

Evaluation of five major mycotoxins co-contaminating two cereal grains from Nigeria

ABSTRACT

Mycotoxin production in food crops and finished food products by filamentous fungi is favoured by high temperatures, humidity and improper storage and handling practices. Rice and maize samples were collected from stores and markets in South Eastern and South Western Nigeria and evaluated for co-contamination by five major mycotoxins- aflatoxins (AFs), ochratoxin A (OTA), fumonisin B₁ (FB₁), Fumonisin B₂ (FB₂), deoxynivalenol (DON) and zearalenone (ZEA). Following three different extraction procedures, mycotoxin occurrence and concentration was determined via thin layer chromatography (TLC) and high performance liquid chromatography (HPLC). Results showed high incidences of all five mycotoxins in both samples with OTA highest in rice samples (92.7%), while AFs and FB₁ had highest incidence in maize samples (94.9%). Data from HPLC analysis showed high levels of FB₁, AFs, OTA and ZEA in food samples above Nigerian and European Union (EU) set limits. The co-occurrence of these mycotoxins in food samples analysed in this study gives further awareness to the health risks consumers of the food commodities are exposed to. This study also contributes to research being done in Nigeria in order to understand the degree of mycotoxins contamination of food consumed by the populace.

Keywords: Mycotoxins, Maize, Rice, Nigeria

1.0 INTRODUCTION

Cereal grains, maize and rice consist of some of the staple foods consumed in most parts of sub-Saharan Africa and the world over [1]. Rich in different nutrients such as carbohydrates, proteins and vitamins, these cereal grains are also susceptible to fungal infestation both on the growing fields and in the stores [2,3]. Subsequent production of mycotoxins by fungi contaminating cereals is a fact that cannot be disputed. These mycotoxins which are secondary metabolites have very negative health effects on humans and animals [4] inducing carcinogenic, teratogenic, mutagenic and immuno suppressive effects [5].

32 In Nigeria, especially in the rural areas most of the foods consumed are staple foods with less
33 consumption of processed foods. Maize and rice are some of the food crops widely consumed
34 across the country. Rice is cultivated in the country as well as imported in other to meets the
35 high demand for rice in the country. It was reported that up to 32.9kg of rice was consumed
36 per person on a yearly basis between 2007 and 2010 in the country [6], with an assumption
37 that this value must have increased between 2010 and now. Maize on the other hand is one
38 food crop that is consumed in different forms in the country. It can be cooked, roasted or
39 processed into a porridge meal (“ogi”) that is most times used as a weaning food for infants.
40 Most of these food crops are cultivated by subsistence farmers who do not implore high tech
41 farming techniques. Also, because food crops are cultivated in seasons they are usually stored
42 for all year round availability in proper and improper storage houses.

43 Mycotoxin production by filamentous fungi mainly *Aspergillus*, *Fusarium* and *Penicillium*
44 species is usually triggered by specific conditions which range from environmental, climatic
45 and human [7]. Extremely high and low temperatures, humidity, improper handling and
46 improper storage have been contributory to mycotoxin production by filamentous fungi in
47 most food crops either on the field, in storage or during transit. There are two major seasons
48 in Nigeria: the rainy and dry (harmattan) seasons, with high temperatures almost all through
49 the year. These climatic conditions alongside other conditions are some of the challenges
50 faced by farmers in this part of the world which has prompted the need for research into
51 mycotoxin contamination of a variety of food crops cultivated in the country [8]. In the past
52 years, due to the prevalence of *Aspergillus* species contaminating most food commodities in
53 Nigeria, much emphasis had been on aflatoxins, fumonisins and ochratoxin A studies in
54 comparison to other mycotoxins but in recent years, mycotoxin research has gone beyond
55 aflatoxins and ochratoxin A to other less common mycotoxins produced by filamentous fungi
56 [9,10].

57 Imploring different methods of extraction and two types of validation technique to determine
58 mycotoxin contamination, this study was done in contribution to other studies being carried
59 out in Nigeria in a bid to understand the extent of exposure of the consumers to some
60 mycotoxins that are known to contaminate cereal grains with the main aim of screening
61 maize and rice samples for mycotoxin co-contamination.

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64 2.0 MATERIALS

65 2.1 Sample collection

66 Maize samples (39) were collected from markets and stores in south-eastern Nigeria whereas
 67 rice samples (41) were collected from south-eastern and south-western Nigeria. They were
 68 transported sealed in sterile sample collection bags and transported to the Food, Environment
 69 and Health Research Laboratory in the University of Johannesburg, South Africa following
 70 strict custom duty procedures. Samples were milled in the laboratory upon arrival using a
 71 sterilized laboratory mill (IKA M20, Merck, Darmstadt, Germany) and stored at -20°C prior
 72 to mycotoxin analysis.

73 2.2 Mycotoxin extraction

74 2.2.1 Multi-mycotoxin extraction

75 Dichloromethane (DCM), acetonitrile, 4% potassium chloride (KCl), saturated sodium
 76 hydrogen bicarbonate (NaHCO₃), sodium sulphate (Na₂SO₄), acetone, 250ml separating
 77 funnels, 250ml conical flasks, round bottom flasks, 1ml screw cap amber vials, glass funnels,
 78 Whatmann no. 1 filter paper, dialysis tubing, boiling test tubes, rotary vacuum evaporator
 79 (Buchi Laboratory-techniques Ltd., Flawil, Switzerland).

80 2.2.2 Fumonisin extraction

81 Strong anion exchange (SAX) cartridge (ANATECH, Gauteng, South Africa), methanol
 82 (CH₃OH), distilled water, acetonitrile, acetic acid (CH₃COOH), sodium hydroxide (NaOH),
 83 Whatmann no. 1 filter paper, 250ml conical flasks, pH meter (MicroSet MS 324), sterile
 84 pipette tips, 1 ml amber vials.

85 2.2.3 Immuno-affinity extraction

86 Immuno-affinity columns (VICAM), fluted filter papers, microfiber filter papers, 250ml
 87 plastic disposable beakers, disposable plastic pipette, HPLC grade methanol, acetonitrile,
 88 PBS, tween-20 solution, sodium bicarbonate, disposable plastic pipette, HPC grade methanol,
 89 acetonitrile, PBS, tween-20 solution, sodium bicarbonate

90 2.2.4 TLC (thin layer chromatography)

91 *mobile phase*- Dichloromethane/Ethyl-acetate/Propan-2-ol (90:5:5 v/v/v) (DEP),
 92 Toluene/Ethyl-acetate/Formic acid (6:3:1 v/v/v) (TEF), Butanol/Water/Acetic acid (12:5:3
 93 v/v/v) (BWA), Dichloromethane/Acetone (90/10 v/v) (DA); aluminium backed thin layer
 94 chromatography plates (20 x 20cm) pre-coated with silica gel G (Merck,Germany), reversed-

95 phase (C18) tlc plates (Merck, Germany), capillary pipettes, chromatography tanks (Camag
96 Ltd), variable hot air drier, UV light in viewing cabinet, Spray gun, fume hood, paper
97 towelling, oven at 120°C; *derivatizing and spraying reagents*- chromotropic acid (CTA),
98 diazotised benzidine and anisaldehyde reagent.

99 2.2.5 HPLC (high performance liquid chromatography)

100 HPLC grade acetonitrile, HPLC grade methanol, sodium di-hydrogen phosphate, potassium
101 bromide (KBr), nitric acid (HNO₃), o-phthaldialdehyde (OPA) reagent prepared by dissolving
102 40 mg of OPA in 1 ml CH₃OH and diluted with 5 ml 0.1M sodium tetraborate (Na₂B₂O₄)
103 and 50µl mercapthoethanol. HPLC Spectra Physics SCM400 SYSTEM (Waters, Milford,
104 MA, USA), Shimadzu Corporation (Kyoto, Japan) LC-20AB liquid chromatograph equipped
105 with CBM-20A communication bus module, LC-20AB degasser, CTO-20A column oven,
106 Nova-Pak 4mm C18 reversed phase analytical column (250×4.6 mm, 5µm), SIL-20A auto
107 sampler, RF-10AxL fluorescence detector, RID-10A refractive index detector and SPD-
108 M20A photodiode array detector linked to LC solutions version 1.22 Software Release.
109 Kobra cell (DR Weber Consulting, Germany).

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111 2.2.6 Mycotoxins standards

112 Aflatoxins B₁, B₂, G₁, G₂, ochratoxin A(OTA), fumonisin B₁, B₂, zearalenone (ZEA) and
113 deoxynivalenol (DON) were procured from the Medical Research Council (MRC), South
114 Africa.

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118 3.0 METHODS IMPLEMENTED

119 3.1 Multi-mycotoxin extraction procedure

120 Following the method implored by Patterson and Roberts [11] and Mwanza [12] aflatoxins,
121 ochratoxin A, zearalenone and deoxynivalenol were extracted from 25g of food samples
122 using isooctane and DCM through an anhydrous Na₂SO₄ bed. Extracts clean-up was achieved
123 through a dialysis clean up step where extracts were left to shake overnight in a dialysis tube
124 inserted in a test tube containing 30% acetone and further extracted with DCM. Neutral
125 fractions and acid fractions were extracted, dried using nitrogen gas stream at 60°C and
126 stored at 4°C for further analysis.

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128 3.2 Fumonisin extraction procedure

129 The method of clean-up and extraction of using solid phase extraction (SPE) isolate strong
130 ion exchange (SAX) columns (International Sorbent Technology, UK) was implemented for
131 all the food commodities following the method of Shephard and Sewram [13] with some
132 modifications. Ten (10) grams of each sample was extracted using 100ml of Methanol/water
133 (70/30, v/v) and pH of filtered extract adjusted between 5.9 and 6.5. This step was followed
134 by diluting extracts with 4 ml methanol/water (70/30) and loaded unto SAX columns which
135 had been pre-conditioned with methanol and methanol/water (70/30). Fumonisins were
136 eventually eluted with methanol/acetic acid (99/1, v/v) after columns had been washed with
137 methanol/water (70/30) and methanol. Extracts were dried under a stream of nitrogen gas at
138 60°C and stored at 4°C for further analysis.

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140 3.3 Immuno-affinity clean up and extraction procedure

141 Clean-up and extraction of each mycotoxin (OTA, AFs, FBs) using immuno-affinity columns
142 was done following the procedure of the manufacturers (VICAM Ltd. Watertown, USA). In
143 this procedure, extracts from food samples were applied to the column and mycotoxins acted
144 as antigens binding to the antibodies present in the column. The mycotoxins were then eluted
145 using an eluting solution which was passed through the column. Final mycotoxin extracts
146 from the columns were dried and stored at 4°C for further analysis.

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148 3.4 Thin layer chromatography of mycotoxin extracts

149 Using silica gel coated aluminium backed 20cm by 20cm TLC plates and specific mobile
150 phases mycotoxin extracts were analysed to determine the presence of toxins investigated.
151 Dried neutral and acid fraction extracts were dissolved in 200µl of acetonitrile, thoroughly
152 mixed and 20 µl of extracts spotted on silicon backed tlc plates. Extracts were run alongside
153 known concentration of standards in different mobile phases and mycotoxins that could
154 florescence were viewed under UV light. Other mycotoxins like ZEA and DON which did
155 not florescence were sprayed with derivatizing solutions (dianisidine and chromotropic acid
156 respectively) and viewed.

157 Fumonisin extracts were dissolved in 200 µl of methanol, vortexed and 20 µl spotted on tlc
158 plates and run in a tlc tank containing a mobile phase (butanol/water/acetic acid 12/5/3,
159 v/v/v). Thin layer chromatography plates were dried, derivatized using anisaldehyde solution,
160 put in an oven for a few minutes and viewed.

3.5 High Performance Liquid chromatography analysis of food extracts

Acid and neutral fractions of mycotoxin extracts from the food samples were dissolved in 500µl of hplc grade acetonitrile prior to hplc analysis, whereas fumonisin extracts were suspended in 500µl hplc grade methanol. Following the methods of Shepherd, et al. [14], Reiter, et al. [15] and Abdulkadar, et al. [16], concentrations of AFs, OTA, ZEA and FBs were determined at different emission and excitation wavelengths alongside standards of known concentrations.

3.6 Recoveries

Samples with known mycotoxin concentrations were spiked with the following different mycotoxin standards of known concentration- (AFs-100µg/ml;OTA-50µg/ml;FBs-100µg/ml; ZEA-50µg/ml and DON-50µg/ml). In order to confirm the effectiveness of the methods implemented for extraction of mycotoxins from samples, mycotoxins were extracted and final mycotoxin concentration determined using the hplc equipment.

3.7 Statistical analysis

Using the IBM SPSS statistical software version 16, descriptive statistics and a one-way analysis of variance (ANOVA) was performed to derive mean values, which were compared by least significant difference using all pairwise multiple comparison procedures. Mean values among treatment groups were deemed to be different if the level of probability was ≤ 0.05 .

4.0 RESULTS

Data obtained from the different analysis showed mycotoxin occurrence in both food samples analysed at varying concentrations. Results showed some of the food samples being contaminated by all five mycotoxins screened for. Thin layer chromatography analysis was able to determine to some extent the occurrence of some of the mycotoxins (AFs, OTA and FBs) and this result is illustrated in table 1. High performance liquid chromatography analysis was able to detect mycotoxin contamination at very low concentrations in the food samples analysed. Incidences of all five mycotoxins were determined in both food samples up to 94.9%. Data obtained from the analysis (table 2 and 3) showed the occurrence of aflatoxins and ochratoxin A in both rice and maize samples in comparison to the other three mycotoxins. The concentration of the different mycotoxins detected in the food samples via HPLC were based on the chromatograms of

AFB₁, AFB₂, AFG₁, AFG₂, OTA, FB₁, FB₂, ZEA and DON standards used as well as calibration curves plotted from the areas of peaks of standards and concentration of standards.

Table 1. Percentage incidence of major mycotoxins in rice and maize samples determined by thin layer chromatography

Mycotoxins	Food samples	
	Rice (n=41)	Maize n= (39)
AFs	14.6	23.1
OTA	4.9	2.6
FBs	n.d	15.4
ZEA	n.d	n.d
DON	n.d	n.d

n- no. of samples, n.d- none detected

Table 2: Analyses of mycotoxins in rice samples as determined by high performance liquid chromatography

Mycotoxins	% incidence	Range	Mean	Std.
				Dev.
AFs	87.8	0.01-6.50	0.76	3.10
OTA	92.7	0.7-180.9	18.2	112.3
FB ₁	90.2	0.9-59.6	18.8	112.6
FB ₂	n.d	n.d	n.d	n.d
ZEA	80.5	0.7-570.6	203.9	440.0
DON	70.7	0.1-0.7	0.3	1.6

Mean*- mean of positive samples. Concentrations of AFs, DON, FBs, OTA and ZEA in samples are in µg/kg. % incidence- no. of positive samples in percentage. Std. Dev. – standard deviation.

Table 3: Analyses of mycotoxins in maize samples as determined by high performance liquid chromatography

Mycotoxins	% incidence	Range	Mean	Std. Dev.
AFs	94.9	0.07-109.78	10.24	24.92
OTA	92.3	0.6-79.0	14.8	16.7
FB ₁	94.9	10.0-3644.0	645.2	919.1
FB ₂	66.6	15.2-1123.9	137.8	252.4
ZEA	64.1	1.83-652.3	139.0	155.8
DON	69.2	0.1-0.7	0.3	0.2

Mean*- mean of positive samples. Concentrations of AFs, DON, FBs, OTA and ZEA in samples are in µg/kg. % incidence- no. of positive samples in percentage. Std. Dev. - Standard deviation

Statistical analysis of results from HPLC analysis showed ranges of concentration of the different mycotoxins screened for. The highest range of mycotoxin contamination in rice samples was ZEA ranging from 0.7- 570.6µg/kg as indicated in table 2. Maize samples had highest concentration range of fumonisin B₁ (10- 3644µg/kg) with a mean value of 645.2. table 3 shows the different concentration range of all mycotoxins screened for in the maize samples along with the mean and standard deviation values derived. Different concentration ranges were recorded for mycotoxins in rice and maize samples from the two collection areas as illustrated in table 4 and 5. Table 6 and 7 shows concentration range of mycotoxins determined by all three method implored and correlation co-efficient (r) analysis of results derived from multi-mycotoxin extraction and SAX columns against results got from VICAM immuno- affinity columns.

236 Table 4: Distribution of mycotoxin contamination in rice samples from both collection areas
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Mycotoxins	Stores		Markets	
	Range (µg/kg)	Mean	Range (µg/kg)	Mean
AFs	0.01-0.18	0.06	0.04-6.50	1.26
OTA	0.7-180.9	24.1	0.8-92.2	9.2
FB ₁	0.9-44.9	18.3	4.4-59.6	15.7
FB ₂	0	0	0	0
DON	0.1-0.6	0.4	0.1-0.7	0.3
ZEA	0.7-317.6	97.7	0.7-570.6	271.8

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Table 5: Distribution of mycotoxin contamination in maize samples from both collection areas

Mycotoxins	Stores		Markets	
	Range (µg/kg)	Mean	Range (µg/kg)	Mean
AFs	0.18-109.78	11.62	0.07-30.85	8.94
OTA	0.6-79.0	15.0	2.5-46.5	13.1
FB ₁	10.0-2401.5	630.3	22.2-3643.6	610.7
FB ₂	15.2-686.6	245.2	27.8-1123.9	145.0
DON	0.1-0.7	0.4	0.1-0.7	0.3
ZEA	88.1-652.3	251.4	1.83-132.6	36.7

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251 Table 6: Mycotoxin HPLC concentration range ($\mu\text{g/kg}$) in food extracts by multi-mycotoxin,
252 SAX column and VICAM immuno affinity column extraction procedure
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Mycotoxins	Rice			Maize		
	Multi-Mycotoxin	SAX column	Immuno-affinity	Multi-Mycotoxin	SAX column	Immuno-affinity
AFs	0.01-6.50		4.76-15.70	0.20-109.78		0.91-123.62
OTA	0.7-180.9		10.4-220.40	0.6-40.3		7.8-60.1
FB ₁		0.90-59.60	3.7-62.0		10.0-3643.6	16.3-3722.7
FB ₂		n.d	n.d		15.2-1123.9	32.1-1343.8

254 n.d- none detected
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261 Table 7: Correlation co-efficients (r) for mycotoxins detected from multimycotoxin and SAX
262 column in comparison with VICAM extraction.
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Correlation co-efficient (r)				
Food samples	AFs	OTA	FB ₁	FB ₂
Maize	0.970	0.969	0.990	-
Rice	0.932	0.992	1.0	0.982
Cocoa	0.996	1.0	-	-
Cocoa beverage	0.992	0.984	-	-

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266 5.0 DISCUSSION

267 Food contamination by mycotoxins is a major problem in the tropics and sub-tropics due to
268 climatic conditions and other conditions that favour the growth of fungi and subsequent
269 production of mycotoxins by these fungi [17]. Nigeria is not left out of this situation because

of its adverse weather conditions, farming practices, storage conditions and mode of transportation of food crops. Out of over 20 commonly occurring mycotoxins in food and agricultural products [18], the aflatoxins, ochratoxins, fumonisins, zearalenone and the trichothecenes have been reported to be major mycotoxins occurring in food.

Rice grain is a highly suitable substrate for the production of the five major occurring mycotoxins mentioned as these mycotoxins have been reported to occur in rice grains at different stages of production worldwide. Mycotoxin contamination of rice often occurs in the field prior to harvest, while post-harvest contamination can occur if drying was delayed and during storage if moisture content is allowed to exceed critical values for mould growth [17]. Results obtained from the mycotoxin screening of rice samples also agree with this views about mycotoxin contamination of rice crops. Although lower incidences were obtained when samples were analysed using TLC which could be attributed to the detection limit of thin layer chromatographic techniques [19-21], very high incidences were determined through HPLC analysis. The occurrence of these mycotoxins AFs, OTA, FBs, DON and ZEA evaluated in this food sample could be attributed to contamination of the samples by mycotoxigenic fungi *Aspergillus*, *Fusarium* and *Penicillium*[22-24].

Maize is also another food commodity that act as good substrate for growth of toxigenic fungi producing mycotoxins. One major mycotoxin associated with maize is fumonisin which is produced mainly by *F.verticillioides* and *F. proliferatum*[25-27]. Other mycotoxins reported to be associated with contamination of maize include OTA, ZEA, AFs and the trichothecenes. Thin layer chromatography data from maize samples showed incidences for AFs, OTA and FBs at 23.1%, 2.6% and 15.4% whereas ZEA and DON were not detected. High performance liquid chromatography analysis data also indicated high incidences of all five mycotoxins with varying concentration ranges. Out of all five mycotoxins present in the maize samples, fumonisins B₁ and B₂ was detected to be of highest concentration. This could very well be attributed to the susceptibility of maize crops to the fungi specie *F.verticillioides* and *F. proliferatum* as was earlier mentioned.

There have been reports of filamentous fungi prevalence and mycotoxin occurrence in many food commodities originating from Nigeria in recent years [22,23,28-30] with many food commodities whether unprocessed or processed being screened for these fungal metabolites. The findings from this study are in agreement with most studies from other parts of the world

where mycotoxin analysis has been done on cereal crops and grains. In this study, OTA was found to occur in most samples at lower concentration although some samples had high concentrations of OTA which was above the set regulatory limit of 5µg/kg for the EU and Nigeria. Ochratoxin A mostly produced by *A. ochraceus* and *P. viridicatum* has been mentioned as a predominant mycotoxin alongside the aflatoxins contaminating many Nigerian food commodities from farm to fork [22,29]. This can be due to the high temperature and humidity of the country which favours the production of these mycotoxins by fungi [7]. One other observation from the study was that samples that were highly contaminated with OTA had lower concentration of AFs and vice versa. This could be explained by the inhibitory effect of ochratoxin A to the production of aflatoxins by *A. parasiticus* reported by Dimitrokallis, et al. [31], although there is no evidence of this inhibitory effect on the production of aflatoxins by *A. flavus*. Another reason could be the divergent uptake behaviour of mycotoxins which indicates the transport of OTA being different from that of AFs in food commodities as a result of chemico-physical effects [32].

Fumonisin contamination of both rice and maize samples with concentration above the EU regulatory set limit of 1000µg/kg was recorded in this study. There was also the prevalence of DON and ZEA in both samples. As it is, most mycotoxin studies on Nigerian food commodities have concentrated on the fumonisins, ochratoxin A and aflatoxins with very little attention given to other mycotoxins even though it is well known that these mycotoxins have very adverse health effects both in humans and animals. The high levels of DON and ZEA in the food samples analysed could be as because cereals are good substrates for contamination by DON and ZEA producing fungi [3,33,34]. Also, ZEA is a heat and pH stable mycotoxin and so can withstand very harsh weather conditions [35,36].

There was a distinctive difference in mycotoxin contamination of food samples from stores and markets. All rice samples collected from the markets were contaminated with high concentrations of mycotoxins in comparison to rice samples collected from d stores. A reason for this variation in mycotoxin contamination could be attributed to the means of transportation and handling of the samples at the market places. Most of these food samples are displayed in big containers or in bags at the market places which are left open for buyers to see and purchase. With the presence of filamentous fungi in the air and soil, some of the food samples in the market places would have been more contaminated by the mycotoxin producing fungi and subsequent mycotoxin production. Evaluation of mycotoxins co-

occurring in the selected food samples showed a prevalence of all five mycotoxins at varying concentration levels which is something to taken seriously.

6.0 CONCLUSION

In this study, we were able to evaluate the incidence of the five mycotoxins in the food samples. These mycotoxins were found to co-occur at different concentrations above the set limits which is a cause for concern in terms of mycotoxin control strategies in Nigeria. As was mentioned earlier in the text much emphasis has been given to aflatoxins, ochratoxin A and fumonisins in Nigerian foods with little attention given to other less common mycotoxins which also have negative health effects. The high incidence of both DON and ZEA in the food commodities analysed is a cause for concern and reason for more research into other mycotoxins contamination of Nigerian food commodities as well control strategies in order to combat mycotoxin contamination.

8.0 CONFLICT OF INTEREST

The authors declare no conflict of interest.

8.0 REFERENCES

1. Chauvin ND, Mulangu F, Porto G. Food production and consumption trends in sub-Saharan Africa: Prospects for the transformation of the agricultural sector. United Nations Development Programme; 2012.
2. Piotrowska M, Śliżewska K, Biernasiak J. Mycotoxins in Cereal and Soybean-Based Food and Feed; 2013.
3. Pereira VL, Fernandes JO, Cunha SC. Mycotoxins in cereals and related foodstuffs: A review on occurrence and recent methods of analysis. Trends Food Sci Technol. 2014;36(2):96-136.
4. Edite Bezerra da Rocha M, Freire FdCO, Erlan Feitosa Maia F, Izabel Florindo Guedes M, Rondina D. Mycotoxins and their effects on human and animal health. Food Control. 2014;36(1):159-165.
5. Richard JL. Some major mycotoxins and their mycotoxicoeses—An overview. Int J Food Microbiol. 2007;119(1–2):3-10.
6. Cadoni P, Angelucci F. Analysis of incentives and disincentives for rice in Nigeria. Technical notes series. Rome: MAFAP, FAO; 2013.
7. Wagacha JM, Muthomi JW. Mycotoxin problem in Africa: Current status, implications to food safety and health and possible management strategies. Int J Food Microbiol. 2008;124(1):1-12.

- 376 8. Atanda O, Makun HA, Ogara IM, Edema M, Idahor KO, Eshiett ME, Oluwabamiwo
377 BF. Fungal and Mycotoxin Contamination of Nigerian Foods and Feeds. In: Makun
378 HA, editor. *Mycotoxin and Food Safety in Developing Countries*. Croatia: Intech;
379 2013.
- 380 9. Fapohunda SO, Anjorin ST, Akueche EC, Harcourt B. Multi mycotoxin profile of
381 gamma-radiated sesame seeds from Abuja markets, Nigeria using LC-MS/MS. *Nature*
382 *and Science*. 2012;10(10):127-134.
- 383 10. Rubert J, Fapohunda SO, Soler C, Ezekiel CN, Mañes J, Kayode F. A survey of
384 mycotoxins in random street-vended snacks from Lagos, Nigeria, using QuEChERS-
385 HPLC-MS/MS. *Food Control*. 2013;32(2):673-677.
- 386 11. Patterson DS, Roberts BA. Mycotoxins in animal feedstuffs: sensitive thin layer
387 chromatographic detection of aflatoxin, ochratoxin A, sterigmatocystin, zearalenone
388 and T-2 toxin. *J Assoc Official Chems*. 1979;62:1265-1267.
- 389 12. Mwanza M. An investigation in South African 461 domesticated animals, their
390 products and related health issues with reference to mycotoxins and fungi. *Biomedical*
391 *Technology*. South Africa: University of Johannesburg; 2007.
- 392 13. Shephard GS, Sewram V. Determination of the mycotoxin fumonisin B1 in maize by
393 reversed-phase thin-layer chromatography: a collaborative study. *Food Addit Contam*.
394 2004;21:498-505.
- 395 14. Shepherd GS, Thiel PG, Sydenham EW, Vleggaar R, Alberts JF. Determination of the
396 mycotoxin fumonisin B1 and identification of its partially hydrolysed metabolites in
397 the faeces of nonhuman primates. *Food Chem Toxicol*. 1994;32:223-29.
- 398 15. Reiter E, Zentek J, Razzazi E. Review on sample preparation strategies and methods
399 used for the analysis of aflatoxins in food and feed. *Mol Nutr Food Res*.
400 2009;53(4):508-524.
- 401 16. Abdulkadar AHW, Al-Ali AA, Al-Kildi AM, Al-Jedah JH. Mycotoxins in food
402 products available in Qatar. *Food Control*. 2004;15(7):543-548.
- 403 17. Kumar V, Basu MS, Rajendran TP. Mycotoxin research and mycoflora in some
404 commercially important agricultural commodities. *Crop Protect*. 2008 27:891-905.
- 405 18. Steyn PS. Mycotoxins, general view, chemistry and structure. *Toxicol Lett*.
406 1995;82/83:843-851.
- 407 19. Xu B-j, Jia X-q, Gu L-j, Sung C-k. Review on the qualitative and quantitative analysis
408 of the mycotoxin citrinin. *Food Control*. 2006;17(4):271-285.
- 409 20. Ono EYS, Silva Md, Ribeiro RMR, Ono MA, Hayashi L, Garcia GT, Hirooka EY.
410 Comparison of thin-layer chromatography, spectrofluorimetry and bright greenish-
411 yellow fluorescence test for aflatoxin detection in corn. *Braz Arch Biol Techn*.
412 2010;53:687-692.
- 413 21. Turner NW, Subrahmanyam S, Piletsky SA. Analytical methods for determination of
414 mycotoxins: A review. *Anal Chim Acta*. 2009;632(2):168-180.
- 415 22. Makun HA, Anjorin ST, Moronfoye B, Adejo FO, Afolabi OA, Fagbayibo G,
416 Balogun BO, Surajudeen AA. Fungal and aflatoxin contamination of some human
417 food commodities in Nigeria. *Afr J Food Sci*. 2010;4(4):127-135.
- 418 23. Adejumo TO, Hettwer U, Karlovsky P. Occurrence of *Fusarium* species and
419 trichothecenes in Nigerian maize. *Int J Food Microbiol*. 2007;116(3):350-357.
- 420 24. Mounjouenpou P, Gueule D, Fontana-Tachon A, Guyot B, Tondje PR, Guiraud J-P.
421 Filamentous fungi producing ochratoxin a during cocoa processing in Cameroon. *Int J*
422 *Food Microbiol*. 2008;121(2):234-241.
- 423 25. Dutton MF. Fumonisin, mycotoxins of increasing importance: Their nature and their
424 effects. *Pharmacol Ther*. 1996;70(2):137-161.

- 425 26. Kpodo K, Thrane U, Hald B. *Fusaria* and fumonisins in maize from Ghana and their
426 co-occurrence with aflatoxins. *Int J Food Microbiol.* 2000;61:147-157.
- 427 27. Sweeney MJ, Dobson AD. Mycotoxin production by *Aspergillus*, *Fusarium* and
428 *Penicillium* species. *International Journal of Food Microbiology* 1998; 43:141-158.
- 429 28. Adejumo TO, Hettwer U, Karlovsky P. Survey of maize from south-western Nigeria
430 for zearalenone, α - and β -zearalenols, fumonisin B1 and enniatins produced by
431 *Fusarium* species. *Food Addit Contam.* 2007;24:993-1000.
- 432 29. Egbuta MA, Chilaka CA, Phoku JZ, Mwanza M, Dutton MF. Co-contamination of
433 Nigerian cocoa and cocoa-based powder beverages destined for human consumption
434 by mycotoxins. *Ethno Medicine.* 2013; 7(3):187-194.
- 435 30. Bankole SA, Schollenberger M, Drochner W. Survey of ergosterol, zearalenone and
436 trichothecene contamination in maize from Nigeria. *J Food Compos Anal.*
437 2010;23(8):837-842.
- 438 31. Dimitrokallis V, Meimaroglou DM, Markaki P. Study of the ochratoxin A effect on
439 *Aspergillus parasiticus* growth and aflatoxin B1 production. *Food Chem Toxicol.*
440 2008;46:2435- 2439.
- 441 32. Raters M, Matissek R. New examinations of mycotoxin carryover to cocoa beans.
442 *Mycotoxin Research.* 2007;23:39-42.
- 443 33. Van Der Fels-Klerx HJ, Klemsdal S, Hietaniemi V, Lindblad M, Ioannou-Kakouri E,
444 Van Asselt ED. Mycotoxin contamination of cereal grain commodities in relation to
445 climate in North West Europe. *Food Additives & Contaminants: Part A.*
446 2012;29(10):1581-1592.
- 447 34. Bernhoft A, Eriksen GS, Sundheim L, Berntssen M, Brantsæter AL, Brodal G, Fæste
448 CK, Hofgaard IS, Rafoss T, Sivertsen T, Tronsmo AM. Risk assessment of
449 mycotoxins in cereal grain in Norway. *Norwegian Scientific Committee for Food*
450 *Safety (VKM);* 2013.
- 451 35. Yazar S, Omurtag G. Fumonisins, Trichothecenes and Zearalenone in Cereals. *Int J*
452 *Mol Sci.* 2008;9(11):2062-2090.
- 453 36. Ryu D, Hanna MA, Eskridge KM, Bullerman LB. Heat Stability of Zearalenone in an
454 Aqueous Buffered Model System. *J Agric Food Chem.* 2003;51(6):1746-1748.

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