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Mycoflora and natural occurrence of aflatoxins and fumonisin B_1 in cassava and yam chips from Benin, West Africa

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Abstract

The presence of fungi, aflatoxins and fumonisin B_1 in cassava and yam chips (during 28 processing and storage) were evaluated during two consecutive seasons in two agroecological zones of Benin (Northern Guinea Savannah, NGS and Sudan Savannah, SS). The Benin samples were assessed for moisture content, fungal infestation and total aflatoxin and fumonisin B_1 contamination. During the two seasons, samples collected from the NGS, had moisture contents ranging from 10.0 to 14.7% in cassava chips and from 11.4 to 15.3% in yam chips. In samples from the SS, moisture content ranged from 10.1 to 14.5% and 11.1 to 14.5% in cassava and yam chips, respectively. *A. flavus* was the predominant fungal species. The maximum cfu/g in cassava and yam chips was 8950 and 6030, respectively. Other fungal species isolated included *P. chrysogenum*, *M. piriformis*, *Phoma sorghina*, *F. verticillioides*, *R. oryzae* and *Nigrospora oryzae*. High performance liquid chromatography analysis of both cassava and yam chips showed no contamination by either aflatoxins or fumonisin B_1.

Keywords: Benin; Cassava; Yam; Chips; Fungi; Aflatoxins; Fumonisin B_1

1. Introduction

Cassava (*Manihot esculanta* Crantz subspecies *esculanta*) and yams (*Discorea* spp) are important starchy root and tuber crops, eaten and used by millions of people in West Africa and parts of Eastern and Central Africa (Amusa et al., 2003). In Benin, cassava 55 and yam are the most important root and tuber crops, with about 4 and 2.5 million tons 56 produced in 2004, respectively (FAO, 2004).

The conservation of cassava and yam is hindered by their highly perishable nature and the roots are easily contaminated by fungi (Wareing et al., 2001) and bacteria (Babajide et al., 2006), or subject to sprouting due to increased metabolic activity (Amusa et al., 2003). In order to minimize postharvest losses, cassava and yams require processing to ensure stability during storage. One way is to process the roots and tubers into dried cassava and yams chips (Diop et al., 1997). Cassava and yam processing into chips is a common traditional activity in Benin during the dry period, especially in the Central and Northern regions of the country (Diop et al., 1997). The chips are then ground into flour used to make common foods such as *lafou* (from cassava chips) and *elubo* (from yam chips) (Diop et al., 1997). Chips are subject to attacks by fungi such as *Aspergillus*, *Fusarium* and *Penicillum* (Wareing et al., 2001; Bassa et al., 2001). Fungal contamination can lead to discolouration of the chips, give rise to a mouldy taste and produce off odours (Gwinner et al., 1996). Some fungal species also produce toxins that can be harmful to animals and humans (Sajise and Ilag, 1987).

Mycotoxin contamination in food products has become a worldwide concern. Although cassava and yams are important dietary staple foods in many countries, not much attention has been given to microbial and toxin contamination of the raw and processed products. The presence of aflatoxins in yam chips from Nigeria (Bankole and Mabekoje, 2004) and Benin (Bassa et al., 2001; Mestres et al., 2004) has been reported. Other

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mycotoxins including sterigmatocystin, patulin, cyclopiazonic acid, penicillic acid and tenuazonic acid have also been detected in cassava chips sampled from Ghana (Wareing et al., 2001). There is the need to assess the extent of fungal and toxin contamination in these products in order to develop methods to minimise and/or eliminate fungal and mycotoxins contamination. This study evaluates the extent of fungal and mycotoxin contamination of cassava and yams chips from two agroecological zones in Benin during two consecutive seasons.

2. Materials and methods

2.1. Agroecological zones and villages

Two countrywide surveys were carried out between 2003 and 2005 in Benin to evaluate the natural occurrence of fungi and mycotoxins (aflatoxins and fumonisins) in cassava and yam chips. Two agroecological zones, previously described by Hell et al. (2000), were chosen: (i) Northern Guinea Savannah (NGS), which lies between latitude 8–11° North and covers parts of the central and north part, and has one rainy season (April to September). The climate of the NGS is characterized by low relative humidity (<70%) most of the year except during the short rainy season between July and September.

Maximum temperatures range between 28 and 35 °C for most of the year. (ii) Sudan Savannah (SS), which lies between latitude 11–12° North, and covers the northern parts of Benin. The SS zone has one rainy season between May and September. The climate is dry with very low average relative humidity of less than 60% during most of the year with high maximum temperatures between 30 and 42 °C, this zone is the limit to the Sahel.

2.2. Survey and sampling procedure

Cassava and yam chips made from all varieties used in Benin were collected from 20 villages (Fig. 1) with 10 villages per agroecological zone. Ten farmers were selected in each village (five farmers for cassava chips and five for yam chips). About 500 g of chips were taken from each farmer at the beginning of storage and after 3 months of storage. The chips were stored by the farmers in various storage structures such as in polyethylene bags, on roof tops (on-spot drying), in traditional granaries or on floor in house. Samples from each village were pooled and ground using a laboratory mortar. The meals obtained after grinding were stored at 4 °C until mycological and mycotoxin analyses.

2.3. Determination of moisture content

Moisture content was determined by heating at 105 °C for 2 h to constant weight (AOAC, 1984).

2.4. Mycological analyses

2.4.1. Fungal enumeration and identification

Fungal genera were enumerated using the dilution plating method. Ground samples (10 g each) were thoroughly mixed with 90 ml of sterile water containing 0.1% peptone water for the 10^{-1} dilution. Further serial dilutions to the 10^{-4} dilutions were made with 0.1% peptone water. Aliquots (1.0 ml) of each dilution were then transferred to Petri dishes containing potato dextrose agar (PDA). The Petri dishes were incubated at 25 °C in alternating 12-h periods of fluorescence light and dark for 5 days (Singh et al., 1991). Colonies developing on plates were counted at the end of the incubation period and recorded as Colony Forming Units per gram (CFU/g) (Bankole and Mabekoje, 2004).

Isolates from PDA were sub-cultured on malt extract agar (MEA) (Oxoid Ltd, Hampshire, UK) for identification. *Fusarium* species were sub-cultured on carnation leaf agar (CLA) for identification. The MEA and CLA plates were incubated at 25 °C for 7 days under alternating 12-h periods of fluorescence light and darkness. Cultures were identified based on macro- and micro-morphology, and on reverse and surface characteristics of colonies. *Aspergillus flavus* and *A. parasiticus* were distinguished from other *Aspergillus* spp. by the bright orange–yellow reverse coloration on *Aspergillus flavus parasiticus* agar (AFPA). Standard texts such as those of Nelson et al. (1983) and Pitt and Hocking (1997) were used in the identification process.

2.5. Mycotoxins analysis

2.5.1. Aflatoxins extraction

Aflatoxins were extracted and analyzed according to the method described by Bankole and Mabekoje (2004) with some modifications. In brief ground samples (10 g) were mixed with 50 ml of methanol/water (85:15 v/v) and blended (PowerGen 125, Germany) for 3 min. The mixture was filtered through Whatman No.1 filter papers and 40 ml of the filtrate was then mixed with 40 ml of 10% sodium chloride. The mixture was transferred to a separator funnel and defatted with 25 ml of *n*-hexane. The hexane layer was discarded and the aqueous layer partitioned with 2×25 ml of chloroform. The chloroform layers were pooled and dried over anhydrous sodium sulphate. The chloroform was then evaporated off on a rotary evaporator (Laborota 4000 WB, Germany) and the residue transferred to an amber vial with 2×0.5 ml of chloroform. The chloroform was then evaporated off under nitrogen and the residue stored at −20 °C until analyzed. The residue was re-dissolved in 200 µl chloroform for TLC and HPLC analysis.

2.5.2. TLC detection of aflatoxins

Thin Layer Chromatography (TLC) as described by Bankole et al. (1996) with some modifications was used as a screening method to identify the positive samples. In brief different volumes (2–10 µl) of sample extracts were applied to pre-coated TLC plates (ALUGRAM®SIL G, Macherey-Nagel Gmbh and CO. KG, Düren, Germany) along with standard aflatoxins mixture (containing aflatoxin B_1 and G_1 at 0.5 µg/ml each and B_2 and G_2 at 0.15 µg/ml each respectively). The plates were developed in chloroform/acetone (9:1, v/v) in glass tanks covered with aluminum foil. After development, the plates were dried and observed under short and long wavelength (254 and 365 nm). Detection limit was 0.25 µg/kg for all the aflatoxins.

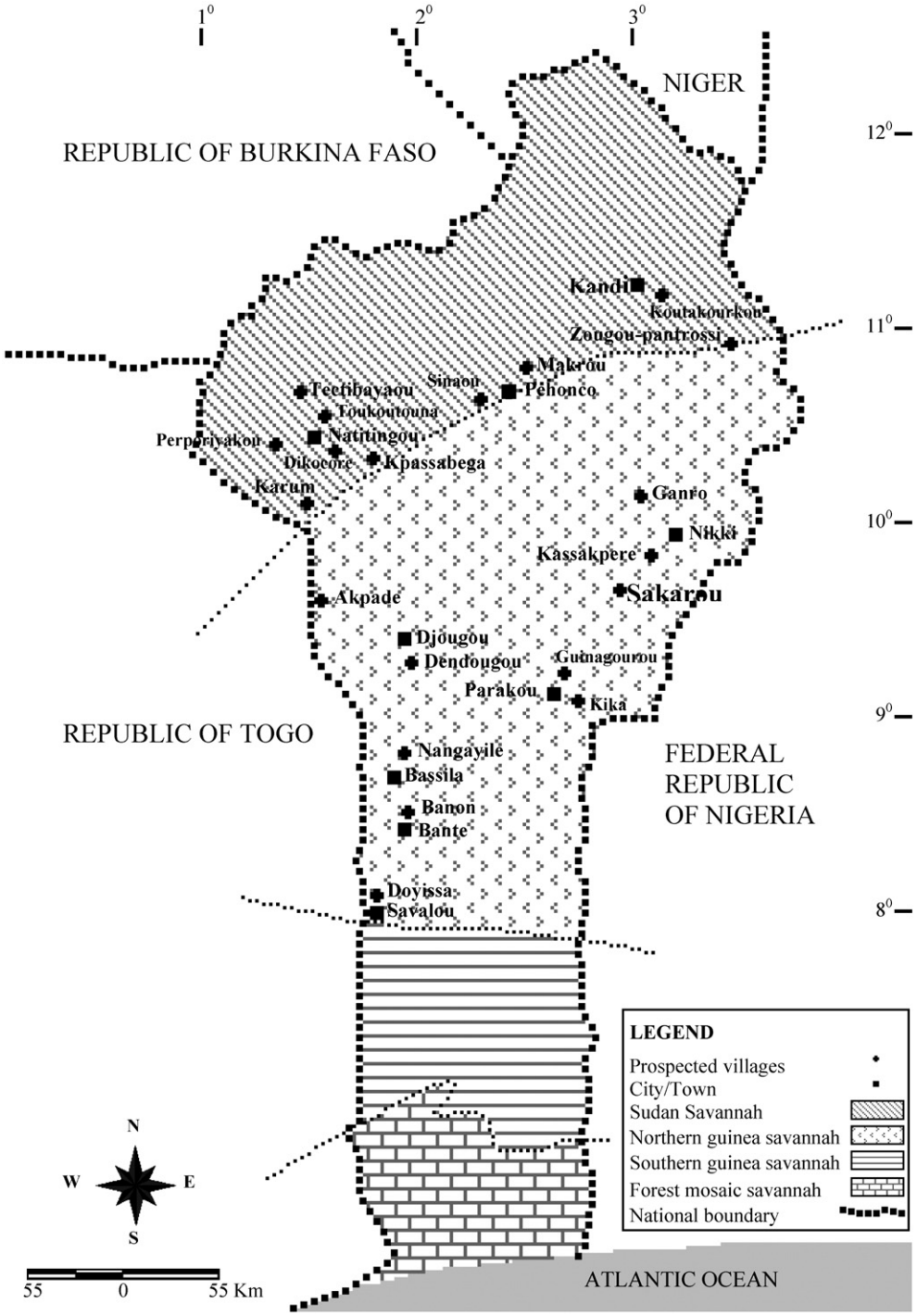

Fig. 1. Map of Benin showing the agroecological zones and the prospected villages (based on Hell et al., 2000).

Suspected aflatoxin positive samples were further analyzed by High Performance Liquid Chromatography (HPLC).

2.5.3. HPLC detection of aflatoxins

HPLC was used to confirm the identity of the aflatoxins and to quantify them. Aflatoxins were derivatized with trifluoroacetic acid (TFA) according to Jaimez et al. (2000) and analyzed with a fluorescence detector. Chloroform from the sample extracts (50 µl) was evaporated off, 200 µl of *n*-hexane and 50 µl TFA added to the residue, and mixed for 30 s on a vortex mixer. The mixture was reconstituted in 1450 µl of ultra pure water (adjusted to pH 4 with hydrochloric acid) to make 1500 µl of the aqueous layer. The top *n*-hexane layer was removed before HPLC analysis. Samples (20 µl) were injected into the Water™ HPLC system with Water™ 474 Scanning fluorescence detector (excitation wavelengh 335 nm; emission wavelengh 440 nm). Separation was carried out on Supelcosil LC-C_{18} column (4.6×150 mm, 3 µm particle size) (Supelco,

Bellefonte, PA, USA). Isocratic elution with methanol:water: acetic acid (45/55/2, v/v/v) was used at a flow rate of 1.0 ml/min. Quantification was by comparing the peak areas with those of the aflatoxin standards (Sigma, St. Louis, MO, USA), using the Apex Chromatography Workstation (Autochrom Inc., Milford, MA, USA). Detection limit was 0.1 μg/kg for all the aflatoxins.

2.5.4. Fumonisin B_1 extraction

Fumonisins were extracted and analyzed according to the method described by Shephard and Sewram (2004) with some modifications. A finely ground sample (10 g) was blended with methanol/water (75:25, v/v, 50 ml) using a PowerGen blender for 1 min. The mixture was filtered using Whatman No.1 filter papers and 10 ml of the filtrate applied to a conditioned SAX solid-phase extraction cartridge (Supelco, Bellefonte, PA, USA). The SAX cartridges (Supelco, Bellefonte, PA, USA) were conditioned by successive application of 5 ml water, 5 ml methanol and 5 ml methanol/water (75:25, v/v) at a flow rate of 1 ml/min. The filtrate (10 ml) was applied to the SAX cartridge and washed with 8 ml of methanol/water (75:25, v/v) followed by 8 ml of methanol. Fumonisin B_1 was then eluted with 14 ml of methanol/glacial acetic acid (99:1, v/v) at a flow rate of 1.0 ml/min. The solvents (14 ml of methanol/glacial acetic acid) were evaporated off on a rotary evaporator and the residue transferred to amber vials using 1 ml of methanol. The solvents were evaporated off under a gentle stream of nitrogen at 60 °C and the dry residue stored at −20 °C until analyzed. The residue was re-dissolved in 200 μl of methanol for HPLC.

2.5.5. HPLC detection of fumonisin B_1

The method by Doko and Visconti (1994) with modifications was used to determine fumonisin B_1 in both cassava and yam chips. Sample extract (50 μl) was derivatized by mixing with 200 μl of *o*-phthaldehyde (OPA) solution. The OPA solution was prepared by dissolving 40 mg OPA in 1 ml of methanol followed by addition of 5 ml of 0.1 M sodium borate solution and 50 μl of 2-mercaptoethanol. The derivatized sample (10 μl) was analyzed by HPLC with a scanning fluorescence detector set at excitation and emission wavelengths of 355 nm and 440 nm, respectively. Separation was carried out using stainless steel Supelcosil LC-C_{18} column (4.×150 mm, 3 μm particle size) at 30 °C. Separation was done using isocratic elution with methanol/0.1 M sodium dihydrogen phosphate (80/20, v/v), (adjusted to pH 3.35 with phosphoric acid). The flow rate was 1 ml/min. Quantification was by comparing peak areas with those of the FB_1 standard (Sigma, St. Louis, MO, USA) using the Apex Chromatography Workstation (Autochrom Inc., Milford, MA, USA). Detection limits was 0.025 μg/kg for fumonisin B_1.

2.5.6. Mycotoxins recoveries

Analytical recoveries were determined in triplicate of both cassava and yam chips samples (10 g each) were place in 250 ml conical flask and spiked with aflatoxin and fumonisin B_1 standards at concentration of 1 μg/kg and 2.5 μg/kg, respectively and left to dry overnight. The samples were then extracted according to the above extraction methods and the recoveries calculated. Mean recoveries of added aflatoxins from both cassava and yam chips were 80% and 100% for fumonisin B_1.

2.6. Statistical analyses

SPSS for Windows version 12.0 (SPSS, Chicago, IL) was used for statistical analyses. A multivariate analysis of variance (MANOVA) was performed with Roy's Largest Root test for interactions between season, agroecological zone and time of cassava or yam chips sampling on observed parameters (fungal and mycotoxin occurrence). A univariate analysis of variance (Student-Newman-Keul's test) was used to compare means of fungal occurrence and incidence and means of total aflatoxins and fumonisins per season in the different agroecological zones and per storage period. Pearson correlation test was also performed to determine relationships among the observed parameters.

3. Results

3.1. Moisture content of stored dried cassava and yams chips in Benin

Overall, the moisture content ranged from 9.2±0.0% to 15.3±0.0%. In all cases, higher moisture contents were recorded in the samples at the beginning of storage (Fig. 2). The moisture content in samples from Northern Guinea Savannah (NGS) ranged from 10.0% to 15.3% during the 2003/2004 season and from 9.5% to 13.6% during the 2004/2005 season. In the Sudan Savannah (SS), moisture content ranged from 10.1% to 14.5% in 2003/2004 and from 9.2% to 13.8% in 2004/2005. The moisture content differed significantly from one zone to another and throughout the storage period ($p<0.01$). In general, the mean moisture contents were significantly higher in yam chips than in cassava chips but decreased significantly after 3 months ($p<0.01$).

3.2. Mycological analyses

The incidence of fungal infection on stored cassava and yam chips are presented in Tables 1 and 2. The toxigenic species isolated were *Aspergillus flavus* Link, *Fusarium verticillioides* (Sacc.) Nirenberg, *Penicillium chrysogenum* Thom and *Phoma sorghina* (Sacc.) Boerema. The most prevalent species on cassava and yam chips were *A. flavus* and *Mucor piriformis*. With respect to cassava chips, during the 2003/2004 season, *A. flavus* was isolated from 60% and 50% of the samples collected from NGS and SS zones, respectively (Table 1). The incidence decreased to 30% in both agroecological zones after three months of storage. *M. piriformis* was isolated from 40% and 60% of the samples collected from the NGS and SS at the beginning of storage, respectively and its incidence rather increased to 60% in the samples from NGS but decreased to 30% in the samples collected from SS after three months of storage. *F. verticillioides* was not detected in samples from both

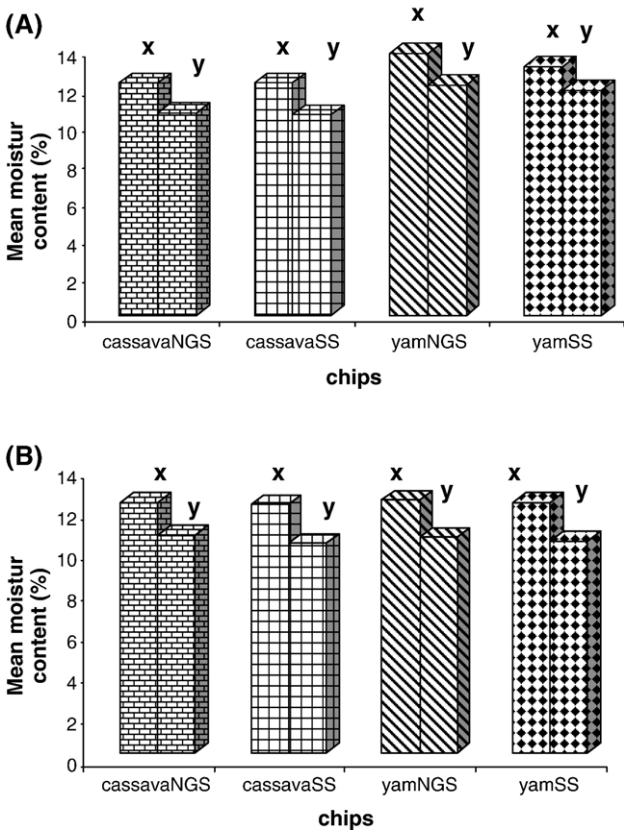

Fig. 2. Moisture content in stored cassava and yam chips during the (A) 2003/2004 and (B) 2004/2005 storage period. *x*=start; *y*=end. CassavaNGS=Cassava chips samples collected from Northern Guinea Savannah, CassavaSS=Cassava chips samples collected from Sudan Savannah, YamNGS=Yam chips collected from Northern Guinea Savannah and YamSS=Yam chips collected from Sudan Savannah.

NGS and SS zones during the 2003/2004 season. Similar trends were observed during the 2004/2005 sampling season with *A. flavus*. *M. Piriformis* was found to be the predominant species and 90% and 100% of the samples were contaminated in the NGS and SS, respectively. The percentage decrease to 30% and 40% at the end of storage in the samples collected from NGS and SS, respectively. *F. verticillioides* was isolated from 10% of the samples in NGS at both start and end of storage period. In the SS, this fungus was isolated in 20% of the samples at the start of storage but none was detected at the end of storage (Table 1).

Regarding yam chips fungal incidence varied significantly within and between agroecological zones, across sampling seasons and with storage period ($p<0.01$). The highest fungal incidences were observed at the beginning of storage in both zones, slightly higher in NGS than the SS (Table 2). During the 2003/2004 season, 80% of the yam samples were infected with *A. flavus* at the beginning of storage in NGS whereas 50% of the samples were infected at the end of storage. In the SS zone, 70% and 20% of the samples were infected with *A. flavus* at the beginning and after three months of storage, respectively. All samples (100%) were infested with *Aspergillus* spp in the NGS while 80% in the SS at the beginning of storage. *A. flavus*, *M. piriformis* and *P. chrysogenum* were the most prevalent fungal species. Similar trends were observed in the 2004/2005 sampling season. *F. verticillioides* was also isolated from 70% and 30% of the samples at the beginning of storage in NGS and

Table 1
Fungal contamination of stored cassava chips during the 2003/2004 and 2004/2005 seasons

Fungal species	Cassava chips [a]			
	2003/2004 season (% incidence)		2004/2005 season (% incidence)	
	Start	End	Start	End
Northern Guinea Savanna (NGS)				
A. flavus	60	30	90	30
Aspergillus. Spp	80	40	20	20
F. verticillioides	0	0	10	10
P.chrysogenum	30	0	30	0
P. sorghina	30	10	10	0
M. piriformis	40	60	70	10
R. oryzae	40	50	10	0
N. oryzae	10	0	0	0
Sudan Savanna (SS)				
A. flavus	50	30	100	40
Aspergillus. Spp	40	20	50	10
F. verticillioides	0	0	20	0
P.chrysogenum	10	0	10	0
P. sorghina	0	10	0	0
M. piriformis	60	30	60	10
R. oryzae	30	10	10	0
N. oryzae	0	0	0	0

[a] The number of samples contaminated (as a percentage of the total) was determined at the beginning ($N_{start}=100$) and after three months of storage ($N_{end}=100$).

Table 2
Fungal contamination of stored yam chips during the 2003/2004 and 2004/2005 seasons

Fungal species	Yam chips [b]			
	2003/2004 season (% incidence)		2004/2005 season (% incidence)	
	Start	End	Start	End
Northern Guinea Savanna (NGS)				
A. flavus	80	50	100	90
Aspergillus. Spp	100	70	70	20
F. verticillioides	10	0	70	0
P.chrysogenum	80	20	80	30
P. sorghina	40	0	10	0
M. piriformis	100	70	90	20
R. oryzae	60	50	30	20
N. oryzae	10	10	30	10
Sudan Savanna (SS)				
A. flavus	70	20	100	80
Aspergillus. Spp	80	30	50	10
F. verticillioides	10	0	30	0
P.chrysogenum	60	20	70	30
P. sorghina	20	0	10	0
M. piriformis	60	60	100	50
R. oryzae	10	30	10	0
N. oryzae	0	30	0	20

[b] The number of samples contaminated (as a percentage of the total) was determined at the beginning ($N_{start}=100$) and after three months of storage ($N_{end}=100$).

SS, respectively. However, the incidence decreased significantly after 3 months of storage (Table 2).

The numbers of isolates (as colony forming units per gram, cfu/g) for each fungal species was compared to the total of fungal species isolates (Tables 3 and 4). In general, the cfu/g in most samples decreased over the storage period. Regarding cassava chips, during the 2003/2004 sampling season, the number of *A. flavus* isolates in NGS decreased from 6030 cfu/g at the start of storage to 210 cfu/g at the end of storage. In the SS samples, a similar decrease was observed in the number of *A. flavus* isolates from 3380 cfu/g to 460 cfu/g at end of storage. However, there was an increase in the level of *M. piriformis*, the number of isolates increasing from 1700 cfu/g at the beginning of storage to 2480 cfu/g at the end of storage in samples from NGS. Overall, the numbers of isolates varied significantly within and between agroecological zones, from one season to another and throughout the storage period ($p<0.01$) for cassava chips. The number of *A. flavus* isolates in cassava chips during the 2004/2005 NGS sampling season decreased from 8950 cfu/g at the start of storage to 600 cfu/g at the end of storage (Table 3). *M. piriformis* isolates also decreased from 3430 cfu/g at the start to 500 cfu/g at the end of storage. Samples with *F. verticillioides*, were also observed during this season and fungal contamination with this species decreased from 60 cfu/g at the start to 20 cfu/g at the end of storage. Similar decreases were observed in *A. flavus*, *M. piriformis* and *F. verticillioides* isolates in the SS zone (Table 3). Significant differences were observed in the number of isolates of fungal species within the zone and throughout the storage period ($p<0.01$) but, no significant differences were observed between agroecological zones ($p>0.05$) in 2004/2005.

Table 3
Major fungal species encountered in stored cassava chips during the 2003/2004 and 2004/2005 seasons

Fungal species	Cassava chips [c]			
	2003/2004 season [CFU/g (% occurrence)]		2004/2005 season [CFU/g (% occurrence)]	
	Start	End	Start	End
Northern Guinea Savanna (NGS)				
A. flavus	6030 (47.8)	210 (4.5)	8950 (66.2)	600 (49.6)
Aspergillus spp	2700 (21.4)	210 (4.5)	160 (1.2)	90 (7.4)
F. verticillioides	0 (0)	0 (0)	60 (0.4)	20 (1.7)
P.chrysogenum	620 (4.9)	0 (0)	170 (1.2)	0 (0)
P. sorghina	780 (6.2)	60 (1.3)	700 (5.2)	0 (0)
M. piriformis	1700 (13.4)	2480 (52.9)	3430 (25.4)	500 (41.3)
R. oryzae	730 (5.8)	1730 (36.8)	50 (0.4)	0 (0)
N. oryzae	60 (0.5)	0 (0)	0 (0)	0 (0)
Sudan Savanna (SS)				
A. flavus	3380 (43.3)	460 (18.1)	4980 (67.9)	440 (86.2)
Aspergillus. spp	2370 (30.3)	360 (14.2)	430 (5.9)	20 (4.0)
F. verticillioides	0 (0)	0 (0)	20 (0.3)	0 (0)
P.chrysogenum	60 (0.7)	0 (0)	30 (0.4)	0 (0)
P. sorghina	0 (0)	120 (4.7)	0 (0)	0 (0)
M. piriformis	1590 (20.4)	1350 (53.2)	1620 (22.1)	50 (9.8)
R. oryzae	410 (5.3)	250 (9.8)	250 (3.4)	0 (0)
N. oryzae	0 (0)	0 (0)	0 (0)	0 (0)

The samples were analyzed at the beginning (start) and after 3 months of storage (end).

[c] The % occurrence was calculated out of the total CFU/g per season, per zone and per time of storage.

Table 4
Major fungal species encountered in stored yam chips during the 2003/2004 and 2004/2005 seasons at the beginning (start) and after 3 months of storage (end)

Fungal species	Yam chips [d]			
	2003/2004 season [CFU/g (% occurrence)]		2004/2005 season [CFU/g (% occurrence)]	
	Start	End	Start	End
Northern Guinea Savanna (NGS)				
A. flavus	6290 (24.2)	770 (9.0)	5980 (54.5)	660 (53.6)
Aspergillus. spp	11,510 (44.4)	580 (6.8)	620 (5.7)	60 (4.9)
F. verticillioides	90 (0.3)	0 (0)	170 (1.5)	0 (0)
P.chrysogenum	3510 (13.5)	1740 (20.4)	930 (8.5)	140 (11.4)
P. sorghina	110 (0.4)	0 (0)	50 (0.5)	0 (0)
M. piriformis	4110 (16.0)	4080 (47.8)	3010 (27.4)	300 (24.4)
R. oryzae	260 (1.0)	1280 (15.0)	130 (1.2)	50 (4.1)
N. oryzae	60 (0.2)	80 (1.0)	80 (0.7)	20 (1.6)
Sudan Savanna (SS)				
A. flavus	2200 (22.8)	520 (7.6)	2470 (38.6)	410 (21.4)
Aspergillus. spp	2840 (29.4)	110 (1.6)	270 (4.2)	70 (3.6)
F. verticillioides	350 (3.6)	0 (0)	80 (1.3)	0 (0)
P.chrysogenum	2050 (21.2)	350 (5.1)	1530 (23.9)	390 (20.3)
P. sorghina	150 (1.5)	0 (0)	10 (0.2)	0 (0)
M. piriformis	1890 (19.5)	4450 (65.2)	1980 (31.0)	980 (51.0)
R. oryzae	180 (2.0)	600 (8.8)	50 (0.8)	0 (0)
N. oryzae	0 (0)	800 (11.7)	0 (0)	70 (3.6)

[d] The % occurrence was calculated out of the total CFU/g per season per zone and per time of storage.

Regarding the yam chips, the number of fungal isolates in samples also varied within and between zones, across the season and throughout the storage period. In all cases, the number of isolates decreased throughout the storage period (Table 4). During the 2003/2004 season, *A. flavus*, *F. verticillioides* and *M. piriformis* isolates decreased from 6290 cfu/g to 770 cfu/g, from 90 cfu/g to 0 cfu/g, and from 4110 cfu/g to 4080 cfu/g in samples collected from NGS, respectively (Table 4). In the samples collected from SS the number *A. flavus* isolates decreased from 2200 cfu/g at the start to 520 cfu/g to the end of storage. As for *F. verticillioides*, the number of isolates decreased from 350 cfu/g at the start to 0 cfu/g at the end of storage. However, there was an increased in the number of isolates of *M. piriformis* from 1890 cfu/g to 4450 cfu/g (Table 4). During the 2004/2005 sampling season, the number of isolates decreased throughout the storage period in all cases (Table 4). Significant differences were observed in the number of isolates within zone and throughout the storage period ($p<0.01$), but no significant differences were observed between agroecological zones ($p>0.05$).

Overall, no significant interactive effects of all factors together such as season, agroecological zone and time of sampling were observed on *A. flavus* and *F. verticillioides* occurrence ($p>0.05$). However, using Roy's Largest Root test, significant interaction between season and time of sampling ($p<0.05$) were observed. *A. flavus* occurrence was positively and significantly correlated with season in cassava chips ($r=0.7$, $p<0.05$) and in yam chips ($r=0.8$, $p<0.05$).

3.3. Mycotoxins analyses: aflatoxins and fumonisin B_1

None of the cassava and yam chips samples collected from the two zones in both seasons i.e. 2003/2004 and 2004/2005 contained detectable amounts of aflatoxin or fumonisin B_1.

4. Discussion

Variations were observed in the moisture content of both cassava and yam chips from one agroecological zone to another and from one growing season to the other. The moisture content of the chips was slightly lower in 2004/2005 than in 2003/2004 season, implying that the 2004/2005 season was much drier than the 2003/2004 season. This was supported by data from the Meteorological Services of Benin which showed seasonal average temperature of 32 °C and relative humidity of 15% for the northern part of Benin in the 2004/2005 season compared to average temperature of 30 °C and relative humidity of 34% for the 2003/2004 (Meteorological data, http://www.wunderground.com/global/stations/65344.html). Low moisture content in stored commodities can result from high temperature (30–42 °C) and low relative humidity (less than 60%) (Hell et al., 2000), conditions that prevailed in the SS agroecological zone during the sampling period. Overall, the majority of the stored dried cassava and yam chips samples had moisture content below 13%, and were therefore safe for storage. During the storage period the moisture content decreased significantly, the amount of reduction depending on the storage structure, with aerated stores having higher rates of reduction (Mestres et al., 2004; Fandohan et al., 2006). Mestres et al. (2004) have observed an increase of moisture content in yam chips stored on the floor in many houses of farmers. In yam chips, the moisture content at the beginning of storage was high (15.3%). This could be due to the processing method that involves soaking yam chips, in water or other fluids such as the supernatant from *ogi* (maize dough screenings and fermented), after parboiling and before sun drying (Fandohan et al., 2005b).

A wide range of fungal species were isolated from the stored cassava and yam chips. These fungal species have been reported to contaminate a wide variety of food commodities including fruits, vegetables and cereals (Jimenez et al., 1993; Michailides and Spotts, 1990; Logrieco et al., 1995; Qaher, 2005; Fandohan et al., 2005a).

The fungal species *A. flavus*, *F. verticillioides*, *P. chrysogenum* and *R. oryzae* that were isolated from yam and cassava chips in this study have been implicated as major causal agents of rots in living, but dormant yam tubers (Amusa et al., 2003), and cassava tubers (Wareing et al., 2001). Being a soil fungus, direct contact between the tubers and soil could be the primary source of contamination by *A. flavus*. It is a common practice in rural Benin not to wash the tubers before peeling. It has been reported that soil adhering to tubers contains many microorganisms that can infect the surface of freshly harvested tubers (Osagie, 1992). On the other hand, the fungi may come from bruised and already contaminated tubers that are used to prepare the chips. Fungal pathogens can enter the substrate through natural wounds in the tubers. The wounds can be caused by insects, nematodes and poor handling before, during and after harvest (Amusa et al., 2003). *Mucor piriformis* and *Rhizopus oryzae* have been implicated in soft rot of fresh tuber (Amusa and Baiyewu, 1999) and hence it is not surprising that these fungi were isolated from the chips as well.

The most important mycotoxigenic fungal species isolated from both cassava and yam chips were *A. flavus*. The levels of contamination of *A. flavus* were higher than the tolerance limit as given by the International Commission on Microbiological Specification for Food (ICMSF). The level of fungal contamination of cassava and yam chips varied from one agroecological zone to another. Influence of climatic factors on the occurrence of toxigenic fungi such as *A. flavus* and *F. verticillioides* have been reported in Benin (Setamou et al., 1997; Hell et al., 2000; Fandohan et al., 2005a).

No aflatoxins or fumonisin B_1 were detected in the surveyed samples despite the presence of *A. Flavus* and *F. verticillioides*. However, other studies have revealed their presence in yam and cassava chips in Benin (Bassa et al., 2001; Mestres et al., 2004), in Nigeria (Bankole and Mabekoje, 2004) and in Ghana (Wareing et al., 2001).

The absence of mycotoxin in this study could be explained by various factors including climatic conditions, nature of the substrate and processing factors. Indeed, low relative humidity, low moisture content (<13%) and high temperatures have been recorded in the regions where chips were produced during the sampling period. Aflatoxins and fumonisins contamination are usually influenced by the nature of the substrate and the presence of competing fungi (Betina, 1989). The diversified mycoflora showed by the isolation of eight distinct fungal genera indicates a competition for available nutrients in both cassava and yam chips. It has been previously pointed out that fungal interaction due to competition could lead to decreased mycotoxin levels (Velluti et al., 2000). The absence of aflatoxins and fumonisin B_1 in both cassava and yam chips produced in Benin may be as a result of interactions between variables which were not fully taken in account in the present study.

Even though yam chips is a rich carbohydrate source for mould growth, the plants involved in parboiling of yam chips may contain active compounds, which may affect fungal physiology, reduce their growth rate and mycotoxin production (Fandohan et al., 2004; Okigbo and Nmeka, 2005). Furthermore, anti-microbial and fungitoxic compounds such as scopoletin have been known to accumulate in roots and tubers as a result of postharvest physiological deterioration. These compounds may affect the growth of some of the fungal and inhibit mycotoxin production (Gomez-Vasquez et al., 2004).

The absence of mycotoxins in the samples was re-assuring but further investigations are required to carry out a survey of cassava and yam chips offered for sale in Benin markets before definitive statements can be made on the safety of the products. Similarly, the performance of different detection methods for determination of mycotoxins on these products should be reviewed. There is a need to test *A. flavus* isolates for their potential to produce aflatoxins in stored cassava and yam chips and to expand microbiological evaluation to coliforms and enterotoxigenic bacteria to assess the potential of other food

safety risks in Benin. Also a complete organoleptic (colour, odor and taste) and physicochemical (crude fat, crude protein, ash and crude fiber) analysis of the resulting flour after insect attacks is needed, since these flours are still being eaten by the rural population. Furthermore, there is a need to test the efficacy of parts of plants (leaves of mango and pawpaw trees and sorghum straw) used in the parboiling process in reducing fungal infection and analyze yam and cassava chips for other mycotoxins such as ochratoxin, cyclopiazonic acid and citrinin, since fungi like *P. chrysogenum* was observed.

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