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Mycotoxin Profile of 'Tom Bran', a Cereal-legume Weaning Food Preparation

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ABSTRACT

Higher levels of different mycotoxins than their respective tolerable limits exist in infant foods with concomitant far reaching physiological implications. This study aimed to measure the levels of commonly occurring mycotoxins in Tom Bran (a cereal-legume weaning food) and its composite grains (groundnut, soybean, millet, guinea corn and yellow corn) against regulatory standards. Samples of Tom Bran and their respective composite grains were subjected to mycotoxin analysis using LC-MS/MS following initial flouroacylation derivatization. The result obtained showed that the levels of aflatoxins (Af) B_1, B_2, G_1 and G_2; fumonisins (Fum) B_1, B_2 and B_3; ochratoxin (OTA) B; deoxynivalenol (DON) and zearalenone (ZEA) in Tom Bran were all within the respective safe limits set for infant population by Codex Alimentarius Commission and the United States Department of Agriculture. The levels of ZEA in all the sampled composite foodstuffs investigated, save some groundnut samples, were within regulatory limit (<10 µg/kg), just like those of Fum B_1, B_2 and B_3 (< 50 µg/kg each); DON (<50 µg/kg) and OTA B (<0.5 µg/kg). Af B_1 in groundnut samples investigated were respectively above the allowable limit of 2.0 µg/kg set for infant food, except in $R_1$where Af G_1 level in groundnut is safe (1.450µg/kg). The respective levels of Af B_1, B_2, G_1 and G_2 in 'Tom Bran', soybean, millet, guinea corn and yellow corn, but not groundnut, were all within permissible limits. The composite infant formula is therefore considered 'safe' for human consumption and as a weaning food for older infants and young children.

Keywords: Cereals, Complementary Food, Legumes, Mycotoxins, Tom Bran.

Introduction

Mycotoxins' is a term describing a collection of xenobiotics of certain fungal origin with substrates ranging from a variety of crops to crop products, thus constituting food safety and toxicological threats to humans and animals upon consuming contaminated substrates.[1,2] Mycotoxins have been associated with liver cancer, reduced immunity, altered protein metabolism, respiratory dysfunction, nephrotoxicity, teratogenesis, and negative changes in gut microbiota in human and livestock.[3,4] Their prevalence in plant raw materials that are commonly incorporated into weaning food could be relatively high.[5] The most relevant fungal species involved in mycotoxin contamination of food belong to the Genera *Aspergillus*, *Penicillium*, *Fusarium*, *Alternaria*, and *Claviceps*.[2] Mycotoxicity could range from being morbid to fatal depending on the dosage.[2,5,6] Infants and pregnant women are even more at risk because of the not-yet fully developed liver of infants and lowered immunity of both infants and pregnant women.[7-11] Five agriculturally most important mycotoxins have been identified to include aflatoxins (biocarcinogenic and most common),fumonisins, deoxynivalenol, zearalenone, and ochratoxin.[1,12-14] Common mycotoxic food contaminants have been identified to include aflatoxins (such as B_1, B_2, G_1, and G_2), ochratoxin A (OTA), patulin, fumonisins (B_1, B_2 and B_3), zearalenone (Zea), and deoxynivalenol (DON).[15] Till date, over twenty-eight types have been identified.[16-18]

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Several relevant agencies, such as Food and Agricultural Organisation, Codex Alimentarius Commission and European Union, have come up with various tolerable or healthy limits of most mycotoxins for both infants and adults alike.[19-21] The mycotoxins limits in food for infants as set by Codex Alimentarius Commission include 4.0 µg/kg for total aflatoxin, 1000 µg/kg for fumonisin B_1, 200 µg/kg for deoxynivalenol, 2.0 µg/kg (but 5.0 µg/kg by Nigerian standard as adopted from European Union) for ochratoxin A, and 200 mg/kg for zearalenone.[22,23]

Maintaining relative regulatory apparatus and enforcing standards as concerted attempt to address the food safety threat posed by mycotoxins, food wastage and relatively hampered international trade are some of the socioeconomic impacts of mycotixins.[12,24,25] To this end, it becomes imperative to understand the pattern of mycotoxin contamination of food so as to guide consumption and objectively broaden the width of empirical data thereof. Therefore, this study investigated the levels of mycotoxins in selected cereals and legumes that are commonly incorporated as components of composite blends in complementary food formulation in Nigeria.

'Tom Bran' is used operationally herein to describe a cereal-legume based model weaning food made from groundnut, soybean, yellow corn, guinea corn and millet in a given ratio targeted to provide healthy complementary nutrition for older infant and young children sub-population groups. The seemingly wide range of Tom Bran's adoption in complementary feeding evoked an imperative to evaluate both Tom Bran and its commonly adopted composite legumes and cereals for mycotoxins against set regulatory standards.[18,26]Again, significant proportion of Sub-Sahara African complementary foods is drawn from cereals, legumes and nut.[10,18,26-28] The study was justified by the seeming deficit in empirical data on mycotoxins in Tom Bran, considering that Tom Bran constitute 21.9% and 56.60% of total and household formulated complementary foods respectively in Nigeria.[18,26]

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Materials and Methods

Acquisition and processing of samples
Five grain samples made up of two legumes, soybeans (*Glycine max*) and groundnut (A*rachis hypogea*), and three cereals - yellow corn (*Zea mays*), millet *(Pennisetum typhoides)* and sorghum *(Sorghum bicolar)* were obtained from each of three different Markets within South-Western Nigeria; Ilisan Market, Ogun State; Bodija Market, Ibadan and Oja Oba, Akure in December, 2020. In each case, triplicate samples were obtained with each of the triplicates sourced from each of the markets. A sixth food type commonly called 'Tom Bran' was included in triplicate from each of the study locations. The legumes were to complement the nutrients that are deficit in the cereals.

Processing the composite grains
The legume-cereal based complementary food ('Tom Bran') was made up of a roasted, milled composite of soybean, groundnut, yellow corn, guinea corn, and millet in ratio 2:1:1:1:1 respectively, processed and compounded as previously described.[29] Standard procedure was followed in selecting apparently healthy grains for this study. This was followed by washing in distilled water and drying for 12 h at 40^{o}C using a (OHG097 model) Gallenkamp (Loughborough, UK) drying oven. Care was taken to avoid prolonged stay in water during washing so as to forestall the grains absorbing water. The composite grains were then roasted in a (OVB305 model) Gallenkamp hot air oven at previously determined temperature and duration. Further, roasted grains were pulled together and milled into finely particulate texture similar to that of 'powder', using a Bastak (model: 1900 Smart, Ankara, Turkey) laboratory hammer mill. The fine flour was of particular size less than 300 μm. The consistency of the flour particular size was ensured by sifting through a 300μm screen. The sealed 'Tom Bran' samples were packed in portions of 25 g in black polyethylene zip bags and stored for not more than 48 hours in an airtight container at freezing temperature for multi-mycotoxin profiling.

Mycotoxin screening
Liquid chromatography tandem mass spectrometry (LC-MS/MS) was adopted, as previously recommended, to screen the samples for various mycotoxins.[17]The screening was conducted in an SGS Mycotoxin Regulatory Laboratory, Hamburg, Germany.
Procedure: The method was based on the derivatization procedure of flouroacylation aimed at increased volatility and optimized sensitivity. Flouroacylation was adopted for derivatization because it is more suitable for tandem mass spectrometric (MS/MS) detective technique as against trimethysilylation derivatization, which could be used but more suitable for electron capture detective techniques such as electrospray ionization (ESI) or atmospheric pressure chemical ionization (APCI) detection techniques as evidently observed.[11,18,,32] After the derivation process the sample was then aspirated into the chromatograph for separation, and subsequent detection and quantification by liquid chromatography and the tandem spectrometric components respectively (LC-MS/MS), of the hyphenated analytical system.

Statistical analysis
Data were expressed as mean value ± SEM. Descriptive statistics of mean, standard error of the mean and tables were used to present data. One-Way analysis of Variance was adopted for inferential purposes ($p < 0.05$) as values were compared and weighed against those of regulatory standards as benchmark for determining mycotoxin safety of complementary food.

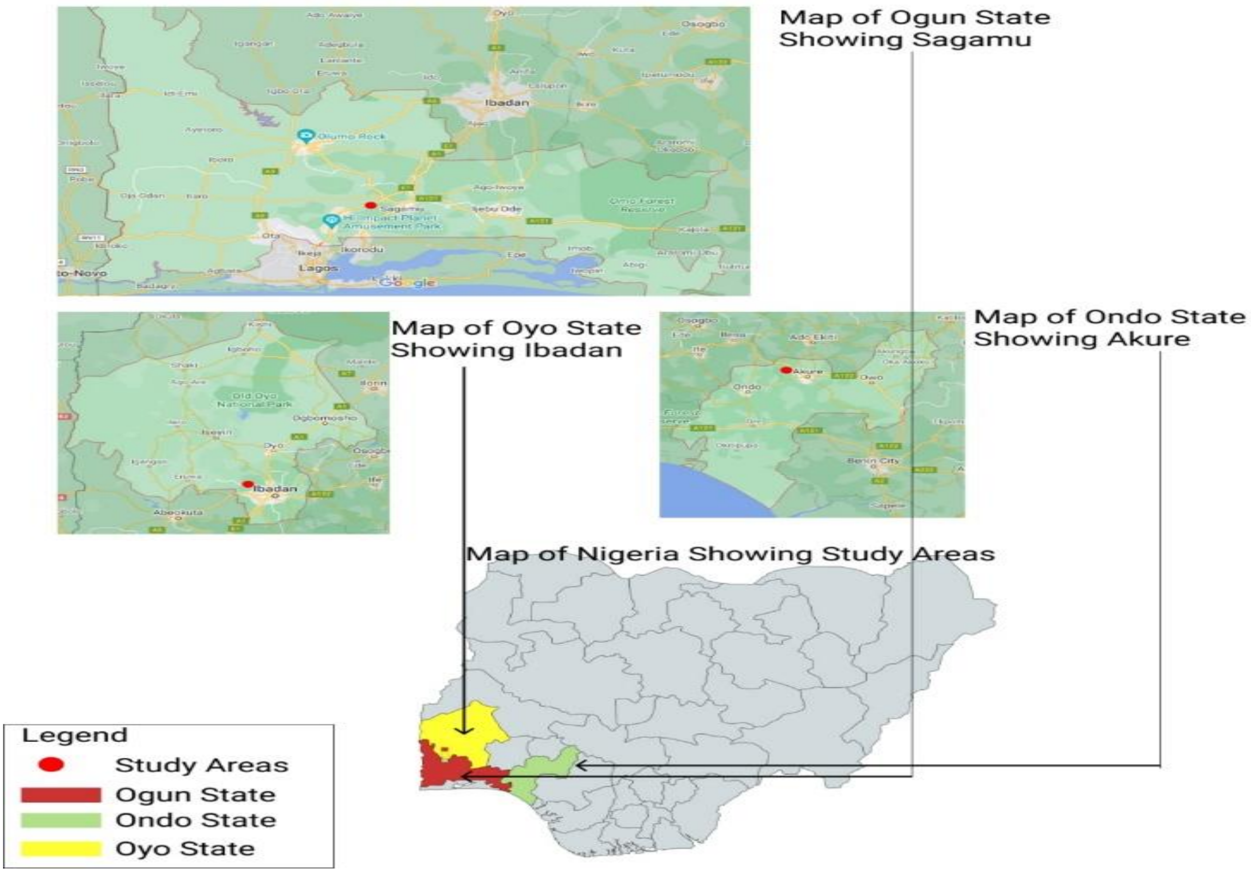

Figure 1: Sample collection sites in Ogun, Ondo and Oyo States in Nigeria

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Results and Discussion

Over the years, researchers,[25-28] corporate organizations, Mycotoxin regulatory bodies (such as Codex Alimentarius Commission and European Union), governmental agencies and world organizations have invested concerted efforts to determine safe limits of the several mycotoxins in food and feed.[26,32-35] Such regulatory limits (as shown in Table 5) constitute the baseline against which the levels of mycotoxins in the various samples were evaluated.

The respective levels of the various mycotoxins investigated in this study are shown on Tables 1-4 and compared with the maximum limit of mycotoxins in food and foodstuffs for infant population on Table 5. ZEA levels in all the samples collected from the six food types investigated were respectively below the minimum detectable limit of <10 µg/kg though; its maximum allowable limit in infant food is 20 µg/kg.[36]Similar studies observed safe levels (0.6-10.3 µg/kg) of ZEA in 30 'TomBran' samples collected from Southwestern (Lagos and Ogun States of) Nigeria previously.[18,26]

It was asserted that the manner of field collection and storage condition of certain grains accounted for reduced ZEA contamination of certain farm produce.[37-40] The Seasonal timing of collection of produce could also contribute to the trend observed in the result, since the samples were obtained during the very dry weather of December. Investigations revealed that climate and weather are also significant determinants of cereals and legumes predisposition to mycotoxin contamination.[41,42] Meanwhile, it has been observed that warm weather condition can reduce ZEA contamination of produce.[43]The foregoing suggests that pre-harvest, harvest and post-harvest treatments of millet, guinea corn, yellow corn and soybean in the various market studied were well managed to least expose such produce to ZEA contamination. Hence, the safe ZEA levels of 'Tom bran' and its composite foodstuffs that were investigated.

The amounts of aflatoxins B_1 and G_1 in groundnut samples obtained from the three locations studied were pretty above the maximum limit of 2.00 µg/kg set for infant population group, except R_2 groundnut sample where amount of aflatoxin G_1 is (1.45 µg/kg) within acceptable limit. The groundnuts from R_1 and R_3 were relatively unsafe for infant consumption. Aflatoxins B_2 and G_2 were within safe limits respectively. The levels of fumonisin B_1, B_2 and B_3 in all the groundnut samples were (<50 µg/kg each) below their respective detectable limits, meanwhile their maximum limit in cereal based infant food is 200 µg/kg each.[36]Similarly, 14-25% of groundnut in Nigerian market has been observed to contain aflatoxin above safe limit.[44]The levels of not only DON but also OTA B in all the groundnut samples studied were below minimum detectable limits of <50 µg/kg and <0.5 µg/kg respectively (Table 1) and are therefore, relatively safe for complementary feeding. Hence, DON and OTA did not constitute any mycotoxic risk in the food samples studied.[45-47] Safe levels of DON in all samples and unsafe levels of OTA in some (but not all) samples of *Tom Bran* in Nigeria have been reported.[18]

Table 1: Levels of Mycotoxins in Groundnut and Tom Bran Samples

Mycotoxin	**Groundnut**			**Tom Bran**		
	R_1	**R_2**	**R_3**	**R_1**	**R_2**	**R_3**
Afla-B_1[µg/kg]	$2.2 \pm 0.40^{*b}$	$2.3 \pm 0.20^{*b}$	$2.5 \pm 0.09^{*c}$	$< 0.2^a$	$< 0.2^a$	$< 0.2^a$
Afla-B_2[µg/kg]	0.24 ± 0.02^b	0.24 ± 0.01^b	0.23 ± 0.00^b	$< 0.2^b$	$< 0.2^b$	$< 0.2^a$
Afla-G_1[µg/kg]	2.8 ± 0.31^d	1.45 ± 0.07^b	2.13 ± 0.11^c	$< 0.2^a$	$< 0.2^a$	$< 0.2^a$
Afla-G_2[µg/kg]	0.3 ± 0.02^c	0.2 ± 0.00^a	0.25 ± 0.01^b	$< 0.2^a$	$< 0.2^a$	$< 0.2^a$
OTA B [µg/kg]	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5
Fumonisin B_1[µg/kg]	< 50	< 50	< 50	< 50	< 50	< 50
Fumonisin B_2[µg/kg]	< 50	< 50	< 50	< 50	< 50	< 50
Fumonisin B_3[µg/kg]	< 50	< 50	< 50	< 50	< 50	< 50
DON [µg/kg]	$< 50^a$	$< 50^a$	$< 50^a$	55 ± 09.3^b	107 ± 32.14^d	81 ± 10.5^c
ZEA [µg/kg]	< 10	$< 10^a$	< 10	< 10	< 10	< 10

* Denotes values above European Union regulatory standard; values with different superscript of alphabets across the row are significantly different ($p < 0.05$) R_1, R_2 and R_3 represent samples obtained from Ilisan Market, Ogun State; Bodija Market, Ibadan and Oba Market, Akure in Nigeria.

Table 2: Levels of Mycotoxins in Millet and Soybean Samples

Mycotoxin	**Millet**			**Soybean**		
	R_1	**R_2**	**R_3**	**R_1**	**R_2**	**R_3**
Afla-B_1[µg/kg]	< 0.2	< 0.2	< 0.2	< 0.2	< 0.2	< 0.2
Afla-B_2[µg/kg]	< 0.2	< 0.2	< 0.2	< 0.2	< 0.2	< 0.2
Afla-G_1[µg/kg]	< 0.2	< 0.2	< 0.2	< 0.2	< 0.2	< 0.2
Afla-G_2[µg/kg]	< 0.2	< 0.2	< 0.2	< 0.2	< 0.2	< 0.2
OTA B [µg/kg]	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5
Fumonisin B_1[µg/kg]	< 50	< 50	< 50	< 50	< 50	< 50
Fumonisin B_2[µg/kg]	< 50	< 50	< 50	< 50	< 50	< 50
Fumonisin B_3[µg/kg]	< 50	< 50	< 50	< 50	< 50	< 50
DON [µg/kg]	< 50	< 50	< 50	< 50	< 50	< 50
ZEA [µg/kg]	< 10	< 10	< 10	< 10	< 10	< 10

R_1, R_2 and R_3 represent samples obtained from Ilisan Market, Ogun State; Bodija Market, Ibadan and Oba Market, Akure in Nigeria.

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Table 3: Levels of Mycotoxins in Guinea Corn and Yellow Corn Samples

Mycotoxin	**Guinea corn**			**Yellow corn**		
	R_1	R_2	R_3	R_1	R_2	R_3
Afla-B_1[μg/kg]	< 0.2	< 0.2	< 0.2	< 0.2	< 0.2	< 0.2
Afla-B_2[μg/kg]	< 0.2	< 0.2	< 0.2	< 0.2	< 0.2	< 0.2
Afla-G_1[μg/kg]	< 0.2	< 0.2	< 0.2	< 0.2	< 0.2	< 0.2
Afla-G_2[μg/kg]	< 0.2	< 0.2	< 0.2	< 0.2	< 0.2	< 0.2
OTA B [μg/kg]	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5
Fumonisin B_1[μg/kg]	$< 50^a$	$< 50^a$	$< 50^a$	$347 \pm 60.38^{*d}$	$271 \pm 49.57^{*b}$	$309 \pm 38.98^{*c}$
Fumonisin B_2[μg/kg]	$< 50^a$	$< 50^a$	$< 50^a$	92 ± 24.11^d	73 ± 21.33^b	83 ± 18.45^c
Fumonisin B_3 [μg/kg]	< 50	< 50	< 50	< 50	< 50	< 50
DON [μg/kg]	< 50	< 50	< 50	< 50	< 50	< 50
ZEA [μg/kg]	< 10	< 10	< 10	< 10	< 10	< 10

* Denotes values above European Union regulatory standard; values with different superscript of alphabets across the row are significantly different ($p < 0.05$). R_1, R_2 and R_3 represent samples obtained from Ilisan Market, Ogun State; Bodija Market, Ibadan and Oba Market, Akure in Nigeria.

Table 4: Mean mycotoxin levels in 'Tom Bran' and its composite grains in Western Nigeria

Food/Mycotoxin	**Yellow corn**	**Millet**	**Guinea corn**	**Groundnut**	**Soybean**	**Tom Bran**
Afla-B_1 [μg/kg]	$< 0.2^a$	$< 0.2^a$	$< 0.2^a$	$2.33 \pm 0.15^{*b}$	$< 0.2^a$	$< 0.2^a$
Afla-B_2[μg/kg]	$< 0.2^a$	$< 0.2^a$	$< 0.2^a$	0.24 ± 0.01^b	$< 0.2^a$	$< 0.2^a$
Afla-G_1[μg/kg]	$< 0.2^a$	$< 0.2^a$	$< 0.2^a$	2.13 ± 0.66^b	$< 0.2^a$	$< 0.2^a$
Afla-G_2 [μg/kg]	$< 0.2^a$	$< 0.2^a$	$< 0.2^a$	0.25 ± 0.05^b	$< 0.2^a$	$< 0.2^a$
OTA B[μg/kg]	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5
Fumonisin B_1 [μg/kg]	$309 \pm 38^{*b}$	$< 50^a$	$< 50^a$	$< 50^a$	$< 50^a$	$< 50^a$
Fumonisin B_2 [μg/kg]	83 ± 9.50^b	$< 50^a$	$< 50^a$	$< 50^a$	$< 50^a$	$< 50^a$
Fumonisin B_3 [μg/kg]	< 50	< 50	< 50	< 50	< 50	< 50
DON [μg/kg]	$< 50^a$	$< 50^a$	$< 50^a$	$< 50^a$	$< 50^a$	81 ± 26^b
ZEA [μg/kg]	< 10	< 10	< 10	< 10	< 10	< 10

* Denotes values above European Union mycotoxin regulatory standard; Values with different superscript of alphabets across the row are significantly different ($p < 0.05$).

The relative observable difference in these findings may be due to the season of sample collection and storage duration of product from which samples were drawn.[41,42,48] Possibility of increased mycotoxin awareness contributing to the observed safe DON level in *Tom Bran* is minimal as mycotoxin awareness level in Nigeria is still very low.[26,42] Notwithstanding, the World Health Organization asserted that OTA (the most toxic variant of ochratoxin) affects human kidney though, evidence of its nephrotoxicity and kidney carcinogenicity only abounds in animal models but remains unclear in human.[21]

The respective amounts of aflatoxins B_1, B_2, G_1 and G_2 in 'Tom Bran' prepared from the samples obtained from all of the three locations were below the minimum detectable limit of 0.20 μg/kg each. Fumonisin B_1, B_2 and B_3 were also observed to be below the minimum detectable limit of <50 μg/kg in all 'Tom Bran' samples studied. Meanwhile, the maximum tolerable limit for each of Fumonisin B_1, B_2 and B_3 for cereal based infant food is 200μg/kg (Table 1). Similar study observed safe mean values of Fumonisin B_1, B_2 and B_3 in 30 'TomBan' samples collected from Nigeria though, a few of the sample contained unsafe levels of fumonisin B_1 and B_2.[18]

All the samples of millet, soybean and guinea corn obtained from the three locations recorded no threat of mycotoxin (Tables 2 and 3). The respective levels of the ten mycotoxins investigated were below their minimum detectable limits in these three food stuffs and by far less than the maximum tolerable limits set for them in complementary foods.[45]This finding agreed with those of Ezekiel *et al* and Akello *et al* who independently observed safe levels of mycotoxins in guinea corn and millet in Nigeria and Zimbabwe respectively.[41,42] Table 4 shows the amount of aflatoxins (B_1, B_2, G_1, and G_2), OTA B, fumonisin (B_1, B_2 and B_3) DON and ZEA in the various samples collected from the three locations in South-Western Nigeria. All the variants of mycotoxins investigated in guinea corn were below their respective detectable limits. However, 347μg/kg, 271 μg/kg and 309 μg/kg of fumonisin B_1 were observed in yellow corn collected from R_1, R_2 and R_3 locations respectively. Fumonisin B_2 levels in yellow corn were 92 μg/kg, 73 μg/kg and 83 μg/kg in samples R_1, R_2 and R_3 respectively (highest in sample R_1 and lowest in sample R_2). The level of fumonisin B_1, but not B_2, in yellow corn collected from all the study locations was above the maximum allowable limit of 200 μg/kg set for infant foods. Hence, yellow corn sampled from those three study locations were not safe to be considered as a component of infant food.Akello *et al* observed similar unsafe mycotoxin levels in 'large grain' cereals like corn and but safe level in 'small grain' cereal like millet and guinea corn and therefore, recommended less consumption of 'large grain' cereals and more consumption of 'small grain' cereals.[41] Notwithstanding, the levels of fumonisin B_3, DON, OTA B and ZEA in the samples investigated in yellow corn are below detectable limits.It is well noted that consuming safe levels of, or minimal exposure to, mycotoxins does not constitute any significant toxicological threat.[21,49,50] The observations made in this study seem to differ from results obtained from previous studies that observed exacerbated mycotoxin contamination of foods and feeds in Nigeria.[22,44]

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Table 5: Maximum Tolerable Limits of mycotoxins in the food type studied as they apply to complementary food

Mycotoxin	Maximum Tolerable Limit					
	Ground nut	**Soybean**	**Yellow corn**	**Guinea corn**	**Millet**	**Comple-mentary food**
Afla-B_1[µg/kg]	2.0[a]	2.0[a]	5.0[a]	5.0[a]	2.0[a]	0.1[a]
Afla-B_2[µg/kg]	ND	ND	ND	ND	ND	ND
Afla-G_1[µg/kg]	ND	ND	ND	ND	ND	ND
Afla-G_2[µg/kg]	ND	ND	ND	ND	ND	ND
OTA B [µg/kg]	3.0[a]	3.0[a]	3.0[a]	3.0[a]	3.0[a]	0.5[a]
Fumonisin B_1[µg/kg]	200[b]	200[b]	200[b]	200[b]	200[b]	200[b]
Fumonisin B_2[µg/kg]	200[b]	200[b]	200[b]	200[b]	200[b]	200[b]
Fumonisin B_3 [µg/kg]	200[b]	200[b]	200[b]	200[b]	200[b]	200[b]
DON [µg/kg]	750[a]	750[a]	750[a]	750[a]	750[a]	200[a]
ZEA [µg/kg]	75[a]	75[a]	75[a]	75[a]	75[a]	20[a]

Note: Complementary food on this table are limited to those made from grains.
ND: No data, implying no maximum tolerable limit yet made public on such parameter.[51,52]
[a]: Data retrieved from.[33]
[b]: Data retrieved from.[23]

The reason for such disparity is strongly predicated upon timing of sample collection as the samples were collected during the dry season. Therefore, there is a strong need to evaluate the extent to which seasonal variation impacts mycotoxin co-contamination of the weaning food and its composite grains reported herein.

Conclusion

Four aflatoxin variants (B_1, B_2, G_1, and G_2), three variants of fumonisin (B_1, B_2, and B_3), ochratoxin B (OTA B), deoxynivalenol (DON) and zearalenone (Zea) were detected in groundnut and in 'Tom bran'. However, the levels of these mycotoxins in 'Tom bran' are within regulatory limits and are therefore, the'Tom bran' samples investigated are considered safe for human consumption.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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