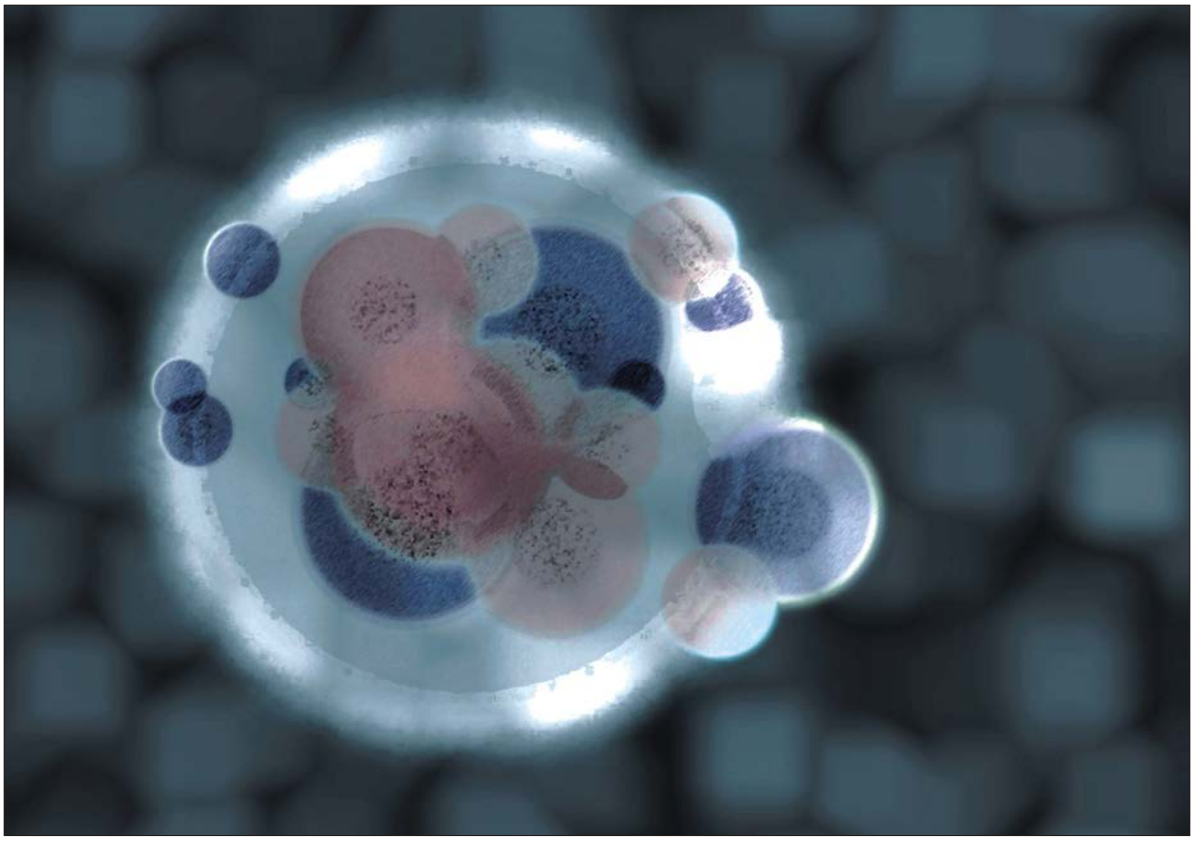

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# Mould and Aflatoxin Contamination of Dried Cassava Chips in Eastern Uganda: Association with Traditional Processing and Storage Practices

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**Abstract:** This study was conducted to establish the level of contamination of dry cassava chips by moulds and aflatoxins and the associated factors. The study was conducted in Kumi district, Eastern Uganda where respondents from ninety households provided information on the cassava processing and storage practices. Samples of dried cassava chips were also obtained from the households for moisture, mould and aflatoxin analyses. The factors that impact on mould and aflatoxin contamination of these products were established using regression analysis. Cassava chips had mean moisture content of 10.14%. Mean mould count was $5.0\times10^4$ cfu $g^{-1}$ and higher counts were associated with extent of visible mouldiness of the products. *Rhizopus* sp. were the most prevalent (66.7%) moulds identified, followed by *Mucor* (37%), *Penicillium* (22.2%), *Aspergillus* (20.4%) and *Fusarium* species (5.6%). *A. flavus* was the most predominant mycotoxigenic mould isolated and occurred on 18.5% of the samples. Thirty percent of cassava samples tested positive for aflatoxin contamination with a range of 0- 4.5 μg $kg^{-1}$ and mean of 0.51 μg $kg^{-1}$. Drying cassava chips on bare ground; storing by heaping on bare floor and storage in old containers such as Jerricans were among the practices positively associated with aflatoxin contamination. Improved practices like drying on tarpaulin were negatively associated with aflatoxin contamination. Since cassava chips are distributed from Eastern to other parts of Uganda, these results show that consumers are exposed to the risk of aflatoxin poisoning. Efforts should therefore be made to improve the quality of cassava by addressing its handling and processing practices in Uganda.

**Key words:** Cassava products, indigenous technology, mycotoxins, moisture, fermentation

## INTRODUCTION

Cassava (*Manihot esculenta* Cranz) is one of the most important staple crops grown in Uganda. In 2005, 5.6 million Metric Tonnes (MT) of cassava were produced in the country, indicating an increase in production from 5 million MT in 2000 (FAOSTAT, 2007). The crop provides 308 kilocalories of energy per person per day making it a significant food crop in Uganda where it is the most important crop for up to 50% of the farmers who cultivate it (FAO, 2005). Most of the cassava in Uganda is produced in the Eastern Region contributing 36% of the total production (MAAIF, 2007).

Despite high levels of production and consumption of cassava in Uganda, dependable methods for preservation have not been developed. The majority of producers do not practice the curing and storage processes that can extend the shelf life of the crop in a fresh form. To extend shelf life, farmers slice the roots into chips and dry them under the sun using different methods Essers (1995). These chips can be stored for several months, depending on moisture conditions and are milled into flour which can be utilised in different forms as food, feed or starch. The majority of people in Uganda utilise cassava in the flour form (Collinson *et al*., 2000).

Some of the major challenges facing cassava processing in Uganda are the inadequate processing and storage equipment and methods used. There are no modern processing equipment and farmers use indigenous technologies such as sun-drying and fermentation which are usually inadequately controlled, labour intensive and produce low quality products. Cassava is sun-dried on virtually any surface in the open air such as flat rocks in the field, on the shoulders of paved roads, on flat rooftops, in a flat basket, or more commonly on bare ground (FAO, 2005). The drying may take from one day to three weeks (Essers *et al*., 1995). During drying, rain or showers may cause the drying pieces to become wet, enabling mould growth to take

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place. After sun drying, the products are stored in traditional storage systems such as granaries and in the huts for a period of up to one year. The storage systems are usually inadequate to assure the safety and quality of the products (FAO, 2005) because rain and various storage pests and moulds may affect the products due to inadequate protection.

One of the serious problems that may arise from processing and storage methods of cassava products in Uganda is the development of aflatoxins. These are metabolites produced by *Aspergillus flavus* and *A. Parasiticus* and have been reported to be hepatic, carcinogenic, immune system suppressing and anti-nutritional contaminants in many food commodities (Williams *et al*., 2004).

Studies in Uganda have shown that cassava can be contaminated with aflatoxins. In 1967, aflatoxin was circumstantially associated with death of a 15-year old boy in Uganda after eating a sample of cassava, which was later found to contain 1700 μg $kg^{-1}$ aflatoxins (Kaaya and Warren, 2005). Since then, no further studies have been conducted to establish mould and aflatoxin contamination of cassava in Uganda and the related factors, which the basis of this study.

## MATERIALS AND METHODS

**Study area:** The study was conducted in Kumi district, which is one of the major producers of cassava on a commercial scale in Eastern Uganda and farmers undertake processing of cassava using traditional technologies, into chips and other products (Collinson *et al*., 2000). The products are transported to several areas of the country especially Kampala, the capital city. The district lies approximately between-latitude 1°10` and 1°35` North and Longitude 33°30` and 34°20` East and has a bi-modal rainfall pattern with peaks in April-May and July-August and average annual temperature of 24°C (Anonymous, 2007).

**Establishment of harvest and post-harvest practices during production and storage of dried cassava products:** A survey to establish the harvest and post-harvest practices of cassava in Kumi district was conducted during January 2007, using a pretested questionnaire. All the three counties of the district (Bukedea, Kumi and Ngora) were included in the survey. In each county one sub county was selected at random for the survey thus a total of 3 sub counties of Mukura, Kumi and Bukedea. In each sub county at least five parishes were sampled and in each, three farmers' households were systematically selected based on the location in the parish. All together 90 households in 18 parishes were surveyed in the three sub counties. Thus, a total of 90 men and women who were heads of the households selected were interviewed using the questionnaire. The information gathered included the practices used in the processing and handling of cassava to produce chips as well as the methods of drying and storage.

In addition to personal interviews, observations were made in the field, drying yards and storage containers to get an understanding of the conditions under which cassava processing takes place.

**Sampling cassava products:** The farmers interviewed were also requested to provide cassava samples. Since some of the farmers didn't have dried cassava products during the survey, a total of 75 samples were obtained. When a respondent had dried cassava products, approximately 1-2 kg of product was collected from containers or heaps where they were stored. Most of the farmers had moderately small stocks ranging from a few kilograms to approximately 300 kg of the product. The sampling protocol for aflatoxin recommended by FAO (1993) was used to collect samples of cassava products. Multiple samples (increments) were taken from different parts of one or more container (s) or heap (s) belonging to the same lot of one farmer and combined to produce approximately 1-2 kg sample. Samples of cassava chips were collected in polyethylene carrier bags to avoid cross contamination and moisture pickup. Historical data on the methods of processing, drying, storage and the length of storage period of each of the samples of cassava was properly recorded.

The samples were transported to the Department of Food Science and Technology, Makerere University, Kampala where they were stored at-18°C to prevent further mould and aflatoxin development (Anderson *et al*., 1995) while awaiting analyses.

**Visual examination and grading of the sampled products:** Each of the samples collected was visually examined for the extent of visibility of moulds on the surface and categorized into four grades, 0, 1, 2 and 3 using a four-point scale adopted from Wareing *et al*. (2001). These authors indicated that the degree of mouldiness of dried cassava products fell into four broad categories: non-mouldy (no visible signs of moulds); slightly mouldy (up to 10% of the surface covered by visible moulds); moderately mouldy (10-50% of the surface covered by visible moulds) and very mouldy (more than 50% of the surface covered by visible moulds). The four categories were subsequently coded 0, 1, 2 and 3 in sequence for data analysis purposes.

**Preparation of cassava samples for laboratory analyses:** The sampled cassava products ranged from about 1 cm in length to more than 20 cm. It was therefore not possible to use the direct plating technique to isolate the moulds. Each of the samples of cassava products was therefore lightly crushed, blended and representative sub-samples of approximately 100 and 200 g flour were used for isolation, enumeration and identification of moulds; and aflatoxin analysis, respectively.

**Determination of moisture content:** Moisture content of cassava samples was analyzed using the air oven method (AOAC, 1999). Approximately 2-3 g of each sample was weighed in duplicate and dried to constant weight at 95°C in an oven (Gallenkampp, Leicestershire, UK). The mean moisture content of duplicate samples was calculated and expressed as percentage on wet weight basis.

**Enumeration of moulds:** Samples of cassava products were analysed for mould counts following the method recommended by ISO (1999). After thorough blending, a 50 g aliquot of each cassava sample was weighed using a digital weighing scale (Satorius AC 112S, Satorius AG, Germany). Malt Extract Agar (MEA) (Oxoid, CM59, Unipath Limited, England) acidified with lactic acid was used to enumerate the moulds using peptone water as a diluent. Ten grams of each sample were weighed in 90 mL diluent and left to soak for about 30 min at ambient temperature (25-28°C) without stirring to form the initial suspension. This suspension was properly blended and further dilutions made aseptically. In each case one millilitre of initial suspension was transferred into 9 mL of diluent successively to make dilutions of $10^{-2}$ to $10^{-5}$.

For each sample of 10 g, 0.1 mL of $10^{-1}$ to $10^{-5}$ dilutions were aseptically spread plated using a 90° glass rod in duplicate on 100 mm Petri dishes. The plates were incubated in an upright position in an incubator (Termarks, Bergen, Norway) for 3-5 days. After incubation, the colonies on the plates were counted with the aid of a colony counter (Stuart, Bibbi, Sterilin Limited, England). Colonies that were flat or fluffy spreading with coloured or sporulating structures were enumerated as moulds (ISO, 1999) and expressed as Colony Forming Units (CFU) per gram of the cassava sample. Data were also transformed in logarithm scale by taking logarithm of $\log_{10}$ (X+1) for each number of mould count, that is $\text{Log}_{10}N = \text{Log}_{10}(X+1)$.

**Isolation and identification of moulds:** Several representative colonies, based on colony colour appearance and shape, grown from cassava were sub cultured on MEA following procedures recommended by Pitt and Hocking (1999) to isolate pure mould cultures. Moulds were identified by macroscopic and microscopic characteristics using keys described by Pitt and Hocking (1999). The percent incidence for each species was calculated by dividing the number of positive samples for the species by the total number of sample of cassava chips analysed (Essono *et al*., 2007).

**Aflatoxin analysis:** Cassava samples were analyzed for aflatoxins using the VICAM AflaTest® immunoaffinity fluorometric method (VICAM, Watertown, MA, USA) that quantifies total aflatoxin concentrations using AflaTest® Fluorometer Series-4 (VICAM L. P, USA) at the Department of Food Science and Technology, Makerere University. The samples were ground to pass through No. 20 sieve and a fifty gram sub sample was weighed, mixed with 5 g sodium chloride and placed in the blender jar. One hundred milliliter of methanol:water solution (80:20, by volume) was added to the jar and the mixture was blended in a Waring blender at high speed for 1 min to extract the aflatoxins. The mixture was filtered through fluted filter paper and the filtrate was diluted (1: 2) with distilled water and filtered again through a glass-microfiber filter paper. Two milliliters of the filtrate was injected into an Aflatest® immunoaffinity column and allowed to elute at a rate of 1-2 drops per second. The column was washed two times with 1 mL distilled water and aflatoxin was eluted from the column with 1 mL High Performance Liquid Chromatography (HPLC) grade methanol. A bromine developer (1 mL) was added to the methanol extract and the total aflatoxin concentration was read in a pre-calibrated VICAM Series-4 fluorometer set at 360 and 450 nm emission. The analyses were done in duplicate and results for each sample were averaged and reported in micrograms per kilogram ($\mu g\ kg^{-1}$).

**Data analysis:** Qualitative data from the questionnaires was cleaned, coded and analysed using SPSS Statistical Programme (SPSS 16, SPSS Inc. Illinois) to investigate frequency distributions (percentages) among categorical variables. Quantitative data (moisture content, mould counts and aflatoxin levels) were subjected to ANOVA using GenStat Statistical Programme. The means were separated using LSD ($p \leq 0.05$).

To examine the effect of farmer processing, drying and storage practices on aflatoxin contamination of cassava products, practices were coded as binary values (yes = 1, no = 0) with 1 representing occurrence of a practice and 0 representing absence of a practice (Hell *et al*., 2003). A total of nine dummy variables were derived from the processing, drying and storage practices reported by farmers. Regression analysis was performed

in order to establish the association between the different processing, drying and storage practices and aflatoxin contamination of dried cassava products.

The measurements of level of aflatoxin in some cassava products showed occurrence of zeros indicating that these samples were aflatoxin negative. Because there were a substantial number of aflatoxin negative samples, the distribution of the data was not normal. Natural logarithmic transformation (Clarke and Cooke, 1983) of the dependant variable, aflatoxin level, was made before the relationship between the aflatoxin levels of the products and the categorical variables was explored. A constant was chosen such that the results of the regression were optimal. The aflatoxin levels in cassava samples (y), were transformed such that: $Y_i$ *cassava* = natural logarithm (y+3). A Poisson (semi logarithmic) regression model was then fitted using GLM Stata (STATA, version 9, StataCorp, 2005) procedure with a bootstrapping command (Afifi *et al*., 2007) for sampling and estimation of standard errors to evaluate the magnitude of association between aflatoxin contamination and the related handling practices used by farmers.

## RESULTS

**Drying and processing of cassava products:** During the survey it was established that farmers in all the counties in Kumi district harvest and dry cassava for home consumption throughout the year. The reason given by farmers for drying throughout the year was that cassava roots can be stored in ground for long and therefore do not require harvesting at once. Farmers therefore, leave the crop in the field after attaining physiological maturity until a processed product is needed.

Two methods of processing cassava roots were reported by respondents in Kumi district Fig. 1. Cassava roots are harvested by hand with the aid of a hand hoe and transported to the homestead. The roots which may reach a diameter of 20 cm and length of up to 50 cm or more are cut into two or more pieces to aid peeling. Small roots may be peeled without cutting. Peeling is done by hand using knives. The peeled roots may be further sliced to reduce the size of the pieces before further processing is done. The peeled, sliced roots are then processed through one of the alternate methods indicated in Fig. 1.

Dried cassava products are either obtained through process A which includes a step of solid-state fermentation (heap fermentation) or step B where cassava is dried without fermentation. In the fermentation method, cassava roots are chopped into fractions using a hoe or a panga and then peeled using knives. After peeling the pieces of cassava root may further be reduced in size

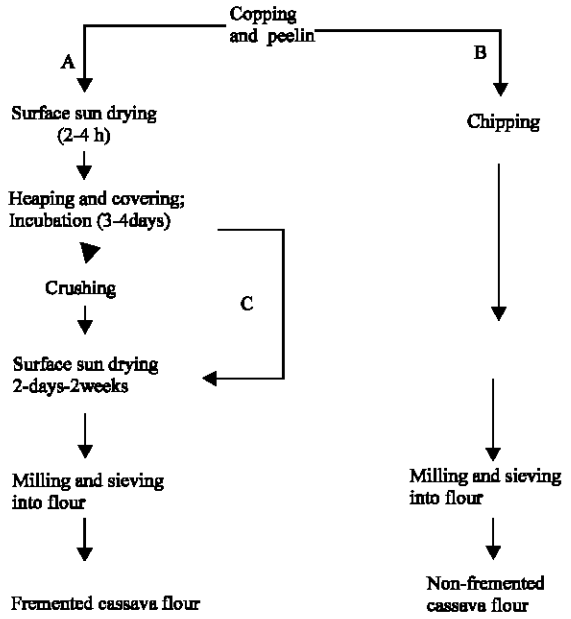


Fig. 1: Stages in the production of dried cassava products

through a slicing process using knives to produce pieces of approximately 10-20 cm long and up to 5 cm thick. The chopping and chipping (slicing) processes are not strictly controlled, so the size of the pieces varies substantially.

The cassava root pieces are then exposed to the sun shine to dry the surface. This process may last 2-4 h but is not strictly controlled. After superficial drying, the cassava is heaped and covered with leaves or using a polyethylene sheet. The cassava may also be put into interwoven polypropylene bags instead of heaping and covering. The cassava is left to ferment for 2-4 days. During this period moulds grow on the surface of the cassava chips. The growth of moulds on the surface of the cassava is considered by farmers as a normal process. Cassava is then crushed to produce crumbs (cassava chips which are irregular in size and shape). The crushing process may be omitted (route C). This is the most likely route when the cassava is intended for storage or for sale. The sliced fermented or crushed pieces are spread in the open air to sundry as chips or crumbs either on bare ground or other surfaces. The chips produced from the sliced pieces and the crumbs from the crushed cassava roots are referred to as dried cassava products.

The alternate process method (B) is known as the direct sun drying route. After peeling and chopping cassava roots, the desired size of the roots is obtained through chipping or crushing in a similar manner as described in the fermentation method. The chips are then spread on bare ground or other surfaces in open air sunshine to dry for 1 to 2 weeks.

Dried cassava chips from both processes are milled to produce cassava flour. The flour is the staple food in Kumi district and is used, singly or in combination with millet or sorghum flour, to prepare atap (stiff bread made by mingling flour in boiling water).

The results of the survey (Table 1) further indicate that the majority of the farmers (50% or more) in all the counties, dry cassava using direct sunshine. On the other hand more than 20% of the farmers in all the counties ferment cassava before sun drying. Other farmers (23.1% in Ngora, 14.7% in Kumi and 20% in Bukedea) practice both methods of processing.

The reasons given by farmers for fermentation of cassava included ease of mingling the flour (the dough is not sticky), does not take a lot of flour to make atap and fermentation imparts good flavour and taste to atap.

**Methods of drying cassava products:** The methods used to dry cassava products reported by farmers in Kumi district are presented in Table 2. Majority of the farmers (86.7% in Bukedea county, 76.5% in Kumi county and 69.2% in Ngora county) reported drying cassava on bare ground. Other farmers, on average 15.8%, indicated drying the produce on rock surfaces. Overall, a total of 93.3% of the farmers depend on natural open surfaces (bare ground and rock surface) to dry cassava products. A small minority of the farmers uses some materials such as tarpaulin or polyethylene sheets (4.3%), old iron sheets (1.1%) and mats (1.3%) to dry the products. The Chi square test of proportion indicated significant differences ($p \leq 0.05$) among the percentages of farmers using the different drying methods.

The farmers reported that the drying process takes between 2 days and 2 weeks and is usually left uninterrupted if the weather permits. During the drying, rains may interfere and the crop will have to be removed into a shelter and returned the following morning depending on weather. These conditions promote mould growth on the cassava products.

**Storage systems used for cassava products in Kumi district:** The results of the survey on the storage systems used by farmers for cassava products are presented in (Table 3). Out of the 90 farmer households surveyed the majority (63.3%) indicated storing cassava products in interwoven polypropylene bags in the huts (grass thatched mud and wooden structures with earth floor used as housing) or semi permanent houses.

This practice varied from 53.8% in Ngora and 64.7% in Kumi to 70% in Bukedea counties. Other farmers indicated storing cassava o r sweet potato products by heaping on the floor in huts or granaries and in polypropylene bags in the granaries.

Table 1: Percentage of respondents fermenting or directly sun drying cassava products in Kumi district

| | % of farmers practicing method | | | |
|---|---|---|---|---|
| Processing method | Ngora county n = 26 | Kumi county n =34 | Bukedea county n = 30 | Mean rank |
| Fermentation | 23.1 | 35.3 | 26.7 | 1.83 |
| Direct sun drying | 53.8 | 50 | 53.3 | 3.00 |
| Both fermentation and direct sun drying | 23.1 | 14.7 | 20 | 1.17 |

Table 2: Methods used by farmers in Kumi district to dry cassava products

| | % farmers practicing method | | | |
|---|---|---|---|---|
| Drying method | Ngora county n = 26 | Kumi county n = 34 | Bukedea county n = 30 | Mean rank |
| Bare ground | 69.2 | 76.5 | 86.7 | 5.00 |
| Rock surface | 23.1 | 17.6 | 6.7 | 4.00 |
| Polyethylene sheet or tarpaulin | 3.8 | 5.9 | 3.3 | 2.67 |
| Iron sheet | 0 | 0 | 3.3 | 1.67 |
| Mats | 3.8 | 0 | 0 | 1.67 |

Table 3: Percentage of farmers reporting the use of different storage systems for cassava products

| | County | | | |
|---|---|---|---|---|
| Storage system | Ngora (n = 26) | Kumi (n = 34) | Bukedea (n = 30) | Mean rank |
| Polypropylene bag inside the hut | 53.8 | 64.7 | 70 | 4.00 |
| Heap on unpaved floor inside hut | 15.4 | 8.8 | 16.7 | 2.33 |
| On the granary floor | 26.9 | 23.5 | 10 | 2.67 |
| Polypropylene bag inside the granary | 3.8 | 2.9 | 3.3 | 1.00 |
| Polypropylene bag inside the hut | 53.8 | 64.7 | 70 | 4.00 |

**Nature of cassava products sampled from farmers:** Cassava products sampled during the survey were either fermented or not fermented by the farmers. The products had been sliced or chipped before sun drying. A comparison of characteristics of the samples of cassava products obtained is presented in Table 4. Samples were nearly equally split between fermented (49.2%) and unfermented (50.2%). The majority of samples were sliced (69.3%) compared to crushed (30.7%).

**Storage history of the sampled cassava products:** During the survey, 72% of the cassava samples obtained from farmers were reported to have been stored in interwoven polypropylene bags inside the huts; 10.7% had been heaped on the floor in huts, 4% in Jerricans (old plastic

containers previously used to carry water) inside the huts, 4% directly on the floor in permanent houses, 2.7% in bags under verandahs, 2.7% in Jerricans under verandahs or in interwoven polypropylene bags in permanent houses (2.7%) and 1.3% in pots (clay containers) in huts (Table 5).

Cassava products had been stored for an average period of 3.4 weeks, with a range of one to 38 weeks (Table 5). The majority of the cassava products (88.2%) had been stored for one to four weeks with 63% having been stored for just one week. Among the samples of cassava collected from the farmers, 4.1% had been stored for 5-8 weeks while 1.5% had been stored for 9-16 weeks, 4.1% for 17-32 weeks and 1.5% for 33 or more weeks.

**Visible mould growth, mould counts and moisture content of cassava products:** The results of grading of cassava products according to the extent of visible mould growth and the findings of moisture content and mould counts are presented in Table 6. After grading cassava samples irrespective of whether they were fermented or not, it was established that the number of samples with no mouldiness, slightly mouldy, moderately mouldy and those that were heavily mouldy were almost the same. Mean moisture content of cassava samples varied significantly and was highest in the heavily moulded samples and lowest in the samples without moulds. After plating on MEA, mould growth was observed in all samples and counts ranged from $4.5\times10^{1}$ to $1.0\times10^{6}$ cfu $g^{-1}$ with an overall mean of $5.0\times10^{4}$ cfu $g^{-1}$. There was a general increase in mould counts with increasing degree of mouldiness and cassava products that were visually judged to be heavily moulded had significantly higher ($p\leq0.05$) counts than the none mouldy cassava products (Table 6).

**Moulds isolated and identified from cassava products:** Results of the incidence of different mould species isolated and identified from fermented and unfermented cassava samples are presented in Table 7. The incidence in fermented and unfermented cassava products were combined and means were computed. *Rhizopus* spp were the most prevalent (66.7%) moulds followed by Mucor (37%), *Penicillium* (22.2%), *Aspergillus* (20.4%) and *Fusarium* species (5.6%). Other moulds that could not be identified using the available keys occurred in 32.5% of the samples. *A. flavus* was the most prevalent mycotoxigenic mould species identified and occurred in 18.5% of the samples. *A.fumigatus* was the only mould that was not isolated in fermented cassava products while *Fusarium* species were not isolated in unfermented products. The majority of moulds isolated and identified co-occurred in both cassava products.

Table 4: Characteristics of cassava products sampled from farmers in Kumi district

| Characteristic | No. samples | % samples | Mean Rank |
|---|---|---|---|
| Fermented | 37 | 49.2 | 2 |
| Un-fermented | 38 | 50.8 | 3 |
| Sliced | 52 | 69.3 | 4 |
| Crushed | 23 | 30.7 | 1 |

Table 5: Storage systems and period for sampled dried cassava products at farm level in Kumi district

| Storage practice | % farmers using method | Storage period (weeks) | % samples collected |
|---|---|---|---|
| Polypropylene bag inside the hut | 72.0 | 1-4 | 88.20 |
| Heap on unpaved floor inside hut | 10.7 | 5-8 | 4.10 |
| Polypropylene bag on the verandah | 2.70 | 9-16 | 1.50 |
| Polypropylene bag inside Permanent House | 2.70 | 17-32 | 4.10 |
| Heap on paved floor inside permanent house | 4.00 | 33+ | 1.50 |
| Jerrican inside the hut | 4.00 | | |
| Jerrican on the verandah | 2.70 | | |

Table 6: Status of mould growth, moisture content and mould count of cassava products sampled from farmers in Kumi district

| Mould category[a] | No. of samples | Mean moisture content (%) | Mean mould count ( cfu $g^{-1}$) |
|---|---|---|---|
| None (0) | 21 | 8.98[a] | $1.8\times10^{4}$ [a] |
| Slight (1) | 17 | 10.05[b] | $2.6\times10^{4}$ [a] |
| Moderate (2) | 21 | 10.14[b] | $5.2\times10^{4}$ [ab] |
| Heavy (3) | 16 | 11.04[b] | $1.2\times10^{5}$ [bc] |
| LSD ($p\leq0.05$) | | 1.03 | 7.0×04a |

None = No visible mould; Slight = Slightly mouldy (<10% of area of the chips covered by visible moulds). Moderate = Moderately mouldy (up to 50% of area covered by visible moulds). Heavy = Heavily mouldy (> 50% of area covered by visible moulds). Means with different superscripts within the same column are significantly different

Table 7: Number of contaminated (Nsc) and % incidence[ab] of mould species in cassava products

| | Type of cassava products[c] | | | |
|---|---|---|---|---|
| | Fermented | | Unfermented | |
| Moukd species | Nsc% | Incidence | Nsc% | Incidence |
| *Aspergillus flavus* | 5 | 18.5 | 5 | 18.50 |
| *A. fumigatus* | 0 | 0.00 | 1 | 3.70 |
| *Penicillium brevicompactum* | 6 | 22.2 | 62 | 2.20 |
| *Fusarium* sp. | 3 | 11.1 | 0 | 0.00 |
| *Mucor* sp. | 11 | 40.7 | 9 | 33.30 |
| *Rhizopus* sp. | 20 | 74.1 | 165 | 9.30 |
| Others[d] | 11 | 40.7 | 17 | 630.00 |

[a]Incidence was calculated as the number of samples contaminated (Nsc) by the mould species divided by the number of samples analysed. [b]Totals add to more than 100% because mould species occurred in more than one sample and at least once in each sample. [c]Moulds that could not be identified using available keys. [d]A total of 27 samples were considered for each of the fermented and non fermented cassava products

**Aflatoxin content in cassava products:** Results of the aflatoxin content of cassava samples obtained from the different counties of Kumi district are presented in

Table 8: Aflatoxins (μg kg$^{-1}$) in cassava products from different counties of Kumi district

| Coutry | Samples analysed | No. positive samples | % positive samples | Range | Mean |
|---|---|---|---|---|---|
| Ngora | 15 | 4 | 26.7 | 0-3.5 | 0.633 |
| Kumi1 | 17 | 6 | 35.3 | 0-2.5 | 0.412 |
| Bukedea | 28 | 8 | 28.6 | 0-4.5[a] | 0.500 |
| LSD ($p \leq 0.05$) | | | | | 0.52 (NS) |

NS = Means were not significantly different

Table 9: Aflatoxin (μg kg$^{-1}$) content in cassava products in relation to degree of moulding

| [a]Visual mould rating | % positive samples | Mean Aflatoxin (μg kg$^{-1}$) |
|---|---|---|
| None | 22.2 | 0.44 |
| Slight | 16.7 | 0.59 |
| Moderate | 41.2 | 0.62 |
| Heavy | 38.5 | 0.38 |
| LSD ($p \leq 0.05$) | | 0.31(NS) |

None: No visible mould. Slight: slightly mouldy (<10% of area of the chips covered by visible mould). Moderate: Moderately mouldy (up to 50% of area covered by visible mould). Heavy: Heavily mouldy (> 50% of area covered). NS: Means within the column are significantly different

Table 10: Correlation coefficients of the relationship between aflatoxin and mould contamination; moisture content and length of storage of cassava products

| Parameter | Moisture content | $Log_{10}N$** | Length of storage |
|---|---|---|---|
| Aflatoxins | 0.151052 | -0.39927 | 0.433458 |
| Moisture content | | 0.160518 | 0.620802* |
| $Log_{10}N$** | | | 0.301277* |

*Significant ($p \leq 0.05$), ** Mould count

(Table 8). Although there was a variation in the range of aflatoxin levels in each county, the means did not differ among the counties.

Aflatoxin levels were further determined in cassava samples in relation to the degree of mouldiness (Table 9). There was a general increase in the percentage of aflatoxin positive samples and the levels of aflatoxin with the degree of mouldiness in cassava. However, although there was a general increase in the aflatoxin levels of cassava samples, the means were not significantly different (Table 9).

**Relationship between aflatoxin content of cassava with moisture content, mould contamination and storage period:** The results of the correlation between aflatoxin levels and moisture content, mould contamination and length of storage of cassava are presented in (Table 10). Aflatoxin levels were positively correlated with moisture content and storage period of cassava products. However, aflatoxin levels were negatively correlated with mould counts. The associations between mould counts with moisture and storage period of cassava products were positive. The association between moisture content and storage period of cassava products was significant ($p \leq 0.05$) while the associations between aflatoxin and moisture content and mould count and length of storage were not significant (Table 10).

**Association between aflatoxin contamination in cassava products with processing, drying and storage practices:** The results obtained from the Poisson regression analysis of aflatoxin levels in cassava samples are presented in (Table 11). Five factors were significantly associated with aflatoxin contamination of cassava.These factors were: drying on tarpaulin and drying on paved surfaces which were negatively associated with aflatoxin contamination. Drying cassava on tarpaulin would lead to 22 times less contamination compared to drying otherwise. Drying on paved surface on the other hand would lead to 23 times less contamination compared to drying otherwise. Storage practices on the other hand, were positively associated with aflatoxin contamination. Storing cassava in Jerricans would lead to 34 times more contamination while storing in polypropylene bags and heaping on bare floor would lead to 19 and 16 times more contamination compared to storing otherwise, respectively.

## DISCUSSION

During the survey it was established that farmers in all the counties in Kumi district harvest and dry cassava for home consumption throughout the year, implying that dried cassava products are important source of food and income for the people of Kumi. It is possible to harvest cassava any time of the year because the crop can be kept in the fields for a long time. The main processing, however, is done during the dry season when there is adequate sunshine, with less rains. During the dry season (December to February) labour is also released from other tasks such as tilling the land, planting and weeding crops to processing cassava. Adequate labour therefore means that farmers can harvest cassava and process it for storage or for marketing purposes.

Drying of cassava throughout the year implies that the products are exposed to varying weather conditions. For example during rainy seasons the products may not dry easily due to high humidity, inadequate sunshine and exposure to rain. This enhances mould growth and aflatoxin formation in the products and could explain the observed levels of aflatoxin in the products.

Results of the study showed that farmers practice both solid-state (heap) fermentation and direct sun drying of cassava. These methods have previously been reported in Uganda (Ameny, 1990; Essers *et al*., 1995) and Tanzania (Muzanila *et al*., 2000).

The cassava storage roots are harvested, peeled and then cut into pieces that vary in size from about 10-20 cm. The pieces of cassava roots are either spread immediately on open surface to dry under the sun (direct sun drying) or they are heaped to ferment before the drying process. Before drying, the cassava roots may also be crushed by

Table 11: Association between aflatoxin levels and processing, drying and storage methods in cassave samples ($R^2$ (Adg) = 13.1%, F = 9.26, p>F = 0.004)

| Processing, drying and storage methods | Coefficient (β) | Bootstra p Std. Err. | Z | P>z | $g = 100*[e^{\beta} -1]$ |
|---|---|---|---|---|---|
| Fermenting cassava (X1) | -0.017 | 0.012 | 1.39 | 0.165 | 1.7 |
| Slicing (X2) | -0.106 | 0.055 | -1.92 | 0.055 | -10.0 |
| Drying on bare ground (X3) | -0.162 | 0.100 | -1.63 | 0.103 | -15.0 |
| Drying on bare rock (X4) | -0.207 | 0.130 | -1.59 | 0.112 | -18.7 |
| Drying on tarpaulin (X5) | -0.250 | 0.124 | -2.02 | 0.043* | -22.1 |
| Drying on paved surface (X6) | -0.260 | 0.129 | -2.02 | 0.043* | -22.9 |
| Storage in polypropylene bags (X7) | 0.148 | 0.058 | 2.55 | 0.011* | 16 |
| Storage in Jerricans (X8) | 0.289 | 0.143 | 2.02 | 0.043* | 33.5 |
| Storage on bare floor (X9) | 0.176 | 0.074 | 2.37 | 0.018* | 19.2 |
| Constant | 0.284 | 0.093 | 3.05 | 0.002* | 32.8 |
| Number of observations | 56.000 | | | | |

g: Estimated effect of a unit change in variable X.; *Effects were significant ($p \leq 0.05$)

hitting with sticks or stones to produce crumbs. This method of processing was also observed by Essers (1995) who reported that the crushing process produces pieces of small sizes and leads to faster drying.

When fermentation is the processing method of choice, the cassava pieces are first exposed to sunshine to dry the surface. Drying is then stopped and delayed for 2-4 days while the roots undergo incubation. Substantial amounts of moulds observed on cassava chips are likely to have grown on the products during this stage. Moulds observed on fermented products were white, black or orange in colour. According to Essers (1995) greenish mould could also occur and was attributed to an insufficient first surface drying step. Such moulds may be removed before or after drying the cassava chips in order to improve on the quality of the final product although at this time, mycotoxins production may have already occurred making the products hazardous to the consumer (Westby *et al*., 1995). Similarly Wareing *et al*. (2001) reported mould growth on kokonte, a Ghanaian dried fermented cassava product. However, the farmers interviewed during this study did not indicate any deliberate efforts to remove moulds either before or after drying of the products.

Majority of the cassava was found to be chipped compared to crushed, although the fermented cassava was more likely to be crushed. This could be attributed to the ease of crushing fermented cassava due to disintegration of the root tissues Essers (1995). Crushing also aids drying and may be practiced when the drying is to be achieved fast.

The survey further revealed that over 90% of the farmers dry the cassava products on bare ground or bare rocks. Indeed, sampling confirmed that more than 90% of the cassava had been dried on the bare ground or rock surface. Very few farmers in Kumi district have appropriate equipment or materials such as tarpaulins, polyethylene sheets or concrete paved surfaces for drying cassava chips. The findings in this study are consistent with the FAO (2005) report which indicated that cassava in Uganda is dried on any open surface including bare ground, bare rock and on shoulders of roads. Kaaya *et al*. (2006) reported that majority (80%) of farmers in Uganda dry maize on bare ground. Thus, this method of drying produce appears to be the most predominant across the farmers in Uganda. This could be attributed to the low income status of the farmers who may not be able to afford new technologies such as the paving of the floor or purchase of polyethylene sheets.

Drying cassava on the ground exposes the crop to contamination with soil, dust, moulds and other foreign matter because this practice promotes contact between the products and the soil which is the primary source of moulds (Diener *et al*., 1987). Aflatoxin has been reported to increase when maize cobs were dried on the ground (Hell *et al*., 2003; Kaaya *et al*., 2006). This is exacerbated in cassava because the crop will have been peeled removing any natural protection. Natural protection such as grain husks has been reported to protect maize and rice from weevils and mould infestation and aflatoxin contamination (Udoh *et al*., 2000; Liu *et al*., 2006). Such protection ultimately lowers the risk of aflatoxin in produce. This kind of protection is not available in cassava after processing into products making them more vulnerable to attack by moulds and possibly subsequent contamination by mycotoxins. Additionally, cassava chips are hygrospcopic and tend to pick up moisture during storage which promotes moulds and other deterioration agents (Knoth, 1993).

In the current study, drying of cassava was reported to last as long as two weeks depending upon weather conditions. Such an extended drying period promotes insect and mould infestation and can lead to discolouration and changes in flavour (Knoth, 1993). The temperatures in the studied region are on average between 22-30°C (Anonymous, 2007). This indicates that the products stay at moisture content and temperatures that favour mould growth and aflatoxin production for a long time. Availability of water is essential for both mould growth and aflatoxin production (Klich, 2007). Westby *et al*. (1995) observed that *A. parasiticus* produced large amounts of aflatoxin on processed

cassava products at 40% moisture content. *A. flavus* has been observed to produce the highest aflatoxin levels at water activity of 0.996 and temperature of 30°C (Gqaleni *et al*., 1997) between 5-15 days of storage. Optimum temperatures for aflatoxin production are between 24 and 30°C with variation between strains and substrates (Klich, 2007).

It is evident that most farmers in Kumi store dried cassava in the huts which double as housing and kitchens in order to protect the produce against theft. In these structures, cassava is either heaped or stored in various containers like jerricans, pots or polypropylene bags. Such structures are built using mud and wood and may have little or no ventilation (Fandohan *et al*., 2005). Essers and Nout (1989) reported similar storage practices for dried cassava products in Mozambique. Storage in the huts is known to be inadequate as it may expose the product to rain due to inadequate or leaking roofs. Rain causes the dry pieces to become wet, enabling a more or less profuse mould growth to take place (Essers and Nout, 1989). FAO (2005) observed that structures and methods for storage of cassava chips in Uganda were inadequate and unsafe for ensuring the quality and safety of the foodstuffs. For example, heaping produce on the bare ground promotes insect and mould proliferation compared to storage on platform and has been associated with aflatoxins contamination in maize (Fandohan *et al*., 2005; Kaaya *et al*., 2006). Similarly, Mestres *et al*. (2004) observed that heaps of yam chips in a poorly ventilated room was not favourable for moisture loss and favoured mould growth and insect infestation. The practice of heaping cassava products on the bare floor, whether cemented or made of mud, could therefore, promote aflatoxin contamination due to unfavourable moisture conditions and insect and mould proliferation. Baumgartner *et al*. (2005) reported a significant association between such practice and occurrence of aflatoxicosis in maize in Kenya.

Farmers in Kumi district store cassava products for varying periods ranging from one week to one year depending on several factors. Essers (1995) reported similar storage period for cassava chips in Uganda which was said to be stored from a few days after drying to one year into the following year's harvest. Similar storage period averaging 8-12 weeks was reported among majority of farmers in Ghana who store kokonte, a Ghanaian dried fermented cassava product (Wareing *et al*., 2001). It is probable that storing cassava products for long is one of the factors predisposing the products to aflatoxin contamination since an increase of aflatoxin contamination with storage time has been reported in similar products like yams (Mestres *et al*., 2004). Aflatoxin increased in yam chips during storage for 5-7 months (Mestres *et al*., 2004) in Benin due to storage conditions like high humidity and moisture build-up. The conditions under which these yams are handled on the farm are comparable to those of cassava chips in Uganda. Essono *et al*. (2007) reported a significant correlation between the incidence of recovery of *Aspergillus* moulds from cassava chips and the moisture contents and duration of storage of the chips. These authors suggested that duration of storage should be considered as an important factor allowing the spread and establishment of fungi in stored food commodities.

It is encouraging to note that the levels of moisture content of cassava products observed in this study were low. This implies that farmers were able to achieve good moisture content during drying cassava. Aryee *et al*. (2006) observed that at 12% moisture cassava products had potential for long shelf life but moisture content greater than 12% allows microbial growth. Safe moisture content is one of the prerequisites in preventing mycotoxins using the Hazard Analysis and Critical Control Point (HACCP) approach (FAO, 2001).

Dried cassava products obtained from farmers in Kumi could be ranked basing on the extent of visible mould growth. This was consistent with the findings of Wareing *et al*. (2001), who reported that dried cassava products could be classified into four categories basing on the degree of visual mould growth. This shows that it is possible to distinguish between cassava products that have more visible mould on the surface from the product that has less or no mould.

In the current study, fermented cassava products were more likely to be scored as moderately or heavily mouldy compared to the non fermented cassava chips. This could be attributed to growth of moulds on the fermented cassava products which is favoured by the conditions of fermentation applied by farmers although they consider it a normal process. These results are consistent with the findings of Essers *et al*. (1995) who reported that farmers in Uganda use the level of mould growth as a parameter to measure the extent of fermentation.

On the contrary direct sun drying of cassava products is likely to shorten the drying period since drying is started immediately after peeling and chipping the cassava roots. Although moulds still grow on the non fermented cassava products, the level of growth is lower than the level of growth in the fermented products thus the observed results.

*Rhizopus*, *Mucor*, *Penicillium*, *Aspergillus* and *Fusarium* species have been observed in cassava products (Wareing *et al*., 2001; Essono *et al*., 2007) as have been reported to occur in other food commodities

dried by farmers like stored maize in Uganda (Kaaya *et al*., 2006), Nigeria (Bankole and Mabekoje, 2004) and Ghana (Kpodo *et al*., 2000).

The species of *Aspergillus* isolated in this study i.e., *A. flavus* and *A. fumigatus* have been reported in cassava elsewhere (Essers, 1995; Wareing *et al*., 2001; Essono *et al*., 2007) with *A. flavus* as the most predominant of the *Aspergillus* species isolated. Essers (1995) reported isolating four species namely A. *fumigatus*, *A. niger*, *A. parasiticus* and *A. oryzae* from six samples of heap-fermented cassava in Masindi-Uganda, with *A. fumigatus* occurring most often. More recently, Essono *et al*. (2007) recovered 13 species of *Aspergillus* from 72 samples of dried cassava chips from Cameroon. Of the *Aspergillus* species reported in cassava from Cameroon, three aflatoxin producing species of *A. flavus, A. nomius* and *A. parasiticus* were isolated with *A. flavus* being the most predominant. Most of the moulds identified in cassava products are soil borne; implying that farmer practice of drying these products on bare ground predisposes them to fungal infection. Besides, the majority of these moulds are known to produce mycotoxins. *A. flavus* is a known aflatoxin producing mould species (Klich, 2007). *T. viride* is a known toxigenic mould that produces Trichodermin; *A. niger* produces oxalic acid and malformin; *A. fumigatus* produces gliotoxin, fumagilin and veruculogen, while *A. ochraceous* produces ochratoxins, Dextruxin B and Penicillic acid, (Adler, 2002). *P. expansum* produces patulin (Moake *et al*., 2005).

The results of aflatoxin analysis in cassava show a relatively high (30%) incidence of aflatoxin contaminated cassava samples although the mean contamination (0.51 μg $kg^{-1}$) is moderately low. The established aflatoxin range of 0-4.5 μg $kg^{-1}$ during cassava sample analysis showed that cassava products had been contaminated with low levels compared to the limit of 20 μg $kg^{-1}$ recommended by Codex *Alimentarius* Commission for food intended for human consumption (FAO, 2004).

The incidence levels in this study were higher than those reported by Essers (1995); Muzanila *et al*. (2000) and Wareing *et al*. (2001). Muzamila *et al*. (2000) reported no aflatoxin in 18 samples of cassava processed by smallholder farmers using sun drying and solid state fermentation in Tanzania. Wareing *et al*. (2001) similarly reported no aflatoxin in 2 samples of kokonte, a Ghanaian fermented dried cassava product. Essers (1995) had earlier reported no aflatoxin in 14 samples of dark mouldy cassava in Mozambique and 10 samples of cassava flour screened in Uganda. Other authors have however, reported even higher incidence levels than the current observations. For example, Bulatao-Jayme *et al*. (1982) screened 142 samples of cassava and cassava products from the Philippines and reported aflatoxin incidence of 100% at a mean of 467.5 μg $kg^{-1}$.

The occurrence of aflatoxins in cassava indicate that the Ugandan population can be at risk of aflatoxicoses because cassava products are staple foods in Kumi district and are often sold to other markets in Uganda especially in Kampala, the capital city of the country. Butalao-Jayme *et al*. (1982) showed that increased consumption of aflatoxin-loaded foods caused higher intake among cases of cancer in the Philippines even when the level of contamination was moderately low.

## CONCLUSION

The results of this study have shown that dried cassava from Kumi district are contaminated with moulds and aflatoxins. Thus, consumers of products from this crop are at a risk of chronic aflatoxicosis. Since cassava products are also distributed from Kumi district to other parts of Uganda, especially Kampala, the most populated city in Uganda, it is possible that even more people are consuming contaminated foodstuffs in the country. It is therefore important that the Government of Uganda helps the poor resource farmers who are the majority of cassava growers and processors, to handle the produce adequately to reduce the chances of contamination. In addition, an informed population is able to reduce the risks of exposure to aflatoxins. It is important therefore, that the information concerning the level of mould and aflatoxin contamination of cassava and other foodstuffs is disseminated at all levels of society in the country.

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