Natural co-occurrence of aflatoxins and ochratoxin A in ginger (*Zingiber officinale*) from Nigeria

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Abstract

The natural co-occurrence of aflatoxins (AFB_1, AFB_2, AFG_1 and AFG_2) and ochratoxin A (OTA) in dried split ginger purchased from different local markets in Lagos, South West Nigeria has been investigated. A total of 120 ginger samples, 31 collected during the rainy season and 89 during the dry season, were analyzed. Mycotoxins were determined according to the AOAC Official Method 2008.02 based on multi-toxin immunoaffinity column clean up and liquid chromatography quantification. The incidence of contamination with aflatoxins (AFs) and OTA was significantly higher during the rainy season (81% and 77%, respectively) than the dry season (46% and 37%, respectively). Average levels of AFs and OTA in positive samples were 3.13 and 5.10 µg/kg in the rainy season (range 0.11-9.52 µg/kg and 0.20-9.90 µg/kg) and 1.18 and 2.76 µg/kg (range 0.20-3.57 µg/kg and 0.17-12.02 µg/kg) in the dry season, respectively. Furthermore, the levels of AFB_1 detected in 7 out of

31 samples (23%) collected during the rainy season were above the European Union (EU) maximum permitted level (i.e. 5 µg/kg). No samples were found above the EU regulatory limits established for OTA in ginger (i.e. 15 µg/kg). Moreover, a higher co-occurrence of AFs and OTA was observed in samples collected during the rainy season (65%) than the dry season (21%). Data showed that high humidity and temperature occurring during storage, which are prevalent in the rainy season, offer favorable conditions for AFs and OTA fungal production. This is the first report on the co-occurrence of AFs and OTA in ginger samples from Nigeria. Our results demonstrate that, in order to minimize the risks for consumers, the monitoring of the co-occurrence of these mycotoxins in ginger is highly recommended.

Keywords: aflatoxins, ochratoxin A, co-occurrence, ginger (*Zingiber officinale*), Lagos-Nigeria

1. Introduction

Ginger (*Zingiber officinale*) is a perennial root crop cultivated nearly tropical and subtropical areas of the world. The rhizomes, which contain several bioactive constituents, are widely used both as a spice and for medicinal purposes. Concerning this aspect, ginger rhizome has been used extensively for more than 2500 years in China to treat headaches, nausea and colds and in Mediterranean and Western countries as an alternative therapy for the treatment of rheumatological conditions, dyslipidemia and muscular discomfort (Bordia *et al.*, 1997; Langner *et al.*, 1998; Sharma & Clark, 1998; Grant & Lutz, 2000). Ginger also has a high content of minerals, vitamins and phytochemicals. Due to these properties ginger has gained considerable attention in the USA and Europe in recent years as a botanical dietary supplement, especially for its use in the treatment of chronic inflammatory conditions (Shukla *et al.*, 2007).

The largest producer of ginger in the world is India, followed by China, Nepal, Nigeria and Thailand (Onu *et al.*, 2014). Even though Nigeria ranks fifth for ginger production, the country contributes significantly to ginger export all over the world (Zakka *et al.*, 2010). In particular, Nigeria and China are the major suppliers to Europe (Onu *et a*l., 2014). In Kaduna State, the major producing area in Nigeria, ginger is usually planted in March, harvested in November, and then sun-dried (Nmadu & Marcus, 2013).

In world trade, the rhizome is marketed in several forms such as fresh or dried product, liquid or solid extract, tablets or capsules, bulk powder and in tea bags (Whitaker *et al.*, 2009). Spices, including ginger, can be subjected to contamination during harvesting, handling, storage and distribution by several fungi responsible for spoilage and production of mycotoxins, in particular aflatoxins (AFs) and ochratoxin A (OTA) (Ozbey & Kabak, 2012; Kabak & Dobson, 2015). Aflatoxins (AFB_1, AFB_2, AFG_1 and AFG_2) are the most toxic group of mycotoxins produced by some *Aspergillus* species (*A. flavus*, *A. parasiticus* and more rarely by *A. nomius*) (Pitt, 2000). Aflatoxin B_1 is among the most carcinogenic substances known and has been classified by the International Agency for Research on Cancer as Group 1 (IARC,1993). Ochratoxin A is produced mainly by *Penicillium verrucosum* in temperate climates and by *Aspergillus ochraceus* (and more rarely by *A. carbonarius*) in warm and tropical climates. It can contaminate agricultural products prior to harvest or, much more commonly, during storage (EFSA, 2006). Ochratoxin A has been shown to cause several toxic effects in animals including immunotoxicity, nephrotoxicity and teratogenicity. Furthermore, the IARC, based on sufficient evidence of its carcinogenicity in animal studies, has classified OTA as a possible human carcinogen (Group 2B) (IARC, 1993).

Due to their health hazards for humans, many countries have established regulations for mycotoxins in spices. Concerning ginger, the European Commission has established maximum levels of 5 µg/kg for AFB_1, 10 µg/kg for total AFs (the sum of AFB_1, AFB_2, AFG_1 and AFG_2) and 15 µg/kg for OTA (European Commission, 2006; European Commission,

2012). Due to environmental conditions favorable to colonization of toxigenic fungi in tropical countries, both groups of mycotoxins could co-contaminate spices both in the field and storage. In vitro and in vivo studies have shown that mycotoxin mixtures may exert synergistic or additive toxic effects (Oliveira and Correa, 2010). The consumption of food contaminated with more mycotoxins could be a significant threat to human health.

Reliable methods for the simultaneous determination of AFs and OTA in ginseng and other botanical roots have been developed and validated (Trucksess et $al.$, 2006; Trucksess et $al.$, 2007, Trucksess et $al.$, 2008). In particular, a method for the determination of AFB_1, AFB_2, AFG_1, and AFG_2 and OTA in ginseng and ginger has been validated by collaborative study (Trucksess et $al.$, 2008) and subsequently approved by the AOAC International as Official Method (AOAC Official Method 2008.02).

In order to assess health risks for humans and animals due to the simultaneous exposure to AFs and OTA in spices, the co-occurrence of these mycotoxins in red chilli, paprika, black pepper, cumin and cinnamon has been widely reviewed in the literature in several countries such as Brazil (Shundo et $al.$, 2009), Hungary (Fazekas et $al.$, 2005), India (Saha et $al.,$ 2007), Malaysia (Jalili et $al.$, 2012; Ali et al., 2015), Spain (Hernández Hierro et $al.,$ 2008; Santos et $al.$, 2010), Turkey (Ozbey et $al.$, 2012), Pakistan (Iqbal et $al.$, 2013) and Italy (Prelle et $al.$, 2014). However, only a few studies concerning the co-occurrence of OTA and AFs in ginger powder have been reported (Trucksess et $al.$ 2007; Wen et $al.,$ 2013; Wen et $al.$, 2014). To date, no survey on the co-occurrence of AFs and OTA in ginger from Nigeria has been reported. Therefore, the aim of this study was to investigate the natural co-occurrence of AFs and OTA in ginger samples purchased from local markets in Lagos, South West Nigeria during the dry season (November - March) and the rainy season (April - October), in order to compare the detected levels with permissible limits established by the European Commission. The possible influence of storage conditions occurring during the

dry and rainy seasons on the incidence and levels of AFs and OTA contamination in ginger

samples is discussed.

2. Materials and Methods

2.1. Sample collection

A total of 120 dried split ginger samples were purchased randomly from different local

markets in Lagos Nigeria. In particular, 89 samples were collected during the dry season

(January – April 2014, equivalent to 2-5 months of storage) and 31 samples during the rainy

season (July - October 2014, equivalent to 8-11 months of storage). Samples were kept at

4°C until chemical analysis.

2.2. Reagents and Chemicals

Acetronitrile and methanol (HPLC grade) were purchased from Carlo Erba Reagents (Milan,

Italy). Ultrapure water was produced by a Waters Milli-Q system (Waters, Milford, MA, USA).

Ochratoxin A and AFs (AFB_1, AFB_2, AFG_1, AFG_2) analytical standards, TWEEN® 20, sodium

chloride (NaCl), phosphate-buffered saline (PBS) and sodium bicarbonate ($NaHCO_3$) were

purchased from Sigma-Aldrich (Milan, Italy). Glass microfiber filters (Whatman GF/A) and

paper filters (Whatman N. 4) were obtained from Whatman (Maidstone, UK). *AflaOchra*

HPLC™ immunoaffinity columns were purchased from VICAM, A Waters Business (Milford,

MA, USA).

2.3. Preparation of Standard Solutions

An OTA stock solution was prepared at concentration of 1 mg/mL by dissolving the solid

toxin in toluene:acetic acid (99:1, *v/v*). An OTA standard solution, at the concentration of

about 10 µg/mL, was prepared by dissolving in methanol an adequate amount of the stock

solution previously evaporated to dryness under a stream of nitrogen. The standard solution

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128 was tested spectrophotometrically (ε= 6330 cm^2/mmol, at λ= 333 nm in methanol). For the

129 preparation of standard solutions for HPLC calibration curves, a solution of OTA was

130 prepared in methanol at a concentration of 250 ng/mL. Aliquots of this solution were

131 evaporated to dryness under a stream of nitrogen and dissolved in a mixture

132 acetonitrile/water/acetic acid 99:99:2 (*v/v/v*). The 250 ng/mL OTA standard solution was also

133 used as the spiking solution for recovery experiments.

134 Standard solutions of AFB$_1$, AFB$_2$, AFG$_1$, and AFG$_2$ were prepared separately according to

135 the AOAC Official Method 971.22 by dissolving the solid toxins in toluene/acetonitrile (90:10,

136 v/v) at a concentration of 100 µg/mL for each aflatoxin. A working solution for each aflatoxin

137 at the concentration of 10 µg/mL was prepared in toluene/acetonitrile 90:10 (*v/v*) and tested

138 spectrophotometrically (ε= 19300, 21000, 16400 and 18300 cm^2/mmol for AFB$_1$, AFB$_2$,

139 AFG$_1$ and AFG$_2$, respectively, at λ= 350 nm). For the preparation of a mixed solution for

140 HPLC calibration, a mixed aflatoxins solution (containing 1000 ng/mL of AFB$_1$ and AFG$_1$,

141 and 200 ng/mL of AFB$_2$ and AFG$_2$ in toluene/acetonitrile 90:10 (v/v)) was prepared by

142 appropriate dilution of AFB$_1$, AFB$_2$, AFG$_1$ and AFG$_2$ working solutions. In particular, aliquots

143 of the mixed aflatoxins solution (1000 ng/mL of AFB$_1$ and AFG$_1$, 200 ng/mL of AFB$_2$ and

144 AFG$_2$) were evaporated to dryness under a stream of nitrogen and reconstituted in a mixture

145 of water/acetonitrile 60:40 (*v/v*). For recovery experiments, an AF spiking solution (250

146 ng/mL of AFB$_1$ and AFG$_1$, 50 ng/mL of AFB$_2$ and AFG$_2$) was prepared by evaporating to

147 dryness (under a stream of nitrogen) the mixed aflatoxins solution, which was then

148 reconstituted in acetonitrile.

149 *2.4. Sample extraction and clean up*

150 Dried split ginger samples were finely ground (particle size ≤0.5 mm) using a Cyclone

151 sample mill (PBI International, Milan, Italy). Extraction and clean up of the powdered ginger

152 were performed according to the AOAC Official Method 2008.02. In particular, 5 g of sample

153 was weighted into a 50 mL centrifuge tube, 1 g of NaCl was added and the sample was

154 extracted with 25 mL methanol/0.5% $NaHCO_3$ (70:30, *v/v*) by shaking at 400 rpm for 10 min

155 (KS 4000i, IKA Werke GmbH & Co. KG., Staufen, Germany). The extract was centrifuged

156 for 10 min at 7000 rpm (SL 16 Centrifuge Series, Thermo Fisher Scientific Inc, Waltham,

157 MA, USA). Seven milliliter of supernatant were diluted with 28 mL 0.1 M PBS containing 1%

158 Tween® 20, mixed and filtered through a glass microfiber paper. Twenty-five milliliter of

159 diluted extract (equivalent to 1 g sample) were passed through an immunoaffinity column

160 (*AflaOchra HPLC™,*Milford, MA, USA) at a flow rate of about one drop per second. The

161 column was washed with 5 mL of 10 mM PBS and 5 mL of distilled water at a flow rate of

162 one or two drops per second. Ochratoxin A and AFs were eluted from the column with

163 methanol (2 × 1 mL) at flow rate of one drop per second. The cleaned extract was collected

164 in a 4 mL screw-cap amber vial, allowing the column to run dry by forcing air through column.

165 The eluate was diluted with 1 mL of water before HPLC analysis.

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167 *2.5. HPLC analysis*

168 Two separate HPLC analyses were performed for the determination of AFs and OTA,

169 respectively. In particular, separate aliquots (100 µL) of the purified extract were injected

170 into the HPLC apparatus, which consisted of an Agilent 1260 Series Chromatographic

171 System (Agilent Technologies, Palo Alto, CA, USA) equipped with a fluorometric detector

172 (model G1321B). Data acquisition and instrument control were performed by the OpenLAB

173 data software (Agilent Technologies).

174 For determination of AFs, the analytical column used was a the Luna® PFP(2) (3 µm, 150 ×

175 4.6 mm, Phenomenex, Torrance, CA, USA). The chromatographic separation was

176 performed by isocratic elution at a flow rate of 0.8 mL/min with a mobile phase of

177 acetonitrile/water 30:70 (*v/v*). A post-column derivatization with a photochemical derivatizer

178 (UVE™, LC Tech, Dorfen, Germany) was performed to enhance the fluorescence intensity

179 of AFB_1 and AFG_1 (photochemical radiation with UV light at 254 nm). The fluorometric

detector was set at excitation and emission wavelengths of 365 nm and 435 nm, respectively. The column was kept at a temperature of 30°C. The retention times of AFG_2, AFB_2, AFG_1, and AFB_1 were between 7 and 13 min.

For OTA determination a Zorbax SB-C18 analytical column (5 μm, 150 × 4.6 mm, Agilent Technologies) was used. The chromatographic separation was performed by isocratic elution at a flow rate of 1 mL/min with a mobile phase composed of acetonitrile/water/acetic acid 99:99:2 (*v/v/v*). The column was kept at a temperature of 30° C and the fluorometric detector was set at excitation and emission wavelengths of 333 nm and 460 nm, respectively. The retention time of OTA was about 7 min.

Typical HPLC chromatograms of AFB_1, AFB_2, AFG_1 and AFG_2 (Fig. 1a) and OTA (Fig. 1b) of standard solutions, blank and naturally contaminated ginger samples are reported in Figure 1.

Limits of detection (LODs), defined as a signal-to-noise ratio 3:1, were 0.1 μg/kg for OTA, AFB_1 and AFG_1 and 0.02 μg/kg for AFB_2 and AFG_2. Limits of quantification (LOQs), defined as a signal-to-noise ratio 10:1, were 0.3 μg/kg for OTA, AFB_1 and AFG_1 and 0.06 μg/kg for AFB_2 and AFG_2.

In three replicate experiments, uncontaminated (blank) ginger samples were spiked simultaneously with 100 μL of the AFs spiking solution and 100 μL of the OTA spiking solution, to obtain a spiking level of 12 μg/kg for AFs (i.e. 5 μg/kg AFB_1, 1 μg/kg AFB_2, 5 μg/kg AFG_1, 1 μg/kg AFG_2) and 5 μg/kg for OTA. Spiked samples were left to dry for 1 hour at room temperature to allow solvent evaporation. Samples were then extracted for mycotoxin analysis. This procedure is commonly used in in-house and in interlaboratory validation studies. Average recoveries of AFs and OTA from ginger were 75% and 86%, respectively with relative standard deviations less than 6%.

2.6. *Statistical analysis*

To compare the evaluation of difference incidence of contamination of OTA and AFs in ginger samples between the rainy and dry seasons the software Sigma Plot™ (Version 12.3, Statistic software Inc, USA) was used. The Shapiro Wilk test was used to determine the normality of the contamination data distribution of two seasonal surveys. Due to the non-normal distribution of the data, the non-parametric Mann-Whitney U test was used, with the level of confidence fixed at $p < 0.05$ (95%). The Mann-Whitney U test is used to determine whether or not two population medians are equal.

3. Results and discussion

The Nigerian climate is generally characterized by high temperatures alternating dry and wet seasons. The rainfall usually starts in April and ends in October, while the dry season starts in November and ends in March. Lagos, Nigeria, lies within latitudes 6°4' and 7°5' N and longitudes 3°5' and 8°8' E and has a bimodal annual rainfall averaging between 1,300 and 2,000 mm and maximum temperatures ranging from 26-28°C (Adetunji et al., 2014). These climatic conditions play a key role in fungal growth and mycotoxin contamination during storage of commodities, mainly when inadequate storage conditions are used (Heperkan, 2006). This study reports, for the first time, the occurrence of AFs and OTA in dry ginger samples from Nigeria, collected at two different periods of storage during the dry and rainy seasons.

3.1. Occurrence of Aflatoxins

Among the 120 samples analyzed, 54 samples (45%) were below the limit of detection (LOD) of 0.1 µg/kg for AFB_1 and AFG_1 and 0.02 µg/kg for AFB_2 and AFG_2. Of these, six samples collected during the rainy season (19%) and 48 samples during the dry season (54%). Sixty-six samples (55%) were contaminated by AFs at levels up to 9.52 µg/kg. In particular, during the rainy season, 25 ginger samples (81% of samples collected in the rainy

season) were contaminated in the range from 0.11 to 9.52 µg/kg with mean value of positive samples of 3.13 µg/kg. However during the dry season, a lower percentage of samples (46% of samples collected in the dry season) were contaminated by AFs, in the range 0.20-3.57 µg/kg, with mean concentration of positive samples of 1.18 µg/kg (Table 1).

Furthermore, AFB_1 was detected in all aflatoxin-positive ginger samples, in the range 0.11-8.76 and 0.11-3.30 µg/kg, during rainy and dry seasons, respectively. In the rainy season AFB_2 was detected in 17 samples (55%), ranging from 0.13 to 1.01 µg/kg. In the dry season AFB_2 was detected in 27 samples (30%) ranging from 0.13 to 0.79 µg/kg (Table 1). The mean values of positive samples determined in the rainy and dry seasons were 2.87 and 0.99 µg/kg for AFB_1 and 0.38 and 0.29 µg/kg for AFB_2, respectively. No AFG_1 and AFG_2 were detected in either season. This could be explained by the colonization of ginger samples by *Aspergillus flavus,* a producer of only type-B aflatoxins in most of cases. In this regard, recently, Jeswal and Kumar (2015) reported that A. *flavus* was the most dominant fungal species among all fungi invading several spices, including ginger. In either season no sample was contaminated by AFs above the regulatory limits fixed in the EU for the sum of aflatoxins in ginger (i.e. 10 µg/kg). However, in the rainy season 7 samples (23%) were found to be contaminated with AFB_1 above the limit of 5 µg/kg (up to 8.76 µg/kg). Statistical analysis with the Mann-Whitney U statistic test showed that AFB_1 and AFB_2 contamination levels were significantly higher ($p < 0.05$) during rainy season than during the dry season. Similarly, the incidence of AFB_1 and AFB_2 occurrence (%) was higher for samples collected during the rainy season with respect to the dry season (Figure 2). Probably, the climatic conditions such as high humidity and high temperature favored mold growth and aflatoxin production (Atanda *et al.*, 2013). These conditions, which are prevalent in wet seasons, combined with poor manufacturing practices during handling and storage, may account for the higher mycotoxin contamination recorded during the rainy seasons than the dry seasons.

The occurrence of AFs in ginger powder and finished products has been previously reported. In particular, in agreement with our results a survey carried out in the USA showed that 67% of 39 samples were contaminated by AFs at levels of up to 31.2 µg/kg with 10 samples above the EU regulatory limits (Trucksess *et al.,* 2007). In Turkey, it has been observed that two of four ginger samples analyzed were contaminated with AFB_1 above the EU limits (Tosun and Arslan, 2013). On the contrary, even though in Morocco it was reported that 86% of 55 ginger powder analyzed samples were contaminated by aflatoxins, the total AFs and AFB_1 were below the regulatory limits established by the European Commission, with maximum levels of 9.10 µg/kg and 3.50 µg/kg, respectively (Zinedine *et al.,* 2006). Similarly, in surveys carried out in the UK in 1996, in Poland in 2011 and 2012 and in China in 2013 and 2014, the observed levels of AFs were below the EU limits (McDonald and Castle, 1996; Twarużek *et al.*, 2013; Wen *et al.*, 2013; Wen *et al.*, 2014).

3.2. Occurrence of OTA

Among the 120 ginger samples analyzed, 62 samples (52%) resulted below LOD of 0.1 µg/kg. Of the samples below the LOD, six were collected in the rainy season (23%) and 56 (63%) were collected in the dry season. With regard to the rainy season, 24 ginger samples (77% of samples from the rainy season) were contaminated by OTA ranging from 0.20 to 9.90 µg/kg with average concentration of positive samples of 5.10 µg/kg. Among the 89 samples collected during the dry season, 33 (37%) were contaminated by OTA in a range 0.17-12.02 µg/kg, with a mean value of positive samples of 2.76 µg/kg (Table 1). Furthermore, OTA contamination levels were significantly higher (Mann-Whitney U test, $p < 0.05$) in samples collected during the rainy season relative to those collected during dry season. In addition, the incidence of OTA occurrence (%) was higher for samples collected during the rainy season relative to the dry season (Figure 2). Thus, these results with OTA showed the same trend as was observed with AFs occurrence.

In our survey, no sample was found above the regulatory limits established by European

Commission for OTA in ginger (i.e. 15 µg/kg) (European Commission, 2012). However, in

India high levels of OTA were found in two samples of ginger powder, i.e. 23 and 80 µg/kg

(Thirumala-Devi *et al.*, 2001). In agreement with our findings, in recent surveys carried out

in China (Wen *et al.*, 2013; Wen *et al.*, 2014) and in the USA (Trucksess *et al.*, 2007), no

samples were observed above the EU limits. In the first survey, carried out in China, all 25

analyzed samples were below the LOD (< 0.3 µg/kg). In the second survey 5 out of 30

samples were contaminated with OTA ranging from 0.3 to 5.2 µg/kg (Wen *et al.*, 2014). In

addition, in the USA survey, 29 out of 39 samples were contaminated, with a range of 1-10

µg/kg (Trucksess *et al.*, 2007).

3.3. Co-occurrence of AFs and OTA

Among the 120 ginger samples collected, 41 samples (34%) were simultaneously

contaminated by AFs and OTA. In particular, data showed that in the rainy season 22 ginger

samples (71%) were simultaneously contaminated by AFs and OTA in the concentration

range 0.11-9.52 and 0.20–9.90 µg/kg, respectively, while in the dry season 19 samples

(21%) were simultaneously contaminated by AFs and OTA in the concentration range 0.11-

3.18 and 0.18–5.41 µg/kg, respectively. In the rainy season, the mean values of positive

samples observed were 5.57 µg/kg for OTA and 3.40 µg/kg for AFs, whereas in the dry

season the mean values of positive samples were 2.14 and 1.40 µg/kg, respectively.

Concerning the comparison of both seasons, a trend was identified between the incidence

of simultaneous occurrence of AFs and OTA and the season. This is in line with the trend

reported above for the single mycotoxins. In fact, the incidence of co-occurrence (Figure 2)

and contamination levels (Mann-Whitney U test, $p < 0.05$) of AFs and OTA were higher in

samples collected during the rainy season than the dry season. In the literature, it has been

highlighted that a combined intake of both mycotoxins may increase the risk of adverse

effects in humans (Speijers & Speijers, 2004). In this regard, Sedmikova *et al*. 2001, demonstrated that OTA could increase the mutagenicity of AFB_1 when they occurred simultaneously in the same matrix. A synergistic effect due to these combined mycotoxins was also found by Huff and Doerr (1981).

During recent decades, the co-occurrence of AFs and OTA in ginger samples has been evaluated in only a few surveys (Trucksess *et al.*, 2007; Wen *et al.,* 2013; Wen *et al.,* 2014). In particular, in agreement with our findings, 67% of ginger samples from the United States were found to be co-contaminated with AFs and OTA in the range 0.6-31.2 μg/kg and 1.2-10.3 μg/kg, respectively, with averages values of 12.3 and 4.4 μg/kg (Trucksess *et al.,* 2007). On the contrary, two recent surveys carried out in China did not show co-occurrence of AFs and OTA in ginger and ginger products (Wen *et al.,* 2013; Wen *et al.,* 2014).

4. Conclusions

The natural co-occurrence of AFs and OTA in ginger samples collected from local markets in Lagos (Nigeria) has been shown for the first time. A higher incidence of co-occurrence of AFs and OTA was observed in samples collected during the rainy season (65%) in comparison with those collected during the dry season (21%). These findings could be explained by the high humidity and temperature which are prevalent in Nigeria in the rainy season, and by inadequate storage practices that offer favorable conditions for mold growth and mycotoxin production.

The high incidence of co-occurrence of AFs and OTA in ginger indicates the importance of their monitoring such co-occurrence on a global scale. In order to evaluate and prevent health risk due from their potential synergistic effects there is an urgent need to creating public awareness of this issue.

Acknowledgments

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Highlights

Occurrence of aflatoxins (AFs) and ochratoxin A (OTA) in ginger from Nigeria was investigated

High incidence of AFs and OTA co-occurrence was found in samples collected in the rainy season

Monitoring of AFs and OTA co-occurrence is recommended to assess human risk exposure

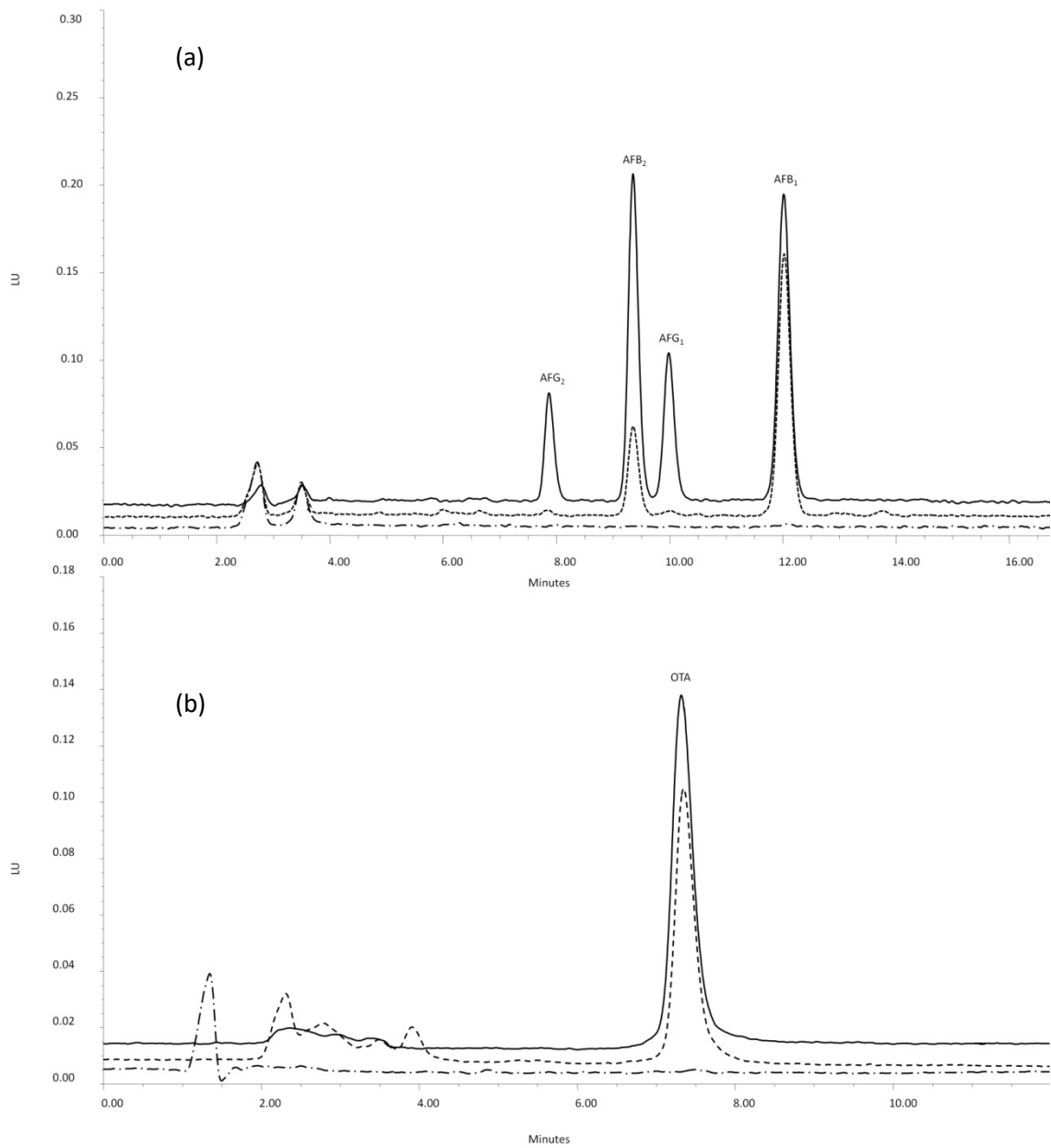**Figure 1**

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512 **Figure 1.** Chromatograms of the HPLC analyses for AFs (a) and OTA (b) determinations of:

513 (———) standard solutions; (- -) naturally contaminated samples; (- ·· -) blank

514 samples. Chromatographic conditions are reported in Section Materials and
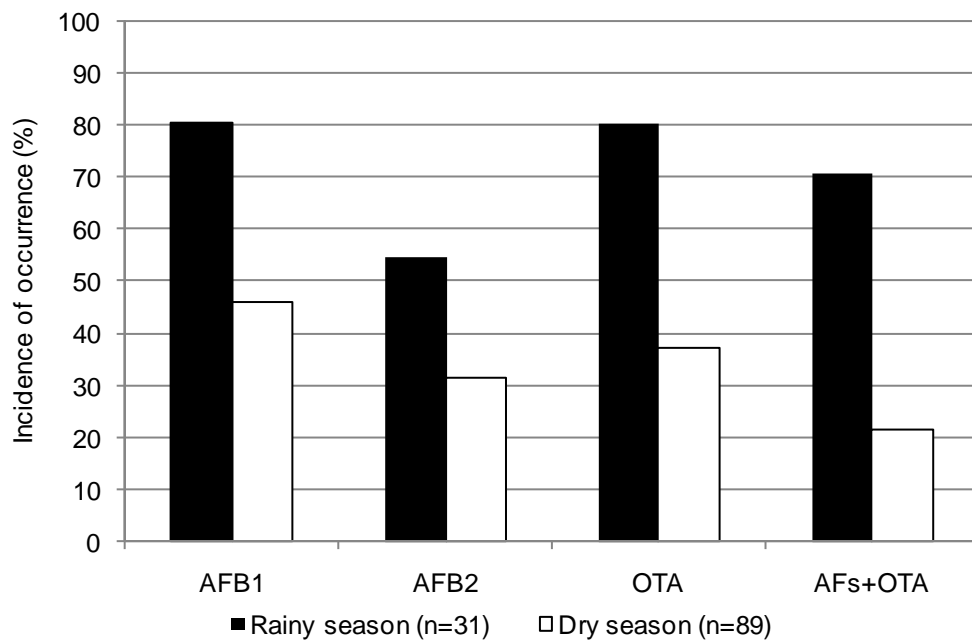
515 Methods.

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519 **Figure 2**

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523 **Figure 2.** Incidence of occurrence (%) of AFB1 and AFB2 and OTA in ginger samples collected in

524 rainy and dry season in Nigeria in 2014.

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534 **Table 1.** Occurrence of aflatoxins (AFB$_1$, AFB$_2$, AFG$_1$ and AFG$_2$) and OTA in ginger samples,

535 collected from Nigeria during rainy and dry seasons in 2014.

Season	Descriptive statistics	Mycotoxin contamination						
		AFG$_1$	AFG$_2$	AFB$_1$	AFB$_2$	AFs[a]	OTA	AFs + OTA
Rainy (n=31)	No. of positives[b] (%)	0 (0)	0 (0)	25 (81)	17 (55)	25(81)	24 (77)	22 (71)
	Range (µg/kg)	<LOD[c]	< LOD[c]	0.11-8.76	0.13-1.01	0.11-9.52	0.20-9.90	0.20-9.90 (OTA) 0.11 -9.52 (AFs)
	Mean of positives (µg/kg)	n.d.[d]	n.d.[d]	2.87	0.38	3.13	5.10	5.57 (OTA) 3.40 (AFs)
	Mean (µg/kg)	n.d.[d]	n.d.[d]	2.32	0.21	2.52	3.94	
	Median (µg/kg)	n.d.[d]	n.d.[d]	1.62	0.13	1.88	1.07	
	No. samples> EU ML[e] (%)	-	-	7 (23)	-	0	0	
Dry (n=89)	No. of positives[b] (%)	0 (0)	0 (0)	41 (46)	27 (30)	41(46)	33 (37)	19 (21)
	Range (µg/kg)	< LOD[c]	< LOD[c]	0.11-3.30	0.13-0.79	0.20-3.57	0.17-12.02	0.18-5.41 (OTA) 0.11 -3.18 (AFs)
	Mean of positives (µg/kg)	n.d.[d]	n.d.[d]	0.99	0.29	1.18	2.76	2.14 (OTA) 1.40 (AFs)
	Mean (µg/kg)	n.d.[d]	n.d.[d]	0.46	0.09	0.54	1.02	
	Median (µg/kg)	n.d.[d]	n.d.[d]	0.00	0.00	0.00	0.00	
	No. samples> EU ML (%)	-	-	0	-	0	0	

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537 AFs: sum of AFB$_1$, AFB$_2$, AFG$_1$ and AFG$_2$; [b] No. of positives: mycotoxin level >LOD.

538 [c]LOD: limit of detection (0.1 µg/kg for OTA, AFB$_1$, AFG$_1$; 0.02 µg/kg for AFB$_2$, AFG$_2$)

539 [d]n.d.: not detected

540   [e]ML: maximum permitted level (5 µg/kg for AFB_1; 10 µg/kg for total AFs; 15 µg/kg for OTA)

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