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Research Article

Mycological, Toxigenic and Nutritional Characteristics of Some Vended Groundnut and Groundnut Products from Three Northern Nigerian Ecological Zones

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ABSTRACT

Groundnut (Arachis hypogaea L.) and groundnut products are important, street-vended, energy-rich sources of protein and oils useful in human and animal diets although fraught with microbial contaminations. Fungi associated with vended samples of roasted groundnut, Kulikuli, Donkwa, peanut butter and Yaji obtained from Kano, Kaduna, Minna and Ibadan were isolated using pour-plate method. These were qualitatively screened for presence of mycotoxin on palm kernel agar medium and the concentrations of aflatoxin and deoxynivalenol content in the samples quantified through immunoassay. The fungal load of the samples was highest between 1.3X103 and 1.6X104 TFU/g while the frequency of occurrence of Aspergillus, Fusarium, Rhizopus and Penicillium species in the samples were 36%, 33%, 20% and 11%, respectively. Qualitatively, the highest aflatoxin intensity producers were two strains of Aspergillus flavus from a Yaji and Kulikuli sample. The highest aflatoxin concentration (115ppb) was recorded in the Kaduna Yaji sample and 65% of the samples had aflatoxin concentration above the FDA-prescribed 20ppb. The highest deoxynivalenol concentration (0.7ppm) was recorded in Kaduna Donkwa sample which was still lower than the 1.0ppb prescribed recommendation. Kano Yaji and Kaduna Kulilkuli had the highest protein content (60% and 44% respectively) while all samples were high in calcium and potassium (725.16-1292.75 and 325-1280mg/100g) respectively. There was fungal contamination of vended groundnut product samples and the detection of mycotoxins in all the samples. Regulatory bodies, especially in developing countries, need to set quality standards and ensure compliance of the same in street vended food products for product and consumer safety.

Keywords: *Groundnut products, Mycotoxigenic properties, Deoxynivalenol, Aflatoxin, Nutritional composition*

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INTRODUCTION

Processed groundnut products are major snacks in many countries of the world (Ostadrahimi et al. 2014; Guchi, 2015) and play significant roles both as food and oil crop (Oladimeji and Kolapo, 2008). For more than two centuries, groundnut has been cultivated and sold as a major economic crop in Nigeria (Shokpeka and Nwaokocha, 2009). Due to a high protein and omega 6 fatty acid content, many industries have incorporated groundnuts as a major ingredient in the formulation of different cereals and weaning foods (Iro et al., 1995). Groundnut products, majorly cultivated and processed in the northern part of the country, are consumed by all age groups in the geo-ecological zones of Nigeria as Ready-To-Eat (RTE) foods and also serve as a key component in the formulation of poultry feed (Akano and Atanda, 1990; Oladimeji and Kolapo, 2008).

In Nigeria, some groundnut product derivatives include Groundnut/ peanut butter (a paste made by milling roasted groundnut and used as spreads in many common diets, local dishes and social gatherings), Kulikuli (the fried residue obtained after groundnut oil extraction), Yaji (a barbecue condiment containing a mixture of milledKulikuli and different spices) Donkwa (a kneaded ball of dry-milled mixture of roasted groundnut and roasted corn with additions of dried red pepper and sugar as desired), husked and de-husked groundnuts (roasted/boiled), etc. These products are retailed in super, mini and local markets as well as frequently hawked in many streets and motor parks of urban and rural Nigerian streets (Adebesin et al., 2001; Aletor and Ojelabi,

2007; Donkor et al., 2009). These products and many other RTE foods are usually contaminated with microorganisms especially fungi through sample handling, packaging, unhygenic storage environment and cross contamination with other contaminated vended products (Oranusi and Olorunfemi, 2011). Over the years, the nutrition, health safety, microbiological and chemical hazards attending street/market vended food products have been of great concern owing to the hygienic practices of the vendors and the processing methods enlisted (Amusa and Odunbaku, 2009). The effective regulation of production, packaging and sales of processed groundnut and its products is a herculean task since these vended groundnut products are commonly prepared the Nigerian populace in almost all localities. Coupled with this is the development of food borne toxins through microbial contamination.

The World Health Organization (WHO) has characterized the contamination of food and feed by mycotoxins (secondary toxic products of oxidative metabolism of fungi on agricultural products), phycotoxins and plant toxins among chemical hazards which result in food-borne illnesses (WHO, 2002). Mycotoxins, fungal products of secondary metabolism, have received the most attention out of these three groups of natural toxins, since they are highly immunotoxic, detectable in the faecal and urine samples of infected subjects and associated with diseases in animals and man (Peraica et al. 1999). Mycotoxins have become a major food safety issue in several parts of the world because of the risks involved in their ingestion, and so scientists continue to study them. Sharma et al., (2018), also commented on the commented on the advertently or inadvertently exposure of people to high aflatoxin contents in food. The European Union set a 'severe aflatoxin tolerance standard' at 2 µg/kg aflatoxin B1 and 4 µg/kg total aflatoxins for nuts and cereals for human consumption (Dimanchie, 2001, Bankole and Adebanjo, 2003). The allowable aflatoxin levels set by U.S. Food and Drug Administration (FDA) is 20 ppb for food, feeds, and groundnut products while that for milk was set at 0.5 ppb (Labuza, 1983). Deoxynivalenol (DON), a toxin of Fusarium species, is commonly associated with grains and nuts, affects receptors in the brain and induces vomiting. While the mycotoxin content of different food grains and nuts harbouring mycotoxigenic fungi have been extensively analysed (Bankole and Adebanjo, 2003), such investigations on Donkwa (a mixture of groundnut and milled grains), Yaji and other groundnut derivatives are yet to be given similar priority especially with reference to the deoxynivalenol content. Due to the importance of these groundnut products in daily living, this work sought to evaluate the fungal species associated with vended groundnut products, obtain information concerning the level of mycotoxins present in these products as an indication of what consumers might be exposed to and determine the nutritional and mineral composition of the samples studied.

MATERIALS AND METHODS

Sample collection: One hundred and sixty-six samples of different groundnut products (*Donkwa*, Peanut butter, *Kulikuli*, *Yaji* and Roasted groundnuts) were sourced at random from local markets and bus terminals/car parks from three northern Nigerian agro-ecological zones {Southern Guinea Savanna (Niger); Northern Guinea Savanna (Kaduna) and Sudan Savanna (Kano)} and compared with those obtained from a Derived Savanna (Ibadan). All samples collected were then transported to the laboratory and subjected to mycological and mycotoxin analysis.

Isolation of and identification of mycoflora present in the different groundnut samples

Triplicates (10g) of crushed groundnut product samples, were each mixed with 90ml sterile distilled water, agitated using a vortexer and serially diluted. The pour plate method, with 1ml of diluted aliquots, was used to determine the total fungal count contained in the samples on sterile, molten and cooled Potato dextrose agar (PDA) supplemented with 0.01% streptomycin sulphate (Samson *et al.* 1995) to repress bacterial growth. Petri dishes were incubated at 28±2ºC over seven days and the number of distinct developed colonies recorded as Total Fungal Count per gramme of sample (TFC/g). Morphologically different fungal colonies were subcultured severally and thereafter stored as pure cultures on PDA slants at 4ºC. The selected pure fungi were identified based on the macroscopic morphological characteristics exhibited by the fungi such as mycelia colour (and reverse plate colour), hyphal characteristic as well as microscopic analysis of teased lactophenol cotton blue stained hyphae and spores of 5 day old fungi (Singh *et al.,* 1991; Samson *et al.,* 1995).

Q**ualitative Screening to Detect Toxigenic Fungi Associated with Groundnut Product Samples :** Five-millimeter (5mm) agar discs of the isolated fungal colonies (5-day old cultures) were transferred unto solidified compounded Palm Kernel Agar medium (Atanda *et al.* 2005) supplemented with 100µg/ml streptomycin (Davis *et al.,* 1987) to presumptively screen for toxigenicity of the fungal isolates. These were incubated at 28±2ºC for 7 days. Aflatoxin production by positive toxigenic fungi was detected in the plates by production of a yellow-orange pigmentation on the lower side of the fungal cultures over the incubation days.

Preparation and Mycotoxigenic Analysis of Groundnut and Groundnut Product Samples: Sample preparation followed the method of Adebayo-Tayo *et al.,* 2015 and Veratox® mycotoxin test kits instructions (Neogen Corporation). The milled groundnut product samples (in triplicates of 5g samples each) were put in different 125ml sterile ready to use covered plastic containers and 25ml aliquot of 70% (v/v) methanol introduced. The mixture was shaken for 5 minutes and the liquid portion was filtered using Whatmann filter paper and kept for further analysis. Enzyme Linked Immunosorbent Assay (ELISA) was used in determining the concentrations of aflatoxin and deoxynivalenol (DON) using the prefabricated Veratox® mycotoxin test kits for Aflatoxin and deoxynivalenol extraction, purification and quantification. Extracts of test samples and kit control standards (50 µl equivalent to 2mg product) were introduced into test wells of microtitre plates coated with antibodies specific to the aflatoxin and deoxynivalenol to be detected. After the reactions, the colour

changes in the wells were subsequently measured at 630nm in a microtitre plate reader. If the mycotoxin content in the sample is high, fewer conjugates would bind to the antibody coat in the microwells hence colour intensity would be lesser than that of the control standards. For aflatoxin, the results of the optical density reading obtained were compared to the readings of the standards used to generate a standard curve at aflatoxins concentration range of 0 to 50ppb while that for deoxynivalenol was generated using DON standard controls ranging between 0-2ppm.

Physico-Chemical and Nutritional Analysis of Groundnut and Groundnut Product Samples: The methods of A.O.A.C. (1990) were used to determine moisture, ash, crude protein, total fat, carbohydrate crude fibre and nutrient content (phosphorus, potassium, calcium, iron).

Statistical Analysis:
The data obtained were average of triplicate readings and subjected to descriptive statistics and one way analysis of variance (ANOVA) at a significant difference of $\alpha_{0.05}$.

RESULTS

Fungal load and Mycoflora: Fungi were recorded in all groundnut product samples studied. The total fungal counts in the groundnut product samples ranged between 1300 and 15000 fungal colonies per gramme of the samples studied (Table 1). The Kano peanut butter and the Ibadan roasted groundnut samples had the highest fungal counts of 1.5 $x10^4$ CFU/g and 1.6 $x10^4$ CFU/g respectively. The least fungal count (1.3 x 10^3 TFU/g) was realised in *Donkwa* sample from Ibadan. The mycoflora of groundnut and groundnut product samples were found to belong to the genera *Aspergillus, Penicillium, Rhizopus* and *Fusarium* (Table 2).

Mycotoxin Determination: Qualitative aflatoxin screening was most intensely orange-yellow pigmentation positive in strains of *Aspergillus flavus* associated with *Kulikuli* samples from Ibadan and Kaduna, and in *Aspergillus parsiticus* isolated from a *Yaji* sample obtained from Kaduna (Table not shown).

Quantitatively, the highest aflatoxin concentration was recorded in the Kaduna *Yaji*, followed decreasingly by the samples of Ibadan *Kulikuli*, Kano *Donkwa* and Ibadan *Donkwa* 115.47, 93.37; 69.73 and 69.73 ppb respectively (Fig. 1). The Kano and Kaduna *Yaji* samples, Kaduna and Ibadan *Kulikuli*, Kano, Kaduna and Ibadan *Donkwa* all had above 30 ppb aflatoxin content. Although, the presence of deoxynivalenol was detected in eleven samples (Fig. 1), the Kano *Donkwa* was the most contaminated sample, with a concentration of 0.67ppm

Physicochemical and Nutritional Analysis of Groundnut Products: The mean moisture content in the different vended samples ranged from 1.15±0.05 to 11.59±0.03% (Table 3). There were no significant differences in roasted groundnut samples from the different regions. The moisture content observed in groundnut butter was found to be relatively low (1.15-2.8 g/100g).

Table 1:
Total fungal count (TFC/g) of groundnut product samples

Products/Zones	Derived Savanna (Ibadan)	Southern Guinea Savanna (Niger)	Northern Guinea Savanna (Kaduna)	Sudan Savanna (Kano)
Peanut butter	-	1.1 x 10^4	-	1.5 x 10^4
Kulikuli	3.4 x 10^3	3.0 x 10^3	3.0 x 10^3	1.5 x 10^3
Donkwa	1.3 x 10^3	1.5 x 10^3	2.7 x 10^3	5.0 x 10^3
Yaji	-	2.0 x 10^3	1.7 x 10^3	1.5 x 10^3
Roasted groundnuts	1.6 x 10^4	3.0 x 10^3	3.5 x 10^3	5.2 x 10^3

Table 2:
Probable identities of fungi isolated from the groundnut product samples

Products / Zones	Derived Savanna (Ibadan)	Southern Guinea Savanna (Niger)	Northern Guinea Savanna (Kaduna)	Sudan Savanna (Kano)
Peanut butter		*Penicillum citrinum, Rhizopus* species *Fusarium oxysporum,*		*Aspergillus flavus Aspergillus terreus*
Kulikuli	*F. oxysporum, A. terreus*	*F. compacticum, P. citrinum, R. nigricans*	*P. citrinum. A. terreus, F. oxysporum,*	*A. terreus, F. oxysporum, Rhizopus* sp., *Aspergillus* sp.
Donkwa	*F. oxysporum, R. nigricans, Aspergillus* sp.	*F. oxysporum, Rhizopus* sp., *A. flavus*	*F. oxysporum,*	*R. nigricans, P. citrinum, F. oxysporum, A. flavus, A. niger,*
Yaji		*F. oxysporum, A. flavus R. nigricans,*	*F. oxysporum, A. flavus*	*F. oxysporum, A. niger*
Roasted groundnuts	*A. flavus, A. niger*	*F. oxysporum, A. niger, R. nigricans*	*A. niger, F. oxysporum, R. nigricans*	*A. flavus, P. citrinum*

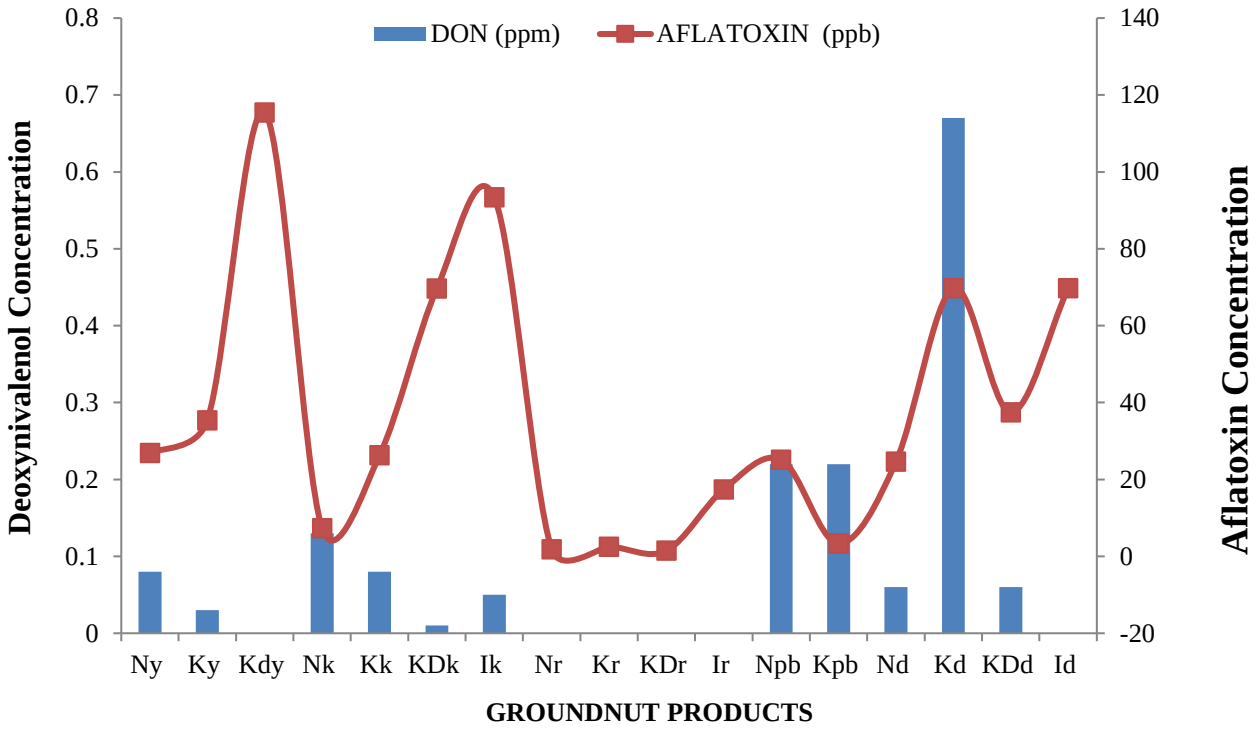

Fig. 1:
Aflatoxin and Deoxynivalenol concentrations in groundnut product samples (*Yaji*, *Kulikuli*, Roasted groundnut, Peanut butter and *Donkwa)*

KEY: N= Niger, K= Kano, Kd= Kaduna, I=Ibadan
Y= *Yaji*, K= *Kulikuli*, R = Roasted groundnut, PB= Peanut butter, D= *Donkwa*

Table 3:
Proximate composition (%) of representative ready–to-eat groundnut product samples

PARAMATER	MOISTURE	PROTEIN	FAT	ASH	CARBOHYDRATE
Niger Y	4.66±0.03^{e}	18.95±0.03^{e}	23.48±0.03^{b}	17.86±0.04^{d}	35.06±0.04^{a}
Kano Y	9.14±0.03^{d}	19.76±0.03^{e}	22.08±0.04^{b}	10.46±0.04^{c}	37.02±0.03^{a}
Kaduna Y	11.59±0.03^{d}	31.22±0.03^{a}	26.08±0.03^{b}	8.42±0.04^{e}	22.09±0.04^{c}
Niger K	5.31±0.01^{d}	34.95±0.01^{a}	33.51±0.03^{b}	3.46±0.03^{e}	22.75±0.03^{c}
Kano K	4.73±0.03^{d}	40.45±0.03^{a}	25.36±0.03^{b}	4.91±0.06^{d}	24.35±0.03^{c}
Kaduna K	4.55±0.05^{d}	43.93±0.05^{a}	27.47±0.05^{b}	4.54±0.04^{d}	19.41±0.04^{c}
Ibadan K	5.02±0.20^{e}	40.43±0.20^{a}	28.41±1.20^{b}	6.17±1.20^{d}	19.97±0.04^{c}
Niger R	3.36±0.06^{d}	29.61±1.05^{b}	49.11±1.05^{a}	2.75±0.50^{e}	15.17±0.50^{c}
Kano R	3.36±0.06^{d}	24.45±0.06^{b}	50.37±0.06^{a}	3.70±0.40^{d}	18.09±0.40^{c}
Kaduna R	3.62±0.25^{e}	30.69±0.25^{b}	48.14±2.50^{a}	4.15±2.5^{d}	13.35±2.50^{c}
Ibadan R	3.98±0.40^{d}	24.45±0.40^{b}	46.21±0.40^{a}	3.43±1.10^{d}	19.63±1.10^{c}
Niger PB	2.80±0.10^{e}	36.19±1.5^{b}	48.32±1.50^{a}	4.03±0.03^{d}	8.65±0.05^{c}
Kano PB	1.15±0.05^{e}	34.20±2.5^{b}	51.72±2.50^{a}	4.46±1.30^{d}	8.46±1.30^{c}
Niger D	3.12±0.02^{d}	12.63±3.5$^{c`}$	24.98±3.50^{b}	1.75±0.04^{e}	57.50±0.30^{a}
Kano D	4.12±0.05^{d}	14.33±0.45^{c}	25.49±0.45^{b}	2.04±0.45^{e}	54.10±0.10^{a}
Kaduna D	5.48±0.14^{d}	22.69±1.40^{b}	57.93±0.03^{a}	2.59±0.50^{e}	11.31±0.50^{c}
Ibadan D	3.37±0.02^{d}	15.39±0.06^{c}	26.61±0.04^{b}	2.31±0.01^{e}	52.32±2.0^{a}

Key: Y=*Yaji*, K=*Kulikuli*, R= roasted groundnut, PB= Peanut butter, D= *Donkwa*

The proximate composition analysis of the groundnut products in the study revealed an overall high protein content with the crude protein content ranged from 12.63±3.5 to 43.93±0.03% with the highest protein content found in the Kaduna *Kulikuli* sample. The general percentage range of crude protein recorded in the *Kulikuli* samples (34.95-43.93%) was high while the least crude protein values were observed in the *Donkwa* samples. Fat content range from 23.48±0.03 to 57.93±2.50% and the result obtained were significantly different. The highest fat content (>50.0%) were recorded in Kano peanut butter and Kaduna *Donkwa* sample. The ash content ranged from 1.75±0.04 to 17.86±0.04 while the carbohydrate content ranged from 8.46±0.04 to 57.5±0.3. The carbohydrate content was observed to be relatively high in *Donkwa* samples. The result of mineral

analysis showed that groundnut samples had a high composition of Calcium and Potassium (Table 4). Values for Calcium composition ranged from 725-1292.74 mg/100g and the highest content was observed in the Kano *Kulikuli* sample. Potassium content ranged from 325-1280 mg/100g with the highest content observed in Niger *Yaji* sample. Overall, Potassium content in the *Yaji* samples, (1280-1207.75 mg/100g), was relatively high compared to other groundnut product samples. *Donkwa* samples had the least Potassium content (325.0-490.75 mg/100g) among all the different samples analyzed. Furthermore, Phosphorus content ranged from 197.61-494.15 mg/100g, while the Iron content ranged from 227.75-326.75 mg/100g, with the highest Iron content observed in Kano roasted groundnut sample.

DISCUSSION

Microbial analysis of a food material provides insight into the number and types of microorganisms present therein, affording an indication of the sample's quality and the gravity of possible risk to susceptible consumers. Fungi were recorded in all groundnut product samples studied, a finding in accordance with the report of Akano and Atanda (1990), Adebesin *et al*. (2001), and Boli *et al.* 2013. The mycoflora of commonly associated with groundnut and groundnut product samples belong to the genera *Aspergillus, Penicillium, Rhizopus* and *Fusarium* and there were found present in the samples studied. Often times, *Rhizopus* species may also occur as a common saprophyte of many foods both at pre-harvest and post-harvest stages. Boli *et al.,* (2013) recorded the occurrence of these species of fungi among others in their work on peanut butter from Benin. These fungal species are reported to be commonly associated with the raw material (groundnut), amongst other materials, in their spore forms either in the field during plant propagation, nut storage, product preparation, packaging or storage of finished product. They may also end up initiating deterioration of the food material and/or produce spores which assist their survival (Mupunga *et al.,* 2017).

It was instructive that in all the pooled samples of the different regional groundnut and groundnut products, the presence of aflatoxin was detected. While the aflatoxin content in the samples from Niger *Kukikuli,* Kano peanut butter and all the roasted groundnut samples were below 20 ppb, the remaining samples had above this FDA recommended concentration. The Kano and Kaduna *Yaji* samples, Kaduna and Ibadan *Kulikuli*, Kano, Kaduna and Ibadan *Donkwa* all had above 30 ppb aflatoxin content. These findings are worrisome, indicating that in 64.7% of the samples analysed, aflatoxin content was above the acceptable universal limit. Continual ingestion of these aflatoxin-contaminated RTE foods would impair health over time and cause a country loss in man-power. Seeing that out of all the groundnut products investigated for Aflatoxin concentration, only 35% of total sample were below the Minimum allowed limit, according to the regulatory levels issued by the Food Drug Administration (FDA) of United States (The FDA levels for aflatoxin intake for humans is maximum of 20ppb), this study also lends weight to other previous studies which reported high incidence of aflatoxin in these snacks. Even though the fungal count of the Ibadan roasted groundnut samples was relatively high, this did not directly correlate to its aflatoxin concentration which was very low. Conversely, while the least fungal counts were recorded in Ibadan *Donkwa*, the sample still recorded the third highest aflatoxin content which might be as a consequence of the association of aflatoxigenic fungi with the product right from the raw material, storage, product handling and packaging stage (Mupunga *et al.* 2017). This also showed that the fungal load cannot be directly correlated to mycotoxin content. Ogunsanwo *et al*., (2004), reported that positive correlations occurred between loss of aflatoxins in the groundnut seeds and the roasting conditions, and concluded that the production/preservation methods used by traders might promote/otherwise the growth of fungi and aflatoxin production. While the occurrence of deoxynivalenol was detected in eleven samples, all these samples still contained lower than the permissible 1.00ppb limit with the Kano *Donkwa* being the most contaminated sample (0.67ppm). This may be as a result of the presence of a mixture of corn and groundnut (which might readily be contaminated by *Fusarium* species) contained in the sample. Onilude *et al*., (2012) also reported the occurrence of toxigenic fungi (majorly *Aspergillus* and *Fusarium* species) in maize samples in Ibadan. It is possible that the detection method employed enhanced the detection of these low deoxynivalenol values indicating its precision. Adebayo-Tayo *et al.,* (2015), also reported the production of deoxynivalenol by some strains of *Fusarium* species isolated from samples of smoke-dried frog.

By virtue of their immediate handling, packaging and storage conditions, groundnut products are usually exposed to the environment and these may contribute to the different moisture content levels recorded in the study. The moisture content of groundnut butter was low and was in agreement with the report of Boli *et al*. (2013). Except for two *Yaji* samples, moisture content values were lower than that recorded in groundnut seeds (7.48 %) by Ayoola and Adeyeye, (2010) and this fact could be explained by the decrease of moisture content during the roasting which is an important step of groundnut butter processing (Campos-Mondragón *et al*., 2009). The proximate composition analysis of the groundnut products in the study revealed an overall high protein content with the crude protein content highest in the Kaduna *Kulikuli* sample (43.93±0.03%). The percentage range of crude protein recorded in the *Kulikuli* samples (34.95-43.93%) was slightly higher than that recorded (32.4%) by Aletor and Ojelabi (2007). *Donkwa* samples, a blend of groundnut, sugar and any grain, especially maize recorded the least value. Fat content range were significantly different and the general trend of a high crude protein and fat content in the samples studied was as a result of the inherent content in the starting raw material (Groundnut), which, even after the processing techniques was still available in the finished RTE product. Even in *Kulikuli* processing, which involves extraction of some of the oil during the kneading process, the chunk is still deep fried inside the groundnut oil, hence its high fat content. The protein and fat content of roasted groundnut realized in this work conformed to the report of Ayoola and Adeyeye (2010). Fat content of peanut butter obtained in this report shows a high correlation with the values reported by

Boli *et al*. (2013) while protein content was higher than what Boli *et al*. (2013) reported. The carbohydrate content relatively high in *Donkwa* samples, maybe as a result of the carbohydrate based cereal mixture (maize and sugar) used during its production. The differences in nutritional composition may possibly be due to their various ingredients/composition.

From this study, result of mineral analysis showed that groundnut samples had a high composition of Calcium, Phosphorous, and Magnesium thus depicting the richness of groundnut in supplying nutrients to man.

In conclusion, the roasted groundnut, *Kulikuli*, *Yaji*, peanut butter and *Donkwa* samples used in this study were contaminated with moulds, and the probable proliferation of these microbes under favourable conditions might predispose the snacks to become vehicles of food intoxication through mycotoxin production. Since some food samples showed a high incidence of fungal contamination but had low mycotoxin concentration, the fungal contamination/load did not correlate with aflatoxin/deoxynivalenol concentration in the samples. The study revealed the incidence of aflatoxin in groundnut-based snacks relatively higher than the acceptable level for human consumption (20ppb). This implies that consumption of these products, although highly nutritional, may pose a threat to the health of the general public, since groundnut snacks are an essential part of daily diet.

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