Moulds, Proximate Mineral Composition and Mycotoxin Contamination of Banda ("kundi"/ "tinko") Sold in Ibadan, Oyo State, Nigeria

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Abstract

Mycotoxin is an important toxin whose consumption could cause foodborne diseases. The moulds, proximate mineral composition and mycotoxin contamination of Banda samples purchased from different markets in Ibadan, Oyo state, were investigated. A total of 90 samples were collected during July - September and a total of 69 fungi isolates were obtained. The fungal isolates found in samples were Aspergillus niger, A. flavus, A. fumigatus, A. candidus and A. piperis among which A. niger had the highest frequency of occurrence (36.23%) followed in order by A. flavus (30.23%), A. fumigatus (13.04%), A. candidus and A. piperis (10.14%). The samples were found to contain adequate amount of proximate mineral contents. Proximate and mineral analysis carried out showed that the percentage crude protein in Banda samples collected in July is higher than that of August and September. The percentages of most of the mineral elements are higher in samples collected in August. It was observed from the analysis that the proximate and mineral composition in the dried meat was higher than that of the control (Raw meat sample). Mycotoxin analysis revealed that the samples were heavily contaminated with aflatoxins B_1, B_2, fumonisin (FB_1 and FB_2) and ochratoxin (OTA). There was a significant difference ($P \leq 0.05$) in mycotoxin concentration among the samples. The AFB_1 and AFB_2 were found in 10% and 90% of the dried meat samples, while 10% were contaminated with FB_1 and FB_2, respectively. 13.3% were contaminated with OTA. AFB_1 was not detected in all of the samples except samples J1, A1 and S4, respectively. For the samples collected in the month of July, the AFB_2 concentration ranged within 0.00 - 201.50, FB1, FB_2 and OTA concentrations ranged within 0.00i - 1.909a, 0.00i - 1.037a, and 0.00i - 2.00a, respectively. For the samples collected in August, the AFB_2, FB_1, FB_2 and OTA concentrations ranged within 0.00i - 234.20a, 0.00i - 2.327a, 0.00i - 1.003a, and 0.00 - 1.600a in which sample A_7 had the highest concentration. The AFB_2 concentration ranged within 0.00 - 167.20a in which sample S_1 had the highest concentration. The FB_1, FB_2 and OTA concentrations ranged within 0.373h - 2.199a, 0.167h - 1.965a, and 0.00f - 2.133a for the samples collected in September. The levels of mycotoxin contamination in all the samples exceeded the maximum limit permitted in most countries. The detection of AFB_1 and AFB_2 in the dried meat samples could be of public health significance and hence there is an urgent need for concerned regulatory bodies to impose necessary measures to safeguard health of consumers. Conclusively, the presence of mycotoxin producing fungi and the level of AFB_1 and AFB_2 in the dried meat samples call for serious attention in the country and there is a need for some form of quality control and proper preservation before usage.

Keywords: *Banda, protein, aflatoxins, ochratoxin and fumonisin.*

Introduction

Banda is one of the most popular traditional hard-smoked meat products, mostly from rejected cattle and discarded transport beasts (donkeys, horses, camels, buffaloes and elephants), widely consumed in Africa. The word "Banda" is of northern origin while similar names in Igbo and Yoruba are "Kundi" and "Tinko", respectively. Banda is the most commonly produced traditional African dried meat. The production processes involve the use of carcass, cutting, cooking for 15-30 min., drying and smoking for about 18-30 hrs, cooling, storage and packaging in sacks and jute/mat bags. Banda is a stable product with a shelf-life of 6-12 months or even up to two years under ambient temperature (Idufueko 1984; Okonkwo and Obanu 1984). "Banda" is an ideal source of animal protein and its inclusion in a balanced diet should go a long way towards improving nutrition in Nigeria. It also adds to the ecstatic appeal of the food. The high quality of meat protein in Banda is well established and is essential in the maintenance of a healthy population (Okonkwo 1987). Microbial quality of meat products plays an important role in an increasing public health issue all over the world. Microbial and mycotoxin analysis must be carried out in "Banda" aimed for human consumption because of its high mycoflora contamination due to inadequate handling practices, as well as fungi contamination during preservation.

Mycotoxins are a group of secondary metabolites produced by filamentous fungi which may contaminate foods, feeds or the raw materials used to produce them. They also produce mycotoxicoses in humans and animals. The genera of mycotoxigenic fungi are mainly represented by *Aspergillus*, *Penicillium* and *Fusarium*, but *Trichoderma*, T*richothecium* and *Alternaria* are also important as food contaminants or pathogens for plants, among others (Smith and Moss 1985; Moss 1994). Mycotoxins, particularly aflatoxins (AFTs) and ochratoxin A (OTA), pose a significant threat to human health. Aflatoxins are potent carcinogens and, in association with hepatitis B virus, are responsible for many thousands of human deaths per annum, mostly in non-industrialized tropical countries (Shephard 2006). Ochratoxin A is a probable human carcinogen and it was reported to cause urinary tract cancer and kidney damage in people from Eastern Europe. Exposure to OTA seems to be the biggest hazard correlated to microscopic fungi for the European consumers of cereals (EC 2006). Fumonisins are a group of *Fusarium* mycotoxins occurring worldwide in maize and maize-based products destined for human and animal consumption. Fumonisins are known to be the cause of equine leukoencephalomalacia (a brain disease that is often fatal) and porcine pulmonary oedema syndrome (swelling of lungs and thorax), both associated with the consumption of corn-based feeds.

This research was embarked upon to investigate the moulds, proximate mineral composition and mycotoxins in the Banda samples from different markets in Ibadan for a period of three months.

Materials and Methods

Sample collection

A total of 30 samples were purchased from different markets in Ibadan (Aleshinloye, Bodija, Bere and Sango). The samples were obtained during July, August and September. The samples were divided into three parts, mainly for aflatoxin extraction, mycological analysis and proximate analysis.

Isolation of Fungi

The associated fungi were isolated using a standard pour plate technique. Ten grams of the dried meat sample were homogenized in sterile distilled water. Also, 1 ml of the homogenate was serially diluted and 1 ml of selected dilution (10^{-3}, 10^{-4}, and 10^{-5}) was plated in duplicate on a sterile Sabouraud Dextrose agar containing 1% streptomycin. Inoculated plates were incubated at 28 ± 2°C for 5-7 days. Discrete colonies were isolated in pure culture by sub-culturing the cells. Fungal isolates were identified based on their morphological and cultural characteristics as recommended by Samson *et al.* (1984) and Frazier and Westholf (1998).

Proximate and mineral composition

Proximate mineral analyses were carried out using AOAC (1990) methods which included protein determination using Kjeldahl method, fat extraction via Soxhlet method, crude fiber determination using digestion with sulphuric acid, moisture determination by drying: the sample for 16-18 hours at 100-102°C in an oven; and the ash by ashing the sample at 550°C for 9 hours in a furnace oven.

Mycotoxin Analysis

Aflatoxin detection and quantification

The aflatoxin analysis was carried using a combination of AgraQuant kit. Using a shaker at room temperature, 10 g of each meat sample was extracted with 20 mL methanol: water (70:30). The residue was dissolved in 1 ml of methanol: water (3:1, v/v) and 200 ml of diluted extract was applied to the enzyme immunosorbent assay (ELISA) plate (Romer Lab. AgraQuant) in order to determine the total aflatoxin content. Each one of both samples and standards were applied in duplicate. The ELISA was performed according to the manufacturer's instructions. The intensity of the resulting yellow colour was measured in the ELISA plate reader with an absorbance filter of 450 nm and evaluated according to the RIDAWIN program. The optical densities (ODs) were then compared to those of the standards. Aflatoxin concentration in each sample was expressed as parts per billion (ppb).

Ochratoxins detection and quantification

About 1 ml of chloroform and 0.2 ml of the reconstituted extract was spotted on a pre-coated 20 x 20 cm thin layer chromatography (TLC) plate along with ochratoxin standards of known concentration. The spotted TLC plate was developed in an equilibrated tank containing toluene: acetylated: 90% formic acid (5:4:1v/v/v). The developed TLC was air-dried at an ambient temperature (28 ± 2°C) and ochratoxins were identified on the basis of co-migration with ochratoxin standards (Fluka) and by their characteristic fluorescent color under ultra-violet (UV) illumination at 366 nm and upon exposure to sulphuric acid (50:50v/v). Preparative TLC plates (0.5 µm thick) were employed for the quantification. To perform chromatography on the maximum amount of sample at the same time, 0.8 ml stored extract was applied to a given plate as a band rather than a spot. The preparative TLC plates were developed in an equilibrium tank as in ochratoxin extraction. The solvent front was allowed to rise to about $^3/_4$ of the total length of the plate; the plate was examined under the UV light. The area containing toxin of interest was scrapped off, eluted with chloroform and filtered with Whatman No. 1 filter paper. The extract was evaporated to dryness over a hot water bath and reconstituted with 3 ml of chloroform. The 3 ml reconstituted solution and ochratoxin standard of 10 µg/ml concentration (Sigma and Aldrich, St. Louis, MO, USA) were used to read the absorbance on an Ultraviolet Spectrophotometer (Cecil Instrument CE505) at wavelength of 366 nm. The ochratoxin concentration in µg/kg was calculated using the analytic expression:
(Absorbance of sample x Conc. x Standard x Dilution factor) / (Absorbance of standard).

Fumonisin determination and quantification

The fumonisin was extracted from the food samples using 85% acetonitrile and then 20 g alumina was added. The mixture was filtered using Whatman No. 1 filter paper. A standard of known concentrations of fumonisin B_1 and B_2 was prepared. An aliquot of the sample extract was prepared and 2 ml of dimethyl-formamide (DMF) was added to different concentrations of the known standard and aliquot of the sample extract. The absorbance was taken after color development with 95% DMF at a wavelength of 560 nm. The fumonisin FB_1 and FB_2 in µg/kg was calculated using the analytic expression:
(Absorbance of sample x Gradient x Dilution factor) / (Weight of the sample).

Results

A total of 69 mould isolates were obtained from the dried meat samples for the three months from July to September. The mould isolates were identified as *Aspergillus niger*, *Aspergillus flavus*, *Aspergillus fumigatus*, *Aspergillus candidus and Aspergillus piperis* as shown in Table 1.

Table 1. Moulds associated with the dried meat samples (Banda).

Source	*A. niger*	*A. flavus*	*A. fumigatus*	*A. candidus*	*A. piperis*
J1	+	+	-	+	-
J2	+	-	+	-	+
J3	+	+	-	-	-
J4	+	+	-	-	+
J5	+	+	-	-	+
J6	+	+	-	-	-
J7	+	-	+	+	-
J8	+	-	-	-	-
J9	+	-	+	-	-
J10	+	+	-	-	-
A1	+	+	-	-	-
A2	+	+	-	-	+
A3	+	+	-	-	-
A4	+	+	-	-	-
A5	-	-	+	+	-
A6	+	-	+	-	-
A7	+	-	-	+	-
A8	+	+	-	-	-
A9	+	-	+	-	-
A10	+	+	-	-	-
S1	-	+	+	-	-
S2	+	-	-	+	+
S3	+	+	-	-	+
S4	-	+	+	-	-
S5	+	+	-	-	-
S6	+	-	-	-	+
S7	-	+	-	+	-
S8	+	+	-	-	+
S9	+	+	-	-	-
S10	-	+	+	-	-

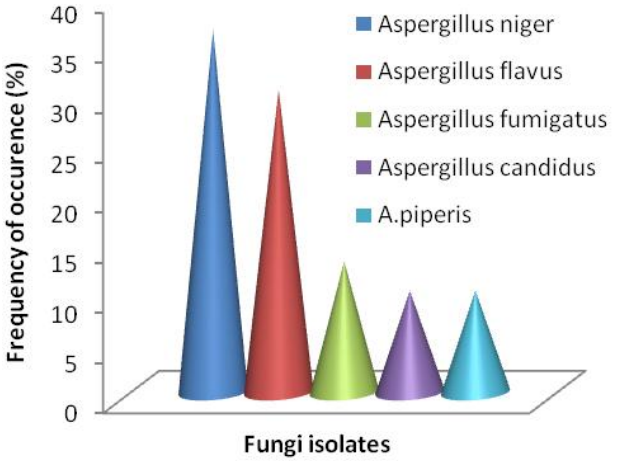

Fig. 1. Fungal isolates and their frequency of occurrence (%).

Aspergillus flavus was predominantly isolated from all the meat samples. From the July samples, *A. flavus* was not isolated from samples J2, J7, J8 and J9, respectively. Also, *A. flavus* was isolated from some of the August and September samples except for samples A5, A6, A7, A9, S2, and S6. The frequency of occurrence of the mould isolates is shown in Fig. 1. *Aspergillus niger* had the highest frequency of occurrence (36.23%), followed in order by *A. flavus* (30.43%), *A. fumigatus* (13.04%), *A. candidus* and *A. piperis* with (10.14%), respectively.

There were significant differences ($P \leq 0.05$) in proximate mineral composition of the dried meat samples collected from July to September. The crude protein, fat, fiber, moisture contents of the samples collected in July ranged within 21.68 - 54.73%, 1.86 - 6.35%, 0.00 - 0.92%, and 7.54% - 70.63%, the least value was obtained in the raw meat sample and the highest value was recorded in sample J9 as shown in Table 2. The sodium, calcium, phosphorus, magnesium, potassium, copper, manganese, zinc and iron content of the samples collected in July ranged within 0.35 - 1.84%, 1.24 - 6.76%, 0.8 - 3.45%, 0.12 - 0.43%, 0.09 - 0.53%, 1.5 - 8.6%, 2.6 - 9.1%, 120 - 449%, and 132 - 443%, respectively. The highest value was obtained from sample J9, except for magnesium, and the least value was recorded from the raw meat samples (Table 2). Table 3 shows the crude protein, fat, fiber, and moisture contents of the samples collected in August, it ranged within 21.68 -53.96% , 1.86 - 6.12%, 0.00 - 1.03%, and 6.53 - 70.63%. The least value was obtained in the raw meat sample and the highest value was recorded in sample A6. The sodium, calcium, phosphorus, magnesium, potassium, copper, manganese, zinc and iron content of the August samples ranged within 0.35 - 1.78%, 1.24 -6.89%, 0.87 - 3.58%, 0.12 - 0.29%, 0.09 - 0.57%, 1.5 - 7.3%, 2.6 - 8.7%, 12.0 - 45.2%, and 132 - 439%. The highest value was obtained from sample A6 and the least value was recorded from the raw meat samples (Table 3). Table 4 shows the crude protein, fat, fiber, and moisture contents of the samples collected in September, it ranged within 21.68 -53.16%, 1.86 - 6.5%, 0.00 - 1.0%, and 7.48% - 70.63%. The least value was obtained in the raw meat sample and the highest value was recorded in sample A6. The sodium, calcium, phosphorus, magnesium, potassium, copper, manganese, zinc and iron content of the September samples ranged within 0.35 - 1.84%, 1.24 - 6.52%, 0.87 - 1.80%, 0.12 - 0.29%, 0.09 - 0.51%, 1.5 - 8.9%, 2.6 - 9.3%, 12.00 - 38.71% and 132 - 447%. The least mineral composition was recorded from the raw meat samples (Table 4).

Mycotoxin analysis revealed that most of the samples were heavily contaminated with aflatoxins B_1, B_2, fumonisin (FB_1 and FB_2) and ochratoxin (OTA). There was a significant difference ($P \leq 0.05$) in mycotoxin concentration among the samples. The AFB_1 and AFB_2 were found in 10% and 90% of the dried meat samples while 10% were contaminated with FB_1 and FB_2, respectively. 13.3% were contaminated with OTA. AFB_1 was not detected in all the samples except samples J1, A1 and S4. There was a significant variation ($P \leq 0.05$) in AFB_2, FB_1, FB_2 and OTA concentration in the samples collected in the month of July as shown in Table 5. AFB_2 concentrations ranged within 0.00 - 201.50^{a}, FB_1, FB_2 and OTA concentrations ranged within 0.00^{i} - 1.909^{a}, 0.00^{i} - 1.037^{a}, and 0.00^{i} - 2.00^{a}, respectively.

Table 6 shows the AFB_2, FB_1, FB_2 and OTA concentrations of the samples collected in August. They ranged within 0.00^{i} - 234.20^{a}, 0.00^{i} - 2.327^{a}, 0.00^{i} - 1.003^{a} and 0.00^{i} - 1.600^{a}, respectively. Sample A7 had the highest concentration. Significant difference ($P \leq 0.05$) was equally observed in mycotoxin concentrations of the samples.

Table 7 shows the mycotoxin concentrations of the meat samples collected in September. There was a significant difference ($P \leq 0.05$) in the mycotoxin concentrations of the samples.

Table 2. Proximate and mineral composition of samples collected in July.

Sample	Crude Protein	Crude Fat	Fibre	Ash	DM	Moisture	Na	Ca	P	Mg	K	Cu	Mn	Zn	Fe
J1	54.16^{b}	6.21^{b}	0.87^{b}	23.86^{b}	92.38^{b}	7.62^{j}	1.55^{b}	4.11^{b}	1.85^{b}	0.21^{e}	0.35^{c}	5.7^{c}	6.3^{c}	34.2^{b}	289^{e}
J2	48.95^{i}	3.32^{g}	0.56^{f}	19.85^{f}	90.44^{e}	9.56^{g}	1.33^{e}	3.51^{h}	1.48^{h}	0.33^{b}	0.21^{f}	4.1^{f}	5.5^{f}	26.3^{h}	274^{g}
J3	49.51^{g}	4.53^{c}	0.51^{h}	19.66^{i}	90.21^{h}	9.79^{d}	1.51^{c}	3.72^{d}	1.63^{e}	0.22^{d}	0.38^{b}	5.2^{d}	6.1^{d}	29.2^{e}	308^{b}
J4	51.38^{c}	3.65^{f}	0.68^{e}	21.79^{c}	90.65^{d}	9.35^{h}	1.48^{d}	3.95^{c}	1.79^{c}	0.43^{a}	0.32^{d}	5.3^{d}	5.9^{e}	33.6^{c}	269^{h}
J5	48.59^{j}	4.21^{e}	0.72^{d}	19.94^{e}	90.09^{j}	9.91^{b}	1.28^{f}	3.26^{j}	1.42^{j}	0.23^{d}	0.21^{f}	2.6^{h}	3.8^{i}	19.8^{j}	269^{h}
J6	49.66^{f}	3.34^{g}	0.53^{g}	19.68^{h}	90.24^{g}	9.76^{e}	1.23^{g}	3.62f	1.57^{f}	0.11^{h}	0.24^{e}	3.8^{g}	4.7^{h}	26.7^{g}	243^{i}
J7	49.24^{h}	4.4^{d}	0.45^{i}	19.58^{j}	90.13^{i}	9.87^{c}	1.34^{e}	3.55^{g}	1.46^{i}	0.11^{h}	0.16^{h}	3.8^{g}	4.9^{g}	23.7^{i}	278^{f}
J8	50.06^{d}	3.17^{h}	0.79^{c}	20.26^{d}	91.28^{c}	8.72^{i}	1.34^{e}	3.49^{i}	1.52^{g}	0.18^{f}	0.18^{g}	4.3^{e}	4.9^{g}	27.4^{f}	306^{c}
J9	54.73^{a}	6.35^{a}	0.92^{a}	24.12^{a}	92.46^{a}	7.54^{k}	1.84^{a}	6.76^{a}	3.45^{a}	0.26^{c}	0.53^{a}	8.6^{a}	9.1^{a}	44.9^{a}	443a
J10	49.79^{e}	3.29^{g}	0.55^{f}	19.76^{g}	90.31^{f}	9.61^{f}	1.28^{f}	3.68^{e}	1.69^{d}	0.15^{h}	0.21^{f}	5.9^{b}	6.8^{b}	32.6^{d}	293^{d}
Raw	21.68^{k}	1.86^{i}	0.00^{j}	8.29^{k}	29.37^{k}	70.63^{a}	0.35^{h}	1.24^{k}	0.87^{k}	0.12^{h}	0.09^{i}	1.5^{i}	2.6^{j}	12.0^{k}	132^{j}

Table 3. Proximate and mineral composition of samples collected in August.

Sample	Crude Protein	Crude Fat	Fibre	Ash	DM	Moisture	Na	Ca	P	Mg	K	Cu	Mn	Zn	Fe
A1	49.58^{i}	4.67^{c}	0.49^{g}	20.09^{d}	91.33^{d}	8.67^{h}	1.31^{f}	3.57^{g}	1.61^{f}	0.21^{bc}	0.31^{d}	3.6^{g}	4.5^{i}	27.8^{c}	295^{f}
A2	48.61^{h}	3.15^{f}	0.47^{g}	19.37^{i}	90.15^{j}	9.85^{b}	1.29^{g}	3.63^{e}	1.55^{h}	0.15^{cd}	0.18^{g}	2.7^{h}	4.7^{h}	24.8^{h}	297^{e}
A3	48.62^{h}	3.26^{f}	0.58^{f}	20.15^{d}	90.25^{i}	9.75^{c}	1.29^{g}	3.76^{c}	1.81^{c}	0.18^{bcd}	0.22^{f}	4.8^{d}	5.1^{g}	25.6^{g}	311^{c}
A4	50.24^{c}	3.21	0.59^{e}	19.76^{g}	90.38^{h}	9.62^{d}	1.53^{b}	3.75^{c}	1.66^{d}	0.20^{ab}	0.36^{c}	4.9^{d}	6.3^{d}	27.9^{c}	311^{c}
A5	49.87^{e}	3.21	0.61^{de}	19.88^{f}	91.37^{c}	8.63^{i}	1.42^{d}	3.38^{h}	1.64^{e}	0.15^{cd}	0.26^{e}	4.5^{e}	5.3^{f}	26.9^{d}	302^{d}
A6	53.96^{a}	6.12^{a}	1.03^{a}	24.37^{a}	93.47^{a}	6.53^{k}	1.78^{a}	6.89^{a}	3.58^{a}	0.29^{a}	0.57^{a}	7.3^{a}	8.7^{a}	45.2^{a}	439^{a}
A7	51.57^{b}	4.29^{d}	0.63^{d}	22.47^{c}	91.16^{f}	8.84^{f}	1.38^{e}	3.42^{g}	1.59^{g}	0.23^{ab}	0.26^{e}	3.9^{f}	4.8^{h}	25.8^{i}	302^{d}
A8	45.23^{i}	6.10^{a}	0.85^{c}	20.00^{e}	90.52^{g}	9.48^{e}	1.21^{h}	3.71^{d}	3.12^{b}	0.21^{bc}	0.31^{d}	5.1^{c}	7.5^{b}	19.3^{j}	252^{h}
A9	49.21^{g}	4.00^{e}	0.62^{de}	19.50^{h}	91.31^{e}	8.69^{g}	1.43^{d}	3.21^{i}	1.82^{c}	0.15^{cd}	0.42^{b}	4.4^{e}	5.5^{e}	29.6^{b}	287^{g}
A10	50.22^{d}	5.25^{b}	0.90^{b}	23.21^{b}	92.14^{b}	7.86^{j}	1.45^{c}	3.78^{b}	1.45^{i}	0.20^{ab}	0.26^{e}	5.7^{b}	6.9^{c}	26.4^{e}	315^{b}
Raw	21.68^{j}	1.86^{h}	0.00^{h}	8.29^{j}	29.37^{k}	70.63^{a}	0.35^{i}	1.24^{j}	0.87^{j}	0.12^{d}	0.09^{h}	1.5^{i}	2.6^{j}	12.0^{j}	132^{j}

Table 4. Proximate and mineral composition of samples collected in September.

Sample	Crude Protein	Crude Fat	Fibre	Ash	DM	Moisture	Na	Ca	P	Mg	K	Cu	Mn	Zn	Fe
S1	52.16^{c}	4.23^{c}	1.00^{a}	21.50^{e}	91.46^{d}	8.54^{g}	1.61^{b}	3.28^{g}	1.67^{b}	0.19^{c}	0.22^{f}	5.1^{b}	5.1^{d}	21.2^{j}	447^{a}
S2	44.21^{g}	3.21^{e}	0.52^{d}	24.00^{b}	90.32^{f}	9.68^{e}	1.63^{b}	3.51^{f}	1.63^{b}	0.15^{e}	0.34^{c}	2.4^{h}	6.3^{c}	30.5^{c}	300^{d}
S3	49.22^{e}	3.28^{e}	0.71^{b}	19.21^{h}	92.33^{c}	7.67^{h}	1.80^{a}	4.00^{c}	1.78^{a}	0.21^{b}	0.51^{a}	3.5^{d}	4.3^{f}	25.81^{e}	281^{e}
S4	48.16^{f}	4.21^{c}	0.62^{c}	19.52^{g}	90.28^{g}	9.72^{d}	1.38^{c}	3.91^{d}	1.81^{a}	0.13^{f}	0.37^{b}	3.2^{d}	3.5^{g}	22.71^{h}	260^{g}
S5	50.16^{d}	5.21^{b}	0.72^{b}	22.14^{d}	92.52^{a}	7.48^{j}	1.29^{f}	3.61^{e}	1.50^{cd}	0.21^{b}	0.24^{e}	4.4^{c}	6.2^{c}	33.1^{b}	270^{f}
S6	46.06^{i}	3.65^{d}	0.45^{e}	19.15^{h}	90.25^{h}	9.75^{c}	1.43^{c}	3.31^{g}	1.53^{d}	0.22^{b}	0.19^{g}	8.9^{a}	5.2^{d}	21.51^{i}	290^{e}
S7	52.38^{b}	4.21^{c}	0.61^{c}	22.15^{d}	90.22^{i}	9.78^{b}	1.35^{de}	3.11^{h}	1.62^{b}	0.29^{a}	0.15^{h}	5.1^{b}	6.5^{b}	24.30^{f}	440^{a}
S8	53.16^{a}	6.50^{a}	0.72^{b}	23.67^{c}	92.47^{b}	7.53^{j}	1.28^{f}	4.21^{b}	1.47^{d}	0.17^{d}	0.38^{b}	3.0^{f}	3.2^{h}	23.10	315^{c}
S9	47.23^{h}	4.21^{c}	0.45^{e}	24.20^{a}	90.24^{h}	9.76^{a}	1.84^{a}	6.52^{a}	1.61^{b}	0.19^{c}	0.23^{e}	2.6^{g}	4.5^{e}	38.71^{a}	415^{b}
S10	48.06^{g}	5.10^{b}	0.75^{b}	20.14^{f}	91.44^{e}	8.56^{f}	1.31^{de}	4.00^{c}	1.80^{a}	0.16^{c}	0.31^{d}	2.1^{i}	9.3^{a}	29.11^{d}	243^{h}
Raw	21.68^{k}	1.86^{f}	0.00^{f}	8.29^{j}	29.37^{j}	70.63^{a}	0.35^{g}	1.24^{i}	0.87^{e}	0.12^{f}	0.09^{i}	1.5^{j}	2.6^{i}	12.00^{k}	132^{i}

The AFB_2 concentrations ranged within 0.00^{i} - 167.20^{a} in which sample S1 had the highest value. The FB_1, FB_2 and OTA concentrations ranged within 0.373^{h} - 2.199^{a}, 0.167^{h} - 1.965^{a}, and 0.00^{f} - 2.133^{a}. FB1 and FB2 were not detected in samples J7 and A9 and OTA was not detected in samples J1, A9 and A7 (Table 7).

Table 5. Mycotoxin analysis of samples collected in July.

Sample code	AFB_1	AFB_2	FB_1	FB_2	OTA
JI	235.00^{a}	0.00^{j}	0.207^{i}	0.084^{h}	0.356^{f}
J2	0.00^{b}	95.00^{g}	0.622^{h}	0.334^{e}	0.267^{g}
J3	0.00^{b}	201.50^{a}	1.286^{d}	0.251^{f}	1.511^{b}
J4	0.00^{b}	113.8^{f}	0.747^{g}	0.251^{f}	1.022^{d}
J5	0.00^{b}	135.60^{d}	1.784^{b}	0.878^{b}	1.289^{c}
J6	0.00^{b}	69.20^{i}	1.037^{f}	1.037^{a}	1.556^{b}
J7	0.00^{b}	92.40^{h}	0.000^{j}	0.000^{i}	0.933^{e}
J8	0.00^{b}	130.20^{e}	1.162^{e}	0.376^{d}	0.178^{h}
J9	0.00^{b}	141.70^{c}	1.909^{a}	0.209^{g}	2.000^{a}
J10	0.00^{b}	151.60^{b}	1.411^{c}	0.543^{c}	0.000^{i}

Table 6. Mycotoxin analysis of samples collected in August.

Sample code	AFB_1	AFB_2	FB_1	FB_2	OTA
A1	234.20^{a}	0.00^{i}	0.000^{h}	0.000^{g}	1.156^{e}
A2	0.00^{b}	217.70^{b}	0.871^{f}	0.627^{e}	0.267^{h}
A3	0.00^{b}	217.70^{b}	1.494^{d}	0.711^{d}	1.556^{b}
A4	0.00^{b}	165.30^{e}	0.207^{g}	0.878^{b}	1.422^{c}
A5	0.00^{b}	211.80^{c}	1.784^{c}	0.836^{c}	0.977^{f}
A6	0.00^{b}	198.00^{d}	1.950^{b}	0.627^{e}	1.378^{c}
A7	0.00^{b}	139.40^{f}	2.327^{a}	1.003^{a}	1.600^{a}
A8	0.00^{b}	234.20^{a}	1.037^{e}	0.376^{f}	0.667^{g}
A9	0.00^{b}	234.40^{a}	0.000^{h}	0.000^{g}	0.000^{i}
A10	0.00^{b}	80.30^{g}	0.871^{f}	0.627^{e}	1.200^{d}

Table 7. Mycotoxin analysis of samples collected in September.

Sample code	AFB_1	AFB_2	FB_1	FB_2	OTA
S1	0.00^{b}	167.20^{a}	1.452^{c}	0.627^{e}	1.156^{c}
S2	0.00^{b}	100.20^{f}	0.373^{h}	0.125^{i}	2.133^{a}
S3	0.00^{b}	102.70^{e}	1.037^{e}	0.920^{c}	1.600^{b}
S4	261.60^{a}	0.00^{j}	0.747^{g}	0.167^{h}	1.067^{d}
S5	0.00^{b}	80.20^{h}	1.535^{b}	0.627^{e}	2.178^{a}
S6	0.00^{b}	66.10^{i}	1.037^{e}	0.334^{f}	1.600^{b}
S7	0.00^{b}	87.20^{g}	2.199^{a}	1.965^{a}	1.022^{d}
S8	0.00^{b}	112.20^{d}	1.162^{d}	1.087^{b}	0.000^{f}
S9	0.00^{b}	118.60^{c}	1.535^{b}	0.251^{g}	0.000^{f}
S10	0.00^{b}	145.30^{b}	0.871^{f}	0.711^{d}	0.311^{e}

Note: In Tables 5-7, each value represents a mean of three replicates. Means followed by the same letter are not significantly different by Duncan's multiple range tests.

Discussion

The occurrence of different mould isolates in the samples is of a major public health concern. Food and feed contamination is an important but unrecognized risk to public health and can have long-term health implications (Coulter *et al*. 1986; Bucci *et al.* 1996). Mould spoilage of the hot-smoked meat product is expected to occur more often than bacteria spoilage due to the low water activity. This is not because moulds grow faster than bacteria but because the competitive effects of the vast majority of bacteria are generally inhibited at low water activity. The occurrence of moulds in the samples may be a result of the storage temperature and high nutrient content in the products. All the fungal organisms isolated and identified are saprophytic, widely distributed in nature and have their habitat mostly in the soil and decaying organic matter (Raper and Fennell 1965). The low moisture content of the samples indicates that Banda is sufficiently dried to minimize microbial growth. However, the higher microbial load may be due to moisture absorption from the environment, which in turns increases the growth of moulds on the meat surface. Egbunike and Okubanjo (1999) reported that intermediate moisture meats (IMM) are low in moisture and are shelf-stable under tropical climates without refrigeration.

The high protein content found in the samples is in agreement with the report of Egbunike and Okubanjo (1999) that IMM are low in moisture content and contain three to four times the raw protein equivalent, which makes them to be less bulky. The high ash levels obtained from the dry meat samples may be due to the condiments used during the boiling of the meat for the Banda production. It may also be due to the resultant dirt on the meat pieces during sun drying on the ground in the open market. Torres *et al*. (1994) reported that ash content at the end of storage differs significantly to that at the onset. The mean ash content agreed with the report of Igene and Ekenen (1985). These authors found that ash content of meat increased with heat application. The moisture contents of the samples collected in the month of July is higher than that of

August and September samples. This may be due to the high relative humidity during the storage period. Prolonged storage and improper drying should be discouraged because it could lead to deterioration and infestation by mycotoxigenic moulds.

The mineral content of the dried meat was higher than the raw meat. This increase is a reflection in the ash content and is mainly due to moisture loss during sun drying of the raw meat.

The occurrence of aflatoxin in the Banda samples may be due to contamination of the dried meat by aflatoxigenic moulds during sun drying and storage. Aflatoxin contamination of the dry meat occurs when aflatoxigenic species of the *A. flavus* group successfully colonize the meat, grow in it and subsequently produce aflatoxins as secondary metabolites. The species of the *A. flavus* group that produce aflatoxins include *A. flavus, A. parasiticus, A. nomius, A. tamarii* and *A. bombycis* (Fennell *et al*. 1973; Goto *et al*. 1996; Peterson *et al*. 2001; Wilson and Payne 1994). Incidence of aflatoxins in some other food products, such as tiger nut, dried yam chips, bush mango, smoked fishes, and soy milk powder, has been reported as well (Bankole and Eseigbe 1996; Bassa *et al*. 2001; Adebayo-Tayo *et al*. 2006).

Detection of OTA in the samples can be a result of contamination by *Aspegillus* sp. Production of OTA by black *Aspergillus* species has been reported by many authors (Urbano *et al*. 2001; Dalcero *et al*. 2002; Magnoli *et al.* 2003). Ochratoxin A has been found in wheat, corn, and oats having fungal infection and in cheese and meat products of animals consuming ochratoxin-contaminated grains (Aish *et al*. 2004). *A. ochraceus* is found on dry foods, such as dried and smoked fish, soybeans, garbanzo beans, nuts, and dried fruit. OTA is immunosuppressive, teratogenic, genotoxic and mutagenic, and IARC (1993) has classified it in group 2B as possibly carcinogenic to humans. OTA is a genotoxic carcinogen, and it was proposed that its levels in foods should be reduced to the lowest level that can be technologically attained (COT 1997). The joint expert Committee on Food Additives of the WHO and FAO set a provisional maximum intake of 100 µg/kg body weight (bw), while the Scientific Committee on Food of the European Union proposed that the maximum daily intake of OTA should not exceed 5 µg/kg bw (WHO 1996). The results showed that the levels of aflatoxin contamination in all the samples tested exceeded the maximum AFB1 residue limit of 20 µg/kg permitted in Nigerian foods (Bankole *et al*. 2004).

Ochratoxin has been detected in blood, other animal tissues and in milk, including human milk (Marquardt and Frohlich 1972). It is frequently found in pork intended for human consumption. The OTA is believed to be responsible for a porcine nephropathy that has been studied intensively in the Scandinavian countries.

Fumonisins are mycotoxins mainly produced by *Fusarium* V*erticillioides*, the most prevalent molds associated with corn worldwide (Jackson and Jablonski 1996). Human exposure to the fumonisins occurs mainly by the consumption of contaminated corn or corn-based foods. These compounds have been implicated as the causative agents in several naturally occurring animal diseases, including leukoencephalomalacia in horses and pulmonary edema in pigs (Marasas *et al*. 2000). Recently, the U.S. Food and Drug Administration has announced guidance levels for total fumonisins levels in corn products for foods and feeds ranging between 2 and 4 ppm in human food and 5 ppm up to 100 ppm in animal feed depending upon the species and the proportion of the contaminated material in the total diet (USFDA 2000). According to the animal studies and epidemiological studies on humans, fumonisins have been classified as a possible human carcinogen by IARC (1993).

The presence of these moulds and mycotoxins in the food samples can cause serious health problems for Banda consumers, which may result in a suppressed immune system and cancer. An accurate prediction of the possible health impact of individual mycotoxins in foods for the vulnerable group is difficult. Possible additive and synergistic effects of multiple mycotoxins make the task even more complex and the long-term effects are beyond foresight. Climatic and environmental conditions during growth,

harvest and storage have great influence on mycotoxin levels, which are probably also reflected in the levels in Banda samples.

Conclusion

In conclusion, the present work indicated that the examined Banda meats were contaminated with several moulds of the genus *Aspergillus*. Many of these moulds are capable of producing mycotoxin such as aflatoxins, ochratoxins and fumonisins. These findings indicate that there may be a risk of human exposure to mycotoxins through the consumption of the dried meat. Strict hygienic microbiological measures must be applied during the processing and storage of the meat samples.

This research work has shown that Banda, an example of an intermediate moisture meat, is nutritionally richer than the raw meat as shown from the proximate and mineral analysis carried. Also, most of the dried meat (Banda) presently on sale in our markets is partially acceptable for human consumption. This is due to the concentration of aflatoxins detected in them which is far above the WHO standard (20 ppb for food samples) and also due to the presence of ochratoxin and fumonisin. It is therefore important that both the dried meat producers and the marketers take necessary precautions in preventing contamination of the meat to reduce possible contamination and hence reduce the risk of aflatoxins and other mycotoxins that are deleterious to human health.

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