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PII:	S0956-7135(18)30625-X
DOI:	10.1016/j.foodcont.2018.12.025
Reference:	JFCO 6449
To appear in:	*Food Control*
Received Date:	22 January 2018
Accepted Date:	18 December 2018

Please cite this article as: Abass, Adebayo Busura, Adegoke, Gabriel Olaniran, Awoyale, Wasiu, Gaspar Audifas, Mlingi, Nicholas, Andrianavalona, Voahangy, Randrianarivelo, Roger, Sulyok, Michael, Mneney, Agnes, Ranaivoson, Lalao Roger, Enumeration of the microbiota and microbial metabolites in processed cassava products from Madagascar and Tanzania, *Food Control* (2018), doi: 10.1016/j.foodcont.2018.12.025

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Enumeration of the microbiota and microbial metabolites in processed cassava products from Madagascar and Tanzania

Abass, Adebayo Busura[1†]; Adegoke, Gabriel Olaniran[2]; Awoyale, Wasiu [3,4]; Gaspar Audifas[1]; Mlingi, Nicholas[1]; Andrianavalona, Voahangy[5]; Randrianarivelo, Roger[5]; Sulyok, Michael[6]; Mneney, Agnes[7]; Ranaivoson, Lalao Roger[5]

[1]International Institute of Tropical Agriculture, Regional Hub for Eastern Africa, Dar es Salaam, Tanzania.

[2]Department of Food Technology, University of Ibadan, Ibadan, Nigeria.

[3]International Institute of Tropical Agriculture, PMB 5320 Oyo Road, Ibadan, Oyo State, Nigeria

[4]Department of Food Science and Technology, Kwara State University Malete, PMB 1530, Ilorin, Kwara State, Nigeria

[5]Centre National de Recherche Appliquée au Développement Rural (DRT/FOFIFA), BP 1444 – Ambatobe, 101 Antananarivo, Madagascar.

[6]Department of Agrobiotechnology (IFA-Tulln), University of Natural Resources and Life Sciences, Vienna, (BOKU), Konrad Lorenzstr. 20, A-3430 Tulln

[7]Tanzania Bureau of Standards, Dar es Salaam, Tanzania.

†Corresponding author: a.abass@cgiar.org

ABSTRACT

Cassava processing practices vary among communities and countries with implications for food safety. The study examined the microbiota and microbial metabolite profiles of 126 samples of sun-dried cassava products: grits, improved chips, improved flour, *kivunde*, and *makopa* from Tanzania, and *mangahazo maina* from Madagascar. All samples were free of *Salmonella* spp. Only 12.5% *makopa*, 6.7% of mechanically processed flour, and 25% of chips conformed to yeast/mold regulatory limits (10^3 cfu/g). Among the most agriculturally important mycotoxins, aflatoxins (B1, B2, G1, and M1) were detected in 6.3–11.9%, fumonisins (B1, B2 and B3) in 3.2–41.3%, and zearalenone in 41.3% of the samples. A few samples of improved chips, improved flour, and *makopa* contained high aflatoxin B1 content. Some emerging mycotoxins: emodin, beauvericin, moniliformin, sterigmatocystin, alternariol methyl ether, nivalenol, mycophenolic acid, enniatin B, and enniatin B1 were detected. The most prevalent microbial metabolites were emodin (75.4%), tryptophol (67.5%), equisetin (61.9%), and beauvericin (51.6%), at mean concentrations of 8.8 µg/kg, 794.1 µg/kg, 277.2 µg/kg, and 29.5 µg/kg, respectively. Emodin and Beauvericin are the only emerging mycotoxins in this group and the mean concentrations are the lowest. Nevertheless, regular surveillance along the cassava food chain is recommended for early detection of emerging mycotoxins to prevent health problems associated with ingestion of unexpected toxins in foods.

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Highlights

- Yeast/mold was a common contaminant in sun-dried cassava products
- Fusarium is a common mycotoxin producer in the sun-dried cassava products
- Sporadic presence of aflatoxins may occur in some sun-dried cassava products
- Processing with machines did not guarantee absence of mycotoxins in cassava products
- Strict hygiene measures are needed to guarantee conformity of products to standards

Keywords: cassava, sun-drying, microbial metabolites, mycotoxins, regulatory standards.

1.0 Introduction

Cassava, *Manihot esculenta* (Crantz), is ranked third, after rice and maize, among the most important foods in tropical Africa. Several methods are used in different geographical regions to process roots to obtain intermediate and food products (Nambisan, 1994). In West and East Africa, raw cassava is eaten as a major snack (Nweke and Bokanga, 1994).

Dry, fermented products such as granules or chips are regarded as being more suitable for long-term storage than fresh roots (Hahn, 1989; Nweke, 2005). To produce chips, various technological pathways including fermentation by soaking or heaping, and sun-drying or smoking are combined with a wide range of other practices such as peeling, washing, dewatering, slicing, roasting, milling, and sifting. The different practices lead to a wide range of intermediate products and different types of chips or *cossettes* (Nweke et al., 2000a, 2000b). Quality is affected by fermentation and drying method, drying environment, water quality, general hygiene, and other factors (Nweke et al., 2000a, 2000b). The type of fermentation or its absence influences the taste, color, flavor, consistency, and sensory preferences for the products (Campbell-Platt, 1994). Hence, fermentation is a key to product differentiation. Soaking or heap-fermentation leads to different types of fermented chips or *cossettes* (COSCA, 1996; Nweke, 1994).

In Tanzania *kivunde (*or *Bada)* and *inyange* in Burundi are made through heap-fermentation during which the roots soften (Kimaryo et al., 2000; Ndunguru, 2001; Nzigamasabo and Nimpagaritse, 2009). *Rhizopus* spp., *Cladosporium* spp., *Penicillium* spp. *Fusarium* spp., *Mucor* spp., and *Aspergillus* spp. (including *A. flavus*) were involved in the heap-fermentation process (Chacha, 2014). Lafun (Nigeria), *Cossettes* (DR Congo), *udaga* and *kondowole* (Tanzania), and *ikivunde* (Burundi) are processed by soaking roots in still water, streams or rivers for some days until the roots are soft. The soft roots are then sun-dried on mats, rocks, road-shoulders or roof tops (Oyewole and Odunfa, 1988; Nweke, 1994; Ndunguru, 2001; Nzigamasabo and Nimpagaritse, 2009). *Bacillus* spp., *Lactobacillus* spp., *Leuconostoc* spp., *Corynebacterium* spp., *Weissella* spp., *Klebsiella* spp., *Saccharomyces* spp., *Candida* spp., *Kluyveromyces* spp., *Hanseniaspora* spp., *Rhodotorula* spp., *Penicillium* spp., *Hansenula* spp., *Geotrichum* spp., *Pichia* spp., *Candida* spp., and *Aspergillus* spp. are known to be involved in

the fermentation of cassava roots by soaking (Nwachukwu and Edwards, 1987; Oyewole and Odunfa, 1988; Padonou et al., 2009).

The diversity in the microorganisms involved in the fermentation of different types of dried cassava products may influence the composition and concentration of the microbial metabolites in the products, some of which may be of public health concerns. Gibb et al. (2015) noted that cyanide and aflatoxins are associated with diseases with high case-fatality ratios. Hundreds of mycotoxins are known (Adegoke, 2004; Leslie et al., 2008). The five major groups of mycotoxins that have the highest negative impact on human health—aflatoxins (e.g., aflatoxin B1, AFB1), trichothecenes (e.g., deoxynivalenol), fumonisins (e.g., fumonisin B1, FB1), ochratoxin A, and zearalenone—occur in the field and during harvesting, handling, storage, and processing (Neme and Mohammed, 2017). These groups of mycotoxins, the maximum levels in food and feed are enforced, and are commonly enumerated in agricultural commodities.

Poor production, postharvest handling, and storage practices may expose humans to high levels of these mycotoxins, especially aflatoxins. In developing countries where the foods consumed are rarely inspected for these toxins (Williams et al., 2004; Strosnider et al., 2006), health problems associated with the foodborne chemical toxins are high. For example, high levels of aflatoxins in home-grown maize have been implicated in devastating health-related outbreaks in Kenya (Azziz-Baumgartner et al., 2005).

In addition to the mycotoxins that the levels are controlled in foods and feeds, "emerging mycotoxins", that contaminate food and feed, are of public health concerns due to their potential toxicity (Streit et al., 2013; Gruber-Dorninger et al., 2016). There is little knowledge about this group of mycotoxins (Kovalsky et al., 2016). Therefore, emerging mycotoxins are of interest with respect to enumeration to ascertain the safety of food and feed (Gruber-Dorninger et al., 2016, Kovalsky et al., 2016). Jestoi (2008) and Gruber-Dorninger et al (2016) recently presented data on the occurrence as well as acute and chronic toxicity for some emerging mycotoxins: enniatins, beauvericin, moniliformin, fusaproliferin, fusaric acid, culmorin, butenolide, sterigmatocystin, emodin, mycophenolic acid, alternariol, alternariol monomethyl ether, and tenuazonic acid. Gruber-Dorninger et al. (2016) concluded that current knowledge suggests little or no health concerns for some of the compounds, but knowledge gaps still exist for some compounds and such gaps need to be closed.

As subsistence farmers could be exposed to toxins in improperly processed and stored foods (Shephard et al., 2013), it is vital to sustain the scrutiny of traditional and newly introduced processing practices regarding their effectiveness to produce food and feed products that conform to regulatory safety limits for the most agriculturally important mycotoxins (Hird et al., 2009) as well as generate data on the occurrence and levels of the little understood emerging mycotoxins to allow a proper risk assessment. For this purpose, the analytical methods to be used for the quantitation of the toxins must be sensitive, accurate, and robust (Shephard et al., 2011). This report therefore presents our findings on the assessment of the microbial population, and multi-mycotoxin assessment of dried cassava products from different traditional and improved processing practices in Madagascar and Tanzania.

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2.0 Materials and methods

2.1 *Samples*

A total of 126 sun-dried cassava samples were collected from farmers, processors, and markets in Tanzania and Madagascar. The sun-dried samples are in six categories: Grits, 12; improved chips, 9; improved flour, 15; *kivunde*, 6; *makopa*, 48 from Tanzania, and *mangahazo maina* 36 from Madagascar. The traditional practices of *kivunde* production involve slightly drying slices of peeled roots for 1–2 days in the sun, under the roof, or by smoking, followed by heaping on a rock surface or cemented floor, and covering with leaves, cassava peel, straw, or any other covering material to ferment for about 5 days (heap fermentation). Mold covers the surface of the fermented roots that become soft and are scraped off before sun-drying for 5–14 days (Kimaryo et al., 2000; Nzigamasabo and Nimpagaritse, 2009). On the other hand, Tanzanian *makopa* and Malagasy *mangahazo maina* are processed without fermentation. Roots or root-chunks, manually peeled or unpeeled (*maina* in Madagascar), are dried on rock surfaces, plant materials, or any other material laid on the ground. The roots are left until dry before packing; drying period depends on the root size and weather conditions. A wet climate could however cause chance fermentation slightly before the chips are fully dry. Grits and improved chips are processed by newly introduced mechanized processing methods that involve peeling and washing of freshly harvested roots followed by mechanical grating or chipping but exclude the fermentation step (Nweke, 2005; Abass et al., 2013; Amaza et al., 2016). Grated cassava is then dewatered mechanically. Smallholders dry the dewatered cassava or chips in the sun (Abass, 2006) while mechanized processors use mechanical dryers (Amaza et al., 2016). None of the samples collected was dried using a dryer.
Between 850 g and 1000 g per lot was collected from the field, kept in sealed polyethylene bags, and stored at –20 ^{o}C. For analysis, samples were allowed to equilibrate to room temperature and carefully milled into fine flour using a Warring® blender and thereafter sub-sampled for the various analyses (20 g for microbial analysis, 5 g for microbial metabolites analysis).

2.1 *Enumeration of microbial population*

All chemicals and agars were obtained from Fisher Scientific UK Ltd, and Oxoid Ltd, Hampshire, UK. Fungi (yeast/mold) were enumerated following the method described by the International Standards Organization (ISO 1990 & 2008) and East African Community (EAC, 2008a). About 20 g of the subsamples was mixed in 180 ml diluent and processed by further decimal dilutions. The dilutions were poured in the plates containing the agar media and incubated in an inverted position at 25–30 ^{o}C for 3 to 5 days. All colonies were counted on the suitable highest dilution plates containing 25 to 300 colonies and the results were expressed as colony forming units per unit weight of sample (cfu/g).

The detection and enumeration of presumptive *Escherichia coli* was carried out in accordance with ISO (2005) using the liquid-medium culture technique and incubation at 37 °C, then at 44 °C. The detection of *Salmonella* in the samples was done in four stages following the method described by EAC (2008b) in accordance with ISO (2002) and its updates (ISO 2004; 2007).

Confirmation of *E. coli* and *Salmonella* was done by means of biochemical and serological tests as described by ISO (2002).

2.2 *Analysis of microbial metabolites using the liquid chromatography tandem mass spectrometry (LC-MS/MS) technique*

Chemicals: All chemicals including the reference fungal metabolites used were pure and of standard grade. Standard stock solutions of each analyte were prepared as previously described by Sulyok et al. (2015). All solutions kept at –20 °C were allowed to equilibrate to room temperature before being used.

Extraction: Five g of each ground sample was extracted using 20 ml of acetonitrile: water: acetic acid mixture [79:20:1, *v/v/v*] in 50 ml polypropylene centrifuge tubes as described by Sulyok et al. (2015). The supernatant from each raw extract was transferred to auto-sampler vials and diluted using the same volume of dilution solvent—acetonitrile: water: acetic acid (20: 79: 1 *v/v/v*). Diluted extracts were vortexed and 5 µl was used for injection into the LC-MS/MS equipment.

Quantitation: The 'dilute and shoot' multi-mycotoxin method for LC-MS/MS as described by Vishwanath et al. (2009) and by Sulyok et al. (2015) was employed for mycotoxin quantification. Analysis was carried out with a Q Trap 5500 MS/MS system (Applied Biosystems, Foster City, USA) fitted with a Turbola spray electrospray ionization source and a 1290 UHPLC system (Agilent Technologies, Waldbronx, Germany). Chromatographic separation was done at 25 °C on a Gemini® C18-Coloumn, 150 × 4.6 mm i.d., 5 µm particle size, equipped with a C18 security guard centridge, 4 × 3 mm i.d. (Phenomerex, Torrance, CA, USA), and elution was carried out in a binary gradient mode as described by Sulyok et al. (2015).

The 126 sun-dried cassava samples were analyzed for 69 metabolites. In two separate chromatographic runs, ESI-MS/MS was carried out in the scheduled multiple reaction monitoring (sMRM) both in positive and negative polarity with the sMRM detection window of each analyte set to the respective retention time, ± 27 sec in positive mode and ± 42 sec in negative mode. Target scan time was then set to 1 sec. The following parameters were used for settings of the ESI-source: source temperature, 550 °C; curtain gas, 30 psi (206.8 kPa of max 99.5% nitrogen); ion source gas 1 (Sheath gas), 80 psi (551.6 kPa of nitrogen); ion source gas 2 (drying gas); 80 psi (551.6 kPa of nitrogen); ion-spray voltage, –4.500 V and +5.500 V, respectively; collision gas (nitrogen) medium. Positive analyte confirmation in line with European Commission decision 2002/657/EC was obtained by the acquisition of two sMRMs per analyte. Automatic and manual integration of peaks was carried out using MultiQuart 2.0.2. LC-MS/MS machine controlled using Analyst® software version 1.5.1 (AB Sciex, Foster City, CA, USA), and linear $^{1}/_{x}$ weighed calibration functions deriving external calibration was employed for qualification of analytes. The external calibration was done by using serial dilutions of a multi-analyte stock solution. Results were corrected for apparent recoveries, which have been determined by spiking experiments using 9 different types of cassava samples as described by Kovalsky et al, 2016.

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2.3 *Statistical analysis*

Statistical analysis of data was carried out using the Statistical Analysis System© (SAS) ®software version 9.3 (SAS Institute Inc, NC, USA). The calculated mean of fungal species were for positive samples only. Mycotoxin data analysis was conducted using the Proc Means procedure**.**

3.0 Results and Discussion

3.1 Microbial population

Table 1 shows the conformity of the products to microbial specifications. The maximum allowable level for yeast and mold contents in cassava flour is 10^3 (cfu/g); *E. coli* must be absent in 1 g of sample and *Salmonella* must be absent in 25 g (FAO/WHO, 1995; EAC, 2010a & b). None of the dried products from Tanzania was fully compliant with *E. coli* or yeast/mold regulatory limits but *Salmonella* spp. was not detected in any of the 90 samples from Tanzania. More than 10^3 cfu/g of yeast/mold were detected in 75% of samples of grits, 87.5% of *makopa*, 100% of improved chips, 93.7% of improved flour, and 100% of *kivunde. Kivunde* was prone to high funga contamination possibly due to the heap-fermentation method used for its production. The 100% non-compliance by improved chips, however, suggests poor hygiene during mechanical processing, possibly from the use of unclean water, use of unhygienic processing utensils, use of plant-based materials during sun-drying, or complete absence of using water to clean the roots during processing. Some farmers skip entirely the root-washing step. Hence, despite the fact that *Salmonella* appears not to be a problem in the sun-dried products, contamination by fungi and *E. coli* reduced their conformity to microbial quality and safety regulations.

3.2 Microbial metabolites

Modern analytical techniques have made it possible to accurately quantify most mycotoxins, but the emerging mycotoxins can now be also easily and accurately quantified (Gruber-Dorninger et al., 2016). The use of LC-MS/MS in this study facilitated the detection and quantification of multitude of metabolites of *Aspergillus, Fusarium, Alternaria,* and *Penicillium* in the cassava products (Fig. 1). A total of 94 metabolites were detected in more than one sample of cassava products collected from Tanzania; 69 microbial metabolites were detected in more than one such sample from Madagascar. The most agriculturally important mycotoxins—commonly found in the sun-dried cassava samples from some traditional and improved processing practices—were aflatoxins (B1, B2, G1, and M1) (Table 2), fumonisins, and zearalenone (Table 3). A total of 31 emerging mycotoxins and other mycotoxin precursors were detected at varying concentrations in at least 5% of the samples investigated (Table 4).

3.2.1 Aflatoxins

All the four types of aflatoxin observed in this study (B1, B2, G1, and M1) were present in at least one or more samples of makopa, improved chips, and improved flour (Table 2). Both aflatoxins B1 and M1 are of significance to the safety of foods for infant and young children. aflatoxin M1 is a hydroxylated metabolite of aflatoxin B1 and is commonly found in milk after aflatoxin B1 intake in feeds or foods. While the possibility of ingestion of aflatoxin M1 is real for children who have not been weaned and still are consuming mothers' milk, weaning onto local foods, which frequently contain aflatoxins, has been linked to the onset of growth

retardation in children. The presence of aflatoxin M1 in *makopa*, improved chips, and improved flour suggest possible contamination of the processed cassava products with excretion products from household livestock, indicating the likelihood and risk of aflatoxin M1 going into the human food chain. Hence, careful storage of dried cassava products away from household livestock is advisable. Also, the concentrations of aflatoxins B1 and M1 and total aflatoxins are important in monitoring food safety. Generally, the average concentration of aflatoxin M1 was lower than that of aflatoxin B1 in the cassava products. Madagascar chips (*mangahazo maina*) were free from B1, B2 and M1, and Tanzania grits were free from B2 and M1 while *kivunde* was free from B2 and M1. Grits contained the least average total aflatoxins: 0.3 µg/kg in the form of aflatoxin B1 in 8.3% of the samples. The regulatory maximum limits are 10 µg/kg for total aflatoxins and 5 µg/kg for aflatoxin B1 in cassava products (Codex, 1995; FAO, 2003 & 2004; EAC, 2010a & b). The total aflatoxins (sum of B1, B2, G1, and M1) were 38.3 µg/kg in 2/9 samples of improved chips, 29.2 µg/kg in 5/15 of improved flour samples, and 24.8 µg/kg in 3/48 of *makopa* samples. Hence, contrary to prior expectation, some samples of improved cassava products (chips and flour), which were produced using machines and were not fermented, contained mean aflatoxin B1 concentrations that were above the limits, an indication that fermentation contributes to suppression of B1 accumulation and that the use of machines does not guarantee safety unless strict hygiene practices are applied. Percentage prevalence of aflatoxins B1, B2, G1, and M1 were low: 8.7%, 11.9%, 6.3%, and 6.3%, respectively. In total, only 11.1% of all the samples investigated contained at least one form of aflatoxin.

The results suggest that sporadic aflatoxin contamination may occur in mechanically processed cassava products, possibly resulting from poor hygiene conditions even when machines were used or with improper drying. On the other hand, storage period has been observed to influence an increase in the mean concentration of aflatoxin B1 in cassava chips (Manjula et al., 2009).

3.2.2 Fumonisins and zearalenone

Some 31.3–77.8% of the samples contained either or both fumonisins and zearalenone (Table 3). Fumonisins B1, B2, and B3 were absent in *mangahazo maina,* B2 and B3 were absent in grits while B3 was absent in *kivunde*. *Makopa* contained the highest mean concentration of fumonisin B1 (218.3 µg/kg 12/48 of the samples) while grits contained the lowest (11.8 µg/kg; 1/12 of the samples). The mean concentrations of fumonisins B2 and B3 detected in at least one sample of improved chips and flour, *kivunde,* and *makopa* were much lower than B1. In their examination of cassava chips from Brazzaville, Congo, Manjula et al. (2009) noted an occurrence of fumonisin with a mean concentration of 8 µg/kg. Zearalenone is an estrogenic mycotoxin produced by some species of *Fusarium*, mainly *F. graminearum* and *F. culmorum* (Adegoke, 2004). Zearalenone was found at mean concentration range of 16.3–286.7 µg/kg with a mean value of 83.8 µg/kg (Table 3). Zearalenone was detected in *mangahazo maina* (12/36), grits (3/12), improved chips (4/9), improved flour (4/15), *kivunde* (1/6), and *makopa* (28/48). Hence, there seemed to be a higher possibility of zearalenone occurrence in *makopa* than in the other products but with a lower mean concentration (16.5 µg/kg) than in *mangahazo maina* (286.7 µg/kg), *kivunde* (77.3 µg/kg), and improved chips (64.3 µg/kg).

The mean concentration of fumonisin B1 obtained from cassava should be of interest to food safety authorities since in north-eastern Italy where polenta (a maize product) is a staple food,

Franceshi et al. (1990) noted the existence of oesophagal cancer, and Doko and Visconti (1994) later detected high concentrations of fumonisins (500 to 4570 µg/kg) in polenta samples. Many *Fusarium* toxins have a wide range of toxicological and other effects such as immunosuppression and influence on the gastrointestinal system. The recent report of the Joint FAO/WHO Expert Committee on Food Additives recommended 2000-4000 µg/kg as the maximum levels of fumonisins achievable in maize and other cereal-based foods through good agricultural and manufacturing processes (WHO, 2018). Although, no safe levels of fumonisins have been established specifically for cassava products in many African countries, based on the current result, there seems to be a lower risk of fumonisin exposure in processed cassava products, and it is unlikely to obtain higher levels of safety for cassava than that already established for maize. Nevertheless, a risk assessment of exposure to the mycotoxins in cassava products would be necessary.

The co-occurrence of zearalenone with fumonisins was 78% in improved chips, 50% in grits, and 40% in improved flour while prevalence of zearalenone in other products ranged from 31 to 33% (Table 3). Therefore, zearalenone is not only associated with cereals, which is in agreement with Kuiper-Goodman et al. (1987) and Gilbert (1989). While no safe levels have been established for zearalenone in cassava products, the maximum limits allowed in maize and other cereals are between 50 and 1000 µg/kg (FAO, 2003).

3.2.3 Other agriculturally important mycotoxins and precursors

Some aflatoxin precursors were identified in some of the sun-dried cassava products: sterigmatocystin (32.5%; 7.4 µg/kg), averufin (34.1%; 3.8 µg/kg), averantin (19.0%; 1.5 µg/kg), versicolorin C (16.7%; 1.3 µg/kg), averufanin (10.3%; 1.3 µg/kg), and versicolorin A (10.3%; 0.8 µg/kg) (Table 4). The precursors were present at higher mean concentrations and greater prevalence than previously reported for cassava products from Nigeria (Abass et al., 2017). The presence of aflatoxin B1 precursors agrees with the findings of Adjovi et al. (2014) that observed that fresh cassava was safe from aflatoxin B1 but certain processing practices such as heating, freezing, and sun-drying, could limit the ability of fresh roots to block toxigenesis, thereby exposing it to secondary contamination. The presence of aflatoxin B1 precursors and the presence of aflatoxin B1 in some cassava chips show that more knowledge is required to understand the factors that might promote aflatoxin B1 synthesis in some cassava products subjected to specific processing techniques or processed under specific sanitary conditions. Zearalenon-4-Sulfate is the only metabolite of zearalenone detected in the current study in 13.5% of the samples at a mean concentration of 3.6 µg/kg.

The emerging mycotoxins detected at various concentrations in at least 50% of the samples were emodin (75.4%; 8.8 µg/kg), beauvericin (51.6%; 29.5 µg/kg), moniliformin (41.3%; 17.5 µg/kg), sterigmatocystin (a toxic precursor of aflatoxins, especially B1; 32.5%; 7.4 µg/kg), alternariol methyl ether (31.00%; 0.7 µg/kg), nivalenol (a trichothecene; 19.0%; 7.9 µg/kg), fusaric acid (19.0%; 466.2 µg/kg), mycophenolic acid (13.5%; 98.3 µg/kg), enniatins B (7.1%; 2.6 µg/kg), and enniatins B1(6.3%; 1.9 µg/kg). The most prevalent emerging mycotoxin, emodin, is a metabolite of *Aspergillus* spp. and could also be produced by *Penicillium* spp. Moniliformin, beauvericin, and enniatins are well known *Fusarium* metabolites. Toxicologically, toxins of *Fusarium* have been reported to be potentially carcinogenic to

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humans (Franceshi et al., 1990; Sydenham et al., 1990; IARC, 1993; Miller, 2001; Rumbeiha and Oehme, 2005). In addition, moniliformin is a metabolite of *Penicillium melanoconidium*.

Continuous data collection on the potential effect of human exposure to the microbial metabolites is important. Some of the emerging mycotoxins—moniliformin, beauvericin, and enniatins—were recently observed by Gruber-Dorninger (2016) to have no or only low toxicity and acute exposure may pose no serious risk to humans. On the other hand, the little data available suggest that high doses of butenolide fusaric acid or fusaproliferin could be a risk to humans.

The co-occurrence of zearalenone with other *Fusarium* toxins, such as nivalenol, is not unusual in cereals as Tanaka et al. (1988) noted the co-existence of zearalenone with trichothecenes—nivalenol and deoxynivalenol—in some cereals. Kovalsky et al. (2016) recently reported the co-occurrence of mycotoxins with enniatins, and moniliformin in finished feed, maize, and maize silage samples collected in some countries of Europe. Their report revealed median concentration values of 1400 µg/kg for deoxynivalenol in Austria, 350 µg/kg in Germany, and median enniatin's concentration value of 250 µg/kg in Europe. The mean concentrations for enniatin B (1.9 µg/kg), enniatin B1 (2.6 µg/kg), moniliformin (17.5 µg/kg), nivalenol (7.9 µg/kg), tryptophol qual (70.0 µg/kg), tryptophol (794.1 µg/kg) found in this study on sun-dried cassava were lower than the concentrations found in cereals in Europe.

While tryptophol and emodin are anthraquinone derivatives that may be of plant origin (Mueller et al., 1999), alternariol methyl ether is an *Alternaria* metabolite. Toxigenic activities have been reported in *A. alternata* found in apples and maize (Robiglio and Lopez, 1995; Torres et al., 1998). Alternariol which is associated with *Alternaria* spp. has also been reported to be cytotoxic and teratogenic (Burge and Ammann, 1999). Thus, despite the low concentrations of the emerging toxins found in the dried cassava products, attention should continue to be focused on the microbial metabolites along the cassava food chain, particularly when it is realized that literature on mycotoxins in cassava is just beginning to grow owing to the recently available sensitive multi-toxin detection methods (Vishwanath et al., 2009; Ediage, et al. 2011; Sulyok et al., 2015; Abass et al., 2017). Furthermore, available data is still limited when compared with that on cereals and cereal products (Adegoke et al., 1993; Gnonlonfin et al., 2008).

4.0 Conclusions

This study showed that both the traditional and improved processing practices resulted in some cassava products sporadically not conforming to regulatory standards with respect to mold/yeast and some mycotoxins. Although most mycotoxin precursors were detected at generally low concentrations, the exact toxicity limits for the majority of the precursors are not yet known. Also, unlike for maize and other cereals, regulatory limits for some known mycotoxins in cassava have not been established. Hence, attention should continue to be focused on the microbial metabolites through regular surveillance for the early detection of mycotoxins in cassava products. Finally, strict quality control procedures need to be introduced to cassava processors to ensure that the processed products conform to regulatory standards for

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both quality and safety (Dziedzoave et al., 2006). This will improve transboundary movement of cassava products and enhance regional trade, thereby ultimately contributing to increased incomes, employment generation, and improved welfare for the value chain actors.

Acknowledgments

We acknowledge the contribution of the IITA Research Assistants (Mwanahamisi Msangi, Fadhili Yahya, Lazaro Karson, and Eliza Nyamisana) who carried out the survey of cassava products in Tanzania. We thank Dr Michael Waithaka and Ms Miriam Kyotalimye of the Association for Strengthening Agriculture Research in East and Central Africa (ASARECA) for their partnership.

Funding

This work was supported by the Association for Strengthening Agriculture Research in East and Central Africa [sub-grant agreement number ASARECA-RC12-PAAP-01]; the Common Fund for Commodities of the Netherlands [Project number CFC/FIGG/43]; and the CGIAR Research Program on Agriculture for Nutrition and Health (A4NH).

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Figure

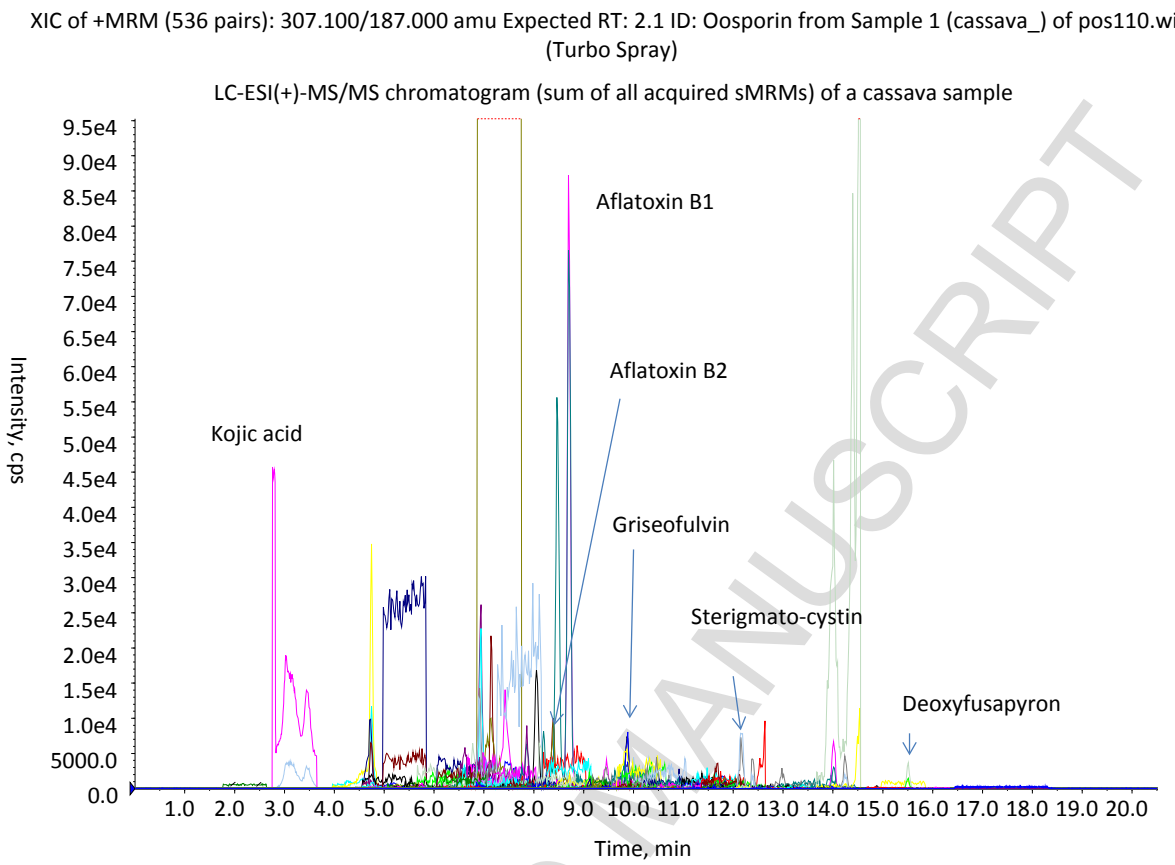


Figure 1: LC-MS/MS chromatogram of a cassava sample

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Tables

Table 1. Conformity with regulatory microbial specifications of sun-dried cassava samples from Madagascar and Tanzania.

Regulatory limit	N	*E. coli*	Salmonella	Yeast/Mold	*E. coli*	Salmonella	Yeast/Mold
		Nil *(MPN/g)*	Nil (/25g)	<1x10^3 (cfu/g)			
		Sample conformity (%)			Microbial population in non-compliant samples		
Mangahazo maina	36	NA	NA	NA	NA	NA	NA
Grits	12	83.3	100	25	4.6×10^1–4.6×10^3	–	2.9×10^3 –2.8×10^7
Improved chips	9	0	100	0	2.4×10^1–1.1×10^5	–	5.0×10^3–5.1×10^6
Improved flour	15	46.7	100	6.7	4.6×10^1–4.6×10^4	–	1.7×10^3–2.4×10^6
Kivunde	6	33.3	100	0	2.1×10^2–2.4×10^4	–	2.7×10^4–6.0×10^5
Makopa	48	39.6	100	12.5	2.0×10^4–4.6×10^4	–	2.9×10^3–3.5×10^6

Maximum regulatory limit was based on FAO/WHO 1995 & EAC 2010a & b; NA: Data not available. Percentage conformity was calculated as: 100*n/N , where n = number of samples with occurrence of microorganism below the maximum regulatory limit, N = total number of investigated samples. MPN = most probable number of *E. coli,* cfu = colony forming unit.

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Table 2. Concentration of aflatoxins in sun-dried cassava samples from Madagascar and Tanzania.

Products	N	Aflatoxin B1 (µg/kg)	Aflatoxin B2 (µg/kg)	Aflatoxin G1 (µg/kg)	Total Aflatoxins (B1, B2, G1)		Aflatoxin M1 (µg/kg)
R (%) ± STD		82.9 ± 7.3	91.8 ± 5.4	86.5 ± 8.5	Mean concentration (µg/kg)	# of samples <LOQ	84.1 ± 6.8
LOD (µg/kg)		0.1	0.2	0.2			0.3
LOQ (µg/kg)		0.3	0.6	0.6			0.9
Mangahazo maina	36	<LOD	<LOD	2.6 (2)	2.6 (2)	0	<LOD
Grits	12	0.3 (1)	<LOD	<LOD	0.3 (1)	1	<LOD
Improved chips	9	31.9 (2)	7.3 (1)	5.5 (1)	38.3 (2)	0	3.4 (1)
Improved flour	15	23.0 (5)	5.1 (3)	5.3 (3)	29.2 (5)	2	1.6 (2)
Kivunde	6	4.9(1)	<LOD	1.6 (1)	6.5 (1)	0	<LOD
Makopa	48	32.1(2)	8.3(1)	1.8 (1)	24.8 (3)	0	3.6 (1)
Mean	126	22.6 ± 31.1	6.2 ± 5.3	3.7 ± 2.9			2.6 ± 1.5
Range	126	0.3–32.1	5.1–8.3	1.6–5.5			1.6–82.9
Prevalence (%)	126	8.7	11.9	6.3	11.1		6.3

R is the percentage apparent recovery obtained with nine test samples; LOD is the limit at which a toxin is detectable; LOQ is the minimum concentration at which quantitative concentrations were reported; Figures in parentheses are a number of samples with concentration figures used for mean estimation; N is the total number of investigated samples.

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Table 3. Concentration of fumonisins and zearalenone in sun-dried cassava samples from Madagascar and Tanzania.

Products	N	Fumonisin *B1* (µg/kg)	Fumonisin *B2* (µg/kg)	Fumonisin *B3* (µg/kg)	Zearalenone (µg/kg)	Co-occurrence of fumonisins and zearalenone (%)	# of samples < LOQ
R (%) ± STD		*86.3 ± 9.4*	*92.2 ± 7.0*	*92.4*	*100.1 ± 16.4*		
LOD (µg/kg)		*3*	*1.5*	*2*	*0.1*		
LOQ (µg/kg)		*9*	*4.5*	*6*	*0.3*		
Mangahazo maina	36	<LOD	<LOD	<LOD	286.7 (12)	33.3	2
Grits	12	11.8 (1)	<LOD	<LOD	16.3 (3)	50.0	1
Improved chips	9	31.4 (3)	13.2 (2)	16.3 (1)	64.3 (4)	77.8	2
Improved flour	15	73.4 (4)	26.0 (4)	10.8 (1)	18.0 (4)	40.0	2
Kivunde	6	47.7 (1)	20.1 (1)	<LOD	77.3 (1)	33.3	0
Makopa	48	218.3 (12)	20.9 (4)	62.1 (2)	16.5 (28)	31.3	1
Mean	126	141.1 ± 437.7	20.5 ± 16.1	37.8 ± 44.7	83.8 ± 469.5		
Range	126	11.8–218.3	13.2–26.0	10.8–62.1	16.3–286.7		
Prevalence (%)	126	19.1	9.5	3.2	41.3		

R is the percentage apparent recovery obtained with nine test samples; LOD is the limit at which a toxin is detectable; LOQ is the minimum quantitable concentration; Figures in parentheses are a number of samples with quantitable concentrations used for mean estimation; Prevalence was calculated as 100*n/N where n is the number of samples with quantitative concentration; N is the total number of investigated samples.

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Table 4. Emerging mycotoxins and other microbial metabolites detected in a least 5% of the sun-dried cassava samples from Madagascar and Tanzania.

Analytes	Prevalence (%)	Mean [µg/kg]	LOD [µg/kg]	Samples detected (< LOQ)	R ± Std (%)	Analyte	Prevalence (%)	Mean [µg/kg]	LOD [µg/kg]	Samples detected (< LOQ)	R ± Std (%)
Emodin†	75.4	8.8	0.1	19	105.8 ± 3.6	Averantin	19.0	1.5	0.1	11	79.8 ± 11.4
Tryptophol	67.5	794.1	15.0	3	96.7 ± 13.0	Nivalenol†	19.0	7.9	0.5	5	70.8± 13.5
Equisetin	61.9	277.2	0.2	11	136 ± 18.4	Versicolorin C	16.7	1.3	0.1	7	119.6 ± 8.4
Beauvericin†	51.6	29.5	0.1	28	97.6 ± 8.1	Dechlorogriseofulvin	16.7	36.4	0.2	NA	96.5 ± 7.7
Moniliformin†	41.3	17.5	0.8	13	82.4 ± 24.9	Zearalenon-4-Sulfate	13.5	3.6	0.0	NA	107.1±10.8
3-Nitropropionic acid	36.5	277.7	0.7	7	36 ± 1.7	Mycophenolic acid†	13.5	98.3	1.0	NA	95.1 ± 7.4
Deoxyfusapyron	34.9	238.5	0.3	1	95.4 ± 7.5	Macrosporin A	12.7	10.9	0.2	16	115.1 ± 10.8
Averufin	34.1	3.8	0.1	9	71.2 ± 16	Siccanol	11.9	7165	NA	5	100.0
Kojic acid	32.5	1,417	15.0	6	72.3 ± 33.6	Averufanin	10.3	1.3	0.1	8	63.3 ± 6.2
Sterigmatocystin†	32.5	7.4	0.0	2	96.2 ± 7.1	Versicolorin A	10.3	0.8	0.1	2	119.6 ± 8.4
Griseofulvin	32.5	35.6	0.2	NA	95.8 ± 6.1	Physcion	7.9	477.9	5.0	3	92.2 ± 13.2
Alternariol methyl ether†	31.0	0.7	0.0	7	97.9 ± 6	Enniatin B†	7.1	2.6	0.0	3	99.4± 8.9
Fusapyron	29.4	323.8	0.3	1	95.8 ± 17.2	Curvularin	6.3	16.9	0.4	3	109.3± 9.6
Chrysogine	26.2	168.3	0.4	NA	90 ± 1.7	Enniatin B1†	6.3	1.9	0.0	1	96.6 ± 10.1
Dihydrogriseofulvin	23.0	19.4	0.2	NA	94.1 ± 5.5	Diacetoxyscirpenol	5.6	6.1	0.2	NA	96.3 ± 7.1
Apicidin	21.4	49.7	0.1	4	98.3± 8.8						

Emerging mycotoxin†; R is the percentage apparent recovery obtained with nine test samples; Prevalence was calculated as 100*n/126, where n is the number of quantitable samples and 126 is the total number of investigated samples; Calculation of means was based on concentration values of quantitated samples; LOD is the limit of detection; NA: Not applicable

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