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

3 Vincenzo Lippolis^{1*}, Olubukola Irurhe^{2,3}, Anna Chiara R. Porricelli¹, Marina Cortese¹,
4 Roberto Schena¹, Tayo Imafidon^{2,3}, Afolabi Oluwadun², Michelangelo Pascale¹

5
6 ¹ Institute of Sciences of Food Production, National Research Council of Italy (ISPA-CNR),
7 via G. Amendola 122/O, 70126 Bari, Italy

8 ² Department of Medical Microbiology & Parasitology, College of Health Sciences, Olabisi
9 Onabanjo University, P.M.B. 2001, Sagamu, Ogun State, Nigeria

10 ³ National Agency for Food and Drug Administration and Control (NAFDAC), 3/4 Apapa via
11 Oshodi Expressway, Oshodi-Lagos, Nigeria

12 *Corresponding author: vincenzo.lippolis@ispa.cnr.it

13
14 **Abstract**

15 The natural co-occurrence of aflatoxins (AFB₁, AFB₂, AFG₁ and AFG₂) and ochratoxin A
16 (OTA) in dried split ginger purchased from different local markets in Lagos, South West
17 Nigeria has been investigated. A total of 120 ginger samples, 31 collected during the rainy
18 season and 89 during the dry season, were analyzed. Mycotoxins were determined
19 according to the AOAC Official Method 2008.02 based on multi-toxin immunoaffinity
20 column clean up and liquid chromatography quantification. The incidence of contamination
21 with aflatoxins (AFs) and OTA was significantly higher during the rainy season (81% and
22 77%, respectively) than the dry season (46% and 37%, respectively). Average levels of
23 AFs and OTA in positive samples were 3.13 and 5.10 µg/kg in the rainy season (range
24 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
25 0.17-12.02 µg/kg) in the dry season, respectively. Furthermore, the levels of AFB₁ 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).

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

58 In world trade, the rhizome is marketed in several forms such as fresh or dried product,
59 liquid or solid extract, tablets or capsules, bulk powder and in tea bags (Whitaker *et al.*,
60 2009). Spices, including ginger, can be subjected to contamination during harvesting,
61 handling, storage and distribution by several fungi responsible for spoilage and production
62 of mycotoxins, in particular aflatoxins (AFs) and ochratoxin A (OTA) (Ozbey & Kabak,
63 2012; Kabak & Dobson, 2015). Aflatoxins (AFB₁, AFB₂, AFG₁ and AFG₂) are the most
64 toxic group of mycotoxins produced by some *Aspergillus* species (*A. flavus*, *A. parasiticus*
65 and more rarely by *A. nomius*) (Pitt, 2000). Aflatoxin B₁ is among the most carcinogenic
66 substances known and has been classified by the International Agency for Research on
67 Cancer as Group 1 (IARC,1993). Ochratoxin A is produced mainly by *Penicillium*
68 *verrucosum* in temperate climates and by *Aspergillus ochraceus* (and more rarely by *A.*
69 *carbonarius*) in warm and tropical climates. It can contaminate agricultural products prior to
70 harvest or, much more commonly, during storage (EFSA, 2006). Ochratoxin A has been
71 shown to cause several toxic effects in animals including immunotoxicity, nephrotoxicity
72 and teratogenicity. Furthermore, the IARC, based on sufficient evidence of its
73 carcinogenicity in animal studies, has classified OTA as a possible human carcinogen
74 (Group 2B) (IARC, 1993).

75 Due to their health hazards for humans, many countries have established regulations for
76 mycotoxins in spices. Concerning ginger, the European Commission has established
77 maximum levels of 5 µg/kg for AFB₁, 10 µg/kg for total AFs (the sum of AFB₁, AFB₂, AFG₁

78 and AFG₂) and 15 µg/kg for OTA (European Commission, 2006; European Commission,
79 2012). Due to environmental conditions favorable to colonization of toxigenic fungi in
80 tropical countries, both groups of mycotoxins could co-contaminate spices both in the field
81 and storage. In vitro and in vivo studies have shown that mycotoxin mixtures may exert
82 synergistic or additive toxic effects (Oliveira and Correa, 2010). The consumption of food
83 contaminated with more mycotoxins could be a significant threat to human health.

84 Reliable methods for the simultaneous determination of AFs and OTA in ginseng and other
85 botanical roots have been developed and validated (Trucksess *et al.*, 2006; Trucksess *et al.*,
86 2007, Trucksess *et al.*, 2008). In particular, a method for the determination of AFB₁,
87 AFB₂, AFG₁, and AFG₂ and OTA in ginseng and ginger has been validated by
88 collaborative study (Trucksess *et al.*, 2008) and subsequently approved by the AOAC
89 International as Official Method (AOAC Official Method 2008.02).

90 In order to assess health risks for humans and animals due to the simultaneous exposure
91 to AFs and OTA in spices, the co-occurrence of these mycotoxins in red chilli, paprika,
92 black pepper, cumin and cinnamon has been widely reviewed in the literature in several
93 countries such as Brazil (Shundo *et al.*, 2009), Hungary (Fazekas *et al.*, 2005), India (Saha
94 *et al.*, 2007), Malaysia (Jalili *et al.*, 2012; Ali *et al.*, 2015), Spain (Hernández Hierro *et al.*,
95 2008; Santos *et al.*, 2010), Turkey (Ozbey *et al.*, 2012), Pakistan (Iqbal *et al.*, 2013) and
96 Italy (Prelle *et al.*, 2014). However, only a few studies concerning the co-occurrence of
97 OTA and AFs in ginger powder have been reported (Trucksess *et al.* 2007; Wen *et al.*,
98 2013; Wen *et al.*, 2014). To date, no survey on the co-occurrence of AFs and OTA in
99 ginger from Nigeria has been reported. Therefore, the aim of this study was to investigate
100 the natural co-occurrence of AFs and OTA in ginger samples purchased from local
101 markets in Lagos, South West Nigeria during the dry season (November - March) and the
102 rainy season (April - October), in order to compare the detected levels with permissible
103 limits established by the European Commission. The possible influence of storage

104 conditions occurring during the dry and rainy seasons on the incidence and levels of AFs
105 and OTA contamination in ginger samples is discussed.

106

107 **2. Materials and Methods**

108 *2.1. Sample collection*

109 A total of 120 dried split ginger samples were purchased randomly from different local
110 markets in Lagos Nigeria. In particular, 89 samples were collected during the dry season
111 (January – April 2014, equivalent to 2-5 months of storage) and 31 samples during the
112 rainy season (July - October 2014, equivalent to 8-11 months of storage). Samples were
113 kept at 4°C until chemical analysis.

114

115 *2.2. Reagents and Chemicals*

116 Acetonitrile and methanol (HPLC grade) were purchased from Carlo Erba Reagents
117 (Milan, Italy). Ultrapure water was produced by a Waters Milli-Q system (Waters, Milford,
118 MA, USA). Ochratoxin A and AFs (AFB₁, AFB₂, AFG₁, AFG₂) analytical standards,
119 TWEEN[®] 20, sodium chloride (NaCl), phosphate-buffered saline (PBS) and sodium
120 bicarbonate (NaHCO₃) were purchased from Sigma-Aldrich (Milan, Italy). Glass microfiber
121 filters (Whatman GF/A) and paper filters (Whatman N. 4) were obtained from Whatman
122 (Maidstone, UK). *AflaOchra HPLC*[™] immunoaffinity columns were purchased from
123 VICAM, A Waters Business (Milford, MA, USA).

124

125 *2.3. Preparation of Standard Solutions*

126 An OTA stock solution was prepared at concentration of 1 mg/mL by dissolving the solid
127 toxin in toluene:acetic acid (99:1, v/v). An OTA standard solution, at the concentration of
128 about 10 µg/mL, was prepared by dissolving in methanol an adequate amount of the stock
129 solution previously evaporated to dryness under a stream of nitrogen. The standard

130 solution was tested spectrophotometrically ($\epsilon = 6330 \text{ cm}^2/\text{mmol}$, at $\lambda = 333 \text{ nm}$ in methanol).
131 For the preparation of standard solutions for HPLC calibration curves, a solution of OTA
132 was prepared in methanol at a concentration of 250 ng/mL . Aliquots of this solution were
133 evaporated to dryness under a stream of nitrogen and dissolved in a mixture
134 acetonitrile/water/acetic acid 99:99:2 (v/v/v). The 250 ng/mL OTA standard solution was
135 also used as the spiking solution for recovery experiments.

136 Standard solutions of AFB_1 , AFB_2 , AFG_1 , and AFG_2 were prepared separately according to
137 the AOAC Official Method 971.22 by dissolving the solid toxins in toluene/acetonitrile
138 (90:10, v/v) at a concentration of $100 \text{ }\mu\text{g/mL}$ for each aflatoxin. A working solution for each
139 aflatoxin at the concentration of $10 \text{ }\mu\text{g/mL}$ was prepared in toluene/acetonitrile 90:10 (v/v)
140 and tested spectrophotometrically ($\epsilon = 19300, 21000, 16400$ and $18300 \text{ cm}^2/\text{mmol}$ for
141 AFB_1 , AFB_2 , AFG_1 and AFG_2 , respectively, at $\lambda = 350 \text{ nm}$). For the preparation of a mixed
142 solution for HPLC calibration, a mixed aflatoxins solution (containing 1000 ng/mL of AFB_1
143 and AFG_1 , and 200 ng/mL of AFB_2 and AFG_2 in toluene/acetonitrile 90:10 (v/v)) was
144 prepared by appropriate dilution of AFB_1 , AFB_2 , AFG_1 and AFG_2 working solutions. In
145 particular, aliquots of the mixed aflatoxins solution (1000 ng/mL of AFB_1 and AFG_1 , 200
146 ng/mL of AFB_2 and AFG_2) were evaporated to dryness under a stream of nitrogen and
147 reconstituted in a mixture of water/acetonitrile 60:40 (v/v). For recovery experiments, an
148 AF spiking solution (250 ng/mL of AFB_1 and AFG_1 , 50 ng/mL of AFB_2 and AFG_2) was
149 prepared by evaporating to dryness (under a stream of nitrogen) the mixed aflatoxins
150 solution, which was then reconstituted in acetonitrile.

151

152 *2.4. Sample extraction and clean up*

153 Dried split ginger samples were finely ground (particle size $\leq 0.5 \text{ mm}$) using a Cyclone
154 sample mill (PBI International, Milan, Italy). Extraction and clean up of the powdered
155 ginger were performed according to the AOAC Official Method 2008.02. In particular, 5 g

156 of sample was weighted into a 50 mL centrifuge tube, 1 g of NaCl was added and the
157 sample was extracted with 25 mL methanol/0.5% NaHCO₃ (70:30, v/v) by shaking at 400
158 rpm for 10 min (KS 4000i, IKA Werke GmbH & Co. KG., Staufen, Germany). The extract
159 was centrifuged for 10 min at 7000 rpm (SL 16 Centrifuge Series, Thermo Fisher Scientific
160 Inc, Waltham, MA, USA). Seven milliliter of supernatant were diluted with 28 mL 0.1 M
161 PBS containing 1% Tween[®] 20, mixed and filtered through a glass microfiber paper.
162 Twenty-five milliliter of diluted extract (equivalent to 1 g sample) were passed through an
163 immunoaffinity column (*AflaOchra HPLC*[™], Milford, MA, USA) at a flow rate of about one
164 drop per second. The column was washed with 5 mL of 10 mM PBS and 5 mL of distilled
165 water at a flow rate of one or two drops per second. Ochratoxin A and AFs were eluted
166 from the column with methanol (2 × 1 mL) at flow rate of one drop per second. The
167 cleaned extract was collected in a 4 mL screw-cap amber vial, allowing the column to run
168 dry by forcing air through column. The eluate was diluted with 1 mL of water before HPLC
169 analysis.

170

171 2.5. HPLC analysis

172 Two separate HPLC analyses were performed for the determination of AFs and OTA,
173 respectively. In particular, separate aliquots (100 µL) of the purified extract were injected
174 into the HPLC apparatus, which consisted of an Agilent 1260 Series Chromatographic
175 System (Agilent Technologies, Palo Alto, CA, USA) equipped with a fluorometric detector
176 (model G1321B). Data acquisition and instrument control were performed by the OpenLAB
177 data software (Agilent Technologies).

178 For determination of AFs, the analytical column used was a the Luna[®] PFP(2) (3 µm, 150
179 × 4.6 mm, Phenomenex, Torrance, CA, USA). The chromatographic separation was
180 performed by isocratic elution at a flow rate of 0.8 mL/min with a mobile phase of
181 acetonitrile/water 30:70 (v/v). A post-column derivatization with a photochemical

182 derivatizer (UVE™, LC Tech, Dorfen, Germany) was performed to enhance the
183 fluorescence intensity of AFB₁ and AFG₁ (photochemical radiation with UV light at 254
184 nm). The fluorometric detector was set at excitation and emission wavelengths of 365 nm
185 and 435 nm, respectively. The column was kept at a temperature of 30°C. The retention
186 times of AFG₂, AFB₂, AFG₁, and AFB₁ were between 7 and 13 min.

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

193 Typical HPLC chromatograms of AFB₁, AFB₂, AFG₁ and AFG₂ (Fig. 1a) and OTA (Fig. 1b)
194 of standard solutions, blank and naturally contaminated ginger samples are reported in
195 Figure 1.

196 Limits of detection (LODs), defined as a signal-to-noise ratio 3:1, were 0.1 µg/kg for OTA,
197 AFB₁ and AFG₁ and 0.02 µg/kg for AFB₂ and AFG₂. Limits of quantification (LOQs),
198 defined as a signal-to-noise ratio 10:1, were 0.3 µg/kg for OTA, AFB₁ and AFG₁ and 0.06
199 µg/kg for AFB₂ and AFG₂.

200 In three replicate experiments, uncontaminated (blank) ginger samples were spiked
201 simultaneously with 100 µL of the AFs spiking solution and 100 µL of the OTA spiking
202 solution, to obtain a spiking level of 12 µg/kg for AFs (i.e. 5 µg/kg AFB₁, 1 µg/kg AFB₂, 5
203 µg/kg AFG₁, 1 µg/kg AFG₂) and 5 µg/kg for OTA. Spiked samples were left to dry for 1
204 hour at room temperature to allow solvent evaporation. Samples were then extracted for
205 mycotoxin analysis. This procedure is commonly used in in-house and in interlaboratory
206 validation studies. Average recoveries of AFs and OTA from ginger were 75% and 86%,
207 respectively with relative standard deviations less than 6%.

208

209 2.6. Statistical analysis

210 To compare the evaluation of difference incidence of contamination of OTA and AFs in
211 ginger samples between the rainy and dry seasons the software Sigma Plot™ (Version
212 12.3, Statistic software Inc, USA) was used. The Shapiro Wilk test was used to determine
213 the normality of the contamination data distribution of two seasonal surveys. Due to the
214 non-normal distribution of the data, the non-parametric Mann-Whitney U test was used,
215 with the level of confidence fixed at $p < 0.05$ (95%). The Mann-Whitney U test is used to
216 determine whether or not two population medians are equal.

217

218 3. Results and discussion

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

229

230 3.1. Occurrence of Aflatoxins

231 Among the 120 samples analyzed, 54 samples (45%) were below the limit of detection
232 (LOD) of 0.1 µg/kg for AFB₁ and AFG₁ and 0.02 µg/kg for AFB₂ and AFG₂. Of these, six
233 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, AFB₁ 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 AFB₂ was detected in 17 samples (55%), ranging from 0.13 to 1.01 µg/kg. In the dry season AFB₂ 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 AFB₁ and 0.38 and 0.29 µg/kg for AFB₂, respectively. No AFG₁ and AFG₂ 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 AFB₁ above the limit of 5 µg/kg (up to 8.76 µg/kg). Statistical analysis with the Mann-Whitney U statistic test showed that AFB₁ and AFB₂ contamination levels were significantly higher ($p < 0.05$) during rainy season than during the dry season. Similarly, the incidence of AFB₁ and AFB₂ 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

260 prevalent in wet seasons, combined with poor manufacturing practices during handling
261 and storage, may account for the higher mycotoxin contamination recorded during the
262 rainy seasons than the dry seasons.

263 The occurrence of AFs in ginger powder and finished products has been previously
264 reported. In particular, in agreement with our results a survey carried out in the USA
265 showed that 67% of 39 samples were contaminated by AFs at levels of up to 31.2 µg/kg
266 with 10 samples above the EU regulatory limits (Trucksess *et al.*, 2007). In Turkey, it has
267 been observed that two of four ginger samples analyzed were contaminated with AFB₁
268 above the EU limits (Tosun and Arslan, 2013). On the contrary, even though in Morocco it
269 was reported that 86% of 55 ginger powder analyzed samples were contaminated by
270 aflatoxins, the total AFs and AFB₁ were below the regulatory limits established by the
271 European Commission, with maximum levels of 9.10 µg/kg and 3.50 µg/kg, respectively
272 (Zinedine *et al.*, 2006). Similarly, in surveys carried out in the UK in 1996, in Poland in
273 2011 and 2012 and in China in 2013 and 2014, the observed levels of AFs were below the
274 EU limits (McDonald and Castle, 1996; Twarużek *et al.*, 2013; Wen *et al.*, 2013; Wen *et*
275 *al.*, 2014).

276

277 3.2. Occurrence of OTA

278 Among the 120 ginger samples analyzed, 62 samples (52%) resulted below LOD of 0.1
279 µg/kg. Of the samples below the LOD, six were collected in the rainy season (23%) and 56
280 (63%) were collected in the dry season. With regard to the rainy season, 24 ginger
281 samples (77% of samples from the rainy season) were contaminated by OTA ranging from
282 0.20 to 9.90 µg/kg with average concentration of positive samples of 5.10 µg/kg. Among
283 the 89 samples collected during the dry season, 33 (37%) were contaminated by OTA in a
284 range 0.17-12.02 µg/kg, with a mean value of positive samples of 2.76 µg/kg (Table 1).
285 Furthermore, OTA contamination levels were significantly higher (Mann-Whitney U test, p

286 < 0.05) in samples collected during the rainy season relative to those collected during dry
287 season. In addition, the incidence of OTA occurrence (%) was higher for samples collected
288 during the rainy season relative to the dry season (Figure 2). Thus, these results with OTA
289 showed the same trend as was observed with AFs occurrence.

290 In our survey, no sample was found above the regulatory limits established by European
291 Commission for OTA in ginger (i.e. 15 µg/kg) (European Commission, 2012). However, in
292 India high levels of OTA were found in two samples of ginger powder, i.e. 23 and 80 µg/kg
293 (Thirumala-Devi *et al.*, 2001). In agreement with our findings, in recent surveys carried out
294 in China (Wen *et al.*, 2013; Wen *et al.*, 2014) and in the USA (Trucksess *et al.*, 2007), no
295 samples were observed above the EU limits. In the first survey, carried out in China, all 25
296 analyzed samples were below the LOD (< 0.3 µg/kg). In the second survey 5 out of 30
297 samples were contaminated with OTA ranging from 0.3 to 5.2 µg/kg (Wen *et al.*, 2014). In
298 addition, in the USA survey, 29 out of 39 samples were contaminated, with a range of 1-
299 10 µg/kg (Trucksess *et al.*, 2007).

300

301 3.3. Co-occurrence of AFs and OTA

302 Among the 120 ginger samples collected, 41 samples (34%) were simultaneously
303 contaminated by AFs and OTA. In particular, data showed that in the rainy season 22
304 ginger samples (71%) were simultaneously contaminated by AFs and OTA in the
305 concentration range 0.11-9.52 and 0.20–9.90 µg/kg, respectively, while in the dry season
306 19 samples (21%) were simultaneously contaminated by AFs and OTA in the
307 concentration range 0.11-3.18 and 0.18–5.41 µg/kg, respectively. In the rainy season, the
308 mean values of positive samples observed were 5.57 µg/kg for OTA and 3.40 µg/kg for
309 AFs, whereas in the dry season the mean values of positive samples were 2.14 and 1.40
310 µg/kg, respectively. Concerning the comparison of both seasons, a trend was identified
311 between the incidence of simultaneous occurrence of AFs and OTA and the season. This

312 is in line with the trend reported above for the single mycotoxins. In fact, the incidence of
313 co-occurrence (Figure 2) and contamination levels (Mann-Whitney U test, $p < 0.05$) of AFs
314 and OTA were higher in samples collected during the rainy season than the dry season. In
315 the literature, it has been highlighted that a combined intake of both mycotoxins may
316 increase the risk of adverse effects in humans (Speijers & Speijers, 2004). In this regard,
317 Sedmikova *et al.* 2001, demonstrated that OTA could increase the mutagenicity of AFB₁
318 when they occurred simultaneously in the same matrix. A synergistic effect due to these
319 combined mycotoxins was also found by Huff and Doerr (1981).

320 During recent decades, the co-occurrence of AFs and OTA in ginger samples has been
321 evaluated in only a few surveys (Trucksess *et al.*, 2007; Wen *et al.*, 2013; Wen *et al.*,
322 2014). In particular, in agreement with our findings, 67% of ginger samples from the United
323 States were found to be co-contaminated with AFs and OTA in the range 0.6-31.2 µg/kg
324 and 1.2-10.3 µg/kg, respectively, with averages values of 12.3 and 4.4 µg/kg (Trucksess *et*
325 *al.*, 2007). On the contrary, two recent surveys carried out in China did not show co-
326 occurrence of AFs and OTA in ginger and ginger products (Wen *et al.*, 2013; Wen *et al.*,
327 2014).

328

329 **4. Conclusions**

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

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

341

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345

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485 **Figure 1.** Chromatograms of the HPLC analyses for AFs (a) and OTA (b) determinations
486 of: (—) standard solutions; (---) naturally contaminated samples; (-.-) blank
487 samples. Chromatographic conditions are reported in Section Materials and
488 Methods.

489

490

Figure 2

Figure 2. Incidence of occurrence (%) of AFB1 and AFB2 and OTA in ginger samples collected in rainy and dry season in Nigeria in 2014.

Table 1. Occurrence of aflatoxins (AFB₁, AFB₂, AFG₁ and AFG₂) and OTA in ginger samples, 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)	20 22 (7165)
	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	

503

504 AFs: sum of AFB₁, AFB₂, AFG₁ and AFG₂; ^b No. of positives: mycotoxin level >LOD.

505 ^cLOD: limit of detection (0.1 µg/kg for OTA, AFB₁, AFG₁; 0.02 µg/kg for AFB₂, AFG₂)

506 ^dn.d.: not detected

507 ^eML: maximum permitted level (5 µg/kg for AFB₁; 10 µg/kg for total AFs; 15 µg/kg for OTA)

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