Fungal and insect contamination of yam and cassava chips in Benin

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Abstract

In 2003/2004 and 2004/2005 in two agroecological zones fungi, mycotoxins and insects in cassava and yam chips were evaluated. Interaction of season, zone and time with fungal occurrence and incidence (cfu/g) and aflatoxin and fumonsin level. Mean moisture content was 11.9% and 12.4% with levels in the Northern Guinea Savannah (NGS) exceeded the recommended limit of 9-10% (2003-04). In the Sudan Savannah (SS), moisture content ranged from 10.1 to 14.5% and 11.1 to 14.5% in cassava and yam chips, respectively. During the 2004/2005 moisture ranged from 9.5 to 13.6% in the NGS and from 9.2 to 13.8% in the SS. The insects' species identified included *Prostephanus truncates*, *Cathartus quadricollis, Carpophilus dimidiatus*, *Tribolium casteneum* and *Sitophilus zeamais*. *P. truncates* and *C. quadricollis* were more prevalent in cassava than yam. After three months, all stored cassava chips were infested by *P. truncates* and *C. quadricollis,* whereas no yam chips in the SS showed insects. *A. flavus* was the most predominant fungal species its prevalence varied with season, during storage period and agroecological zone. Fungal levels in cassava and yam chips reached 8950 cfu/g and 6030 cfu/g, respectively, exceeding the tolerance limit in foodstuffs (10^2). Other fungal species included *Penicillium chrysogenum*, *Mucor piriformis*, *Phoma sorghina*, *Fusarium verticillioides*, *Rhizopus oryzae* and *Nigrospora oryzae*. High performance liquid chromatography (HPLC) analysis of both cassava and yam chips showed that they were not contaminated by either aflatoxins or fumonisin B_1. The limits of detection were 0.1µg/kg for all the aflatoxins and 0.025µg/kg for fumonisin B_1.

Keywords: Benin, agroecological zones, cassava and yam chips, fungi, insects.

Introduction

Cassava and yam are important starchy root crops, eaten and used by millions of people in West Africa, and parts of East and Central Africa. In Benin, cassava and yam are the most important crops with about 2.2 and 1.6 million tons produced in 2007, respectively (FAO, 2007). The production of cassava and yam is hindered by storage problems sine they are a highly perishable commodities and are easily contaminated by fungi (Ikotun, 1989) and bacteria (Ikotun, 1983) or are subject to sprouting due to increased metabolic activity (Ugochukwu *et al.,* 1974).

In order to minimize the above problems, cassava and yams require processing. One way is to process tubers into dried cassava and yams chips. The processing of cassava and yams into chips is a common traditional activity in Benin during the dry period, especially in the Central and Northern regions of the country. Chips are subject to attacks by fungi of which the most important are *Aspergillus, Fusarium* and *Penicillum* (Wareing *et al.*, 2001; Bassa *et al.*, 2001). Fungal contamination can lead to the discolouration of the chips, mouldy taste, production off odours (Gwinner *et al.*, 1996) and possibly the production of toxins that are harmful to animals and humans (Constant *et al.,* 1984; Sajise and Ilag, 1987).

The presence of aflatoxins in yam chips produced in Nigeria (Bankole and Mabekoje, 2004) and Benin (Bassa *et al.*, 2001; Mestre *et al.*, 2004) has been reported. Insect infestation and quality changes brought about by insects in cassava chips have been reported in India (Prem Kumar *et al.*, 1996). Little information exists in this respect on cassava and yams chips. Therefore, there is need to establish the relationship between insects, fungi dissemination and mycotoxins contaminated cassava and yam chips in Benin. The main purpose of the present work is to evaluate the magnitude of cassava and yams chips contamination by mycotoxigenic fungi and to

evaluate the level of aflatoxins and fumonisins contamination of cassava and yam chips in Benin. A survey of fungi, mycotoxins, and insect contaminating stored cassava and yams chips in two different geographical regions of Benin including the Northern Guinea Savannah and the Sudan Savannah was undertaken.

Objectives

6. Identify and enumerate the fungal species on cassava and yam chips from two geographic zones in Benin.
7. Identify and enumerate the insects present on the cassava and yam chips.
8. Determine the aflatoxin and fumonisin levels in stored cassava and yams chips.

Materials and methods

Agro-ecological zones and villages

Two countrywide surveys were carried out between 2003 and 2005 in two agro ecological zones [Northern Guinea Savannah (NGS) and Sudan Savannah (SS)] of Benin have been described by Hell et al. (2000) were chosen for the survey to evaluate the natural incidence of insects, fungi and mycotoxins. The fungi of interest were *Aspergillus* spp and *Fusarium* spp whereas the mycotoxins to be studied were aflatoxins (B_1, B_2, G_1, and G_2) and fumonisin B_1.

Traditional processing of Cassava and Yam chips

The following flow diagrams show the processing of fresh cassava and yam to chips as described by the processors (Figure 1).

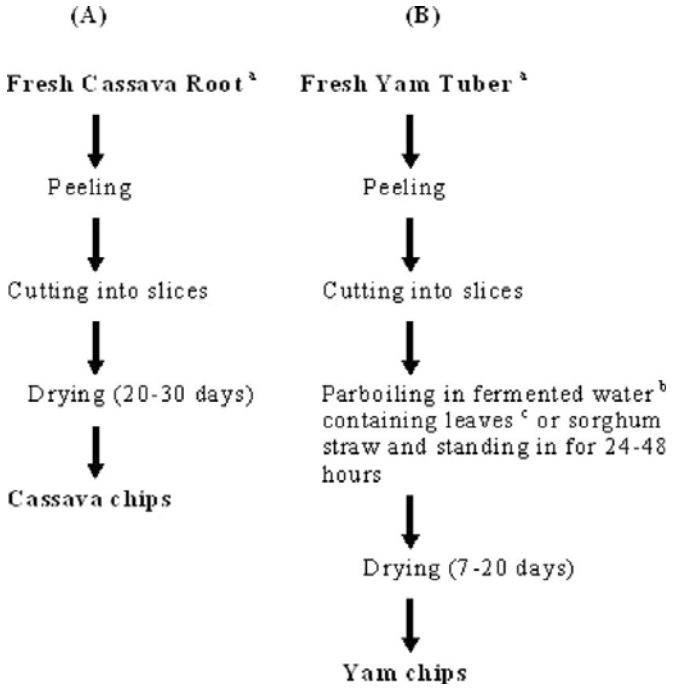

Figure 1. Flow diagrams of the traditional processing methods of cassava (A) and yam (B) chips in Benin. [a], fresh harvested cassava root and yam tubers. [b], supernatant of *ogi* (based on maize or sorghum). The fermented supernatant was obtained from *ogi* that was prepared following the traditional processing method described by Fandohan et al. (2005). [c], from pawpaw or mango tree.

Survey and sampling procedure

The surveys were conducted in 20 villages (10 villages per agro ecological zone). Ten farmers were randomly selected in each village (5 processors of cassava chips and 5 processors of yam chips). The same farmers were involved in both surveys. Cassava and yam chips were sampled at the beginning of the storage (after drying for 20-30 days). At least 500g of the cassava and yam chips were randomly selected from each farmer's stock. Samples from each village were pooled together. Part of the 500g sample (200g) was treated as outline in section 2.4 to identify the insect pests. The rest of the sample was ground using a traditional mill stored at 4°C until mycological and mycotoxins analyses. Samples were again collected from the same farmers after three months of storage.

Insects collection and identification

The insects were collected from the unmilled samples, enumerated and the common insects identified in the field. All the insects were taken to the laboratory for confirmation of identity using the keys by Weidner and Rack, (1984) and Dobie *et al*. (1991). They were classified as primary (meaning that is able to attack the raw commodity and causes entrance for other pests) and secondary (that can infest the commodity after the damage of the primary pest) pests. All insect identifications were done at IITA.

Determination of moisture content

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

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.

Mycotoxins analysis

Aflatoxins extraction and detection: Aflatoxins were extracted and analyzed according to the method described by Bankole and Mabekoje (2004) with some modifications, for thorough description please refer to Gnonlonfin et al. (2008).

Fumonisin B1 extraction and detection: Fumonisins were extracted and analyzed according to the method described by Shephard and Sewram (2004) with some modifications, for thorough description please refer to Gnonlonfin et al. (2008). The method by Doko and Visconti (1994) with modifications was used to determine fumonisin B1 in both cassava and yam chips.

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 B1 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 B1.

3. Results

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. 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 ($p<0.01$).

Mycological analyses

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 1). *M. piriformis* isolates also decreased from 3430 cfu/g at the start to 500 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 1).

Table 1. Major fungal species encountered in stored cassava chips during the 2003/2004 and 2004/2005 seasons. The samples were analyzed at the beginning (start) and after three months of storage (end)

Fungal species	Cassava chips[a]			
	2003/2004 season [CFU/g (% occurrence)]		2004/2005 season [CFU/g (% occurrence)]	
Northern Guinea Savanna (NGS)	**Start**	**End**	**Start**	**End**
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)

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

Regarding 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 2). 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 2). 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 increase in the number of isolates of *M. piriformis* from 1890 cfu/g to 4450 cfu/g (Table 2). 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 ($p>0.05$). *A. flavus* occurrence was positively and significantly correlated with season in cassava chips ($r=0.7$, $p<0.05$) and in yam chips ($r=0.8$, $p<0.05$).

Table 2. Major fungal species encountered in stored yam chips during the 2003/2004 and 2004/2005 seasons at the beginning (start) and after three months of storage (end)

Fungal species	Yam chips[b]			
	2003/2004 season [CFU/g (% occurrence)]		2004/2005 season [CFU/g (% occurrence)]	
Northern Guinea Savanna (NGS)	**Start**	**End**	**Start**	**End**
A. flavus	6290 (24.2)	770 (9.0)	5980 (54.5)	660 (53.6)
Aspergillus. spp	11510 (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)

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

Mycotoxins analyses: aflatoxins and fumonisin B1

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 B1.

Insects encountered in stored cassava and yam chips

Overall, the number of infested chip samples varied from one season to another and across agroecological zones. The percentage of infested cassava chips varied from 10% at the beginning of storage to 100% after 3 month of storage (Table 3).

Although infestation was observed in yam samples, most of the samples did not have insects at the beginning of storage, and very few samples (30%) were infested even after 3 month of storage. The observed insect species included *P. truncatus,Carpophilus dimidiatus* (F.) (Coleoptera: Nitidulidae), *C. quadricollis, Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae), and *S. zeamais.*

Table 3. Principal insects encountered in stored cassava chips at the beginning and end of storage during the 2003/2004 season in Northern Guinea Savannah (NGS; N= 100) and Sudan Savannah (SS; N= 100)

NGS zone Insects species	Beginning of storage				End of storage			
	Total insects	% occurrence	# infested samples	% Infested samples	Total insects	% occurrence	# infested samples	% infested samples
Prostephanus truncatus	7	24.1b	2	20	838	48.4a	10	100
Carpophilus dimidiatus	0	0c	0	0	115	6.6c	9	90
Cathartus quadricollis	22	75.9a	3	30	719	41.6b	9	90
Tribolium castaneum	0	0c	0	0	53	3.1c	3	30
Sitophilus zeamais	0	0c	0	0	5	0.3c	1	10
Total	29	100	3	30	1730	100	10	100
% infested samples	30				100			
SS zone Insects species								
Prostephanus truncatus	1	6.2b	1	10	650	69.8a	10	100
Carpophilus dimidiatus	0	0c	0	0	80	8.6c	3	30
Cathartus quadricollis	15	93.7a	1	10	190	20.4b	2	20
Tribolium castaneum	0	0c	0	0	11	1.2d	1	10
Sitophilus zeamais	0	0c	0	0	0	0d	0	0
Total	16	100	1	10	931	100	10	100
% infested samples	10				100			

The % occurrence was calculated as the insect population out of the total observed per zone.
Means in a column followed by the same letter are not significantly different from each other (SNK, P = 0.05)

Discussion

A wide range of fungal species were isolated from the stored cassava and yam chips. 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 and root (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).

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 (Hell et al., 2000; Fandohan et al., 2005).

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) and in Congo and Tanzania (Manjula et al., 2009).

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.

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. 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 primary pest *P. truncatus* was the most prevalent in both stored cassava and yam chips and has previously been reported as the most common insect problem in these products (Wright, 1993; Birkinshaw et al., 2002; Borgemeister et al., 2003). The secondary pest *C. quadricollis* was also regularly observed. This species can survive on maize powder, such as the one resulting from the attack of *P. truncatus* (Birkinshaw et al., 2002; Borgemeister et al., 2003), we believe that this insect can feed and reproduce on the frass powder resulting from *P. truncatus* damage.

The current study showed that the length of drying time during the processing of cassava chips with a mean of 25 days and a shorter period for yam chips with a mean of 14 days may have an impact on insect infestation longer drying periods increased the risk of infection. Mestre et al. (2004) observed that drying of yam chips in Benin took about 6 days and the moisture content of chips was around 20%. In the present study it was observed that the longer the drying time, the higher the rate of insect infestation. In addition the size of the chips influences the drying time and as a consequence the level of insect infestation (Wareing et al., 2001). Traditional cassava and yam chips in Benin are rather large with lengths measured of a mean of 30 cm (Fandohan, personal observation). Cutting roots and tubers into smaller pieces could help in reducing the drying time and consequently levels of insect infestation (Wareing et al., 2001). Environmental conditions such as. relative humidity and temperature that prevailed in both agroecological zones may have been very conducive to infestation. On cassava chips insect infestation appears to start during drying, persist and increase during the storage period. In the current study, all of the cassava chips samples were infested with the primary pest *P. truncatus* after three months of storage, leading to weigh loss and making the product inappropriate for human consumption, but it has been observed that even flour resulting after heavy insect infestation was collected by farmers and used to make a porridge (K.Hell, personal observation).

Chips destined for human consumption in Benin were found to be infested by various insect genera that included *Prostephanus*, *Cathartus*, *Carpophilus*, *Tribolium* and *Sitophilus*. The results of this study showed that the primary pest such as *P. truncatus* population density increased over the storage period as compare to the secondary pest population density which decreased with storage time. This suggested the competition within insects species. Vowotor et al. (2005) in their study showed the interspecific interaction between *P. truncatus* and *S. zeamais* in the stored maize. These two insects species were sparsely aggregated and not associated with each other. The observed poor storage conditions can be the sources of insect infestation as well as a potential source of proliferation of microorganisms that can produce mycotoxins that are dangerous to animal and human health (Marasas, 1995)

Insects were also encountered on stored yam chips, but the observed infestation levels were much lower than those that occurred in cassava chips, this might have been due to the parboiling process (Rajamma et al., 1994). The plants used during parboiling (Vernier et al., 2005; Eze et al., 2006) might have had an insecticidal or repellant effect. It has been reported that parboiling results in partial gelatinisation of starch and the subsequent binding of the gelled starch onto the surface of the yam chips which hardens them (Rajamma et al., 1994) probably making them more storable.

Conclusion

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. Furthermore, there is a need to investigate on the isolation and inhibitory effect of scopoletin in Beninese cassava and yam varieties.

The results of this study show that cassava and yam chips in Benin were infested by various insect species, mostly Coleopterans. Amongst these insects the specie *P. truncatus* was the more prevalent in both stored cassava and yam chips and also the most destructive. Parboiling the chips, the use of plants during the parboiling process and good storage practices were associated with lower levels of insect infestation. Consequently, any action undertaken to reduce insect infestation during the drying and storage period could help to limit losses to yam and cassava chips, thus increased their storability and marketability.

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