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Mould and mycotoxin exposure assessment of melon and bush mango seeds, two common soup thickeners consumed in Nigeria

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HT-2 toxin
Moniliformin
Mycophenolic acid
Nivalenol
Ochratoxin A
T-2 toxin
Tenuazonic acid

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ABSTRACT

An examination of the mould and fungal metabolite pattern in melon and bush mango seeds locally produced in Nigeria was undertaken in order to understand the mycotoxicological risk posed to consumers of both of these important and commonly consumed soup thickeners. The variation in mycotoxin levels in graded categories of both foodstuffs were also determined. *Aspergillus*, *Fusarium*, *Penicillium*, Mucorales and *Trichoderma* were the recovered fungi from the foodstuffs with *Aspergillus* species dominating (melon = 97.8%; bush mango = 89.9%). Among the *Aspergillus* species identified *Aspergillus* section *Flavi* dominated (melon: 72%; bush mango: 57%) and *A. flavus*, *A. parasiticus*, *A. parvisclerotigenus* and *A. tamarii* were the recovered species. About 56% and 73% of the *A. flavus* isolates from melon and bush mango seed samples, respectively were aflatoxigenic. Thirty-four and 59 metabolites including notable mycotoxins were found in the melon and bush mango seeds respectively. Mean aflatoxin levels (μg/kg) in melon (aflatoxin B_1 (AFB_1) = 37.5 and total aflatoxins = 142) and bush mango seeds (AFB_1 = 68.1 and total aflatoxins = 61.7) were higher than other mycotoxins, suggesting potential higher exposure for consumer populations. Significantly ($p < 0.05$) higher levels of mycotoxins were found in hand-peeled melon and discoloured bush mango seeds than in machine-peeled melon and non-discoloured seeds except for HT-2 and T-2 toxins which occurred conversely. All melon and bush mango seeds exceeded the 2 μg/kg AFB_1 limit whereas all melon and 55% of bush mango seeds exceeded the 4 μg/kg total aflatoxin EU limit adopted in Nigeria. This is the first report of (1) mycotoxin co-occurrence in bush mango seeds, (2) cyclopiazonic acid, HT-2 toxin, moniliformin, mycophenolic acid, T-2 toxin and tenuazonic acid occurrence, and (3) mycotoxin exposure assessment of both foodstuffs.

1. Introduction

Food forms an important part of the cultural identity of people. In Nigeria, while some foods are consumed as part of cultural or religious festivals, others are consumed as part of regular culinary practices. Some of the latter category of food include condiments and soup thickeners such as melon and bush mango seeds (Odunfa, 1981; Ainge and Brown, 2004).

Melon (*Colocynthis citrullus* L.) seeds (locally regarded as *egusi*) constitute an important soup thickener consumed widely in Nigeria and across West Africa. The seeds are usually harvested from fruits of *C. citrullus* which are heaped and allowed to rot to enable the extraction of the melon seeds in-shell. The freshly harvested seeds are washed, air dried and stored in-shell in bags. The stored seeds may then be sold as in-shell seeds or shelled seeds after seed-coat removal. Shelling of melon seeds may be by hand peeling or machine peeling; it is the common practice to hand-peel in the local setting whereas machine peeling is often associated with large/bulk bags of the seeds especially when intended for large scale markets. Melon seed is consumed popularly as *egusi* soup, snack (Ajuru and Okoli, 2013), protein substitute in diets (Nwokolo and Sim, 1987) and as a fermented condiment called *ogiri* (Odunfa, 1981). *Egusi* is an important oil seed with more than 50% oil. It is also a good source of essential amino acids, vitamins and

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micronutrients (Akobundu et al., 1982). Nigeria contributes about 75% of the global melon seed production, which was estimated at 530,350 tonnes in 2014 (FAOSTAT, 2015). There is high demand for egusi, both locally in Nigeria and internationally, and recently, there has been increased shipment of melon seeds from Nigeria to Europe and USA.

Bush mango (*Irvingia gabonensis*) seeds (locally referred to as *ogbono*) are obtained from bush mango fruits, which are heaped and allowed to ferment spontaneously. The fermented fruits are de-pulped, seeds extracted and then sun-dried (Ejiofor, 1994). Dried bush mango seeds are ground into powdery form and the flour is used to prepare a thick and slimy *ogbono* soup, accompanied with vegetables, meat, fish or other protein sources and eaten with main carbohydrate meals (Ekpe et al., 2007). *Ogbono* soup is a delicacy which constitutes an important part of the diets in southern parts of Nigeria. Bush mango seeds are rich in essential amino acids (Ekpe et al., 2007) and dietary fibers that are effective in ameliorating blood glucose and lipid metabolism of diabetics (Omoruyi and Adamson, 1994). Defatted flour of *I. gabonensis* is rich in crude protein (25.19%) (Ogunsina et al., 2012) while the fruit pulp has been reported to be suitable for wine production (Akubor, 1996). Bush mango is the most valuable non-timber forest product in cross-border trade between Nigeria and Cameroon (Sunderland and Isoni, 2001). It was reported that collection and sale of bush mango accounted for as high as 85% of annual income for some households in Nigeria (Asaha et al., 2006).

Bush mango seeds, just like those of melon, are seasonal crops, hence they are stored in calabashes, pots or sacks at ambient temperatures for up to 8 months (Chinaka and Obiefuna, 1999) in order to make them available during the off-season. Long storage periods, especially when the seeds are not properly dried, coupled with the humid tropical climate provide an optimal environment for fungal infestation and mycotoxin accumulation during storage (Bankole et al., 2006b). These could induce physical (discolourations) and chemical loss in seed quality.

Very few studies have focused on both fungi and aflatoxin contamination of either melon or bush mango seeds in Nigeria (Bankole et al., 2004a, 2004b; Adebayo-Tayo et al., 2006; Bankole et al., 2006a) although others have considered either moulds (Aboloma and Ogunbusola, 2012; Sanyaolu et al., 2014) or aflatoxins in the seeds (Williams et al., 2015). The implicated moulds include *Aspergillus*, *Penicillium*, *Trichoderma viride* and *Mucor mucedo* in bush mango seeds (Adebayo-Tayo et al., 2006; Aboloma and Ogunbusola, 2012; Sanyaolu et al., 2014), while *Aspergillus* and *Penicillium* species have been found to predominantly contaminate melon seeds (Bankole et al., 2004a, 2006a). Additionally, the only mycotoxin contamination reported for either crop has been aflatoxin and levels reached 55 μg/kg aflatoxin B_1 (AFB_1) in melon seeds from farmers' stores and markets in Nigeria (Bankole et al., 2004b, 2006a; Williams et al., 2015) and 11.7 μg/kg mean AFB_1 and 63.4 μg/kg mean total aflatoxin in bush mango sold in markets in Calabar, Nigeria (Williams et al., 2015). At present, there is no report on other mycotoxins in these crops in Nigeria, although Somorin et al. (2016) found co-occurrence of aflatoxins, ochratoxin A (OTA) and citrinin (CIT) in melon seeds (mostly originating from Nigeria) consumed in Ireland and the United Kingdom.

This study was designed to assess the mycotoxicological safety of food thickeners regularly and widely consumed in Nigeria through routine surveillance studies, particularly considering the importance of *ogbono* and *egusi* soups to the Nigerian people, and their wide consumption in the diaspora which increases their local and export trade. The study aimed also at enumerating the fungi, especially the aflatoxigenic species of *Aspergillus*, and spectrum of mycotoxins contaminating melon and bush mango seeds sold in local markets in Lagos, Nigeria.

2. Materials and methods

2.1. Food samples

Samples of melon ($n = 16$) and bush mango ($n = 40$) seeds were purchased from local markets in Lagos state, Nigeria between September and October 2014. The melon samples comprised two categories based on the method of peeling/seed coat removal: hand-peeled and machine-peeled; each having an equal number of samples. The hand-peeled melon seeds appeared dirtier and often broken/cracked than the machine-peeled seeds. The bush mango samples also comprised two groups of equal sample size, graded by their physical appearance: discoloured and non-discoloured seeds. The discoloured bush mango seeds were dirty and often mouldy, having colours ranging from dark grey with yellow spots to dark green with blue spots. All the melon and bush mango samples originated from the Southeastern parts of Nigeria. In terms of sample storage durations, the melon seeds were <30 days in retailers' possession/store whereas bush mango seeds were between 15 days (non-discoloured seeds) and 90 days (discoloured seeds) in store.

Sampling and quartering of collected samples to obtain representative subsamples for analysis were conducted as follows: about 1 kg bulk sample size was collected per sample of melon and bush mango seeds. Each bulk sample was collected as a mix of 5–10 random subsamples collected from at least five points of the traders' storage bags or cans. Each bulk sample was quartered two consecutive times to yield sub samples weighing 62.5 g. Two of the eight representative sub samples for each sample were taken, thoroughly mixed to yield 125 g and divided into three equal parts: part A, for moisture content determination; part B for assessment of moulds; part C, for mycotoxin analysis. All sub samples of part A were analyzed immediately while parts B and C sub samples were stored in clean *zip-lock* bags at 4 °C prior to further analysis. All samples were ground to powder before analysis. Moisture content and mycological analysis were conducted at the Microbiology Laboratory, Babcock University, Nigeria while mycotoxin analysis by liquid chromatography tandem mass spectrometry (LC–MS/MS) was carried out at the Center for Analytical Chemistry, Department of Agrobiotechnology (IFA-Tulln), Austria. Samples analyzed for mycotoxins were kept at −20 °C at IFA-Tulln prior to analysis. Samples for spiking experiments were bought in a supermarket specialized on African foodstuffs located in Vienna.

2.2. Determination of moisture content of food samples

The moisture content of each melon and bush mango sample was determined using an OAHUS MB25 MC-173467 moisture analyzer (Ohaus Corp. Pinc Brook, NY, USA). The heating temperature was 105 °C (Commission of the European Communities, 2000). Triplicate samples and readings were taken per sample and mean values obtained per sample.

2.3. Isolation of moulds from melon and bush mango seed samples

Moulds present in both foodstuffs were isolated and enumerated by the dilution plating technique of Samson et al. (1995). Ten (10) grams of each sample was suspended in 90 ml of sterile distilled water and homogenized for 2 min. Aliquots (0.1 ml) of each mixture were spread-plated in triplicate on a set of semi-selective mycological media: one-quarter strength potato dextrose agar [(PDA), 9.57 g/l PDA (Difco) and 20 g/l Bacto™ agar supplemented with 0.002% lactic acid] and modified rose bengal agar (mRBA) (Cotty, 1994). The mRBA plates were incubated for 3 days at 31 °C whereas PDA plates were incubated at 25 °C for 5–7 days. *Aspergillus* colonies were counted on mRBA whereas *Fusarium*, *Penicillium* and other moulds were counted on PDA plates. Fungal load was calculated and expressed as Log_{10} colony forming units per gram (log_{10}CFU/g) of sample analyzed.

Colonies of *Aspergillus* (including members of *Aspergillus* section *Flavi*) were transferred to malt extract agar (MEA, Lab M™) and further purified on neutral red desiccated coconut agar (NRDCA) (Ezekiel et al., 2014a), while *Fusarium* and *Penicillium* were purified on full-strength PDA and water agar (2% agar/L of distilled water) Ezekiel et al. (2014b), respectively. Other moulds were purified on half- and full-