

#### Research Article

# Mycotoxin Profile of Honey and Dry-Cured Meat (Kilishi) for Export in Abuja, Nigeria

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Abstract: Animal products are not immune to contaminants and could render them unsafe for local consumption or unacceptable for export. This study aimed to ascertain the prevalence and profile of Aflatoxins (AFs), Ochratoxins (OTs) and Fumonisins (FBs) contamination in honey and dry-cured meat (kilishi) intended for export in the Federal Capital Territory (FCT) of Nigeria. Ninety (90) samples of each animal product were collected and analyzed. Mycotoxins were determined using High-Performance Liquid Chromatography (HPLC) quantitative techniques. According to the investigation, none of the honey samples for export from the FCT Abuja was positive for Aflatoxin B<sub>1</sub> (AFB<sub>1</sub>) and Aflatoxin B<sub>2</sub> (AFB<sub>2</sub>) contaminants. The occurrence of Ochratoxin A (OTA) was most prevalent in the samples of honey from Abuja East, but with the greatest average concentration (62.87 μg/Kg) in the samples from Abuja South. In samples from the Abuja South zone, the prevalence of AFB<sub>1</sub> and AFB<sub>2</sub> in the kilishi was relatively high (73.33% and 66.67%, respectively). Also, OTA and Ochratoxin B (OTB) were detected at varying levels in the cured meat. Dry-cured meat sold in Abuja Nigeria to consumers and for export is deemed unsafe due to Aflatoxin B<sub>1</sub> and B<sub>2</sub>, OTA and OTB contamination levels which were above the EU maximum limit. The regulatory bodies in the country should always carry out routine monitoring to guarantee that marketed products from animals are consumable and also exportable.

Keywords: animal products, aflatoxin, fumonisin, ochratoxin, HPLC, food safety

## 1. Introduction

Animal products' grade and safety are very crucial since it affects the exporters and local consumers [1, 2]. Honey (zuma), dry-cured meat (kilishi), snail meat, processed milk, and animal skins are some of the most represented animal products for export in Northern Nigeria [3]. The safety of these commodities can be compromised by various contaminants, entering any point of the food chain, including production, storage, processing, and transport. Honey is a viscous, sweet semi-liquid substance made by honeybees from plant nectar. It varies tremendously in quality depending

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on different locations [4]. Most times kinds of honey contain contaminants such as agrochemicals, charcoal, plant part, or adulteration with sugar molasses or starch [5, 6].

Kilishi is often prepared from red meat such as beef, mutton, or goat meat that is dried (3-5 mm thickness), salted, and spices added. It is a traditionally processed, sun-dry, roasted ready-to-eat meat product. It is a version of jerky that is a dry-cured form of meat made from deboned cow, sheep, or goat meat. Kilishi is popular, especially in Northern Nigeria, Cameroun, Chad, Niger Republic, and other countries in the Sahelian region of Africa [7]. It also has been an important export commodity to most Eastern world countries such as Saudi Arabia and UAE, where it is also widely consumed [8]. Kilishi which is mostly from beef is considered a highly desirable and favorable snack in Nigeria probably because of its palatability. It is often used as house snack refreshment, delicacy and shared during special celebration or ceremonies but without bothering of the safety status. However, the snack meat is prone to microbial and chemical contaminants due to exposure to microbial load, poor processing and packaging, poor handling and storage conditions and protracted length storage before being sold [9].

Mycotoxins are toxic secondary metabolites produced by various toxigenic species of fungi in a field and during storage, the most important of them being *Aspergillus*, *Fusarium*, *Alternaria*, and *Penicillium* [10]. Mycotoxin may be biosynthesized in feed and foodstuff. The most relevant mycotoxins for animal production worldwide are Aflatoxins B<sub>1</sub> (AFB<sub>1</sub>), B<sub>2</sub> (AFB<sub>2</sub>), G<sub>1</sub> (AFG<sub>1</sub>), and G<sub>2</sub> (AFG<sub>2</sub>), Deoxynivalenol (DON), Fumonisin B<sub>1</sub> (FB<sub>1</sub>) and Fumonisin B<sub>2</sub> (FB<sub>2</sub>), zearalenone (ZEN), T-2 and HT-2 toxins, and Ochratoxin A (OTA) [11, 12]. Virtually most mycotoxins can cause one or more major health problems and expose consumers to health threats [13]. Diseases caused by the consumption of mycotoxins are known as mycotoxicosis, which does not necessarily require the presence of fungi after the mycotoxin production [14]. According to their hazardous activity under long-term exposure, mycotoxins are classified as either mutagenic, carcinogenic, or teratogenic [15, 16]. Some of these mycotoxins can suppress the immune system, decrease reproductive capacity and can cause allergies in consumers [16, 17]. The amount of mycotoxin within a human body depends on its concentration in specific animal products, frequency of consumption, amount consumed, and the rate of detoxification of such contaminants in the human body [18].

The principal mycotoxins are regulated by the European Union (EU) via Regulation (EC) [19, 20] setting the highest permitted levels in µg/kg in certain foodstuffs including animal products and feedstuff. When evaluating the dangers of consuming animal products, mycotoxins among other contaminants need to be regularly monitored from the farmhouse to end-use product and their interaction must be taken into account [21, 22]. Given the potential harm caused by food pollutants, the use of confirmatory analytical techniques, such as HPLC for mycotoxin detection in food safety control, is crucial for accurate identification and quantification [23-25].

The contamination of food including honey and dry-cured meat with mycotoxins such as AFB<sub>1</sub>, OTA and FBs do not only affects the health of consumers but also exerts an impact on global trade [26]. Poor implementation of regulatory actions against the occurrence and sale of non-quality food commodities, poor monitoring, and inadequate supervision of animal products for consumption, sale, or export can pose some problems. Due to the dearth of information on the prevalence and occurrence of harmful chemicals in animal products for export in Nigeria. This study sought to ascertain the frequency and incidence of mycotoxins contaminants in honey and dry-cured beef (kilishi) intended for export in the FCT, Abuja, Nigeria, and also the suitability of these items for international trade.

#### 2. Materials and methods

## 2.1 The study location

The Federal Capital Territory (FCT), was the area of the study. The FCT is situated in the geographical heartland of Nigeria and has an area extent of 8,000 square kilometers. The FCT lies between Lat. 8.25° N and 9.21° N within the Equator and Long. 6.45° E and 7.39° E within the Prime Meridian. It has a total area of (713 km² i.e., 71,300 ha) with an estimated population of 1.8 million. The territory's borders are Kaduna State to the North, Kogi State to the South, Nasarawa State to the East, and Niger State to the West (Figure 1). The FCT was created to replace Lagos on 3<sup>rd</sup> February 1976, which was considered to be no longer suitable to serve as the national capital. Abuja officially became Nigeria's capital on 12<sup>th</sup> December 1991. FCT is one of Nigerian leading urbanized centers. Due to its centrality, the FCT is well-connected and accessible from the States and Federal highways. Abuja has savannah vegetation, giving

it rich soil for agriculture and a favorable climate that is pleasant year-round and is neither overly hot nor under-cold. Abuja is divided into six area councils; Kuje, Abaji, Bwari, Gwagwalada, Kwali, and Municipal Area Council (AMAC). The quality of animal products sold or intended for export in the six Area Councils of the Territory is the focus of this study.

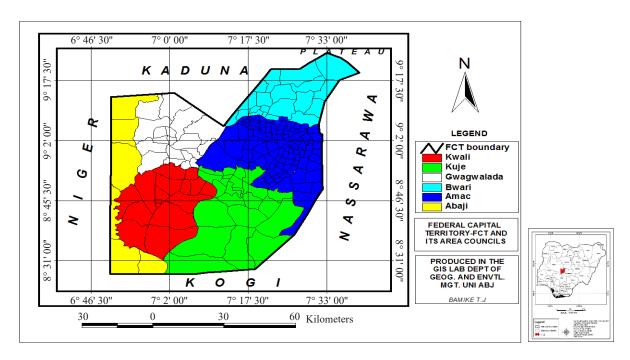


Figure 1. FCT Map displaying the six area councils

#### 2.2 Sampling method

The gathering of the processed honey and dry-cured beef intended for export in the FCT Abuja served as the study's population. The sampling sites represent all the honey products and dry-cured meat available in the market for export thus sampling was focused on locations with a higher number of units where animal products were processed for export. The sample gathering sites were from the suppliers or exporters who sourced their products from different honey and dry-cured meat producers and/or processors in each of the three zones of the FCT, Abuja respectively. Their locations include markets, supermarkets, and exporting warehouses. The 90 collected bottled honey samples were kept in clean polystyrene bags (80 µm thickness) with labels and laboratory seals.

A total of 90 processed dry-cured meat samples; n = 30 for each zone were collected at random from authorized retail markets, supermarkets, and marts in 3 different zones in each of the FCT, Abuja, Nigeria (Table 1). The collected samples were labeled, placed into sterile polystyrene bags, and rapidly transported under complete aseptic conditions in zip lock bags to Central Research and Diagnostic Laboratory, Ilorin Kwara State Nigeria for the mycotoxin analysis and determination. The pictures of some of the bottled honey and packaged dry-cured meat sold or ready for export in Abuja are shown in Figure 2 and 3.

#### 2.3 Mycotoxins determination

#### 2.3.1 Chemical and reagents

Chemicals, Reagents, and Standards Certified standards of mycotoxins were produced by Romer Labs Biopure (Romer Labs, Tulln, Austria). All the grade solutions were stored following the manufacturer's recommendations and tempered to ambient temperature before use.



Figure 2. Bottled honey for sale and export in Kano and Abuja Nigeria



Figure 3. Wrapped/packaged dry-cured meat for sale and export in Kano and Abuja Nigeria

Table 1. Collection of study samples

Location	Zone	Area councils	Honey/Dry-cured meat samples
FCT, Abuja	Abuja East	AMAC and Bwari	30
	Abuja Central	Gwagwalada and Kwali	30
	Abuja South	Kuje and Abaji	30
Total			90 each

#### 2.3.2 Sample preparation

Before analysis, dry-cured meat samples were first broken up using a pestle and mortar followed by of the use Romer RAS mill (Romer Labs, Austria). For each specimen, 25 g were minced aseptically in the grinder through a 4 mm sterilized plate diameter (AC110V, China). The pulverized particles were then kept in a freezer at -18 °C pending analysis. To absorb the evaporating moisture, 10 g of the blended sample was combined with 60 g of anhydrous sodium sulfate in an agate mortar. Thereafter, the homogenate was placed in a 500 mL beaker, and 300 mL of n-hexane was employed in the extraction lasting for 24 hours at ambient temperature, following the cold extraction method adapted from Zaeshahrabadi et al. [27]. The extract was evaporated to dryness at 40 °C through the use of a rotary vacuum evaporator and later clean up using the imunoaffinity affinity column for the AFs and OTs; and C18 column for FBs.

#### 2.3.3 Analytical method

The procedures of analysis to determine mycotoxin levels included extraction of mycotoxins from the samples, the obtained extract is further purified to remove unwanted co-extracted matrix components, and finally, an optional sample concentration step takes place, before the last stages of separation and detection.

#### 2.3.3.1 Extraction

Extraction and HPLC-quantitative measurements of aflatoxin B<sub>1</sub>, B<sub>2</sub>, OTA, OTB, OTC, and FB<sub>1</sub> contaminants in honey and dry-cured meat were carried out. The extraction's reagents and chemicals and HPLC-quantitative measurement of the mycotoxins in the commodities were acquired from Sigma (Sigma, Germany). Phosphate-Buffered Saline (PBS) was prepared by dissolving 8 g of NaCl, 0.2 g of KCl, 0.2 g of KH<sub>2</sub>PO<sub>4</sub>, and 1.2 g of Na<sub>2</sub>HPO<sub>4</sub> in 1,000 mL of water. The pH for PBS was adjusted to 7.0 with 0.1 M HCl. Ten grams of each examined specimen were homogenized with 40 mL of acetonitrile: water (60:40, v/v) and 0.2 g NaCl for 90 s, then blended by a magnetic stirrer for 10 minutes. The mixture underwent filtration using a Whatman No.1 fast filter paper (Whatman Inc., Clifton, NJ, USA). About 4 Ml of the filtrate were mixed with 44 mL of 2% tween-20-PBS solution in a 50 mL Erlenmeyer flask. Then, the filtrate was cleaned up using the liquid/liquid extraction method. It was by diluting 0.5 mL aliquot of the filtrate with 0.5 mL acetonitrile, then 0.5 ml of the mix was spilled into an Alltech 1.5 mL Extract-Clean reservoir packed with 200 mg basic aluminum oxide 9 mm high-layer adsorbent.

#### 2.3.3.2 HPLC techniques

The quantitative detection of the AFs, OTA and FBs was performed by the HPLC system (Thermo Fisher Scientific, Waltham, MA 02451, USA) using 100 microlitres of the extract as before stated by Herzallah (29). Each of the mycotoxins quantities of standards and from samples was determined using HPLC with fluorescent detection. The HPLC system consisted of a pump (Knaur, Germany) and a fluorescence detector (Knauer, Germany). The prepared sample was injected automatically using an injection volume of 20 µl. Aflatoxins were separated in HPLC column with isocratic mobile phase of water: methanol: acetonitrile (60:30:10, v/v/v). The fluorescence sensor was set at an excitation wavelength of 365 nm and an emission wavelength of 435 nm to determine AF. Aflatoxin retention times with

a 1.2 ml/min flow rate were 8-9 min for AFG<sub>2</sub>, 10.5-11.5 min for AFG<sub>1</sub>, 13-14 min for AFB<sub>2</sub> and 16-17 min for AFB<sub>2</sub>. The total run time in the HPLC was 33 min.

For OTA load determination, acetonitrile:water and acetic acid in a ratio 50:48:2 respectively were used as mobile phase at a flow rate 1 ml/min. The OTs excitation and emission wavelengths were 435 nm and 495 nm respectively. The injection volume was  $60 \mu l$ . The retention time for OTA was 1.11 minutes while the detection limit of the machine with regards to the toxin was  $0.01 \mu g$ . Fumonisins (FBs) were extracted twice with acetonitrile:methanol:water (25:25:50) and the combined extracts were diluted with Phosphate Buffered Saline (PBS) and applied to a FBs Test immunoaffinity column. After washing PBS, FUMs were eluted from the column with methanol and reacted with  $\alpha$ -opthaldehyde to form fluorescent derivatives. FBs had an excitation wavelength of 335 nm and an emission wavelength of 385 nm.

To confirm and ensure the truthfulness of the test, approximately 25 g of aflatoxins-free sample (for each sample type) was spiked (enriched) with aflatoxin  $B_1$  at levels of 3, 5, and 10  $\mu$ g/kg. The protocol was performed in three replicates. The spiked specimens were assessed employing the HPLC, followed by the estimation of both the recovery and standard deviation [25]. The % recovery is the level of analyte found divided by the level of standard analyte added to the sample. To confirm and ensure the precision of the quantitative measurement of OTs and FBs, a five-point calibration curve was generated using the following concentrations: 0.5, 2, 5, 10, and 30  $\mu$ g/kg. Also, the signal-to-noise approach was used to detect the Limits of Quantification ( $\mu$ g/g; LOQ) and the Limits of Detection (LOD;  $\eta$ g/g). Also, to ensure the precision of the test, about 25 g OTA-free sample (for each sample type) was spiked with OTA in A at the levels of 1, 5, and 20  $\mu$ g/kg.

The assay was performed in three replicates. The raw concentration level (incidence) of mycotoxins in the honey samples analyzed was obtained and reported in  $\mu g/100g$ , but now multiplied by 10 to convert the 100 g to Kg.

The LOD and LOQ were obtained using the formula:

$$LOD = 3 \times SD + B_{ave} \tag{1}$$

$$LOQ = 10 \times SD + B_{ave} \tag{2}$$

SD = standard deviation of the measurement

 $B_{ave}$  = average concentration of spike samples

#### 2.4 Statistical data analysis

Mycotoxins concentrations were analyzed in triplicates from each location and recorded as means  $\pm$  Standard Errors (SE). Shapiro-Wilk test (i.e.  $x = \mu + Az$ ) was employed to determine the normality test by involving the descriptive statistics of SPSS (version 21.0, SPSS Inc., Chicago, IL, USA). Those data whose significant value of the test were greater than 0.05 were considered as normal and further subjected to ANOVA. Normally distributed data were subjected to a one-way Analysis of Variance (ANOVA) (using SPSS for Windows) Duncan's test was used to establish the differences in mycotoxins load across the different locations. The value of the probability level was set at  $p \le 0.01$  and 0.001 to indicate statistically significant differences in the honey respectively. Some comparisons were made using a graphical representation.

#### 3. Results

The data gathered from the examination of honey and kilishi samples obtained from each of Abuja's three zones are presented and interpreted in this section. The mean recovery rate for the AFs, OT and FB ranged from 90 to 96% with Relative Standard deviation of 0.81, 0.88 and 1.1 respectively. The LOD and LOQ values are indicated in Table 2.

Table 2. Mean recovery rate of AFs, OTs and FB from the spiked samples and LOQ and LOD by the HPLC

Analyte	Mean % recovery	±SD	LOD (mean)	LOQ (mean)	
AFB <sub>1</sub>	95	0.543	1 ng/g	0.1 μg/g	
$AFB_2$	94	0.241			
$AFG_1$	96	1.65			
$AFG_2$	95	0.89			
OTA	90	0.97	0.5 ng/g	8 μg/g	
OTB	92	1.07			
OTC	91	0.83			
$FB_1$	93	0.99	0.2 ng/g	4 μg/g	
$FB_2$	92	1.23			

#### 3.1 Prevalence and incidence of ochratoxins contaminants in honey for export in Abuja

All the samples of processed honey for export from the FCT Abuja were negative for AFB<sub>1</sub> and AFB<sub>2</sub> contaminants. The occurrence of OTA was most prevalent in the honey from Abuja East. Nonetheless, samples from Abuja South had the greatest concentration level (26.92  $\mu$ g/Kg) (Table 3). The average OTB incidence in Abuja South honey was significantly more than that in other zones. The occurrence of OTC in the honey was most prevalent in the samples from Abuja East. However, the samples from Abuja South had the greatest mean concentration (27.09 g/L).

# 3.2 Prevalence and incidence of aflatoxins contaminants in kilishi for export in Abuja

The prevalence of AFB<sub>1</sub> and AFB<sub>2</sub> in the kilishi (73.33% and 66.67%, respectively) was relatively high in samples from the Abuja South zone (Table 4). The average concentration of AFB<sub>2</sub> in the kilishi was highest in samples from Abuja Central (79.73 g/kg). The occurrence of AFG<sub>1</sub> in the kilishi was most prevalent in the samples from Abuja East and Central (40.00%, respectively).

Honey samples for export in the FCT had higher OTC than other types of Ochratoxins but it was only in about 45.56 percent of the samples (Figure 4). The prevalence of AFB<sub>1</sub> and AFB<sub>2</sub> in the kilishi samples from the FCT Abuja was relatively high (Figure 5). As high as 62.22% of the kilishi in Abuja contained varying levels of Aflatoxin B<sub>2</sub> contaminants.

#### 3.3 Detection of ochratoxins contaminants in kilishi for export in Abuja

The occurrence of OTA in all the kilishi samples was relatively low in the study area (Table 5). The mean concentration of OTA in the samples from Abuja South was greater than those from other zones. While mean concentrations of OTB were as high as 188.43 µg/kg, in Abuja Central samples. Ochratoxin C (OTC) was detected in most of the honey samples, but it was undetectable in the kilishi from all three FCT zones.

The OTA was found to be more prevalent in kilishi from the FCT Abuja but Ochratoxin B was less prevalent in the product from the same FCT Abuja (Figure 6). The percentage prevalence was relatively low, the highest being 22.22% in kilishi marketed in the FCT Abuja. AFG<sub>2</sub> and FB<sub>1</sub> were more prevalent in kilishi for export in the FCT Abuja (Figure 7). FB<sub>1</sub> had the highest % prevalence of 37.78% in the kilishi samples from the FCT, Abuja.

Table 3. HPLC-based detection of ochratoxins contaminants in honey for export in Abuja

				Ochra	ıtoxin A, Ocl	nratoxin B, and	d Ochratoxin C	Ochratoxin A, Ochratoxin B, and Ochratoxin C in honey samples	SS			
Location		OT,	OTA (μg/kg)			ОТІ	OTB (μg/kg)			OTC	OTC (μg/kg)	
N = 10  samples/location	No. of + ve samples	% prevalence	Range	Mean ± SE	$\begin{array}{c} No. \\ of + ve \\ samples \end{array}$	$\begin{array}{ll} \text{No.} & \text{00} \\ \text{of + ve} & \text{00} \\ \text{samples} & \text{prevalence} \end{array}$	Range	$ \begin{array}{ll} No. \\ Mean \pm SE & of + ve \\ samples \end{array} $	$\begin{array}{c} \text{No.} \\ \text{of + ve} \\ \text{samples} \end{array}$	% prevalence	Range	$\begin{array}{ccc} No, & 06 \\ of + ve \\ samples & prevalence \end{array}  Range  Mean \pm SE$
Abuja East	8	53.33	5.9-65.77	$5.9-65.77$ $61.88 \pm 0.7^{a**}$	4	26.67	$10.03-40.09$ $33.16 \pm 1.1^{b}$	$33.16\pm1.1^{\text{b}}$	8	53.33	15.1-60.44	53.33 $15.1-60.44   26.92 \pm 0.9^{a}$
Abuja Central	သ	20.0	2.52-69.55	$58.54 \pm 2.1^{\rm a}$	5	33.33	11.06-45.07	$38.56\pm0.9^{\text{b}}$	7	46.67	2.5-60.7	$2.5-60.7$ $24.55 \pm 0.06^{a}$
Abuja South	2	13.33	8.9-69.11	$62.87\pm1.9^{\mathrm{a}}$	5	33.33	8.2-50.11	$49.67\pm1.0^{\rm a}$	4	36.67	2.44-46.7	$2.44-46.7$ $27.09 \pm 1.3^{a}$
*+ ve = sample in which a particular mycotoxin is detected: ** There is no significant variation between the mean in a column followed by identi letters (at n < at 0.01 level of probability)	articular myc	otoxin is deter	cted· ** There	is no significant v	zariation betw	ween the mean	in a column fo	llowed by identi	letters (at n	< at 0.01 leve	al of probabil	itv)

sample in which a particular mycotoxin is detected; There is no significant variation between the mean in a column followed by identi letters (at  $p \le at 0.01$  level of probability)

Table 4. HPLC-based detection of aflatoxins contaminants in Kilishi for export in Abuja

				Afla	toxin B <sub>1</sub> , Aff	latoxin B <sub>2</sub> and /	Aflatoxin G <sub>1</sub> ii	Aflatoxin $B_1$ , Aflatoxin $B_2$ and Aflatoxin $G_1$ in Kilishi samples				
Location		AFB	AFB <sub>1</sub> (μg/kg)			${ m AFB}_2$	$AFB_2$ (µg/kg)			$AFG_1$	$AFG_1(\mu g/kg)$	
N = 13 samples/location)	$_{ m of+ve*}^{ m No.}$ samples	% prevalence	Range	Mean ± SE	$\begin{array}{c} No. \\ of + ve \\ samples \end{array}$	% prevalence	Range	Mean ± SE	$\begin{array}{c} No. \\ of + ve \\ samples \end{array}$	No. $%$ $No.$ $%$ $No.$ $No.$ $%$ $No.$	Range	Mean ± SE
Abuja East	5	33.33	3.2-45.6	$29.99 \pm 1.2^{b*}$	9	60.0	5.1-69.1	$70.54\pm0.6^{\rm a}$	6	40.0	5.1-53.6	40.0 5.1-53.6 $13.76 \pm 1.02^{b}$
Abuja Central	10	66.67	5.5-70.1	$45.56\pm0.8^{\mathrm{a}}$	10	60.0	6.3-71.2	$79.73\pm0.7^{\mathrm{a}}$	5	33.33	2.9-40.1	$2.9-40.1$ $10.87 \pm 1.1^{b}$
Abuja South	=	73.33	60.8-79.3	$30.51 \pm 1.0^{b}$	9	66.67	1.9-42.1	$64.55 \pm 1.3^{\text{b}}$	6	40.0	1.5-49.2	$1.5-49.2$ $12.39 \pm 1.2^{b}$

<sup>\*</sup>There is no significant variation between the mean in a column followed by identical letters (at  $p \le at 0.01$  level of probability). \* +ve = sample in which a particular mycotoxin is detected; \*\* There is no significant variation between the mean in a column followed by identical letters (at  $p \le at 0.01$  level of probability)

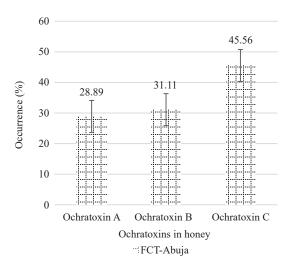


Figure 4. Prevalence of OTA, OTB, and OTC in honey for export in the FCT, Nigeria

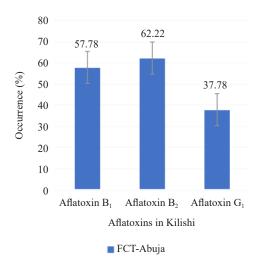


Figure 5. Presence of AFB<sub>1</sub>, B<sub>2</sub>, and G<sub>1</sub> in kilishi for export in the FCT, Nigeria

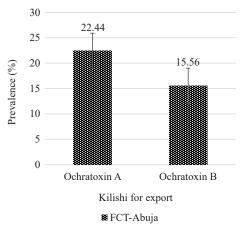


Figure 6. Prevalence of OTA and OTB in kilishi for export in FCT Abuja

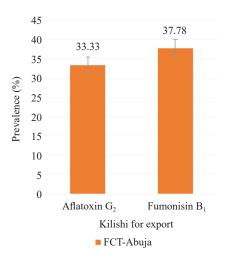


Figure 7. Prevalence of AFG<sub>2</sub> and FB<sub>1</sub> in kilishi for export in FCT Abuja

Table 5. HPLC-based detection of ochratoxins contaminants in kilishi for export in Abuja

		,	Ochratoxin .	A, Ochratoxin B, a	and Ochrator	xin C in kilishi	samples (μg	/kg)	
Location $N = 15$		(	OTA			C	TB		OTC
(samples/location)	No. of + ve samples	% prevalence	Range	Mean ± SE	No. of + ve samples	% prevalence	Range	Mean ± SE	No. of + ve samples
Abuja East	4	26.67	0.5-2.6	1.1 ± 0.02°*	1	6.67	0.1-190.4	$160.45 \pm 2.1^a$	0
Abuja Central	2	13.33	1.3-70.3	$59.91 \pm 1.06^{\text{b}}$	5	33.33	2.3-193.6	$188.43 \pm 2.2^{a}$	0
Abuja South	5	33.33	3.4-314.2	$299.34 \pm 3.11^{a}$	1	6.67	0.4-188.8	$170.91 \pm 1.89^{a}$	0

<sup>\* +</sup> ve = sample in which a particular mycotoxin is detected; \*\* There is no significant variation between the mean in a column followed by identical letters (at  $p \le at 0.01$  level of probability)

# 3.4 Detection of Aflatoxin $G_2$ and Fumonisin $B_1$ Contaminants in kilishi for export in Abuja

The highest prevalence of  $AFG_2$  and  $FB_1$  was noticed in samples from Abuja South and Abuja Central respectively (Table 6). The highest detected level of  $AFG_2$  mean concentration in the kilishi (66.6  $\mu$ g/kg) was noticed in the samples from Abuja central. The greatest mean concentration of  $FB_1$  was from Abuja south samples but did not vary significantly from the mean incidence from other zones.

# 3.5 Safety assessment of mycotoxin contaminants in honey and dry-cured meat for export FCT, Abuja

Every sample of honey from the FCT has no AFB<sub>1</sub> and B<sub>2</sub> contaminants (Table 7), however, the incidence of OTA and ATB contaminants was above the EU Maximum Permitted Limit (MPL). All the dry-cured meat samples from the FCT Abuja have Aflatoxin B<sub>2</sub> (AFB<sub>2</sub>) contaminants above the EU permissible limit (MPL). It should be noted that the maximum permissible limit level according to Regulation (EU) No 1881/2006 setting maximum levels for certain contaminants in foodstuff as regards mycotoxins were 2  $\mu$ g/kg MPL for AFB<sub>1</sub> and 4  $\mu$ g/kg for B<sub>2</sub>, G<sub>1</sub> and G<sub>2</sub>. Sixty per cent (60%) and 66% of the dry-cured meat had OTA and OTB contaminants respectively above the MPL by the EU.

Table 6. HPLC-based detection of Aflatoxin G2 and Fumonisin B1 contaminants in kilishi for export in Abuja

			AFG	<sub>2</sub> and FB <sub>1</sub> in kilis	hi samples	(μg/kg)		
Location $N = 15$		AFC	$\vec{\mathbf{j}}_2$				$FB_1$	
(samples/location)	No. of + ve samples	% prevalence in samples	Range	Mean ± SE	No. of + ve samples	% prevalence in samples	Range	Mean ± SE
Abuja East	3	20.0	2.2-40.2	$30.44 \pm 1.01^{a}$	6	40.0	10.2-80.2	$69.76 \pm 1.22^{a}$
Abuja Central	5	33.33	3.2-66.6	$31.57\pm0.9^{\text{a}}$	8	53.33	8.2-88.2	$73.12 \pm 1.09^{a}$
Abuja South	7	46.67	4.3-46.7	$26.84 \pm 0.81^{\text{a}}$	3	20.0	2.1-40.1	$74.75 \pm 2.01^{a}$

<sup>\* +</sup> ve = sample in which a particular mycotoxin is detected; \*\*Mean followed by the same letters within a column are not significantly different at 0.01 level of probability

Table 7. Safety assessment of mycotoxin in honey and dry-cured meat for export in FCT, Abuja

Mycotoxin contaminant	Animal product	Lower Mean Conc. Limit (µg/Kg)	Upper Mean Conc. Limit/Location	Max. Permissible Limit (MPL) by EU (Ready to eat) (μg/Kg)	Remark
OTA	Honey	40.45	62.87 (Abuja South)	10	Above MPL in all samples, Unsafe
OTB	Honey	33.33	69.55 (Abuja Central)	20	Above MPL in all samples, Unsafe
OTC	Honey	7.89	27.09 (Abuja South)	30	Below MPL in all samples, Safe
$AFB_1$	Dry-cured meat	3.74	79.3 (Abuja South)	2.0	Above MPL in 55% of the samples
$AFB_2$	Dry-cured meat	38.74	79.73 (Abuja Central)	4	Above MPL in all samples, Unsafe
$AFG_1$	Dry-cured meat	0.726	13.76 (Abuja East)	4	Above MPL in most the samples and relatively high in samples from Abuja East
OTA	Dry-cured meat	0.2	33.33 (Abuja South)	10	Above MPL in 60% of the samples
OTB	Dry-cured meat	6.67	188.43 (Abuja Central)	20	Above MPL in 66% of the samples esp. in Abuja Central
$AFG_2$	Dry-cured meat	1.56	66.6 (Abuja Central)	4	Below MPL in most samples, unsafe
$FB_1$	Dry-cured meat	60.64	88.2 (Abuja Central)	200	Below MPL in all samples, safe

# 4. Discussion

The prevalence of mycotoxins in dry-cured meat might result from mould growth, feeding animals with contaminated feed, or poor processing practices [28, 29]. Also, this might be a result of too long storage of the meat that is often brought into Abuja from far distant Northern Western parts of the country, especially from Kano State. Some of the mycotoxins with the highest impact on human health and economic status include AFs, OTs, FBs, trichothecenes (TCs), zearalenone (ZEA), and patulin (PAT) [30, 31]. The possible reasons for the occurrence of Aflatoxin B<sub>1</sub> and B<sub>2</sub>, OTA and OTB in the cured meat, is likely to be due to mycotoxigenic fungi contamination. Generally, AFB<sub>1</sub> and AFB<sub>2</sub> are produced by *Aspergillus flavus*, *A. parasticus*, *A. nomius*. Aflatoxin G<sub>1</sub>, G<sub>2</sub> AFG<sub>1</sub> AFG<sub>2</sub> are produced by *Aspergillus* 

parasiticus and A. nomius. While OTA are produced by A. ochraceus, A. westerdijkiae, A. carbonarius, Penicillium viridicatum, P. cyclopium, P. verrucosum and P. nordicum [32]. The FBs are produced by Fusarium verticilliodes and F. proliferatum and recently by A. nigri (black Aspergillus). The co-occurrence of these fungi in the animal products always differs in the products and could result in significant variation in the mycotoxin prevalence and profile across the location of the collected animal products.

This study showed that AFB<sub>1</sub> and AFB<sub>2</sub> were below detectable levels in all the honey samples analyzed. Ochratoxins (OTs) were however detected in some of the tested honey samples. Original honey is popularly known to have an antifungal property and often has long shelf life thus antimycotoxic [33]. Those honey samples with OTs load might be due to adulteration of the product. Kilishi in Abuja is commonly produced in Kano which is over 420 km to Abuja and oven stored for days/months in an unhygienic condition by the vendors, coupled with poor personal hygiene.

An *in vitro* investigation revealed that AFB<sub>1</sub>, AFB<sub>2</sub>, AFG<sub>1</sub>, and AFG<sub>2</sub> production was increased in the medium containing 32.0% honey concentration but decreased in a medium of high honey concentration without any production of aflatoxin B<sub>2</sub> and G<sub>2</sub> [29]. It was also revealed from the study that honey in Nigeria. It was revealed that honey at varying levels of concentrations was inhibitory against *A. flavus* and *Microsporum gypseum*, while *C. albicans* were the least sensitive. A recent *in vitro* study revealed that the biomass of *A. parasiticus* was enhanced in the medium containing 32% honey concentration. However, the biomass of *A. ochraceus* was decreased in medium containing 32 and 48% honey but Ochratoxin A was not produced at either honey concentration [34].

In another study involving the dilutions of honey ranging from 12-20% were tested *in vitro* against *A. flavus* by paper disc technique and its effects on aflatoxin  $B_1$ , and  $B_2$  residues at various periods by the AOAC for extraction, TLC chromatography, and HPLC chromatography. Results indicated that the dilutions of honey showed various antimicrobial effects (highly and moderately sensitive) on *A. flavus*. Aflatoxin  $B_1$  and  $B_2$  residues in the sample treated with 18% honey were greatly reduced compared to those of the control samples. Also, the aflatoxin  $B_1$  and  $B_2$  profile in the samples treated with 18%  $H_2O_2$  was highly decreased than those of the control sample [35]. The amount of mycotoxin in the human body depends on its concentration in specific animal products, frequency of consumption, amount consumed, and the rate of detoxication of such contaminant in the human body.

Production of mycotoxin in the animal product may occur within different parts of the food chain, including pre-slaughtering, during processing and storage [36]. Poor management practices especially feeding the animals with mouldy feeds, poor processing methods and inadequate curing of the meat, poor packaging, unhygienic transport conditions and storage, can increase fungal growth and the risk of mycotoxin production. The metabolism of mycotoxins in the livestock body can lead to their accumulation in various organs or tissues of the animal and enter the food chain through meat consumption [37].

Certainly, various factors interfere with fungal colonization and mycotoxin synthesis in animal products. These factors are classified into three classes of physical (e.g. temperature, relative humidity and insect infestation of the product), chemical (e.g. use or non-use of preservatives and additives), and biological factors (e.g. fungus species and their co-occurrence, strain specificity, strain variation, and toxigenic properties). Generally, these factors can selectively change the colonization and metabolism of mycotoxin-producing fungi, thereby changing the production of mycotoxin over time and across the varied locations as indicated in the study [30, 38]. This study has indicated that most of the cured meats sold or ready for export in Abuja are contaminated with one mycotoxin or the other and thus signaling the danger of their continuous consumption or exportation [39]. According to the Rapid Alert System for Food and Feed (RASFF) 2012 annual report, mycotoxin contamination is the main reason for the rejection of products in the European Union (EU) borderline and mycotoxin levels are considered a barrier to accessing the EU markets [40]. Strategies to prevent fungal contamination and mycotoxins should be implemented in the entire food production chain (animal growth, processing, storage, and distribution). Control measures must be taken into consideration before any fungal contamination or during the period of the mould invasion and mycotoxin formation [41].

There are some important criteria for choosing an appropriate method to control mycotoxins in animal products, including economical and technical applicability and safety; not inducing a significant change in the nutritional value of food; not generating more toxic compounds; and not releasing hazardous residues [42]. The recommendations of Codex Alimentarius for the prevention and reduction of mycotoxins in animal products are focused on the guidelines of Good Livestock Management Practice (GLMP), Good Processing Practice (GPP), and the application of Hazard Analysis and Critical Control Points (HACCP) [43-45]. Developed countries are less exposed to mycotoxins in comparison with

developing countries with regard to modern processing technologies and restrictive government regulations [46]. In addition, HACCP systems play an important role in the prevention of mycotoxins and the quality of animal products from farm to fork, which currently include preventive and control measures in all stages, such as storage monitoring, leading to the reduction of mycotoxin production [47, 48]. Maintaining desired conditions during storage is extremely important in preventing mould growth and mycotoxin occurrence in foods.

It should be noted that any detoxifying substance used in preserving cured meat should not induce carcinogenic, mutagenic, or toxic effects, be capable of preserving the nutritional value of food, and make no change in the technological and sensorial properties of the product [49]. Irradiation, as a fast, cost-effective, non-thermal technology, is of great interest to apply for microbial inactivation and mycotoxin degradation without damage to the organoleptic properties, nutritional value, and quality of animal products [50]. However, it is worth mentioning that irradiated foods must be labeled and monitored according to the laws. The Joint Experts Committee on Food Radiation (JECFI) recommends a total allowable dose of 10 kGy. Meanwhile, the United States and China allow less than 10 kGy and Japan allows up to 150 kGy [51]. Reducing mycotoxin contamination could be through good storage conditions, additional monitoring of the marketed foods, and implementation of more stringent control and prevention strategies that reduce dietary exposure levels in Nigeria [52].

#### 5. Conclusion

Based on the mycotoxin analysis's findings, there was more health and trade risk concerns on the dry-cured meat sold or ready for export in Abuja, Nigeria than in the honey when compared with the established EU acceptable limit. There was an indication that most of the cured meat meant for export in the FCT Abuja, are contaminated with major mycotoxin of health significance. Though none of the analysed honey's samples were positive for aflatoxin, but OTA and OTB were detected even at a relatively high level above the permissible of EU. The dry-cured meat often sold by hawkers, and in supermarkets and marts were found contaminated with aflatoxins B<sub>1</sub>, Aflatoxins B<sub>2</sub>, OTA, and OTB above the permissible limit of EU and not fit for consumption. Thus the populace of FCT, Abuja should desist from patronizing kilishi sellers for their snacks until they are confirmed safe for consumption. Since the chains of supply for animal products traverse numerous national and regional borders, a close relationship between all the stakeholders, including the governments, farmers, suppliers, distributors, and consumers, will eventually ensure the safety of animal products in the markets. Continuous monitoring of food contaminants especially after processing of animal products is germane to the quality of the commodity and ensure sustainable public health protection.

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#### **Conflicts of interest**

There is no conflict of interest.

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