould growth during drying of these products is common (Clerk & Caurie, 1968; Mota & Lourenco, 1974; Essers & Nout, 1989).

Confusion exists in the literature over the ability of cassava to support aflatoxin production by *Aspergillus flavus* or *parasiticus* because scopoletin, a coumarin accumulating naturally in cassava roots after harvest, has a similar R_f value to aflatoxin B_1 on chromatography plates and gives an intense blue fluorescence (Wheatley, 1984). To clarify the situation and to determine whether toxicogenic *Aspergilli spp* were present on cassava products, isolates from several cassava products were tested for their ability to produce aflatoxins in rice (a known good substrate), sterile cassava and three cassava products. Scopoletin contamination was avoided by using bi-directional high performance thin layer chromatography (HPTLC).

Materials and Methods

1. Cultures

Aspergillus flavus (7 isolates) were isolated from samples of makopa and miette normale from Zaire. *Aspergillus parasiticus* (1 isolate) was isolated from a sample of konkonte from the Ivory Coast. *A. parasiticus* IMI 89717 was a known toxicogenic isolate from groundnut.

2. Growth of cultures on rice, cassava and cassava products

White rice (40% moisture content) was prepared according to Shotwell et al. (1966). Modified Czapek solution was added prior to sterilization according to Schroeder (1969). Imported Colombian cassava, preserved by the method described by Centro Internacional de Agricultura Tropical (1989), was peeled, chipped and dried at 50°C for 24 h. This was divided into 30 g amounts (average chip weight 0.47 g) and sterilized by ionising radiation at 25 kGy (Isotron, Swindon). Samples were hydrated to 40% moisture content with sterile distilled

water. When modified Czapek solution (Schroeder, 1969) and 3% sucrose were added to cassava, it was dissolved in an appropriate amount of distilled water and the solution was used to hydrate the cassava to 40% moisture content.

Gari was purchased from a roadside market north of Accra, Ghana. Acid fermented pieces were made from waxed cassava bought locally in the UK. This was prepared by soaking peeled and chopped roots in water for 2 days at 30°C, breaking up the pieces by hand and drying for 2 x 8 h under artificial sunlight (550-600 kJ/m2; 35-45°C). Dry chips were prepared by chopping cassava (3-4 cm length) and sun drying for 6 x 24 h as described for acid fermented pieces. In each case, pieces were stored at 30°C overnight between drying times (except between days 4 and 5 when stored for 2 days at 18°C). Processed products were hydrated to 40% moisture content in the same way as sterile cassava chips.

Inoculum was prepared by adding a 1 cm2 block from a 7 day old sporing culture on Malt Extract Agar (Pitt & Hocking, 1985) to 10 ml of 0.1% sterile agar + 0.01% sodium lauryl sulphate and mixing vigorously. Duplicate 500 ml conical flasks of each growth substrate were inoculated with 1 ml of spore suspension.

Cultures on sterile rice and cassava were incubated in a shaking incubator (120 rpm) at 30°C for 5 days. Cassava products were incubated for 19 days at 30°C without agitation.

3. Extraction and analysis of aflatoxins

Cultures were damped down with chloroform (50 ml) and left to soak for at least 1 h. The mixture was transferred to 1 l blender (Christison Scientific, Gateshead) using chloroform (100 ml), blended at high speed for 90 s and filtered on a Whatman 541 filter paper. The filtrate and filter paper were re-blended with chloroform and filtered. The volume of chloroform was measured and any water present removed. Samples (50-5000 µl) were evaporated to dryness under N_2 at 45°C and dissolved in benzene/acetonitrile (9:1). Extracts were diluted in the same solvent and spotted on a 100x200 mm HPTLC plate (Merck 5547, Poole). Mixed aflatoxin and scopoletin standards in the same solvent were spotted on the same plate. For extracts from cassava, scopoletin was removed by developing the plate in the reverse direction with ether (20 ml) containing about 0.2% water in a vertical metal tank for 6 minutes and cutting off the bottom 16 mm of the plate. Plates were developed using 20 ml chloroform:xylene:acetone (6:3:1) in two stages each of 20 min with 20 min drying period between developments. Spots of aflatoxins were read densitometrically at 365 nm in fluorescent mode and quantified by comparison with standards.

Results and Discussion

All the species of *Aspergillus* grew well on rice. Five of the seven strains of *A. flavus* produced significant amounts of aflatoxin B_1 and two also produced G_1 on rice with additional nutrients (Table 1). *A. parasiticus* C7 produced large amounts of aflatoxins B_1 and G_1 (Table 1).

Although good visible growth of *A. flavus* and *A. parasiticus* was observed on cassava, aflatoxins were not detectable (probable limit of detection 0.2 mg/kg). Sterile cassava, in comparison with rice, therefore represents a poor substrate for aflatoxin formation by pure cultures of toxigenic *A. flavus* and *A. parasiticus* under the conditions used.

Addition of nutrients (modified Czapek solution and sucrose) stimulated aflatoxin formation on cassava by *A. parasiticus* $C7_7$, but aflatoxin formation by isolates of *A. flavus* was not stimulated (Table 2). Amounts of aflatoxins produced by *A. parasiticus* C7 on cassava with added nutrients were far less than those produced on rice under the same growth conditions (cassava: mean total aflatoxin 17.4 mg/kg of which B_1 1.7 mg/kg and G_1 15.7 mg/kg; rice: mean total aflatoxin 672.4 mg/kg of which B_1 163.0 mg/kg and G_1 277.8 mg/kg). Various nutritional factors are known to affect the ability of *Aspergillus* spp. to produce aflatoxins, but

Table 1

Ability of isolates of Aspergillus flavus and parasiticus from processed cassava products to produce aflatoxins on sterile rice plus nutrients, sterile cassava and sterile cassava plus nutrients

Isolate	Rice + nutrients (**)		Cassava		Cassava + nutrients (**)	
	Aflatoxins		Aflatoxins		Aflatoxins	
	B1	G1	B1	G1	B1	G1
A. flavus A38	+	-	-	-	-	-
A. flavus A39	++	-	-	-	-	-
A. flavus A40	-	-	-	-	-	-
A. flavus A41	++	++	-	-	-	-
A. flavus A42	+	+	-	-	-	-
A. flavus A43	-	-	-	-	-	-
A. flavus A44	+	-	-	-	-	-
A. parasiticus C7	+++	+++	-	-	++	++
A. parasiticus MO39	+++	+++	-	-	ND	ND

** (-) less than 0.2 mg/kg ; (+) 0.2-1 mg/kg ; (++) 1-5 mg/kg ; (+++) > 5 mg/kg ; ND not determined*
Results are means of triplicate determinations on duplicate cultures

the mechanisms that trigger cells into aflatoxin formation are not fully understood (Luchese & Harrigan, 1993). Clearly, there are additional nutritional factors that can stimulate aflatoxin formation by *A. parasiticus*s C7 in pure culture on cassava.

In processed cassava products (gari, acid fermented pieces and dry cassava pieces), *A. flavus A39* was unable to produce aflatoxin after incubation for 19 days at 30°C (40% moisture content). *A. parasiticus*s, however, produced significant amount of aflatoxin particularly after incubation for 19 days (Table 2). It can be hypothesized that the additional nutrients for aflatoxin formation were available for *A. parasiticus* in the processed products. Aflatoxin formation by *A. parasiticus* was greatest on gari which could either be because gari contains gelatinised starch or because the cassava was of different variety.

Confusion has existed in the literature over the ability of cassava to support aflatoxin production because of contamination of assays with scopoletin (Wheatley, 1984; Wheatley and Cock, 1985). In this study it has been demonstrated that isolates of *Aspergillus* spp. from cassava can produce aflatoxins on a suitable substrate (rice with additional nutrients). However, in pure culture on sterile cassava their ability to produce aflatoxin is limited in that none of the *A. flavus* isolates produced aflatoxin under the conditions used and production by *A. parasiticus* could only be stimulated by additional nutrients. Natural means of supplying these additional nutrients such as the growth of other microorganisms during processing is a possibility and could account for aflatoxin formation by *A. parasiticus* on processed cassava products. A potential problem therefore could exist with aflatoxin production in cassava products when conditions are suitable for the growth of *Aspergillus* spp. These laboratory experiments give some indication of the potential for aflatoxin formation, but they do not totally reproduce the real field situation and there is a need to screen naturally contaminated processed products with reliable analytical techniques.

Table 2

Aflatoxin production (mg/kg wet weight basis) by Aspergillus flavus and parasiticus on dried cassava products after incubation for 19 days at 40% moisture content

Isolate	Gari		Acid fermented dry pieces		Dry chips	
	Aflatoxins		Aflatoxins		Aflatoxins	
	B1	G1	B1	G1	B1	G1
A. flavus A39	< 0.2	< 0.2	< 0.2	< 0.2	< 0.2	< 0.2
A. parasiticus C7	53.8	194.5	12.8	45.5	5.0	63.3

References

CENTRO IINTERNACIONAL DE AGRICULTURA TROPICAL, 1989 - Conservation of cassava roots in polythene bags (Series 04SC-07.06), CIAT: Cali, Colombia.

CLERK (G.C.), CAURIE (M.), 1968 - Biochemical changes caused by some Aspergillus species in root tuber of cassava (*Manihot esculenta Crantz*). *Tropical Science*, 10 : 149-154.

ESSERS (A.J.A.), NOUT (M.J.R.), 1989) - The safety of dark moulded cassava flour compared to white - a comparison of traditionally dried cassava pieces in North East Mozambique. *Tropical Science*, 29 : 261-268.

LUCHESE (R.H.), HARRIGAN (W.F.), 1993 - Biosynthesis of aflatoxin - the role of nutritional factors. *Journal of Applied Bacteriology*, 74 : 5-14.

MOTA (T.P.), LOURENCO (M.C.), 1974 - Farinha da mandioca de Mocambique. *Agronomica Mocambicana*, 8 : 47-59.

NATURAL RESOURCES INSTITUTE, 1992 - COSCA Phase I *Processing Component.* COSCA Working Paper no. 7. Chatham, United Kingdom.

PITT (J.I.), HOCKING (A.D.), 1985 - *Fungi and Food Spoilage.* Sydney. Academic Press.

SCHROEDER (H.W.), 1969 - Factors influencing the development of aflatoxins in some field crops. *Journal of Stored Products Research*, 5 : 187-192.

WHEATLEY (C.), COCK (J.), 1985 - Methods of aflatoxin analysis with particular reference to cassava samples. *International Journal of Epidemiology*, 14 : 185.

WHEATLEY (C.), 1984 - Aflatoxin in cassava - is it a real problem? *Cassava Newsletter*, 8 : (2) : 14.