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Original article

Occurrences of Deoxynivalenol, Zearalenone and some of their masked forms in selected cereals from Southwest Nigeria

Bunmi K. Olopade [a,b,*], Solomon U. Oranusi [a], Obinna C. Nwinyi [a,b], Sefater Gbashi [b], Patrick B. Njobeh [b]

[a] Department of Biological Sciences, Covenant University, KM 10 Idiroko Road, Ota, Ogun State, Nigeria
[b] Department of Biotechnology and Food Technology, University of Johannesburg, Doornfontein 2028, South Africa

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ABSTRACT

The study determined the occurrence of deoxynivalenol (DON), zearalenone (ZEN) and their metabolites in staple cereals from Southwest Nigeria. Sixty composite samples of maize, sorghum and millet were evaluated for DON, ZEN and their masked forms; 3-acetyldeoxynivalenol (3Ac-DON), 15-acetyldeoxynivalenol (15Ac-DON), α-zearalenone (α-ZEL) and β-zearalenone (β-ZEL). Deoxynivalenol did not co-occur with ZEN in maize, sorghum and millet samples in contrast to previous reports. Only the masked forms; 3Ac-DON occurred in few maize samples within the range < LOQ-24 μg/kg and one millet sample at a concentration of 16 μg/kg. The levels of ZEN in maize and sorghum samples were lower than the maximum limit of 100 μg/kg set by the European Union for ZEN. However, two millet samples exceeded this limit with concentration of 152 and 396 μg/kg. The percentage incidence for α-ZEL was 100% for maize, sorghum and millet samples while the percentage incidence of β-ZEL was 100% for maize and millet and 95% for sorghum samples. Regardless of the low levels of these mycotoxins, particularly DON, the high incidence rates are of concern as there could be synergistic or additive effects from ZEN and its masked forms.

1. Introduction

Mycotoxins are secondary metabolites from toxigenic fungi that are responsible for the contamination of agricultural commodities both in the field, during transportation and storage under a wide range of climatic conditions [1]. Some of the agricultural commodities contaminated by mycotoxins include maize (*Zea mays*), sorghum (*Sorghum bicolor*) and millet (*Pennisetum glaucum*). Maize is grown throughout the world, with Nigeria producing approximately 10.4 million metric tonnes/year [2]. Sorghum is another staple food consumed across several countries in the world. The United States Department of Agriculture (USDA), projected the yearly output of sorghum production as 8.4 million metric tonnes in the USA, 6.4 million metric tonnes in Nigeria and 6.0 million metric tonnes in Mexico [3]. Nigeria has also been ranked amongst the top five producers of millet in the world [2]. The Forum for Agricultural Research (FARA) [4] stated that maize production in Africa increased at the rate of (2.8% per annum). However, the yields from maize production were lower (1.3% per annum). Mycotoxigenic fungal contamination, pests and other diseases that affect crops, can result in low yield of maize and other cereal grains. The presence of mycotoxins in these cereals generates food safety problems because they are carcinogenic, teratogenic, tremorogenic and immunesuppressive [5].

Zearalenone (ZEN), which, is one of the mycotoxins of economic importance, occurs naturally in many important crops worldwide with high concentrations [6]. Reports have shown that zearalenone co-exists with other mycotoxins, which include deoxynivalenol (DON), 15-acetyldeoxynivalenol (15Ac-DON), 3-acetyldeoxynivalenol (3Ac-DON), nivalenol and Fusarenon X because they are produced by the same fungal species [7,8]. Zearalenone has consistently been detected in Nigerian maize [9], together with its metabolites, i.e., α-ZEL, and β-ZEL in the same commodity and millet [10,11]. Also, DON and ZEN have masked forms which cannot be detected by conventional analytical methods because they exhibit a different chemical behaviour such as their solubility and polarity [12]. These masked forms can be converted back to their parent toxin, thereby increasing the overall toxicity in mammals [13,14].

Fusarium species, particularly *Fusarium graminearum* and *F. culmorum*

* Corresponding author at: Department of Biological Sciences, Covenant University, KM 10 Idiroko Road, Ota, Ogun State, Nigeria.
E-mail address: bunmi.olopade@covenantuniversity.edu.ng (B.K. Olopade).

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are responsible for the production of DON [15].

Deoxynivalenol has been reported in maize from Nigeria [11,16]. Several reports show that its masked forms which are acetyl derivatives, 3-acetyl DON (3Ac-DON) and 15-acetyl DON (15Ac-DON) usually co-occur with DON in grains, but at much lower levels [17]. It is also possible for 15Ac-DON to be metabolised into Deoxynivalenol-3-*O*- β-d-glucoside (DON-3Glc). Deoxynivalenol-3-glucoside (DON-3Glc), the dominant product of DON metabolism amongst other DON metabolites, has been shown to be less toxic amongst other DON metabolites [18–20] but not detected in maize and millet from Nigeria [11]. Deoxynivalenol, commonly referred to as vomitoxin, belongs to a class of mycotoxins known as trichothecenes [21] that co-occur with its acetylated derivatives, i.e., 3Ac-DON and 15Ac-DON mostly in cereals [22]. Toxicity of DON is initiated through oxidative stress, which causes damage to deoxyribonucleic acid (DNA) and the death of cells [23]. Deoxynivalenol inhibits protein synthesis by binding the enzyme; peptidyl transferase, which is situated in the subunit of the 60s ribosome [24]. Deoxynivalenol intoxication could lead to decreased absorption of glucose in the gastrointestinal tract due to the suppression of SGLT1 (glucose transporter) mRNA expression. This glucose transporter- SGLT1 is not only necessary for glucose absorption but also water reabsorption. Thus, diarrhoea is induced by the suppression of SGLT1 transporter [25]. Different species of *Fusarium* that include *Fusarium culmorum* and *F. graminearum* are also responsible for the production of zearalenone [26]. The masked forms of zearalenone include α-ZEL, β-ZEL, zearalenone-14-sulfate (ZEN14Sulf), Zearalenone-14-*O*-β-D-glucopyranoside (ZEN14βDGlc*p*), α-zearalenol 14-*O*-β-D-glucopyranoside (α-ZEL14βDGlc*p*) and β- zearalenol 14-*O*-β-D-glucopyranoside (β-ZEL14βDGlc*p*).

Zearalenone exhibits oestrogenic effects due to its structure which enables it to bind to the oestrogen receptor of mammals [27]. From the structural elucidation, ZEN is a resorcylic acid lactone that is biosynthesised through a polyketide pathway by different species of *Fusarium* [28]. The maximum permissible limits established by EU directives (Commission Regulation (EC) No. 1881/2006) for DON and ZEN in unprocessed cereals are 1250 and 100 μg/kg, respectively, while the limits of DON and ZEN for unprocessed maize are 1750 and 350 μg/kg, respectively [29]. However, there are no set limits for masked mycotoxins in grains. Deoxynivalenol (DON) and zearalenone (ZEN) are the most prevalent mycotoxins produced by the same *Fusarium* species in cereals, animal feed and forages under various environmental conditions [30]. The co-occurrence of DON and ZEN have been reported in maize flour and maize-based products in Italy, Belgium, Czech Republic and Serbia at concentrations higher than the established limits [31]. Both DON, ZEN and their conjugates have shown to be stable compounds even when subjected to treatment by heat [32]. The consumption of DON-contaminated and ZEN-contaminated cereals have a negative influence on the health and reproductivity of humans and farm animals. The entire food chain is compromised because humans feed on cereals and farm animals affected by DON, ZEN and their conjugates, thereby accumulating xenobiotic compounds that impair the immune system and reproductive cycle. The decrease in reproduction in farm animals also generates economic losses [33]. Reports from several studies in Nigeria have focused more on aflatoxins, ochratoxins, fumonisins while there are only few reports on DON and ZEN [34]. Hence, it is important to evaluate DON, ZEN and their conjugates since they are known to have a strong socioeconomic impact on the society.

2. Materials and methods

2.1. Chemicals and reagents

Mycotoxin standards used in this study were purchased from Sigma (Sigma, South Africa) and the National Metrological Institute of South Africa (NMISA); they included deoxynivalenol (DON), 3-acetylydeoxynivalenol (3-AcDON), 15-acetylydeoxynivalenol (15-AcDON), zearalenone (ZEN), α-zearalenol (α-ZEL), and β-zearalenol (β-ZEL). The solvents used included MS-grade acetonitrile, formic acid, and methanol, which were also purchased from Sigma (South Africa). A Milli-Q Gradient A10 dispensing system (Millipore, Billerica, MA, USA) was used to produce the deionized (ultrapure) water.

2.2. Sampling

Three-hundred samples of maize, sorghum and millet intended for consumption as food and feed were obtained from the major grain hubs of the six Southwest states of Nigeria (Ekiti, Lagos, Ogun, Ondo, Osun, and Oyo) which falls into the Humid rain forest agro-ecological zone of Nigeria. This zone is also referred to as the Southwest geographical zone of Nigeria. The area lies between longitude $2^{\circ}31^{1}$ and $6^{\circ}00^{1}$ East and Latitude $6^{\circ}21^{1}$ and 8° 37^{1}N with a total land area of 77, 818 km^2 [35]. Southwest Nigeria has a tropical climate characterised by heavy rainfall (150 and 3000 mm per annum), with an average annual temperature range between 21 and 34 °C. Twenty composite samples each of maize, sorghum and millet were prepared separately to screen for the presence of DON, ZEN and their metabolites. Each representative composite sample of maize across the six states was obtained by combining hundred grams each of five maize samples from the same state, while a representative composite sample of sorghum was obtained by combining hundred grams each of five sorghum samples from the same state. Representative composite samples of millet across each state were obtained by combining hundred grams of five millet samples from the same state. The hand-mixed samples were coarsely ground to pass through a No. 14 mesh screen. Five-hundred grams of the subsamples of the different cereals were ground using a blender (IKA M20, Germany) and sieved using a 1-mm mesh. Fifty grams of the ground sub-samples were taken from each lot and stored in zip-lock bags at −20 °C before analyses.

2.3. Extraction and analysis of DON, ZEN, and their metabolites

The method of the LC-MS/MS procedure [36] was used to determine and quantify DON and two of its masked forms 3Ac-DON and 15Ac-DON, ZEN and two of its masked forms α-zearalenol (α-ZEL) and β-zearalenol (β-ZEL) in sixty composite samples of maize, sorghum and millet. The ground representative samples (5 g) of each lot was transferred to a 50-mL polypropylene tube (Sarstedt, Nümbrecht, Germany) and mixed with 20 ml of the extraction solvent that consisted of acetonitrile/water/formic acid (79:20:1, *v*/v/v). The extraction was carried out for 90 min on a GFL 3017 rotary shaker (GFL, Burgwedel, Germany). Aliquots of 500 μL containing the same extraction solvent (20:79:1) were used to dilute the same volume (500 μL) of the pipetted mixture extracted by (acetonitrile/water/formic acid (79:20:1). Five μL of each diluent was injected into the LC-MS/MS.

The targeted mycotoxins were screened using a Shimadzu LC-MS/MS 8030 System (Shimadzu Corporation, Tokyo, Japan) equipped with an LC-30 CE Nexera chromatograph coupled with an autosampler (SIL-30 AC Nexera). The temperature of the column oven (CTO-20 AC Prominence) was isothermal at 40 °C while chromatographic separation was performed on a Raptor™ ARC-18 column (2.7 μm, 2.1 mm × 100 mm; particle size, ID, & length, respectively) (Restek Corporation, Pennsylvania USA). The mobile phases comprised of an aqueous phase (Solvent A), which contained 0.1% formic acid (FA) in deionized water, while the organic phase (Solvent B) contained 0.1% FA in methanol/acetonitrile (50/50, *v*/v). The LC gradient began with 10% of Solvent B after 0.1 min, then ramped steadily to 95% B within 8.4 min, and kept in this state for 3 min. Thereafter, the previous state, i.e., 10% B was re-established in 1 min and kept in this state for 4.5 min to allow the re-equilibration of the column before the subsequent run. Flow rate for the mobile phase was kept constant at 200 μL/min, with each sample having a total run time of 17 min.

Following LC separation of the analytes, a triple quad MS was used

for the detection and quantification of the analytes. The MS method used was in a multiple reaction monitoring (MRM) mode operated in a positive ionization mode (ESI^+). The desolvation line (DL) temperature was maintained at 250 °C, with the heat block temperature kept at 400 °C. The flow rate for the interface nebulizing gas was set at 3 L/min, while that of the drying gas was 15 L/min. After data acquisition, the obtained results were processed and interpreted using the Shimadzu LabSolutions software.

2.4. Method validation

The method was validated according to the Commission Regulation 401/2006/EC [29]. The values for the limit of detection (LOD), the limit of quantification (LOQ) were determined using the signal-to-noise ratio as described in Eqs. (1) and (2) [37]. The apparent recovery (AR) were obtained by spiking mycotoxin-free samples (blank) with 25, 50 and 100 μg/kg and extracting the residue and results expressed as the percentage ratio of the recovered analytes to that of the initially spiked concentration. The linearity of the analytes were determined by evaluating the coefficient of determination (R^2) of the calibration curves within the linear ranges by a scatter plot of the graph, meanwhile a lack-of-fit test was used to evaluate the linear regression model.

$$\mathrm{LOD} = 3 \text{ x } (\sigma/\mathrm{s}) \quad (1)$$

$$\mathrm{LOQ} = 10 \text{ x } (\sigma/\mathrm{s}) \quad (2)$$

where σ = standard deviations of residuals.
s = the slope of the calibration curve.

3. Results and discussion

3.1. Method performance characteristics of the cereals

The limits of detection (LOD) for the tested mycotoxins ranged from 2.00 to 7.00 μg/kg in all the matrices. The limits of quantification (LOQ) for all analytes in maize ranged from 5.00 to 20.00 μg/kg, while that for sorghum was 7.00–50.00 μg/kg. The apparent recoveries (AR) for all the mycotoxins ranged from 65 to 104% in maize, 79–113% in sorghum and 84–104% in millet as shown in Table 1.

3.2. The occurrence of deoxynivalenol, zearalenone, and their metabolites in maize, sorghum, and millet

Out of the 60 composite samples of maize, sorghum and millet analysed, DON did not occur in any of them. Only its masked form 3Ac-DON occurred in three of the maize samples within the range of <LOQ-24 μg/kg (Table 2). ZEN was detected within the range of <LOQ-16 μg/kg in maize samples, <LOQ-20 μg/kg in sorghum samples and < LOQ-396 μg/kg in millet samples. Only two millet samples exceeded the European Union maximum limit of 100 μg/kg. The concentrations of ZEN recorded in the two millet samples were 152 and 396 μg/kg.

A report showed that maximum set limits for ZEN were exceeded in millet samples from the Northern Guinea Savanna (NGS) and Southern Guinea Savanna (SGS) with a mean concentration of 109 and 481 μg/kg, respectively [11]. It appears that ZEN and its metabolites are usually present at higher quantities in millets compared with maize and sorghum. However, there are very few reports on ZEN in millet and sorghum from Nigeria. A study reported ZEN in mouldy sorghum from Niger State [38] while another study reported ZEN in sorghum and millet from some of the agro-ecological zones of Nigeria [11]. In contrast, most studies in Nigeria have focused on the detection of ZEN in maize, and their results showed that ZEN levels in maize were exceeded in all except one study [9]. Furthermore, the level of ZEN in maize from this study (4–16 μg/kg) did not exceed the set limit of 100 μg/kg by the European Commission (EC) and was similar to the study [9] where ZEN was detected in maize from Nigeria within the range of 2–13 μg/kg.

Another study [39] reported ZEN in maize from Southwest Nigeria within the range of 115–779 μg/kg, which exceeded the limit of 100 μg/kg set by the EC. Some studies [11,16] reported ZEN in maize from the five agro-ecological zones of Nigeria; and Northeastern Nigeria, respectively within the ranges of 0.4–2044 μg/kg and 0.7–570.6 μg/kg which showed that ZEN limits were exceeded. In contrast, lower levels of ZEN were recorded in this study with only two millet samples with concentrations of 152 and 396 μg/kg exceeding the EC limit (100 μg/kg) for ZEN. In this study, the masked zearalenone (α-ZEL); occurred in 100% of maize, sorghum and millet samples. The levels of α-ZEL were within the ranges of <LOQ-64 μg/kg in maize samples, <LOQ-20 μg/kg in sorghum samples and < LOQ-36 μg/kg in millet samples. The percentage incidence of β-ZEL in maize was 100% and within the range of <LOQ-28 μg/kg, 95% in sorghum with a range of <LOQ-44 μg/kg and 100% in millet samples with a range of <LOQ-136 μg/kg. In contrast to a previous study, α-ZEL was detected in maize and sorghum samples with an incidence of 3% and 8% respectively from the SGS and NGS whereas β-ZEL had an incidence of 3% in maize, 3% in sorghum and 3% in millet from NGS, SGS and DS, respectively [11].

Deoxynivalenol and ZEN was recorded in maize, sorghum and millet from the five agro-ecological zones of Nigeria [11]. However, the results from this study revealed that there was no co-occurrence between DON and ZEN in maize, sorghum and millet samples from Southwest Nigeria. As shown in Fig. 1, DON and its modified form, 15Ac-DON did not occur in any maize sample similar to a previous report [11] wherein 15Ac-DON was not detected in maize but detected in few sorghum and millet samples in some parts of the agro-ecological zones. Modified DON precisely 3Ac-DON occurred in 20% of the maize samples. Deoxynivalenol, 3Ac-DON, and 15Ac-DON were not present in any sorghum sample (Fig. 2), while 3Ac-DON was detected in 10% of millet samples (Fig. 3). Higher percentage occurrences were also observed for ZEN and its metabolites (Figs. 1–3). Contrary to a previous study, the percentage of DON and its metabolites were higher than that of ZEN and its metabolites in maize, sorghum, and millet which was purchased between September 2015 and October 2015 [11].

All the positive DON samples detected by Chilaka and co-workers were below the indicative level of 1250 μg/kg for DON set by the European Commission. Although, they did not detect DON in any sorghum sample from the Southern Guinea Savanna (SGS). The low humidity and high temperature at the time of collection of the cereal grains could have

Table 1
Method performance parameters of the different cereal grains.

Mycotoxins	Calibration range (μg/kg)	Maize			Sorghum			Millet		
		LOD	LOQ	AR ± Stdev	LOD	LOQ	AR ± Stdev	LOD	LOQ	AR ± Stdev
Deoxynivalenol	1.57–5000	2	5	84 ± 1.00	2	5	79 ± 27.34	2	50	91 ± 6.98
3-Acetyldeoxynivalenol	1.57–5000	7	20	71 ± 8.56	7	20	80 ± 5.59	7	20	88 ± 4.88
15-Acetyldeoxynivalenol	1.57–5000	3	20	65 ± 15.98	7	20	92 ± 9.05	7	20	84 ± 6.22
Zearalenone	1.57–5000	2	6	104 ± 5.94	2	8	113 ± 1.69	2	6	104 ± 3.68
Alpha-Zearalenol	1.57–5000	2	7	100 ± 0.28	2	7	94 ± 0.57	2	7	89 ± 1.13
Beta-Zearalenol	1.57–5000	3	8	92 ± 3.11	2	9	89 ± 3.82	2	9	91 ± 0.14

LOD: limit of detection (μg/kg); LOQ: limit of quantification (μg/kg); AR: Apparent recovery (%); Stdev: standard deviation.

Table 2
Mycotoxin contamination in maize, sorghum, and millet from Southwest Nigeria.

Mycotoxins	Maize n = 20			% above EU limits	Sorghum n = 20			% above EU limits	Millet n = 20			% above EU limits
	% + ve	Range	Mean		% + ve	Range	Mean		% + ve	Range	Mean	
DON	0	<LOQ	0	0	0	<LOQ	0	0	0	<LOQ	0	0
3Ac-DON	3(15)	<LOQ-240.00	130	0	0	<LOQ	0	0	1(5)	16.00–16.00	16	0
15Ac-DON	0	<LOQ	0	0	0	<LOQ	0	0	0	<LOQ	0	0
Zearalenone	15(75)	<LOQ-16	6	0	18(90)	<LOQ-20	18	0	17(85)	<LOQ-396.00	64	2(10)
Alpha-Zearalenol	20 (100)	<LOQ-64	27	0	20 (100)	<LOQ-20	8	0	20 (100)	<LOQ-36.00	18	0
Beta-Zearalenol	20 (100)	<LOQ-28	11	0	19(95)	<LOQ-44	16	0	20 (100)	<LOQ-136.00	27	1(10)

DON: Deoxynivalenol; LOD: limit of detection (μg/kg); LOQ: limit of quantification (μg/kg); AR: Apparent recovery (%).

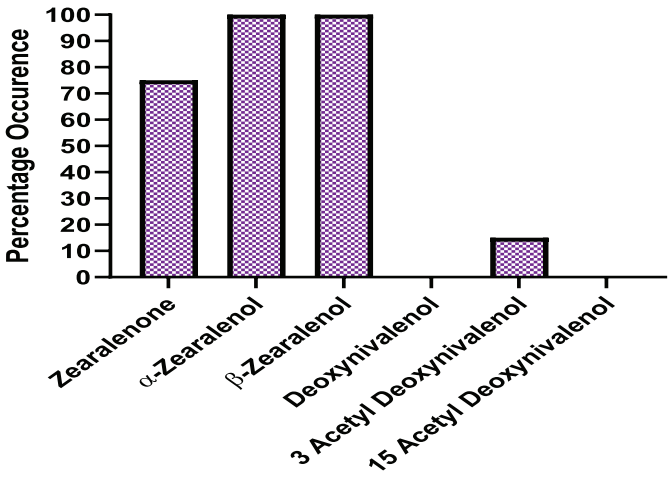

Fig. 1. Percentage of DON, ZEN, and their masked forms in maize samples from Southwest Nigeria.

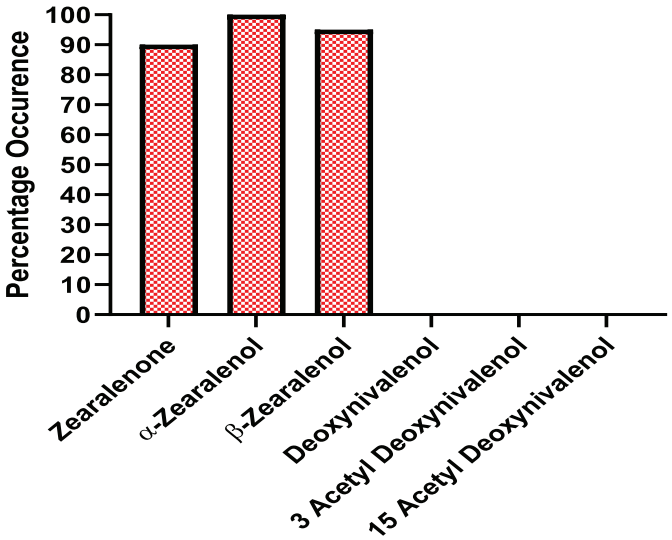

Fig. 2. Percentage of DON, ZEN, and their masked forms in sorghum samples from Southwest Nigeria.

led to the reduction in the proliferation of *Fusarium* moulds such as *F. graminearum* and *F. culmorum.* Deoxynivalenol is relatively stable, but its production can be compromised under unfavourable conditions. Consequently, DON degradation has been reported at high storage temperatures and its production is increased at lower or cooler storage

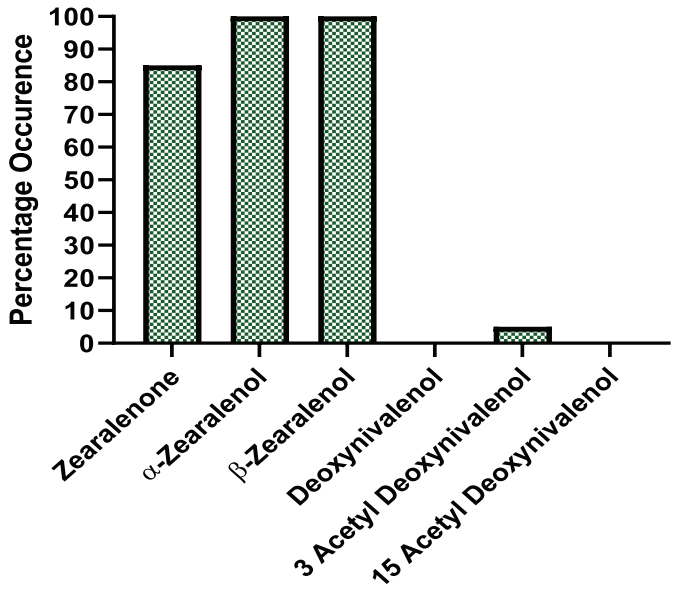

Fig. 3. Percentage of DON, ZEN, and their masked forms in millet samples from Southwest Nigeria.

temperatures. A study reported maximum DON production in wheat to be at a_w 0.97 and 0.99 at 15 and 25 °C, respectively, for *Fusarium culmorum,* while maximum DON production was at a_w 0.99 and 0.98 at 15 and 25 °C, respectively, for *F. graminearum* [40].

3.3. Significance of mycotoxin contamination and co-occurrence in Nigerian staple cereals

Large quantities of maize are circulated in international trade; therefore, contamination by ZEN, DON and their modified forms have an impact on the economy as well as public health. Moreover, significant losses have been accrued by Nigeria and other West African countries due to trade rejections of mycotoxin-contaminated foods. As a result of this, proper control measures are required to reduce the level of these mycotoxins in cereals. Cases of early puberty have also been reported in some regions of Southwest Nigeria. A study on pubertal timing of secondary school children in some urban and rural settlements in Osun State, Nigeria revealed that over 80% of the female respondents within the age range of 10–13 attained puberty much earlier than expected [41]. Several factors, such as place of residence, socio-economic class and living conditions were considered in the study. However, the diet of the respondents cannot be overlooked. Cereal is a staple food consumed by Nigerians of all ages in the form of gruels (ogi), beverages (kunu and pito) and other crunchy cereal snacks, including Kokoro'. These cereals are processed in various ways before consumption such as steeping,

grinding, sieving, cooking and roasting. The wet-milling of cereals enhances the concentration of high level of mycotoxins in the pomace of processed food such as ogi [42]. Therefore, in the cause of processing these cereals, levels of zearalenone may increase in the products. Poverty and food insufficiency have also been identified as a driving factor towards the use of low quality/damaged grains as raw materials that are processed for food at household levels in Nigeria [43].

In this study, α-ZEL was present in all samples of the various grain types. The occurrence of α-ZEL in all the cereal grains is worrisome. According to the European Food Safety Association, the presence of α-ZEL in these cereal grains is a toxicological concern [44] and α-ZEL is known to be more toxic than β-ZEL and its parent toxin, ZEN [45–47]. Studies have shown that α-ZEL and β-ZEL can be more oestrogenic than the parent toxin ZEN depending on the kind of fungal species involved in its biotransformation [48,49]. Zearalenone was also detected in several samples in this study. In contrast to another study, dry-milled maize samples from North West Italy had an incidence of 27% for ZEN, 60% for ZEN14-Sulf, 5% for α-ZEL, and 8% for β-ZEL [50]. Zearalenone is produced before harvest and also under very poor conditions of storage such as high moisture content [51]. Zearalenone is hepatotoxic, haematotoxic, genotoxic and immunotoxic [52] and it causes changes in oestrous cycle and increases uterine weight in humans and animals [53]. Ingestion of ZEN has also been implicated in the development of symptoms such as testicular atrophy, enlarged mammary glands, swelling of the vagina (vulvovaginitis), prolapses of the vagina and rectum, abortion, stillbirths and a reduction in litter size [54,55]. Because mould-damaged grains are often used in animal feed, the risk for zearalenone intoxication is highest for farm animals [56]. Feed and livestock producers are also concerned about the occurrence of ZEN in cereals because their ability to compete and make profits is dependent on the control of these mycotoxins in the diets of animals.

In this study, DON was not present in any of the cereals (maize, sorghum and millet) except its modified form, 3Ac-DON, which was present in few maize samples and a millet sample. Deoxynivalenol has been associated with diarrhoea, nausea, inflammation of the gastrointestinal tract and vomiting when ingested by humans [57]. Also, DON is immunosuppressive in man [57]. Poultry and animals that feed on DON-contaminated feeds accumulate DON in their eggs, milk and tissues [58]. This is a risk for individuals consuming meat and dairy products contaminated with DON. Studies carried out on DON in cereals from Nigeria revealed that most of the cereals were within the set limit of 1250 μg/kg in unprocessed cereals. Deoxynivalenol was detected within the range of 9.6–745.1 μg/kg and its conjugate 3Ac-DON (0.7–72.4 μg/kg) in maize from Southwest Nigeria [34]; while DON was detected in maize within the range of 0.1–0.7 μg/kg from Northeast Nigeria [16]. In another study, DON was not detected in maize samples from the major markets in Ota, Ogun State [59] while DON was detected in maize, sorghum and millet samples, and 15Ac-DON in few sorghum and millet samples from the agro-ecological zones in Nigeria [11]. The levels of DON in the previous studies did not exceed the set limit of DON. Deoxynivalenol does not appear to be a major issue in Nigerian cereals as shown by previous studies and the present study because all DON levels detected were below the European Commission (EC) set limit of 1250 μg/kg in unprocessed cereals. In contrast to another study, dry-milled maize samples from North West Italy had an incidence of 56% for DON, 29% for DON-3Glc, 14% for 3Ac-DON, and 1% for 15 Ac-DON [50]. However, the co-occurrence of DON, ZEN and their metabolites even at low concentrations may have synergistic or additive effects when ingested. Therefore, their occurrence in cereals should not be ignored because they can undergo biotransformation in the presence of other microorganisms, thereby increasing their toxicity levels [48,49]. For example, biotransformation of ZEN performed in vivo by yeasts, reduces the toxin to α-ZEL, which is more oestrogenic than ZEN [60]. Additionally, an in vivo study has demonstrated that the consumption of either DON or ZEN stimulated pro-inflammatory responses, and altered microbial populations and expression of tight junction proteins in piglets, while the simultaneous consumption of both DON and ZEN had a negative effect on body weight gain, average daily food intake and the functioning of the intestine of the piglets [61].

4. Conclusion

The level of ZEN and its metabolites in cereals from Southwest Nigeria were low. Only 3Ac-DON; a modified form of DON was recovered in three maize samples and one millet sample at low concentrations. It is essential to implement proper control measures to prevent the growth of *Fusarium* species responsible for the development of these toxins on the field and during storage. Continuous screening of major staples should be done to ascertain the safety of the food consumed by the populace.

Contributions

All authors contributed to the study conception and design. Solomon Oranusi, Obinna Nwinyi and Patrick Njobeh supervised the work. Data Analysis was performed by Bunmi Olopade and Sefater Gbashi. The first draft of the manuscript was written by Bunmi Olopade, and all authors commented on the manuscript. All authors read and approved the final manuscript.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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