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Full Length Research Paper

Mycoflora and mycotoxins in kolanuts during storage

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The mycoflora, levels of afllatoxins and the presence of ochratoxin A and zearalenone in nuts of *Cola acuminata* and *C. nitida* were determined immediately after curing and after 3, 6 and 9 months of storage in leaf-lined baskets. Five field fungi and 11 storage fungi were isolated. *Aspergillus, Penicillium* and *Fusarium* were the predominant genera. None of the target toxins was detected immediately after curing. Increasing quantities (5 to 160 ppb) of each of the aflatoxins B_1, B_2, G_1 and G_2 were recorded as from the 3rd month while zearalenone and ochratoxin A were detected only after the 6th and 9th month, respectively.

Key words: Kolaunts, mycoflora, mycotoxins, storage.

INTRODUCTION

Kolanuts are the cotyledons of some species of *Cola*, a genus of trees belonging to the family Sterculiaceae (Purseglove, 1974). They are a major stimulating masticatory in West Africa. They are used for pharmaceuticals and for flavouring soft drinks and in the preparation of choca-cola and wine (Opeke, 1982).

The cultivation, processing and storage of the nuts are undertaken in the warm, humid rain forest zone where there is high mould infection. However, so highly esteemed are the nuts that fairly mouldy samples are commonly ingested. This is in spite of the numerous toxic metabolies (Bacha et al., 1988, Jimenez et al., 1991) frequently associated with mould-contaminated foods and the consequent risk (Campbell and Stoloff, 1974; Frazier and Westhoff, 1978) posed to their consumers. The present report is on the evaluation of moulds and mycotoxins (aflatoxins, ochratoxin A and zearalenone) in the nuts of 'abata' kola or *Cola acuminata* (P. Beauv.) Schott and Endl. and 'gbanja' kola or *Cola nitida* (Vent.) Schott and Endl. immediately after curing and during storage.

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MATERIALS AND METHODS

Collection and preparation of samples

Fresh kolanuts were obtained immediately after a 5-day curing period from kola merchants in Ago-Iwoye and Sagamu, both in Ogun State and from Ikorodu, Lagos State, Nigeria. Altogether, 15 samples (approx. 6 kg each) were collected for each of *C. acuminata* and *C. nitida*. On the day of collection, 30 nuts were randomly taken from each sample before the remaining nuts were packaged, following a traditional method, inside a basket lined with leaves of *Thaumatococcus daniellii* (Benn.) Benth. The packages (15) were kept inside a clean and well-ventilated room. After 3, 6 and 9 months, 10 nuts were randomly picked from each of three different points (surface, middle, bottom), making a total of 30 nuts from each basket or sample.

Evaluation of mycoflora

During each investigation, 225 of the 450 nuts obtained randomly from the 15 packages were carefully observed with the naked eye for any evidence of mould infection.

Table 1. Incidence[a] of fungi in nuts of *Cola acuminata* (Ca*)* and *C. nitida* (Cn).

Fungi	Storage period (months)							
	0[b]		3		6		9	
	Ca	Cn	Ca	Cn	Ca	Cn	Ca	Cn
Field fungi								
Botryodiplodia theobromae Pat.	24	21	22	21	3	5	0	0
Cladosporium herbarum Link ex Fr.	7	8	5	5	1	0	0	0
Diplodia sp.	4	5	0	2	0	0	0	0
Fusarium moniliforme Sheldon	19	22	13	16	9	10	4	7
F. oxysporum Schlecht ex. Fr.	11	11	8	5	3	2	2	2
Storage fungi								
Aspergillus clavatus Desm.	1	0	4	2	6	5	16	16
A. flavus Link	3	4	44	41	61	52	73	77
A. niger van Tieghem	3	3	52	37	74	69	79	78
A. ochraceus Wilhem	0	0	5	3	7	6	10	12
A. parasiticus Speare	0	0	1	3	2	3	6	6
A. tamarii Kita	1	1	18	14	28	31	29	33
Penicillium digitatum Sacc.	0	0	0	0	2	0	3	1
P. funiculosum Thom	2	1	14	11	21	20	39	34
Penicillium sp.	0	0	0	0	0	1	2	2
Rhizomucor pusillus Lindt Schipper	0	0	3	5	6	7	11	7
Rhizopus arrhizus Fischer	0	0	0	1	3	3	8	9

a. Isolation % (of 400 cotyledons)
b. Immediately after the curing stage

The cotyledons were then separated by hand and the state of the inner surfaces was also noted. Each cotyledon was subsequently cut into four pieces (about 10 by 18 mm each) and surface disinfected with a 2% aqueous solution of sodium hypochlorite for 2 min. This was followed by washing with six changes of sterile distilled water before the four cotyledon-pieces were plated together equispaced on potato dextrose agar (PDA). The latter contained streptomycin sulphate (5 mg/ml) as an antibacterial antibiotic. Plates were incubated at 28°C for 5 to 10 days during which time the fungi that emerged were counted, isolated and identified with the aid of appropriate manuals. The percentage incidence of the fungal isolate per 400 cotyledons was calculated.

Extraction and determination of mycotoxins

The remaining 225 nuts were similarly observed with the naked eye for signs of mould infection. Subsequently, the split cotyledons were dry-milled and mixed thoroughly before three 25-g portions were taken and independently analysed for mycotoxins. Extraction was carried out and the components were separated by 2-dimensional thin layer chromatography (Patterson and Roberts, 1979). Aflatoxins (B_1, B_2, G_1, G_2), ochratoxin A and zearalenone were detected by their fluorescence under ultraviolet (UV) light. These toxins were positively identified by coincidence of migration in several solvent systems with reference compounds (Robb and Norval, (1983). Confirmation of aflatoxins and ochratoxin A was by reaction with dilute sulphuric acid and dilute sodium hydroxide, respectively. Zearalenone was confirmed by ratio of fluorescence under 254 and 365 nm UV light (Blaney et al., 1984). For aflatoxins only, concentrations were determined by visual comparison of fluorescent intensity of sample spots with standard following suitable dilution.

RESULTS AND DISCUSSION

At collection time, only about 2% of the kolanuts had visible mould infection. After 3, 6 and 9 months the proportions had increased to approximately 15, 39 and 88% respectively. This significant increase in mould growth and development during storage necessitates a closer appraisal of the traditional storage technique with the aim of preventing mould deterioration of the nuts.

A total of 5 field fungi and 11 storage fungi were obtained from the nuts investigated. The frequency of isolation of the former and the latter fungi decreased and increased, respectively with storage time (Table 1). Field fungi usually colonize seeds before harvest. Their water activity (a_w) growth optima are above 0.96 (Hudson

Table 2. Aflatoxin contents (μg/kg) and the incidence of ochratoxin A and zearalenone in nuts of *Cola acuminata* (Ca) and *C. nitida* (Cn).

Mycotoxin	Storage period (months)							
	0[a]		3		6		9	
	Ca	Cn	Ca	Cn	Ca	Cn	Ca	Cn
Aflatoxin B_1	-[b]	-	59	52	70	81	110	160
Aflatoxin B_2	-	-	10	17	22	40	31	55
Aflatoxin G_1	-	-	5	8	10	13	17	30
Aflatoxin G_2	-	-	-	5	5	5	15	11
Ochratoxin A	-	-	-	-	-	-	+	+
Zearalenone	-	-	-	-	+	+	+	+

a: Immediately after curing
b:-, not detected; +, detected.

1986), thus, they gradually die during storage (Christensen and Sauer, 1982) due to decrease in a_w of produce caused by loss of moisture. On the whole, *Aspergillus niger, A, flavus, Penicillium funiculosum, Fusarium moniliforme, A, tamarii, Botryodiplodia theobromae, F. oxysporum and A. orchraceus* were the most commonly isolated fungi (Table 1). *Aspergillus*, *Penicillum* and *Fusarium* are well known producers of potent mycotoxins (Bamburg et al., 1969; Jimenez et al., 1991) and they are considered to be the most significant toxigenic moulds at the present time (Chelkowski et al., 1983). Consequently, control of moisture and temperature levels to prevent mould growth and mycotoxin elaboration (Smith and Moss, 1985; Adebajo, 1992) has became mandatory for safe storage of kolanuts and also to avert possible mycotoxic problems in consumers.

Table 2 which is a summary of the results on the mycotoxin assays shows that none of the target toxins was detected in kolanuts immediately after curing. After 3 months of storage, all the four principal aflatoxins (B_1, B_2, G_1, G_2) were recorded, though at varying levels. Of all the known aflatoxins, B_1 and G_1 are the most toxic (Deacon 1980) and this suggests that an increasing risk of aflatoxicosis is posed to the consumers of kolanuts that had been stored under the traditional method for a long period. *A. flavus* has been implicated in the production of only aflatoxins B_1 and B_2 while *A. parasiticus* may produce aflatoxins G_1 and G_2 in addition to B_1 and B_2 (Wicklow and Shotwell, 1983; Dorner et al., 1984). This, coupled with the low incidence of *A. parasiticus* (Table 1) could account for the comparatively higher levels of B_1 and B_2 obtained in the current work.

Zearalenone and ochratoxin A were detected after 6 and 9 months of storage, respectively, although no quantitative assessment was made (Table 2). According to Hesseltine et al. (1972), ochratoxin A, at least with regards to ducklings has a lethal dose (LD_{50}) within the range of toxicity of aflatoxin and it is produced mainly by *A. ochraceus* although other species of *Aspergillus* and *Penicillium uridicatum* may also be involved in its elaboration. Several reports (Bottalico et al., et al., 1985; Richardson et al., 1985; Di-Menna et al., 1991; Jimenez et al., 1991; Ursula et al., 1992; Merino et al., 1993) show that species of *Fusarium* are the chief producers of zearalenone, a toxic metabolite commonly associated with vulvovaginitis in animals especially pigs (Richardson et al., 1985). So it is best that the levels of these toxins in kolanuts and indeed in all foods should be kept as low as possible by controlling fungal growth.

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