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Mycoflora associated with processed and stored cassava chips in rural areas of southern Cameroon

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A total of 132 home-stored samples of cassava chips were collected in 45 villages situated in three distinct geographical locations of Southern Cameroon for assessment of their mycoflora. Cassava chips were split into small pieces (10 x 10 x 5 mm) and these were plated onto Petri dishes containing water agar at 25°C after a 7 day incubation period to yield of mycoflora. *Aspergillus*, *Fusarium* and *Penicillium* spp. were the main groups of fungi isolated. 14 *Aspergillus*, nine *Fusarium* and 14 *Penicillium* spp. were identified from a total of 3204 isolates obtained. *Aspergillus* spp. ranked first in prevalence at 57%. *Penicillium* spp. represented only 14% of all isolates. Six stored product-insects in five families and two orders were found infesting some samples. These did not appear to have any significant ($P < 0.05$) relationship with the level of recovery of the fungal taxa detected. Only the moisture content and the location of sample collection had statistically significant ($P < 0.05$) impact on fungal infestation of samples. Diversity indices computed using Simpson's index for all fungal genera and for each location showed that these varied greatly. Across locations, Mbalmayo and Yaoundé were sources of greater species diversity. Among the three fungal groups, *Aspergillus* species yielded the most diverse fungal population. The results of the present study could provide a basis for identifying and selecting specific fungi, especially toxigenic species for which intensive efforts should be directed to assess potential mycotoxin problems occurring alone or in combination on stored cassava products.

Key words: *Aspergillus*, *Fusarium*, *Penicillium*, diversity indices, mycotoxin.

INTRODUCTION

Cassava (*Manihot esculenta* Crantz, *Euphorbiaceae*) is one of the most important food staples of Cameroon where it is grown in association with several other food crops such as maize (*Zea mays* L. *Poaceae*), yams (*Dioscorea* spp. L. *Dioscoreaceae*), and sweet potatoes (*Ipomea batatas* L. *Convolvulaceae*). In almost all the cassava growing communities of Africa, the roots (the most widely edible organs of cassava) are consumed mainly in processed forms.

Cassava roots are low in protein, but very high in carbohydrates. Its leaves have a relatively higher protein, and calcium content (Cock, 1985). Further, roots and

leaves of some cultivars tend to be high in cyanogenic compounds (Essers, 1995). In this respect, research has established that cassava processing could successfully reduce or eliminate the cyanogenic compounds found in both the roots and leaves (Bokanga, 1996; Hahn, 1993). However, some authors have reported the association of several micro-organisms with cassava processing, among them are toxigenic taxa. Westby and Twiddy (1991) documented the spectrum of bacteria contaminating gari, a fermented cassava product widely consumed in Western and Central Africa. Among them were toxin-producing species, especially spore-forming

specimen such as *bacillus* spp. Similarly, Essers (1995) isolated 19 fungal species from on-farm fermented cassava products from Uganda and Mozambique, with toxigenic genera such as *Penicillium* and *Aspergillus* being the most representative.

In Cameroon, surveys recently conducted in rural areas indicated that cassava chips, a cassava product obtained after peeling, fermentation, and drying of fresh roots, were the raw material for the bulk of cassava-based foods in the country (Essono, 2008) as it is the case for the majority of African countries where the crop forms the staple (Ugwu and Ay, 1992). In the frame of these surveys, observations were made that chips could be stored for periods exceeding 180 days prior to consumption. In addition, several processing and storage practices were observed, which could impact the microflora profile of the products.

Previously, a study on the microbial composition of derived cassava products sold in market places of former Zaïre (present day Democratic Republic of Congo) reported the occurrence of toxigenic *Penicillium* and *Aspergillus* spp. from an unspecified number of samples analyzed (Liya et al., 1985). These findings are consistent with preliminary results obtained later by Msikita (1995) and Msikita et al. (1998, 2001), while investigating the species composition of the mycoflora of stored cassava chips sampled in Benin and Nigeria.

Although, the three studies mentioned above provided some useful background information with respect to the species composition of the mycoflora of the chips, no mention was made of the processing and storage practices under which the samples examined were collected. In Cameroon, no comprehensive survey has been carried out on the fungal flora of derived cassava products and especially cassava chips.

In this study, three locations covering 45 villages were surveyed to obtain quantitative and qualitative data on the main fungi associated with cassava chips in storage. Due to their relative toxigenicity, emphasis was placed on *Penicillium*, *Aspergillus* and *Fusarium*. In addition, attempts were made to relate the effects of some intrinsic parameters, such as moisture content and levels of insect infestation on the mycoflora composition associated with the samples collected across the locations investigated. Diversity associated with these three fungal genera was assessed using frequencies of occurrence computed on the basis of total isolation figures associated with each of them in the three locations of sample collection.

MATERIALS AND METHODS

Survey areas and samples collection

Surveys were undertaken at household level from December 2010 to June 2011 in 45 villages in Yaoundé, Mbalmayo and Ebolowa: three locations referred to as blocks and situated in southern Cameroon, where cassava is intensively cultivated and processed, and were previously identified as ecologically distinct (Figure 1)

(Anonymous, 1996).

The Yaoundé (Yao) block lies within latitude 3°45' to 4°26'N and longitudes 11°14' to 11°35'E and covers an area of 5200 km². This block has an annual mean temperature of 22.2°C and a relative humidity of 77.7%. The Mbalmayo (Mba) block stretches from latitudes 3°16' to 3°37'N and longitudes 11°6' to 11°47'E, covering 5120 km². In this block, the average relative humidity is 79% and the annual mean temperature is 23°C. The Ebolowa (Ebo) block covers a surface area of 5150 km² and stretches from the southern boundary of Cameroon to Gabon and Equatorial Guinea, within latitudes 2°20' to 3°5'N and longitudes 11°00' to 11°24'E. The block has an average relative humidity of 83.4% and an annual mean temperature of 24.4°C. In all blocks, the annual rainfall is distributed in a bimodal pattern with the greatest accumulation rate in September-October and April, May, averaging 1654 mm in Yaoundé, 1624 mm in Mbalmayo and 1876 mm in Ebolowa.

These surveys were aimed at collecting production practices information from farmers producing cassava chips and to assess the fungal flora composition of such chips. Within each block, 15 villages were selected on the criteria that socio-economic and biophysical characteristics studies have been carried out therein by scientists of the International Institute of Tropical Agriculture (IITA). Three households were randomly selected along a transect cutting across each of the selected villages. From each household, home-stored samples of cassava chips of any types (balls or pellets) each weighing about 4 kg were hand-collected.

Collection of cassava chips was done from the top to the bottom of the sample package so as to obtain a composite sample. These were sealed in polyethylene bags, labelled, placed in a cooler, and brought to the laboratory for mycological analyses. A total of 42 samples were collected in the Yaoundé block, and 45 samples for each of Mbalmayo and Ebolowa. An aggregate of 132 composite samples were thus obtained. The type of chips produced in each location, the methods implemented for their production, and the storage facilities used for their preservation were recorded and are presented in Table 1.

Preparation of cassava chips samples

In the laboratory, samples of cassava chips were divided into four 1 kg batches. The first batch was kept aside as a backup; the second was directly used to determine the water content. The third was used to estimate the insect population per unit of weight of cassava chips by sieving. The fourth for mycological analyses was preserved in sterile plastic bags at 4°C in a cold chamber. The maximum preservation period was 6 weeks.

Moisture content determination and assessment of insect infestation

At collection period, three 100 g samples were weighed out from each second batch of cassava chips using an analytical balance (Model: Sar CP 225D). Samples were subsequently dried in an oven (Model: Gallenkamp Plus II) at 60°C for 72 h. Moisture contents were hence determined on a weight loss basis.

Insect infestation was assessed by sieving three 100 g samples of cassava chips showing visible holes from the fourth batch with a 2.00 mm mesh sieve. Insects (live and dead adults) were collected in a second sieve of 1.00 mm mesh and numbers were recorded. The insects recovered were shipped to the entomology museum at IITA Cotonou (Plant Health Management Division) for identification.

Isolation and identification of fungal flora from cassava chips

To identify fungal flora, cassava chips arbitrary selected from batch

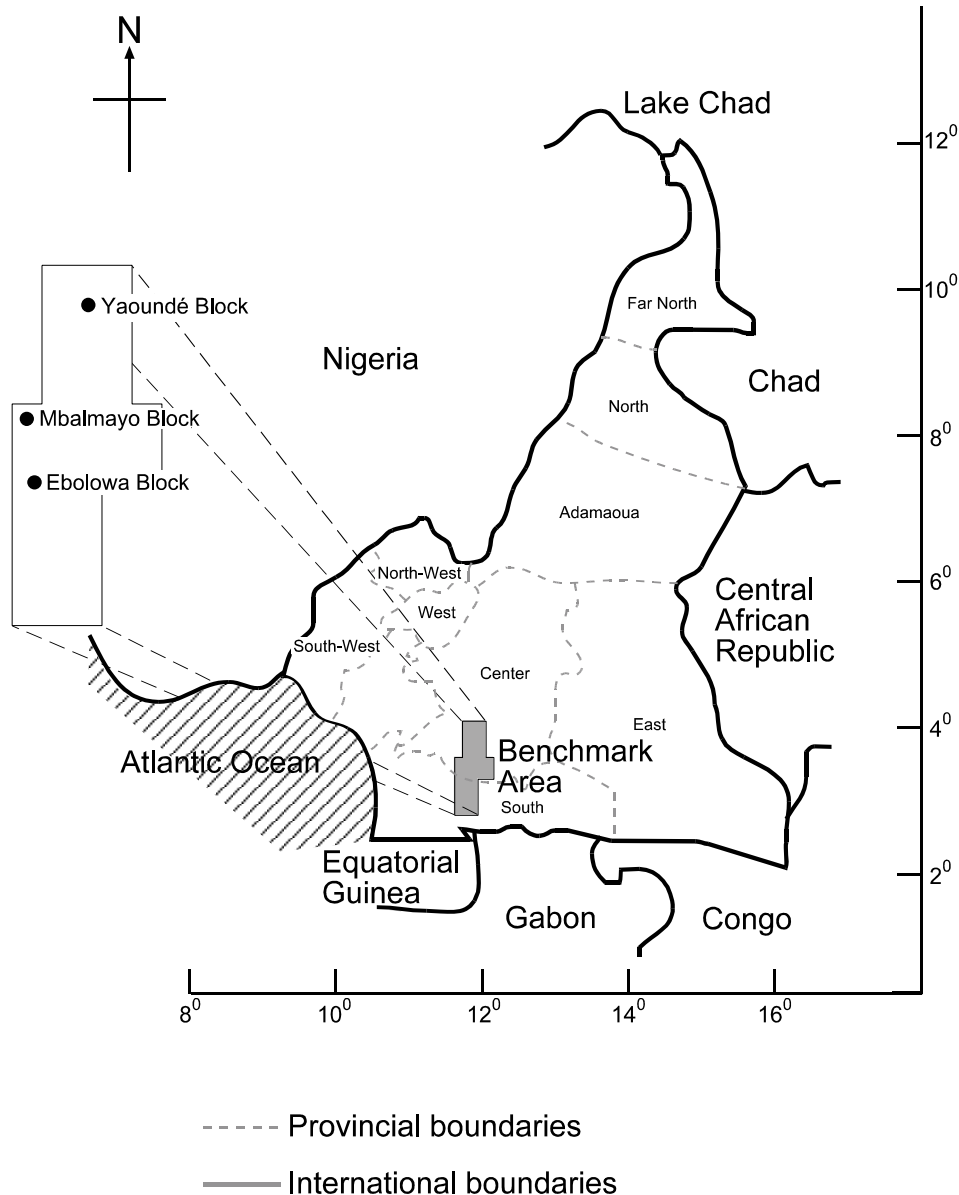


Figure 1. Study site indicating the 3 locations of sample collection.

4 of each sample package were split with a flamed knife into small pieces (10 x 10 x 5 mm) and these were placed in the laminar flow cabinet. Inside the laminar flow, five pieces were removed with sterile forceps and plated in triplicates onto 9 cm diameter Petri dishes (at the center and across two perpendicular diameters of the Petri dishes) containing 20 ml of water agar (BDH Laboratory Supplies) adjusted to pH 4.5 with 0.1 M sulfuric acid to suppress bacterial growth. Preliminary studies (data not shown) carried out on a few samples in the frame of this study using surface sterilization with NaClO as suggested by Msikita (1995), hardly allowed the recovery of fungi from cassava chips' samples even when such samples were observed overgrown with fungi. We attributed this to the lack of a protective shell in cassava chips as is the case for food products such as maize, groundnuts or rice. The presence of a protective barrier is useful in limiting or preventing the sterilizing substance used at appropriated concentration from

diffusing into the inner parts of the analyzed product and killing the internal fungi. In addition, heat sterilization further used did not show any significant differences in the recovery of the fungal species when compared with the non-sterilization method finally adopted in this study. Accordingly, the pieces of cassava chips were not subjected to any prior disinfection. The plates were incubated for seven days at 25°C. Isolated fungi were further sub cultured and purified using single spore cultures onto one-quarter (1/4) strength PDA (Potato Dextrose Agar).

Synoptic keys used in mycology (Raper and Thom, 1949; Raper and Fennel, 1965; Barnet and Hunter, 1972; Nelson et al., 1983; Singh et al., 1991) were employed to identify the fungal isolates recovered from samples. The fungi detected were grouped into similar categories such as *Aspergillus* spp., *Penicillium* spp., *Fusarium* spp., and other miscellaneous fungi. Species initially suspected as belonging to *Penicillium* spp., *Paecilomyces* spp. and

Table 1. Main characteristics of samples of cassava chips collected in three locations.

Location	Chip type	Number of samples ^a	Processing methods ^b	Storage facilities ^c	Storage duration ^d
Yaoundé	Pellets	07	1	Opened containers	< 14 days
Yaoundé	Pellets	05	1	Opened containers	[14-45 days]
Yaoundé	Pellets	01	1	Opened containers	> 60 days
Yaoundé	Pellets	01	2	Closed containers	< 14 days
Yaoundé	Pellets	01	1	Closed containers	< 14 days
Yaoundé	Pellets	02	1	Closed containers	[14-45 days]
Yaoundé	Pellets	03	1	Plastic-jute bags	< 7 days
Yaoundé	Pellets	01	1	Plastic-jute bags	[14-45 days]
Yaoundé	Pellets	10	3	Jute bags	< 14 days
Yaoundé	Pellets	02	1	Jute bags	< 14 days
Yaoundé	Pellets	03	1	Jute bags	[14-45 days]
Yaoundé	Pellets	04	1	Jute bags	> 60 days
Yaoundé	Balls	01	1	Over fireplace	< 14 days
Yaoundé	Balls	01	2	Over fireplace	[14-45 days]
Mbalmayo	Pellets	10	2	Opened containers	< 14 days
Mbalmayo	Pellets	03	3	Opened containers	< 14 days
Mbalmayo	Pellets	07	1	Opened containers	[14-45 days]
Mbalmayo	Pellets	05	3	Opened containers	[14-45 days]
Mbalmayo	Pellets	03	1	Opened containers	> 60 days
Mbalmayo	Pellets	03	1	Closed containers	< 14 days
Mbalmayo	Pellets	02	1	Closed containers	[14-45 days]
Mbalmayo	Pellets	01	3	Closed containers	[14-45 days]
Mbalmayo	Pellets	05	1	Plastic-jute bags	> 60 days
Mbalmayo	Pellets	02	3	Plastic-jute bags	[14-45 days]
Mbalmayo	Pellets	01	1	Jute bags	< 14 days
Mbalmayo	Pellets	01	1	Jute bags	[14-45 days]
Mbalmayo	Pellets	01	1	Jute bags	> 60 days
Mbalmayo	Balls	01	1	Over fireplace	[14-45 days]
Ebolowa	Pellets	07	2	Opened containers	< 14 days
Ebolowa	Pellets	04	3	Opened containers	[14-45 days]
Ebolowa	Pellets	01	1	Closed containers	[14-45 days]
Ebolowa	Pellets	03	1	Jute bags	< 14 days
Ebolowa	Pellets	01	3	Jute bags	< 14 days
Ebolowa	Pellets	04	1	Jute bags	[14-45 days]
Ebolowa	Balls	05	2	Over fireplace	< 14 days
Ebolowa	Balls	11	2	Over fireplace	[14-45 days]
Ebolowa	Balls	05	2	Baskets	< 14 days
Ebolowa	Balls	04	2	Baskets	[14-45 days]

^aTotal number of samples: Yaoundé = 42; Mbalmayo = 45; Ebolowa = 45. ^bProcessing methods: 1: Steeping washed and peeled cassava roots in water for "to 4 days before drying under sunlight for 2 days. 2: steeping washed and peeled cassava roots in hot water for 2 to 3 days before drying over the fireplace for 4 to 6 days. 3: steeping washed and peeled cassava roots in hot water for 2 days using ferments before drying under sunlight for 2 days. ^cStorage facilities: see text for description; ^d Storage duration: as estimated by farmers.

Aspergillus spp. were transferred to PDA, CZ (CzapeckDox Agar) and MEA (Malt Extract Agar) at 25°C for 5 days following Raper and Thom (1949), Raper and Fennell (1965) and Singh et al. (1991). Putative *Fusarium* spp. were later transferred to PDA and MEA at 20 and 25°C for 5 days, according to the synoptic keys of Nelson et al. (1983) and Singh et al. (1991). The other fungal species were cultured on PDA and incubated at 25°C for 5 days for further identifications, according to the general keys of Barnet and Hunter

(1972).

Colony identifications of plates with pure cultures were made under a compound microscope mounted with a photographic apparatus. Pure cultures of *Penicillium* and *Aspergillus* spp. were sent to the Technical University of Denmark for speciation, whereas *Fusarium* spp. were sent to the Program on Mycotoxin and Experimental Carcinogenesis (PROMEC) in South Africa for identification confirmation.

Data analysis

The proportion of pieces of cassava chips within samples contaminated by the fungi was assessed after the cultures had been examined. The total number of samples contaminated (Nsc) by each species and the total number of isolates (Tnil) of individual fungal species recovered per location were recorded. The relative index frequency (RIF) of each fungal species was calculated as the ratio of the number of isolates of individual fungal species obtained from each sample over the total number of all fungal isolates recovered from chips of each replicate, and expressed as percentage. To increase normality, percentage data (X) were transformed to the Arc sin using the function $Y = 180/3.14 \times \text{Arc sin}(X/100)/2$. These were further subjected to analysis of variance using the General Linear Model (GLM) procedure (Gomez and Gomez, 1984; Anonymous, 1997) of SAS (SAS Institute, Inc., Cary, NC, USA, version 9.2). Using moisture content, percentage recovery of individual fungal species in samples and level of insect infestation of samples as class variables, the relationship between the above mentioned parameters and the susceptibility of chips to fungal contamination could hence be assessed.

To determine the locality with the greatest diversity of isolates, diversity indices were calculated for the various species belonging to each group or category of fungi. The diversity index (DI) was calculated using Simpson's index (Simpson, 1949) as follows:

DI = 1 – Simpson's index:

$$\text{With Simpson's index} = \frac{\sum \text{Total number of isolates of a given species within a fungal species}}{\text{Total number of isolates belonging to all fungal genera identified}}$$

It describes both species richness and species equitability. Simpson's index values may range from 1/S to 1, with S being the total number of species recovered. If every isolate belonging to each fungal genus was a different species, Simpson's index would equal 1/S and be very small, resulting in the maximum diversity index. If all isolates were the same species, the index would be equal to 1, resulting in a minimum diversity index.

RESULTS

Mycoflora isolation

Seventy-three (73) fungal species in 34 genera were isolated from the two forms of cassava chips sampled throughout the three research sites. These included saprophytic and toxigenic fungal species as well as presumptive pathogenic species. In order of importance, *Aspergillus*, *Fusarium* and *Penicillium*, were the most common genera of fungi recovered from all samples. Other important species or genera of fungi isolated include *Acremonium* sp., *Armillariamellea* (Vahl & Fr.) Kummer, *Botryodiplodia theobromae* Pat., *Cladosporium* spp., *Colletotricum* spp., *Corticium rolfsii* Curzi, *Curvularia* spp., *Fomes lignosius* (Fr.) Fr., *Geotricum candidum*, *Phytophthora* spp., *Pythium* spp., *Ryzopus nigricans*, *Thamnidium elegans* and *Trichoderma* spp. However, as a result of their involvement in mycotoxicological processes, only *Aspergillus*, *Fusarium*, *Penicillium* sp. are of concern in this paper.

The number of samples contaminated (Nsc), the total

number of fungal isolates (Tnil) obtained for each species, and the percentage (RIF) of each species within each fungal genus are given and presented for each location (Tables 2, 3 and 4).

The genus *Aspergillus* with 1827 isolates was the most important group of fungi isolated (Table 2). It accounted for over 57% of the total fungal population, with the largest number of isolates in Mbalmayo (620) and the lowest in Ebolowa (594) (Table 2, 3 and 4). The *Aspergilli* included 14 species altogether and were most numerous represented by the *Aspergillus flavi* group (35% of all *Aspergillus* isolates), others include *Aspergillus tamarii*, and three other aflatoxin-producing species (*Aspergillus parasiticus*, *Aspergillus nomius* and *A. flavus*).

Within this group, *A. tamarii* was cumulatively isolated from 21 samples in Mbalmayo and Yaoundé, but from only eight samples in Ebolowa. Among members in the aflatoxin-producing species, *A. flavus* was the most commonly encountered species. It represented over 23% of all *Aspergillus* isolates recovered. This species contaminated more samples in Yaoundé (73.8% of samples) than Mbalmayo and Ebolowa. In these two locations, no significant variation in the levels of samples contaminated ($P < 0.05$) by isolates of this species was observed (Table 2). Two additional aflatoxin-producing species, *A. nomius*, and *A. parasiticus* also occurred, but at lower rates (Table 2).

Aspergillus clavatus was the second most important *Aspergillus* species isolated. It was found in all blocks and recovered from 71 out of 132 samples. Its incidence was lower in Yaoundé (75 isolates in 17 samples) and Mbalmayo (93 isolates in 17 samples) than in Ebolowa where 236 isolates were found contaminating 37 samples.

A. niger contaminated over 50% of the sampled chips. Its incidence was more important in Mbalmayo where 131 isolates were obtained in 24 samples. Despite its low level of occurrence in Ebolowa (22 isolates in 4 samples), there was no significant difference ($P < 0.05$) in the incidence of *Aspergillus aculeatus* in Yaoundé (51 isolates in 12 samples) and Mbalmayo (56 isolates in 11 samples). The relative frequency index, the number of isolates and samples contaminated are presented in Table 2 for the remaining *Aspergillus* spp.

The genus *Fusarium*, with 9 species identified was found associated with a total of 929 isolates. With over 32% of the total fungal isolates, *Fusarium oxysporum* was the most commonly isolated species in this group. Its incidence was greater in Yaoundé (more than 100 isolates in 26 samples) and Mbalmayo (more than 100 in 20 samples).

Fusarium semitectum, although not equally abundant in all locations was the second most important *Fusarium* sp. It was isolated in some samples collected from each of the three sites. It accounted for over 22% of the total *Fusarium* isolates, with the greatest expression rate in

Table 2. Occurrence^a and incidence of *Aspergillus* sp. on stored cassava chips.

Species	Yaoundé			Mbalmayo			Ebolowa			Overall		
	Nsc	Tnil	RIF	Nsc	Tnil	RIF	Nsc	Tnil	RIF	Nsc	Tnil	RIF
<i>Aspergillus</i> sp.												
<i>A. aculeatus</i> Liz.	12	51	8.3	11	56	9.0	4	22	3.9	27	129	7.06
<i>A. candidus</i> Link	5	16	2.6	3	16	2.6	3	8	1.4	11	40	2.19
<i>A. clavatus</i> Des.	17	75	12.2	17	93	15.0	37	236	41	71	404	22.1
<i>A. flavipes</i> Th. & Cu.	11	40	6.5	15	62	10.0	3	18	3.2	29	120	6.57
<i>A. flavus</i> Link	31	159	25.5	18	135	21.8	19	127	22	68	421	23.0
<i>A. fumigatus</i> Fres.	16	37	6.0	4	14	2.3	6	13	2.3	26	64	3.50
<i>A. niger</i> Van Thi..	20	77	12.6	24	131	21.1	9	49	8.6	53	257	14.1
<i>A. nomius</i> Kurtz.	11	29	4.7	3	17	2.7	2	2	0.4	16	48	2.63
<i>A. ochraceus</i> Wilh.	12	32	5.2	6	34	5.5	3	13	2.3	21	79	4.32
<i>A. parasiticus</i> Speare	8	19	3.1	2	6	1.0	0	0	0.0	10	25	1.37
<i>A. sydowii</i> Th. & Cu.	3	3	0.5	0	0	0.0	14	52	9.1	17	55	3.01
<i>A. tamarii</i> Kita	21	68	11.1	21	56	9.0	8	32	5.6	50	156	8.54
<i>A. terreus</i> Thom	1	3	0.5	0	0	0.0	0	0	0.0	1	3	0.16
<i>A. versicolor</i> Tir.	2	4	0.7	0	0	0.0	4	22	3.9	6	26	1.42
Total number of isolates		613			620			594			1827	

^a Data were back transformed after analysis of variance; Nsc: Number of samples contaminated; Tnil: Total number of fungal isolates associated with each species per location; RIF: Relative index frequency of the fungus: This parameter was calculated as the ratio of the number of cassava chips pieces with visible contamination by each fungus over the total number of fungal isolates obtained per location for the corresponding fungus: that is 613 isolates in Yaoundé, 620 in Mbalmayo and 594 in Ebolowa.

Table 3. Occurrence^a and incidence of *Fusarium* sp. on stored cassava chips.

Species	Yaoundé			Mbalmayo			Ebolowa			Overall		
	Nsc	Tnil	RIF	Nsc	Tnil	RIF	Nsc	Tnil	RIF	Nsc	Tnil	RIF
<i>Fusarium</i> sp.												
<i>F. chlamydosporum</i> W	2	4	1.44	4	11	4.60	7	28	6.8	13	43	4.63
<i>F. equiseti</i> Sacc.	12	19	6.83	6	18	7.53	12	44	11	30	81	8.72
<i>F. verticillioides</i> Nir.	16	40	14.39	5	28	11.72	12	48	12	33	116	12.49
<i>F. nelsonii</i> Mar. et al.	0	0	0.00	2	7	2.93	3	7	1.7	5	14	1.51
<i>F. oxysporum</i> Schl.	26	105	37.77	20	103	43.10	18	90	22	64	298	32.08
<i>F. sambucinum</i> Fuckel	2	3	1.08	1	6	2.51	10	40	9.7	13	49	5.27
<i>F. semitectum</i> Be. & R	22	63	22.66	5	36	15.06	19	106	26	26	205	22.07
<i>F. solani</i> (Mart.) Sacc.	21	44	15.83	3	23	9.62	12	35	8.5	36	102	10.98
<i>F. stilboides</i> Wollenw.	0	0	0.00	1	7	2.93	1	14	3.4	2	21	2.26
Total number of isolates		278			239			412			929	

^a Data were back transformed after analysis of variance; Nsc: Number of samples contaminated; Tnil: Total number of fungal isolates associated with each species per location; RIF: Relative index frequency of the fungus: This parameter was calculated as the ratio of the number of cassava chips pieces contaminated by each fungus over the total number of isolates obtained per location for the corresponding fungus: that is 278 isolates in Yaoundé, 239 in Mbalmayo and 412 in Ebolowa.

Ebolowa (25.7% of isolates) and the lowest in Mbalmayo (15%).

Fusarium verticillioides (formerly *Fusarium moniliforme*), a *fumonisin*-producing fungus, contaminated 38% of samples in Yaoundé, 11% in Mbalmayo, and almost 27% of samples collected at Ebolowa. This species which formed more than 12% of the total fungal

Fusarium isolates, was the third most important *Fusarium* spp.

Fusarium chlamydosporum, *Fusarium equiseti*, and *Fusarium sambucinum* were also isolated and occurred in all the locations surveyed. *Fusarium nelsonii* and *Fusarium stilboides* were rarely recovered from the samples analyzed. Their incidence was only observed in

Table 4. Occurrence^a and incidence of *Penicillium* sp. on stored cassava chips.

Species	Yaoundé			Mbalmayo			Ebolowa			Overall		
	Nsc	Tnil	RIF	Nsc	Tnil	RIF	Nsc	Tnil	RIF	Nsc	Tnil	RIF
<i>Penicillium</i> sp.												
<i>P. aethiopicum</i> Thom	1	4	3.77	2	4	3.03	0	0	0.0	3	8	1.79
<i>P. brazilianum</i> Thom	0	0	0.00	0	0	0.00	3	9	4.3	3	9	2.01
<i>P. citrinum</i> Thom	25	59	55.66	22	72	54.55	31	104	50.0	78	235	52.46
<i>P. duclauxii</i> Delacroix	5	13	12.3	6	26	20	0	0	0.0	11	39	8.71
<i>P. herquei</i> Bain & Sart	1	2	1.89	3	6	4.55	6	32	15.0	10	40	8.93
<i>P. minioluteum</i> Dierc.	2	3	2.83	3	5	3.79	1	6	2.9	6	14	3.13
<i>P. oxalicum</i> Cu. & Th.	0	0	0.00	1	1	0.76	0	0	0.0	1	1	0.22
<i>P. paneum</i> Boysen	6	12	11.32	2	3	2.27	0	0	0.0	8	15	3.35
<i>P. paxillii</i> Bainier	3	6	5.66	2	3	2.27	1	3	1.4	6	12	2.68
<i>P. piceum</i> Ra. & Fen.	0	0	0.00	1	3	2.27	0	0	0.0	1	3	0.67
<i>P. purpurogenum</i> Th.	4	7	6.60	2	3	2.27	10	14	6.7	16	24	5.36
<i>P. sclerotiorum</i> van Be.	0	0	0.00	0	0	0.00	4	18	8.6	4	18	4.02
<i>P. sumatrense</i> Szilv.	0	0	0.00	2	6	4.55	0	0	0.0	2	6	1.34
<i>P. steckii</i> Zaleski	0	0	0.00	0	0	0.00	11	24	11	11	24	5.36
Total number of isolates		106			132			210			448	

a: Data were back transformed after analysis of variance; Nsc: Number of samples contaminated; Tnil: Total number of fungal isolates associated with each species per location; RIF: Relative index frequency of the fungus: This parameter was calculated as the ratio of the number of cassava chips pieces contaminated by each fungus over the total number of isolates obtained per location for the corresponding fungus: that is 106 isolates in Yaoundé, 132 in Mbalmayo and 210 in Ebolowa.

Mbalmayo and Ebolowa where they contaminated a very low proportion of the samples examined (Table 3).

Within the genus *Penicillium*, 448 isolates representing 14 species were associated with the samples analyzed. Their relative importance varied and Mbalmayo was the location associated with the largest number of *Penicillium* spp. (Table 4).

Penicillium citrinum, *Penicillium herquei*, *Penicillium paneum*, *Penicillium paxillii* and *Penicillium purpurogenum* were found contaminating chips of all locations in some samples. The most commonly isolated species was *P. citrinum*, for which 235 isolates or 52% of all *Penicillium* spp. were found contaminating almost 60% of all samples analyzed. *Penicillium duclauxii* was not found on samples collected from Ebolowa. Isolates of this species along with those of *P. herquei* were hosted by almost an equal number of samples. In this respect, ten samples were associated with the presence of *Penicillium duclauxii*, whereas eleven samples were colonized by isolates of *P. herquei*. An important observation associated with *Penicillium* species occurrence is that *P. herquei* was less abundant where the incidence of *P. duclauxii* was higher (Table 4).

Six other species of *Penicillium* with very low incidence were observed to occur in only one location. These were *Penicillium steckii*, *Penicillium sumatrense*, *Penicillium oxalicum*, *Penicillium sclerotiorum*, *Penicillium piceum* and *Penicillium brazilianum*. The total number of isolates associated with each never exceeded 20, and these could be considered minor components of species

belonging to this genus.

Diversity of fungi isolated and relationships between contamination of chips by fungi and the parameters studied

The diversity indices associated with the fungal groups of concern in this study were computed, and varied between 0.65 and 0.85 depending on locations, and fungal genera (Table 5). The values obtained for each location suggest that Yaoundé and Mbalmayo were sources of greater fungal species diversity. From a generic point of view, *Aspergillus* spp. yielded the largest diversity indices except in Ebolowa. On the other hand, *Fusarium* and *Penicillium* spp. were connected with more diverse populations at Ebolowa, when compared with Yaoundé and Mbalmayo.

Six stored-product insects were found infesting some samples in the course of this study. The taxa detected were *Araecerus fasciculatus* De Geer (Coleoptera: Anthribidae), *Dinoderus bifoveolatus* Wollaston (Coleoptera: Bostrichidae) *Carpophilus dimidiatus* F. (Coleoptera: Nitidulidae) *Carpophilus hemipterus* L. (Coleoptera: Nitidulidae) *Periplaneta americana* L. (Dictyoptera: Blatidae), and *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae). The level of infestation varied between 1 and 20 insects per 100 g of cassava chips in some samples (data not shown). At the same time, the moisture content associated with the

Table 5. Diversity indices associated with *Aspergillus*, *Fusarium* and *Penicillium* spp. isolated from stored cassava chips in three locations of southern Cameroon.

Species	Yaoundé	Mbalmayo	Ebolowa	Total ¹ genus
<i>Aspergillus</i> spp.	0.85	0.77	0.68	0.83
<i>Fusarium</i> spp.	0.76	0.76	0.84	0.79
<i>Penicillium</i> spp.	0.65	0.66	0.70	0.68
Total mycoflora	0.81	0.81	0.78	

Diversity index calculated using Simpson's index. ¹Figures for total represent a separate calculation rather than an average for each location or each fungal genus.

Table 6. Analysis of variance of the relationship between the susceptibility of chips to fungal contamination and the incidence of location of sample collection, chip type, moisture content, levels of insect infestation of individual fungal species.

Source of variation	Degrees of freedom	Means squares	F values	Pr (F)
Location	2	40.0068	3.91	0.0201
Chip type	1	5.747	0.56	0.5702
Moisture content	127	16.549	1.61	0.0001
Insect infestation	5	8.982	0.88	0.5953
Fungal species	35	347.29	33.94	0.0001

samples collected ranged from 4.3 to 29.3% and averaged 12.6% (data not shown). These two parameters (moisture content and level of infestation of samples by insects), along with the type of chips, the incidence of individual fungal species, and the location of sample collection were assessed for their effect on the susceptibility of cassava chips for fungal contamination.

According to information summarized in Table 6, the level of insect infestation, and the type of chips had no significant effect on the level of contamination of cassava chips by fungi ($P < 0.05$). The moisture content ($P < 0.0001$), the locations of sample collection, and the species of fungi ($P < 0.0001$) led to significant different results. This shows that variations in moisture content values and possible differences in environmental factors across the locations surveyed allowed the contamination of cassava chips by different species of fungi.

DISCUSSION

Many fungi have been reported as contaminants of stored food products worldwide. Accordingly, the association of 73 fungal species with stored cassava chips was not unexpected. Our study however provides the first documentation on the distribution and frequency of occurrence of these deteriorogens on stored cassava chips in 45 villages of southern Cameroon. The results obtained suggest that species in the genera *Aspergillus*, *Fusarium* and *Penicillium* are fungi commonly associated

with stored cassava chips.

Among fungi in the genus *Fusarium*, *F. stilboides* and *F. nelsonii* were inconsistently isolated from chips. These species were reported as commonly associated with plant debris and soil samples throughout the world (Marasas et al., 1998). The rest of the *Fusarium* spp. formed part of the mycoflora recovered from transformed cassava products during similar surveys in Benin and Nigeria (Msikita et al., 2001). In this study, *Fusarium* spp. were present in all blocks surveyed but not equally abundant. *F. oxysporum* formed the greatest proportion of this complex. Its incidence increased as that of other *Fusarium* spp. decreased. Similarly, *F. verticillioides* was isolated with highest frequencies where the incidence of *F. solani*, and *F. equiseti* was less important (Table 3). Available arguments accounting for these configurations between species are somewhat limited. However, this could tentatively be explained by a possible competition between species (Kommedhal et al., 1979; Essono, 2008).

In all respects, *Aspergillus* and *Penicillium* spp. obviously constituted the predominant species of the mycoflora associated with cassava chips in storage. These represented over 71% of the species belonging to all three fungal genera. The present results are consistent with data on wheat obtained by Pehlalte (1968), on cotton seeds by Mazen et al. (1990), and on tiger nuts by Bankole and Esegbe (1996) who reported these two genera to be the major components of the mycoflora of stored food products during similar investigations.

In a monographic study, Frisvad and Samson (1991) reported species in the genus *Penicillium* to be more common in temperate areas, and mainly associated with cereal-based foods in storage. Evidence for this ecological specialization was not ascertained from the present study. Instead, besides the frequent isolation of *P. citrinum*, known for its high prevalence in subtropical and tropical zones (Raper and Thom, 1949), thirteen additional *Penicillium* spp. were recovered from our samples and from all the locations investigated, indicating that *Penicillium* spp. might have a distribution not only in colder ecologies, but also in warm tropical zones and other food products. Their inconsistent recovery when compared with *Aspergillus* species, may be because species in this genus do not grow well at the high temperatures that usually characterize tropical areas (Hussaini et al., 2009).

The recovery of *A. flavus* in 68 samples from 36 out of 45 villages surveyed suggests that this fungus is a common contaminant of stored cassava chips. The mean incidence associated with its level of occurrence in the three locations, however, varied greatly. Climatic factors alone such as temperature, rainfall, and relative humidity as suggested in some studies (Doster and Michailides, 1994; Viquez et al., 1994) were not found adequate to explain the fluctuations observed in the incidence of this species among the locations surveyed. In this study, the incidence of *A. flavus* varied in an inverse relationship with high rainfall and temperatures across the benchmark, according to general data associated with these climatic parameters in the area investigated (Anonymous, 1996).

This relationship was reflected by the high percentage contamination means of the fungus in Yaoundé samples where mean values of these climatic factors were low as compared to Ebolowa. It is highly probable that processing practices and storage systems in use in those locations were responsible for the observed variations. To this effect, cassava balls from Ebolowa, hung above a fireplace, were associated with the lowest number of isolates (Table 2). Accordingly, it could be postulated that the temperatures prevailing under those storage facilities in which balls are preserved were presumably higher than the 25°C optimum observed for growth of *A. flavus* (Essonon et al., 2007). This would have prevented this species from developing intensively in samples originating from Ebolowa which were preferably stored under such conditions.

The diversity indices associated with the three fungal genera dealt with in this study greatly varied both between fungal genera, and locations. The high degree of variation observed in their values among fungal groups could be attributed to differences in practices implemented during chips production, or more importantly, to the mode of dispersal and survival of species associated with the fungal genera of concern in this study. The similarity in species diversity observed between locations

in Mbalmayo and Yaoundé may be because these two areas share the same phytogeographical characteristics (Anonymous, 1996). In addition, surveys aimed at collecting information related to processing and constraints aspects of cassava transformation showed that farmers in Yaoundé and Mbalmayo processed and stored cassava chips in the same way, different from that in Ebolowa (Essonon et al., 2008).

The main objectives of this study were to survey and determine the mycoflora complex associated with stored cassava chips traditionally produced in southern Cameroon by rural farmers, and to note the prevalence of fungi in the genera *Aspergillus*, *Fusarium*, and *Penicillium*. The results obtained showed that toxigenic *Aspergillus* spp. were the most prevalent fungi contaminating stored cassava chips. They accounted for over 57% of the total fungal isolates belonging to the three genera of concern in this study. From the present results, there is clear evidence that colonization of cassava chips by all the toxigenic fungi recovered from the samples analysed may have serious consequences for human and animal health as a result of their contamination by their respective mycotoxins. This contamination is reported to be most acute and widespread in the warm and humid areas of Africa, Asia and Latin America, where adequate conditions exist for their subsequent development (CAST, 2003; Williams et al., 2004). As the rate and degree of mycotoxin contamination are often believed to be dependent on a number of factors among which are the moisture content and storage conditions such as damage by insects (Turner et al, 2005), farmers should be trained on how to avoid insect spoilage of stored cassava products used as food or feed so as to limit their infestation by toxigenic fungi. During household surveys, visible insect damages were observed and mainly characterized by holes created on cassava chips. It could therefore be hypothesized that propagules' transmission of mycotoxin-producing fungi is likely, since insect damages are often viewed as access routes for toxigenic fungi penetration in food and feed products while in storage (Hell et al., 2008).

In all respects, because the most taxa detected in the present study are known for their potential capability to produce mycotoxins, the results obtained may provide guidance for better orienting and targeting mycotoxin research on stored cassava products.

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