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Full Length Research Paper

Variety on cassava chips in storage in rural areas of southern Nigeria: their association with storage stage, humidity content and dispensation methods

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A survey was carried out to monitor during a two -month period the incidence of *Aspergillus* in samples of stored cassava chips traditionally produced in southern Nigeria**. Seventy- two samples associated with two forms of chips (cassava balls and cassava pellets) were collected in two locations (Ojuitim and Obubra) and 13 *Aspergillus* species were isolated. In both locations, *Aspergillus versicolor* was seldom isolated, whereas *A. flavus* and *A. clavatus* were most frequently isolated. The level of recovery of isolates obtained was not affected by location and form of chips, but by the duration of storage (P < 0.01) and the moisture content (P < 0.05) . Five core species were identified, which formed more than 70% of the total isolates associated with the samples analyzed. These were *A. clavatus*, *A. flavus*, *A. fumigatus*, *A. niger* and *A. ochraceous*. Correlation coefficients computed between pairs of these species based on total isolation figures for the two locations showed that some were significantly associated. *A. clavatus*, *A. niger* and *A. ochraceous* were positively related to one another in a significant way. Similarly, significant correlations, positive or negative, were observed between the moisture content and all core *Aspergillus* species. The larger number of these toxigenic fungi isolated raises concerns on the potential of stored cassava products as a natural substrate liable to mycotoxin formation.**

Key words: Natural substrate, Variety, Nigeria, Cassava chips and . *Aspergillus versicolor.*

INTRODUCTION

Post-harvest spoilage by filamentous fungi (mould) is one of the most important threats associated with processed and stored food products worldwide. Discoloration, quality deterioration, reduction in commercial value and mycotoxin production has been linked to mouldy contaminated foods (Moreau, 1968; Christensen and Kaufman, 1969). This situation is made worse in the tropics where the warm and humid climates provide these micro organisms with favourable conditions for their spread and subsequent establishment in numerous subs-trates. In sub-Saharan Africa, the phenomenon could be of great concern, especially in areas where food short-ages have compelled people to consume low grade food material, even if moulds are visible as contaminants.

Three genera, *Fusarium*, *Penicillium* and *Aspergillus*, all potential mycotoxin producers, could be considered the most significant toxigenic fungi growing in processed and stored foods. Due to their capability to develop in a wide range of environmental conditions, fungi in the genus *Aspergillus* are comparatively more widespread than others (Chelkowski, 1991). Consequently, special care is to be devoted to them, especially as they could play an important role in food decay and mycotoxin formation under certain storage conditions.

There are many accounts of *Aspergillus* occurring in processed and stored agricultural commodities. Abdel-Gawad and Zohri (1993) and Mazen et al. (1990) documented the spectrum and levels of *Aspergillus* growing on nuts and cotton seeds-based products. The presence and incidence of *Aspergillus* spp. in cereal-based foods, milks, oils and peanuts are also well known (Manabe and Tsuruta, 1991; Adebajo, 1993).

Cassava chips, a derived cassava product, are very popular in Africa where it forms the raw material for the bulk of cassava-based foods (Ugwu and Ay, 1992). The cassava processing cycle leading to their production has several steps, which include fermentation, drying and storage. At each of these steps, contamination by fungi may occur. To date, only tangential references have been made on the incidence of fungal flora in a limited number of derived cassava products (Hahn, 1989; Nout and Essers, 1989; Essers, 1995) and no detailed study asses-

Table 1. Incidence and distribution[a] of *Aspergillus* species in two selected locations of **Ojuitim** and **Obubra**

	Location of sampling									
	Ojuitim					Obubra				
Species	**Nsc**	**Ncpc**	**Rif (%)**	**Pif[1] (%)**	**mean[1] (%)**	**Nsc**	**Ncpc**	**Rif (%)**	**Pif[1] (%)**	**mean[1] (%)**
A. aculeatus	5	20	3.15	13.89	3.70	8	28	4.41	22.22	5.19
A. candidus	14	65	10.24	38.89	12.04	4	9	1.42	11.11	1.67
A. clavatus	31	227	35.75	86.11	42.04	29	198	31.18	80.56	36.67
A. flavipes	1	3	0.47	2.78	0.56	2	6	0.94	5.56	1.11
A. flavus	25	133	20.94	69.44	24.63	28	134	21.10	77.78	24.81
A. fumigates	14	34	5.35	38.89	6.3	12	35	5.51	33.33	6.48
A. niger	18	71	11.18	50.00	13.15	26	121	19.06	72.22	22.41
A. nomius	1	3	0.47	2.78	0.56	14	55	8.66	38.89	10.19
A. ochraceous	9	36	5.67	25.00	6.67	11	38	5.98	30.56	7.04
A. parasiticus	3	8	1.26	8.33	1.48	7	13	2.05	19.44	2.41
A. tamari	9	30	4.72	25.00	5.56	4	11	1.73	11.11	2.04
A. terreus	3	5	0.79	8.33	0.93	11	30	4.72	30.56	5.56
A. versicolor	0	0	0.00	0.00	0.00	1	5	0.79	2.78	0.93

Legend a: data were back transformed after analysis of variance; Nsc = number of samples contaminated; Ncpc = number of cassava chips pieces contaminated by each fungus; Rif = relative index frequency. This parameter was calculated as the ratio of the number of cassava chips pieces infected by each fungus over the total number of isolates obtained per location for the corresponding fungus. That is 635 isolates in Ojuitim and 683 in Obubra; Pif = presence index of the fungus. This parameter was calculated as the ratio of the total number of samples contaminated by each fungus over the total number of samples examined per location that is 36 samples; Mean: the mean percentage was calculated as the ratio of the number of cassava chips pieces infected by each fungus over the total number of pieces of cassava chips submitted to analysis in each location that is 540 pieces ; 1 = samples and pieces of cassava chips were usually infected by multiple species, and thus the sum of individual values associated with mean and presence index parameters in this Table was always in excess of 100%.

sing the contamination of cassava chips by *Aspergillus* spp. has been conducted. The present study is aimed at documenting the spectrum of *Aspergillus* spp. growing on cassava chips in storage in two distinct locations of southern Nigeria, as a function of moisture content, duration of storage and processing methods.

MATERIALS AND METHODS

Study site and sample collection

In 1998, a survey aimed at collecting processing practices and constraints-related information in the production of cassava chips was conducted in 45 villages of southern Nigeria located in the forest margin benchmark area of the National Root Crops Reseach Institute, (NRCRI). During the course of this survey, different processing methods resulting in different forms of chips were observed, hence suggesting probable differences in the microbial profiles of the transformed produce. Within the areas investigated, two villages were identified as producing the greatest quantity of cassava chips., in the zone of **Ojuitim**, specialized in the production of cassava balls;, in the zone of Obubra, mainly produced chips from dried and broken pulp. In both zones, the annual rainfall is distributed in a bimodal pattern with the greatest accumulation in September-October and April-May, averaging 1876 mm at **Ojuitim** and 1654 mm at Obubra.

In 1999, the two villages were revisited from June to August during a second survey to collect samples of cassava chips and to monitor their mycofloral composition and dynamics. In each village, six farmers were randomly selected for us by an (NRCRI) village technician. The farmers were requested to produce cassava chips following their normal routine with a minimum of instructions. From each selected farmer, a 3 kg sample of stored chips was obtained by harvesting from the top, the middle and the bottom of the sample package, kept in Jute bag or over the fireplace. These were mixed so as to obtain a composite sample. Collection of composite sam-ples was done at 7 day intervals during the first 4 weeks of storage, then once every 2 weeks from the fourth to the eighth week of storage. In both locations, 72 samples were obtained and transpor-ted to the laboratory for mycological analysis.

Laboratory measures

In the laboratory, samples of cassava chips were subdivided into three batches. The first batch, weighing 2 kg, was kept aside and preserved in a cold chamber at 4°C. The second batch (500 g) was used for water content determination and the third (500 g) for mycological analyses. Water content analysis was carried out on the day of collection. The mycoflora analysis could also be carried out on the day of collection depending on the quantity of samples to be screened, but generally,

Table 2. Pattern of variation in the incidence[a] of *Aspergillus* species detected from stored cassava chips in two locations of **Ojuitim**) and Obubra during a two-month monitoring period.

Location of sampling												
	Ojuitim						Obubra					
Species	Sampling period (week after storage)						Sampling period (week after storage)					
	1	2	3	4	6	8	1	2	3	4	6	8
A. aculeatus	0.0	0.0	0.0	0.0	11.1	25.9	0 .0	0 .0	0 .0	3.7	13.0	35.2
A. candidus	29.6	33.3	29.6	24.1	3.7	0.0	0 .0	0 .0	3.7	3.7	3.7	5.6
A. clavatus	29.6	48.1	92.6	88.9	83.3	77.8	16.7	44.4	75.9	72.2	81.5	75.9
A. flavipes	0.0	0.0	0.0	0.0	5.6	0.0	7.4	3.7	0 .0	0 .0	0 .0	0 .0
A. flavus	38.9	61.1	48.1	46.3	31.5	20.4	7.4	25.9	55.6	72.2	53.7	33.3
A. fumigates	20.4	5.6	16.7	11.1	5.6	3.7	11.1	3.7	14.8	9.3	13.0	13.0
A. niger	0.0	0.0	13.0	31.5	48.1	38.9	38.9	38.9	44.4	50.0	29.6	22.2
A. nomius	0.0	0.0	0.0	0.0	0.0	5.6	0 .0	0 .0	13.0	27.8	35.2	25.9
A. ochraceous	0.0	0.0	3.7	9.3	24.1	29.6	5.6	7.4	0,0	3.7	22.2	31.5
A.parasitcus	0.0	3.7	9.3	1.9	0.0	0.0	0 .0	0 .0	3.7	1.9	7.4	11.1
A. tamari	0.0	7.4	14.8	16.7	7.4	9.3	0 .0	5.6	0 .0	0 .0	7.4	7.4
A. terreus	0.0	0.0	1.9	1.9	0.0	5.6	0 .0	14.8	7.4	14.8	14.8	3.7
A. versicolor	0.0	0.0	0.0	0.0	0.0	0.0	0 .0	9.3	0 .0	0 .0	0 .0	0 .0

Legend a. the incidence was calculated as the percentage of pieces of cassava chips showing visible fungal growth with the corresponding species. This percentage was calculated, based on a total of 90 pieces of cassava chips plated onto culture media at each sampling occasion and at each location.

these were preserved in sterile plastic bags at 4°C in a cold chamber for further analysis. The maximum preservation period was three days.

Water content determination

At collection, three 100 g samples were weighed out from each of the second batches of cassava chips and dried in an oven (Model: Gallenkamp Plus II, Sanyo Gallenkamp, Leicestershire, UK) at 60°C for 72 h to determine their moisture content on the basis of weight loss according to Lazzari (1994), using the following formula: MC = [(Wi – Wf)/Wi] × 100
Where MC = Moisture content; Wi = Initial weight and Wf = Final weight

Mycoflora remoteness

Under sterile conditions, five pieces of cassava chips from batch 3 at each sampling occasion were plated onto 9 cm diameter 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, and incubated for 7 days at 25□ C. Since the study dealt with human nutrition, the plated chips were not subjected to any prior disinfection. Three replicates were used for each sample. Emphasis was placed on the presence or absence of *Aspergillus* spp. These were identified after purification using single spore cultures on full strength Potato Dextrose Agar (PDA) according to the procedure of Singh et al. (1991), and Raper and Fennel (1965). Some isolates were sent to the Technical University of Denmark for identification and confirmation.

prevalence of variety in the samples examined

The incidence of *Aspergillus* spp. was assessed using the presence index and relative frequency criteria. The presence index is defined here as the percentage of samples within which a given species was found at least once. The relative frequency is related to the number of times a given species was observed to occur in the samples analyzed. Their values were obtained according to Foko (1987), using the following formulae:
Pif = Nsc/Tnse and Rif = Ncpc/Tnil.
Where Pif = Presence index of the fungus; Nsc = Number of samples contaminated; Tnse = Total number of samples examined; and Rif = Relative index of the fungus; Ncpc = Number of cassava chips pieces contaminated by each fungus; Tnil = Total number of isolates obtained per location.

numerical examination

Table 3. Variation in moisture content (MC) over time of 72 samples of stored cassava chips collected from 12 farmers in two selected villages of **Ojuitim** (Nkometou III) and Obubra

Farmers Number	Type of chips	Sampling period (week after storage)					
		1	**2**	**3**	**4**	**6**	**8**
1	Dbp	4.83	7.95	9.11	11.79	10.37	8.28
2	Dbp	8.62	10.52	11.03	11.51	10.23	9.46
3	Dbp	5.88	7.56	7.89	9.05	8.61	9.66
4	Dbp	5.07	8.88	12	10.62	9.88	9.1
5	Dbp	7.68	10.75	10.99	9.69	8.73	8.04
6	Dbp	5.36	7.27	8.3	8.65	8.42	8.19
Mean		**6.24**	**8.82**	**9.89**	**10.22**	**9.37**	**8.79**
7	Balls	14.96	12.66	12.82	9.02	8.62	6.2
8	Balls	14.3	6.49	6.69	5.96	10.87	6.08
9	Balls	18.14	9.5	8.68	7.34	9.42	7.13
10	Balls	31.17	22.39	11.84	10.77	9.05	11.13
11	Balls	28.1	23.23	9.7	8.43	9.44	4.5
12	Balls	46.3	32.15	21.44	18.54	14.77	9.16
Mean		**25.5**	**17.74**	**11.86**	**10.01**	**10.37**	**7.37**

Legend: Dbp = dried and broken pulp; MC = Moisture content.

Table 4. Pearson correlation coefficients for the relationship between core *Aspergillus* species, the moisture content and the duration of storage of cassava chips.

	A. clavatus	***A. flavus***	***A. fumigatus***	***A. niger***	***A. ochraceous***	**Moisture Content**	**Storage Duration**
A. clavatus	-						
	0.098	-					
A. flavus	(**0.1521**)						
	–0.019	0.0248	-				
A. fumigates	(**0.772**)	(**0.717**)					
	0.164	–0.0279	0.043	-			
A. niger	(**0.0159**)	(**0.683**)	(**0.52**)				
	0.195	–0.0889	0.0796	0.265	-		
A. ochraceous	(**0.0039**)	(**0.1928**)	(**0.244**)	(**0.0001**)			
Moisture	–0.209	0.218	0.125	–0.248	–0.184	-	
Content	(**0.0019**)	(**0.0013**)	(**0.066**)	(**0.0002**)	(**0.0068**)		
Storage	0.338	–0.0299	–0.068	0.327	0.464	–0.33	-
Duration	(**0.0001**)	(**0.6614**)	(**0.3183**)	(**0.0001**)	(**0.0001**)	(**0.0001**)	

Figures in regular characters represent correlation coefficients whereas those in parentheses and in bold specify the level of significance.

The mean percentage of recovery of each fungus was calculated as the ratio of the number of cassava chips contaminated by each fungus over the total number of cassava chips submitted to analysis.

To increase normality, percentage data (X) were transformed to the Arc sin using the function $Y = 180/3.14 \times \text{Arc sin } (X/100)^{1/2}$ (Gomez and Gomez, 1984). The resulting data were subjected to analysis of variance. When variation in the dependent variable could not be explained by the main effects, interactions between independent variables (moisture content, location of collection, storage duration) were assessed to verify to what extent the dependent variable was influenced by two or several other independent variables.

Relationships between experimental variables (moisture content, duration of storage, percentage recovery of *Aspergillus* spp., types of chips, or location of sampling) were established by computation of Pearson correlation coefficients (Gomez and Gomez, 1984). Analyses were performed using the Statistical Analysis System software (SAS Institute Incorporation,

Cary, USA, 1996).

RESULTS

Mycoflora examination

A total of 1318 *Aspergillus* isolates in 13 species were obtained from the 72 samples of cassava chips analyzed. The species detected comprised *Aspergillus aculeatus* Lizuku, *A. candidus* Link, *A. clavatus* Desmazieres, *A. flavipes* (Bain. and Sart.) Thom and Curch, *A. flavus* Link, *A. fumigatus* Fresenius, *A. niger* Van Thieghem, *A. Nomius* Kurtzman, *A. ochraceous* Wilhelm, *A. parasiticus* Speare, *A. tamarii* Kita, *A. terreus* Thom, and *A. Versicolor* (Vuill.) Tiraboschi.

prevalence of Variety in the samples examined

With the exception of *A. versicolor*, which occurred only in one sample in the Yaoundé zone, the rest of the spe-cies were found in more than one sample in all locations surveyed. In general, more than one *Aspergillus* spp. could be isolated from the same sample and sometimes from the same piece of cassava chip sub-mitted to analysis.

The predominant *Aspergillus* isolated from the overall samples collected were *A. clavatus*, *A. flavus* and *A. niger* (Table 1). *A. clavatus*, which occurred in 31 out of 36 samples collected at Ebolowa, was the most widely distri-buted species. Comparable figures were observed from samples collected at Yaoundé (Table 1).

Among *Aspergillus* isolates in section *Flavi, A. flavus*, *A. nomius*, *A. tamarii* and *A. parasiticus* were recovered. In this section, *A. tamarii* was not the predominant fungus, but was more frequently isolated from cassava balls collected at Obaghie (25% of samples) than from dried and broken pulp forms of chips produced at **Ojuitim** (11.1% of samples). Within the same section, three aflatoxin-producing fungi, *A. flavus*, *A. nomius* and *A. parasiticus*, were recovered at different frequencies from the samples screened. *A. flavus* dominated the mycoflora in this group of fungi. This species contami-nated over 69% of samples in Obubra and 77.8% in **Ojuitim**
Percentage recovery of isolates of this species from pieces of chips analyzed in Obahetin was 24.8%, similar to levels found in Obubra (24.6%). The incidence of *A. nomius* and *A. parasiticus* was lower in samples and pieces of chips examined. The percentage contamination of samples by *A. nomius* was 38.9% in **Ojuitim** and 2.8% in Obubra. Similarly, *A. parasiticus* contaminated more samples in Obahetin (19.4%) than in Obubra (8.3%) (Table 1).

The dynamics of *Aspergillus* spp. was studied by assessing the pattern of variation in their incidence over time during a two-month monitoring period. Weekly and bi-weekly samples from each location, obtained as previously specified, were plotted against the level of occurrence of individual fungal species from the pieces of cassava chips. After a 7 day storage period, the two locations shared three *Aspergillus* spp. (Table 2). At the end of the observational period (eighth week), 9 out of 13 species were common to both zones. In many cases, certain species were isolated often up to week 3 or 4 of storage following sampling, and then decreased by the end of the observational period. Representative examples are illustrated by *A. clavatus, A. flavus* and *A. niger* in both locations. In other examples, the level of recovery of some fungi increased over the monitoring period. Such patterns of variations were observed for *A. aculeatus* and *A. ochraceous* for all locations (Table 2).

association between the humidity content, the storage duration and the prevalence of remote

During the collection period, the moisture content of balls, dried and stored over the fireplace, was high during the first week of storage. Mean moisture content observed per sample varied between 14.3 and 46.3%, and averaged 25.5% for the samples collected at **Ojuitim** (Table 3). As the storage period was extended, the moisture content decreased and reached values ranging between 4.5 and 11% at the end of the monitoring period (eighth week). Unlike cassava balls, the moisture content of broken pulp forms of chips dried under sunlight was low during the first week of storage (Table 3). Mean moisture contents ranging from 4.8 to 8.6% were obtained in some samples. Increases in their values were observed as cassava chips were stored for prolonged periods (eight weeks).

The analysis of variance on the species detected showed that their level of recovery varied significantly with the duration of storage ($F_{5,\ 2603} = 17.26$; $P < 0.0001$) and the moisture content ($F_{184,\ 2603} = 1.16$; $P < 0.08$), indicating that the variation observed in their incidence occurred as a result of changes in the parameters mentioned above. Conversely, the location of sampling or the type of chips did not bear any significant relationship with the level of recovery of the species isolated ($F_{1,\ 2603} = 2.00$; $P < 0.1576$). This shows that the two forms of chips collected, though location-related, were not associated with any particular *Aspergillus* spp.

We defined a core species based on its occurrence in an arbitrary 6% of pieces of cassava chips submitted to analysis in both locations. When all the samples from the two locations were combined and examined, a total of five core species were found associated with the samples studied: *A. clavatus*, *A. flavus*, *A. fumigatus*, *A. niger* and
A. ochraceous. These species accounted for 78.9% of the total *Aspergillus* spp. isolates detected in Ebolowa and 76.9% in Yaoundé. Correlation coefficients computed between pairs of these species based on total isolation figures for the two locations showed that the fungi iden-tified were significantly associated. In this respect, *A. clavatus* was positively and significantly associated with *niger* and *A. ochraceous*

(Table 4). Positive and significant associations were also found between *A. niger* and *ochraceous*. Similarly, there were positive and signi- ficant correlations between the level of contamination of pieces of cassava chips by *A. flavus* and *A. fumigatus*, and the moisture content. This parameter was negatively related with the rest of core species. Additionally, positive and significant correlations were observed to exist bet-ween pieces of cassava chips contaminated by *A. clava-tus*, *A. niger* and *A. ochraceous*, and the duration of sto-rage (Table 4). This shows that the incidence of these various species was important, as the produce was sto-red for prolonged periods of time.

DISCUSSION

The 13 species of *Aspergillus* mentioned in this paper have been reported in other food commodities elsewhere. Mazen et al. (1990) isolated 12 species of *Aspergillus* from cotton seeds and cotton seeds products in Egypt, eight of which are species detected in our samples. Manabe and Tsuruta (1991) isolated 17 *Aspergillus* spp. from stored rice grains, including 10 associated with our results. Their recovery from the samples examined in this study suggests that stored cassava products are suitable substrates for the growth and development of *Aspergillus* spp. However, the present results differ from those repor-teds from cassava chips-producing communities else-where. In previous studies, Liya et al. (1985), using the dilution plating method, reported the occurrence of six *Aspergillus* spp. from an unspecified number of cassava chip samples collected from different market places of Kisangani (Democratic Republic of Congo) . Likewise, Msikita (unpublished data, 1995) reported the occurrence of *Aspergillus* spp. from 23 samples of cassava chips obtained from different locations of Cross River Nigeria, but did not identify the isolates to species level. Essers (1995) recovered four different *Aspergillus* spp. from 10 samples of fermented cassava crumbs collected in Ugan-da and Mozambique, but the conditions of isolation and sample collection were not specified. Differences in the number of samples analyzed may be one of the factors related to this deviation in results, but it is also possible that differences in methods of isolation had a greater influence Preliminary experiments (data not shown) carried out on a few samples during the course of this study, using surface sterilization with NaClO, as suggested by Msikita (1995), hardly allowed the recovery of mycoflora from samples of cassava chips even when such samples were observed overgrown with fungi. We attributed this to the lack of a protective shell in cassava chips, unlike foods such as maize, groundnuts and rice. The presence of a protective barrier is useful in limiting or preventing the sterilizing substance, used at appropriate concentra-tion, from diffusing into the inner parts of the analyzed product and killing the internal mycoflora. In addition, heat sterilization, using the fire flame method, did not show any significant differences in the recovery of the fungal species when compared to the non-sterilization system finally adopted in this study. Sauer and Burroughs (1986) argued that such a method (absence of sterili-zation) is likely to bring about some overestimation in the actual internal composition of the microflora population when portions of studied samples are to be plated onto culture media for analyses. However, since it is known from reports that mycotoxin formation in food products contaminated by toxigenic fungi requires a minimum of 48 h (Christensen and Meronuck, 1986), it was assumed that cassava chips might have been contaminated with toxigenic fungi and/or their related toxin during the drying period, often lasting at least two days.

During the course of this study, 12 out of the 13 *Aspergillus* species detected were found contaminating cassava chips in both locations. However, it was observed that at the collection period, all the species detected did not occur in the samples examined. In this respect, only *A. flavus*, *A. clavatus* and *A. fumigatus* were found contaminating cassava chips at each sampling occasion in both locations (Table 2). These three species, along with *A. niger*, were each isolated from more than 36% of the samples examined, and could therefore be considered common contaminants of stored cassava chips. A study recently carried out by Qaher (2005) similarly highlighted *A. flavus* , *A. niger* and *A. fumigatus* as the common *Aspergillus* spp. associated with dried spices imported into the Kingdom of Bahrain. Consequently, a probable close relationship between these three fungal taxa cannot be excluded.

However, there were very few significant associations between the most frequently isolated *Aspergillus* spp. In this study, only *A. clavatus*, *A. niger* and *A. ochraceous* were positively related to each other in a significant way. Although Bothast et al. (1976) reported that propagule counts of *A. niger* closely paralleled those of *A. flavus* in maize samples, such patterns of association were not evident with the isolates of these two species in the samples studied. Instead, although not significant, *A. flavus* was negatively related with *A. niger* (Table 4). During a study related with surveys of 238 maize samples collected at harvest in North Carolina, Hesseltine et al. (1981) indicated that *A. niger* was rarely recovered from samples heavily colonized by *A. flavus* . Consequently, the significant correlations obtained in this study should not be regarded as fungal interactions, but, more likely, as the influence of processing and/or storage conditions. In this respect, it was observed that higher percentages of *A. flavus* , *A. clavatus* and *A. fumigatus* were isolated from chips in ball form than from dried and broken pulp form during the first two weeks of storage. It is therefore probable that the values obtained are due to the high moisture content associated with the ball form of chips.

Since most of the fungi start to proliferate at moisture

contents greater than 12% (Christensen and Meronuck, 1986), the moisture contents of cassava balls dried and stored over the fire place and which varied between 4.5 and 46.3%, were sometimes in the range conducive to fu -ngal development. The moisture content of broken pulp forms of chips dried under sunlight ranged from 4.8 to 11.8%. Although low, these values did not prevent fungi from contaminating this form of chips. The present results seem to indicate that lower or upper moisture limits do not seem to exist that might restrict or enhance the growth of fungal flora in stored cassava chips. Accordingly, it could be inferred that the incidence of *Aspergillus* recovered from the samples examined can hardly be explained as a function of the moisture content parameter alone. In similar investigations dealing with stored tubers of *Cyperus esculentus*, Adebajo (1993) reported that the incidence of toxigenic fungi recovered from the samples he examined increased with time in storage. Similar results were obtained in the present study, suggesting that the duration of storage should also be viewed as an important parameter, allowing the spread and subsequent establishment of fungi in stored food commodities.

In general, all the *Aspergillus* spp. referred to in this study are common and distributed in nature worldwide, and have been isolated in a wide array of substrates (Kozakiewicz, 1994). However, the growing interest attached to moulds in the genus *Aspergillus* on cassava is of special concern mainly because of the increasing role of this staple in the diet of an estimated 400 million people in sub-Saharan Africa. Also, mycotoxins arising as a result of the presence of these toxigenic moulds may already be present after the processing of fresh cassava tubers and storage of the resulting products.

A. candidus and *A. flavipes* seem to be rare species associated with this study. Toxicological information about them is still subject to controversy. The production of their respective mycotoxins, candidulin and flavipucin has been demonstrated only in laboratory conditions, and not in natural food substrates (Frisvad and Samson, 1991). Conversely, the aflatoxins, the most widely regulated mycotoxins produced by *A. flavus*, *A. parasiticus* and *A. nomius* (Doster and Michailides, 1994; Moss, 1995) are mutagenic and carcinogenic, and have been implicated in poor resistance to diseases and increased disease susceptibility (Hui et al., 1994). These three aflatoxin-producing species, all of which were recovered from the samples examined in this study, share with *A. tamarii* a common potential capability to produce the toxins cyclopiazonic acid and kojic acid; this is also produced by *A. clavatus* (Frisvad and Samson, 1991). In addition, Flannigan et al. (1984) stated that *A. clavatus* is a causal agent of malt worker's lung syndrome, an intrinsic allergic *alveolitis*, which has been found endemic among malt workers in Europe. Previously, Moreau and Moreau (1960) cited *A. clavatus* as the cause of hepatic degeneration and fatal tremorgenic diseases in cattle. *A. niger*, consistently recovered from our samples, can produce the toxin naphtalo-□-pyrones, which has been reported to cause deaths in ducklings given feed decayed by this species (Mojtahedi et al., 1979). *Aspergillus ochraceus* , a common ochratoxin a-producer, is known to be harmful to the liver and kidneys and to cause nephropathy, enteritis and immunosuppressive symptoms in human and animal systems (Frisvad and Samson, 1991). *A. versicolor*, although inconsistently isolated from the samples examined, produces sterigmatocystin, a mycotoxin closely related to the aflatoxins (Chelkowski, 1991). Considering these, it might be correct to hypothesise that several toxigenic fungal metabolite mixtures could be present at the same time on transformed and stored cassava pro-ducts used as foods or feeds. Accordingly, this would indicate that further research on mycotoxicological asp-ects of derived cassava products is required. Such resea-rch should be aimed at avoiding the co-occurrence of such a number of important toxigenic fungal species in cassava chips intended for human and/or animal con-sumption so as to eliminate or restrict the negative and synergistic effects associated with mycotoxin combina-tions, as Trenholm et al. (1983) highlighted in an earlier study.

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