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MICROBIAL QUALITY AND AFLATOXIN CONTAMINATION OF READY-TO-EAT PEANUT-BASED INDIGENOUS SNACKS VENDED ON THREE HIGHWAYS IN SOUTHWEST NIGERIA

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Abstract
This study investigated exposure level to pathogenic microorganisms and potential risk to aflatoxins for consumers of kulikuli and donkwa vended along three highways in Southwest Nigerian. A total of 162 samples from vendors along Akure-Owo, Akure-AdoEkiti and Ibadan-Ogbomoso highways were subjected to microbiological examination to determine microbial quality, incidence of pathogenic bacteria and aflatoxin contamination. Aerobic colony, staphylococcal, salmonella and fungal counts were $1.19x10^4$-$3.00x10^4$ cfu/g; $1.05x10^2$-$1.80x10^2$ cfu/g; $1.05x10^2$-$2.60x10^2$ cfu/g and $1.2x10^4$-$8.0x10^5$ cfu/g, respectively. Although coliforms and E. coli were not detected, high Salmonella count is above acceptable microbiological quality and may pose public health risk. Thirteen (13) bacterial (Corynebacterium spp. predominated) and seven (7) fungal species, predominated by Aspergillus spp. were isolated. Pathogenic microorganisms present included Staphylococcus aureus subsp. aureus, Listeria monocytogenes, Bacillus cereus var mycoides, Salmonella choleraesuis subsp. Choleraesuis serovar gallinarum, Aspergillus flavus, Aspergillus parasiticus, Fusarium compacticum and Penicillium oxcalicum. High percentage occurrence of 83.33% was obtained for Staphylococcus aureus subsp. aureus, Aspergillus flavus and Aspergillus parasiticus, while Listeria monocytogenes and Salmonella choleraesuis subsp. Choleraesuis serovar gallinarum both had 66.67% occurrence. High exposure levels ≥50% were estimated for Listeria monocytogenes (55.56%), Staphylococcus aureus subsp. aureus (50%) and Aspergillus parasiticus (66.67%), indicating high probability of infection or poisoning on consumption of these snacks. Aflatoxin contents ranged from 3.0-46.0 µg/kg, with AFB1 having highest values especially in the donkwa samples from all three locations. These values which are higher than 15 µg/kg recommended by NAFDAC as the Maximum Allowable Limits of aflatoxins pose serious health risks to consumers of these snacks.

Keywords: *Aflatoxin, Food-borne disease, Food safety, Kulikuli, Mycotoxigenic moulds, Pathogenic microorganisms, Peanut-based snacks, Southwest Nigeria*

Introduction

In Africa including Nigeria, street snack foods constitute a major meal for both urban and rural dwellers due to their availability, affordability and accessibility (Draper, 1996). They are often sold either from temporary or permanent structures since most of the vendors take their wares to places where they can attract large numbers of customers. As such, stalls are set up in busy places, including railway stations, market places, motor parks, schools, street pavements, office centers, and industrial sites; usually in pushcarts, baskets or balance poles (FAO, 2007; Hiamey *et al*., 2015). Particularly, snack foods vended on the highways do not only serve to satisfy hunger pangs during the long stretch of travelling hours on the road; they also serve as a means of relieving boredom associated with long road travels.

Despite the contribution of street foods to meeting the nutritional requirements of a large number of the population (over 2.5 billion people are reported to consume street food on a daily basis worldwide), there are food safety issues surrounding their production, processing, packaging and distribution. Globally, these issues constitute a growing concern (and more so for developing countries) because food-borne diseases caused by biological, physical and chemical contamination do affect the health of millions of consumers of these ready-to-eat street foods (FAO, 2007; Aluko *et al*., 2014; Roesel *et al*., 2015). Specifically, microbiological hazards pose the biggest challenge to safety of these foods and threat to health of consumers in many parts of the world since potentially harmful microorganisms can grow rapidly in street-vended foods due to the supporting environmental conditions. This is even worse with foods/snacks hawked on the highways where the level of exposure to microbial contamination may be higher because of the highly-mobile nature of highway businesses/vendors in comparison with other street

vendors who only display their wares in stationary stalls. Often, the highway vendors run after moving vehicles to catch up with their prospective customers in cars and buses. During this process, most of these foods drop on the ground and are thereafter picked and only dusted off. Also, the snacks are usually packaged in hand-knotted thin polythene bags which predispose the snacks to microbiological contamination (Oko *et al*., 2015). Moreover, the foods are often displayed in the open exposing them to dust and fumes from moving vehicles.

One class of street foods most commonly vended on the highways is peanut products and common among these are peanut cake (known locally in Nigeria as "kulikuli") and spiced peanut-maize balls ("donkwa"). These products are by-products from peanut processing to which flavour-enhancing ingredients such as pepper, alligator pepper, salt, sugar and some spices have been added during preparation (Mayhew and Penny, 1998). Despite being rich sources of cheap and available sources of protein, crude fibre and minerals, their consumption may pose food safety threats to the consumers. This is due to their high susceptibility to aflatoxin contamination which may occur as a result of the infection of the peanut while still in the fields before harvest, during harvesting, sorting and washing of the nuts before storage. During storage, the fungi grow and subsequently produce mycotoxins when not properly handled or stored under conditions such as high temperature and humidity that support their growth and mycotoxin production (Abdel-Hafez and Saber, 1993; Adetunji *et al*., 2014). Aflatoxins produced by *Aspergillus flavus* and *Aspergillus parasiticus*, are of greatest concern among mycotoxins because they have high carcinogenic, teratogenic, and mutagenic activity and are majorly hepatotoxic (of which AFB1 is a very potent hepatocarcinogen). They have been reported to be potent liver toxins in all animals in which they have been tested, carcinogenic in some species and can produce kidney problems and hepatocellular carcinoma (IARC, 2006). Unfortunately, high levels of aflatoxin B1, B2, G1, G2 and citrinin have been reported in various street-vended foods; with positive correlations having been established between consumption of aflatoxin-contaminated foods and increased incidence of liver cancer.

In Nigeria, high concentrations of aflatoxins have been detected in kulikuli and donkwa and deaths of some primary school children resulting from consumption of kulikuli contaminated with incriminating levels of Aflatoxins were recorded in 1988 (Fapohunda, 2011). Also, several authors have reported the presence of bacterial pathogens including *Klebsiella* spp., *Staphylococcus aureus*, *Bacillus cereus*, *E. coli*, *Pseudomonas aeruginosa*, *Streptococcus feacalis*, *Micrococcus* spp., *Enterobacter*, *Salmonella*, *Shigella*, *Proteus* and *Clostridium spp.* (Adebesin *et al.*, 2001; Adjou *et al*., 2012; Ezekiel *et al*., 2011). These studies have focused on street-vended kulikuli and donkwa sold in stationary stalls in markets and streets from various parts of Nigeria. This study has therefore evaluated the microbiological quality and aflatoxin content of peanut-based snacks sold on selected Southwestern highways in Nigeria and potential risks for highway travelers. This is expected to provide baseline information on the level of exposure to aflatoxin for road travelers and other family members (especially children) who often share from leftovers of these snacks when taken home.

Materials and Methods

Sampling Locations and Collection

This study was carried out from May to December, 2018. Three highways in Southwest Nigeria were chosen for sampling because of their busy traffic and link/connectivity to other parts of Nigeria. Akure-Owo (48.4Km) is linked to Benin expressway and the Eastern part of Nigeria; Akure-Ado Ekiti (47.8Km) is linked to some other interior parts of the Southwest, while Ibadan-Ogbomoso (97.7Km) connects the Southwest to Northern Nigeria. Each of the highways was divided into three points. From each of these points, triplicate samples of kulikuli and donkwa were randomly purchased from 3 different vendors, thus making a total of 162 samples (i.e. 9 points in all multiplied by 3 vendors in each of the points multiplied by triplicate samples from each of the vendors = 81 of kulikuli and 81 of donkwa). The samples were collected into clean, dry polyethylene bags, labeled appropriately, placed into an insulated plastic box containing ice flakes and transported to the Food Microbiology Laboratory of the Food Science and Technology Department of Federal University of Technology, Akure, Nigeria for analysis. The samples from the 3 points from each of the highways were mixed together (kulikuli and donkwa separately) to form a composite sample/highway. The composited samples were aseptically blended and 50g were kept in sterile polyethylene bags and labeled appropriately. Samples not immediately analyzed were placed in clean, dry plastic containers with lid to avoid contact with moisture and stored at $0^{o}C \pm 1$ until analyzed.

Microbiological Examination of the Peanut-based Snacks

The aerobic colony, Salmonella, coliform, *E. coli*, Staphylococcal and fungal counts were determined by the dilution plate technique using Nutrient and plate count, Salmonella-Shigella, MacConkey, Eosin methylene blue, Mannitol salt and Potato dextrose (acidified with lactic acid) agars, respectively as described by Olutiola *et al*. (1991). The agars were prepared according to manufacturers' instructions and sterilized in an autoclave at 121^{o} C for 15 min at 0.15MPa. Ten grams (10 g) of each of the samples were homogenized in 90 ml sterile diluent (0.85% NaCl) using a clean Waring blender for 30 secs at normal speed and 1ml from this mixture was serially diluted in 9ml sterile diluent. Aliquots of 0.1ml and 1ml of the diluted samples were dispensed into triplicate sterile petri dishes. Thereafter, 20ml of

sterilized molten nutrient and plate count agars (for aerobic colony count) and acidified PDA (1 ml lactic acid in 100 ml PDA for fungal count) were poured separately into each of the petri dishes and labelled appropriately. The plates were swirled gently and allowed to solidify. NA and PCA plates were incubated at 32°C ±2 for 24 h while PDA plates were incubated at ambient temperature (25° C ±2) for 72 h. Visible colonies which appeared at the end of the incubation period were counted using a digital illuminated colony counter (A. Gallenkamp Co. Ltd.) and the counts expressed in colony forming units per gram (cfu/g) of the samples. Microbial counts were calculated using the formula:

$$\text{cfu/g} = \frac{\text{Number of colonies} \times \text{reciprocal of the dilution factor}}{\text{Plating volume (1 ml)}} \quad (1)$$

Determination of Percentage Occurrence of each Isolate in the Samples and Exposure Assessment of Consumers of the Peanut-based Snacks Consumers to Pathogenic Bacteria/Mycotoxigenic Moulds

The percentage occurrence of each isolate in the samples was determined by dividing the number of samples in which each isolate was detected by the total number of samples (that is 6 – 3 kulikuli and 3 donkwa samples) and expressed as percentage.Percentage occurrence of each isolate =

$$\frac{\text{No. of samples in which isolate occurred}}{\text{Total number of samples (i.e. 6)}} \times 100 \quad (2)$$

The exposure level of the consumers of these snacks to each pathogenic bacterium/mycotoxigenic mould was estimated by dividing the total number of times each known pathogenic bacterium/mycotoxigenic mould was detected in the samples (as identified from the triplicate experiments) by the total number of experiments (which is 18, that is 6 samples poured into triplicate petridishes) and expressed in percentage as illustrated below:

Exposure potential =

$$\frac{\text{Number of times each pathogenic bacterium/mycotoxigenic mould was detected}}{\text{Total expected occurrence of each pathogenic bacterium/mycotoxigenic mould (18)}} \times 100 \quad (3)$$

Quantification of Aflatoxin Content in the Peanut-Based Snacks

Aflatoxin concentration in the samples was extracted using the methods described by William *et al*. (2004) with slight modification as reported by Ezekiel *et al*. (2012). About 5g of each sample was weighed into 250ml conical flask, 25ml of methanol water (60:40 $^{v}/_{v}$) mixture were added and shaken thoroughly for approximately 30 min in a mechanical shaker. The solution was allowed to settle and filtered through a Whatman No. 1 filter paper. The filtrate was transferred into a 250ml separating funnel, 30ml of saturated sodium chloride (NaCl) and 50ml hexane were added, stopped and shaken vigorously for 2 min. The solution was allowed to separate. The lower methanol water layer was collected into another clean dry 250ml separating funnel, 50ml chloroform was added and again shaken vigorously. The chloroform layer was drained into a 250ml conical flask containing 5g of Cupric Carbonate, shaken together and allowed to settle. The mixture was filtered through a Whatman No. 42 filter paper having a bed of anhydrous sodium sulphate into a 250ml beaker. The cupric carbonate was washed off again with 25 ml chloroform and filtered through the sodium sulphate bed into the 250ml beaker. The chloroform extract was evaporated on a water bath to dryness to remove residual water. The residue was reconstituted in 2ml chloroform and transferred into a screw-caped tube for the quantitative estimation. To analyze the extracts for aflatoxin concentration, 40µl extract and 50µl aliquots of 0.50 µg/ml total aflatoxin (TA) standards were spotted and separated on pre-coated TLC plates (silica gel 60 F254; 20 × 10cm; Merck, Germany) in chloroform-acetone-water (88:12:1.5). The plates were dried and visualized under UV light at 365nm. The aflatoxin bands for each sample spot were identified on the basis of characteristic fluorescence and co-migration with aflatoxin standard. Aflatoxin concentrations (µg/kg) in the samples were estimated as described by Atanda *et al*. (2011).

Aflatoxin Concentration in µg/kg was calculated using the formula:

$$\frac{\text{Absorbance of sample x standard concentration dilution factor x 1000}}{\text{Weight of Sample}} \quad (4)$$

Statistical Analysis

All analyses (except otherwise stated) were carried out in triplicate. Means were separated using one-way analysis of variance (ANOVA) and significant differences were determined using Duncan's new multiple range test at $p<0.05$. Colony counts of samples were calculated as cfu/g.

Results and Discussion

Microbial Quantity of Peanut-based Snacks from Some South-Western Highways of Nigeria

Microbiological examination of the peanut-based snacks obtained from three highways in Southwest, Nigeria revealed aerobic colony count of $1.19x10^{4}$-

$3.00x10^4$cfu/g (Table 1). Coliforms and *E. coli* were not detected in the samples. Kuli-kuli (KI) and donkwa (DI) samples from Ibadan-Ogbomoso had the highest aerobic colony counts of $3.0x10^4$ and $2.35x10^4$ cfu/g, while kulikuli from Akure-Owo (KA) and donkwa from Akure-Ado-Ekiti (DE) had the least. This may indicate poor sanitary and unhygienic conditions during processing, handling, distribution and sale of the samples in this area. Also, the heavier traffic and longer distance of this route as compared to the two other routes which may invariably expose the snacks to more dust and vehicle fumes may further be a contributing factor to these higher counts. In addition, control of raw materials, processing and environmental conditions may be critical factors in the prevention of microbial contamination in peanut-based indigenous snacks (Abdullahi *et al*., 2005; Owhe-Oreghe and Afe, 1993). However, aerobic colony counts obtained in all the samples in this study are within limits of acceptable microbiological quality ($\leq 1.0\times10^3$ - $\leq 1.0\times10^7$) as recommended by ICMSF (2007) for ready-to-eat foods.

Table 1: Microbial Count (cfu/g) of kulikuli and Donkwa Vended on Three Highways in Southwest Nigeria

Sample	ACC $x10^4$	Coliform	*E.coli*	*Staphylococcus* spp. $x10^2$	*Salmonella* spp. $x10^2$	Mould $x10^5$
KA	1.38^{d}±0.01	ND	ND	1.80^{a}±0.13	1.55^{c}±0.01	0.12^{c}±0.01
KE	1.93^{b}±0.02	ND	ND	1.05^{c}±0.01	1.05^{d}±0.10	4.00^{b}±0.11
KI	3.00^{a}±0.01	ND	ND	1.30^{b}±0.01	2.60^{a}±0.02	0.16^{c}±0.01
DA	1.44^{c}±0.01	ND	ND	1.80^{a}±0.20	2.00^{b}±0.12	8.00^{a}±0.02
DE	1.19^{e}±0.02	ND	ND	1.25^{b}±0.20	2.60^{a}±0.13	4.00^{b}±0.02
DI	2.35^{a}±0.03	ND	ND	1.05^{c}±0.01	2.05^{b}±0.01	8.00^{a}±0.02

Values are means (n=3) ±SE. Means with different superscript letters on the same column are significantly different at p < 0.05

Keys: KA - Kulikuli (Akure-Owo highway); KE - Kulikuli (Akure-Ado-Ekiti highway); KI - Kulikuli (Ibadan-Ogbomoso highway); DA - Donkwa (Akure-Owo highway); DE - Donkwa (Akure-Ado-Ekiti highway); DI - Donkwa (Ibadan-Ogbomoso highway); ACC = Aerobic Colony Count; ND – Not detected

Staphylococcal counts ranging from $1.05x10^2$-$1.80x10^2$ cfu/g obtained here are classed as satisfactory to marginal and may as such not constitute any potential hazards to human health since these values are within the acceptable microbiological limits for *Staphylococcus* spp. in ready-to-eat foods ($<10^2$ and $<10^2$-10^3 cfu/g for satisfactory to marginal) (European Commission Regulation (EC) No 2073/2005; ICMSF, 2007). Despite these findings, maintenance of these low counts cannot be guaranteed since the samples were collected from each location early enough, stored at low temperatures during transportation to the laboratory and stored at low temperatures in the laboratory during analysis. However, the situation is entirely different from what obtains at the point of sales/locations of these snacks where foods are held at high temperatures under the sun and subjected to unhygienic handling conditions that do not meet food safety standards. Thus, these may support the increase in numbers of coagulase-positive *Staphylococcus* spp. and enterotoxin production in the samples which may result in staphylococcal food poisoning. The presence of *Staphylococcus* spp. in the samples may be an indication of unhygienic handling and direct contact of the snacks with either the processors or vendors since humans are usually the major carriers of *Staphylococcus* spp. (Jay *et al*., 2005).

On the other hand, *Salmonella* spp. count ranging from $1.05x10^2$-$2.60x10^2$ cfu/g is above acceptable microbiological quality and may pose a public health risk since these snacks are usually consumed without further heating. ICMSF (2007) guidelines for microbiological quality of ready-to-eat foods specify that the detection of Salmonella in 25 g of ready-to-eat food is potentially hazardous while its absence or non-detection in 25g food is satisfactory. Thus, ready-to-eat foods are expected to be free from *Salmonella* spp. and their presence, even in small numbers, results in such foods being of unacceptable quality/potentially hazardous. According to ACHC (1997), Salmonella or other pathogens in ready-to-eat foods may not always result in illness, however, several microbiological and epidemiological evidences have established the occurrence of illness resulting from the ingestion of small numbers of pathogens in foods. The salmonellae are small, Gram-negative facultative anaerobic, non-spore forming rods that are widely distributed in nature with most of the serotypes being pathogenic to humans and animals who are their primary reservoirs. Hence, their presence in these samples may suggest cross contamination from either the processors, handlers or vendors of the snacks. They have been reportedly implicated in a number of food borne diseases including typhoid and paratyphoid fevers when they grow in food using it as a vector for their transport to the human GIT (Jay *et al.*, 2007).

High fungal counts ranging from $1.2x10^4$–$8.0x10^5$ cfu/g obtained for all the samples may be attributed to contaminated raw materials which may be from improper harvesting and storage methods/conditions, improper product handling during processing and

vending, storage condition of the finished product and exposure to fungal spores in the air at the vending locations. These high numbers may pose health risks for the consumers since moulds are often implicated in mycotoxicosis. Furthermore, the higher fungal counts obtained for donkwa as compared to kulikuli may be linked to differences in moisture content and type of raw materials used. Usually, preparation of donkwa involves first reducing the moisture content of the raw materials (dried maize grains and roasted peanut) before milling into flour as compared to kulikuli which is usually made from groundnut meal after oil extraction. Moreover, cereals usually carry large numbers of mould and have high mycotoxin content since their rich nutrient composition and low water activity support mould growth and mycotoxin production (Maxwell *et al*., 2000; Jay *et al.,* 2005; Atehnkeng *et al*., 2008). Thus, the inclusion of maize in donkwa may explain the higher fungal count as compared to kulikuli. Among cereals, maize has been specifically reported to contain large numbers of fungi in comparison with the small grains (sorghum, rice, wheat, millet and rice) which are less susceptible to fungi and toxin contamination than the larger grains like maize (Maxwell *et al*., 2000; Atehnkeng *et al*., 2008).

Microbial Ecology and Percentage Predominance of Isolates in Kulikuli and Donkwa Samples

Results of colonial, morphological and biochemical characterization of the microbial isolates from the kulikuli and donkwa samples showed that a total of twenty (20) isolates was obtained which included thirteen (13) bacteria and seven (7) moulds (Tables 2 and 3).

Table 2: Presumptive identification of bacteria isolated from the peanut-based snacks

	Colonial characteristics			Morphological characteristics			Biochemical characteristics												Sugar fermentation						Probable identity
_	Pigmentation	Shape	Edge	Shape	Gram reaction	Spore	Growth on MCA	Motility	Catalase	Gelatin	Oxidase	Urease	Nitrate	Citrate	Indole	H_2S	Coagulase	Hemolysis	Xylose	Sucrose	Lactose	Mannitol	Glucose	O/F	
1	Cream white	Circular	Entire	Long rod	+	+(T)	-	-	+	+	+	-	+	+	-	+	-	-	+	+	-	-	+	O	*Bacillus cereus* var. *mycoides*
2	Cream white	Circular	Entire	Short rod	+	-	+	+	+	-	-	-	-	+	-	-	-	+	-	+	-	-	-	F	*Listeria monocytogenes*
3	Cream white	Irregular	Fimbriate	Slender rod	+	-	-	-	+	-	-	-	+	+	-	+	-	-	-	+	-	-	+	F	*Corynebacterium minutissimum*
4	Cream white	Irregular	Fimbriate	Slender rod	+	-	+	-	+	-	-	-	+	+	-	+	-	+	-	-	-	-	+	F	*Corynebacterium xerosis*
5	Cream white	Irregular	Undulate	Cocci	+	-	+	-	-	-	-	-	+	+	-	-	-	+	-	-	+	+	+	F	*Streptococcus salivarius*
6	White	Irregular	Undulate	Short rod	-	-	+	-	+	+	-	-	+	+	-	-	-	+	-	-	-	A	-	F	*Salmonella choleraesuis* subsp. *Choleraesuis* serovar *gallinarum*
7	Cream white	Circular	Entire	Cocci	+	-	+	-	+	-	-	+	+	+	-	+	+	+	-	+	+	+	+	F	*Staphylococcus aureus* subsp. *aureus*
8	Cream	Circular	Entire	Cocci	+	-	+	-	+	+	+	+	-	-	-	-	-	-	+	+	+	-	-	-	*Micrococcus halobius*
9	Cream white	Circular	Entire	Slender rod	+	-	+	-	+	+	-	+	-	+	-	+	-	+	-	-	-	-	+	F	*Corynebacterium ulcerans*
10	Cream	Circular	Entire	Slender rod	+	-	+	-	+	-	-	+	+	-	-	+	-	-	-	+	-	-	+	F	*Corynebacterium glutamicum*
11	Cream	Irregular	Undulate	Cocci	+	-	+	-	+	-	+	-	-	-	-	+	-	+	+	+	-	-	-	O	*Micrococcus lylae*
12	Cream	Circular	Entire	Cocci	+	-	+	-	+	-	-	-	+	+	-	-	-	-	-	-	-	-	+	F	*Staphylococcus carnosus*
13	Cream	Circular	Entire	Slender rod	+	-	+	-	+	-	+	+	+	+	-	+	-	+	+	-	-	-	+	F	*Corynebacterium cystitidis*

Key: ND – Not determined

Table 3: Characteristics of Moulds Isolated from the Peanut-based Snacks (kuli-kuli and Donkwa)

Isolate	Description of Isolate	Identity of moulds
1	Yellowish-green becoming green with age. The reverse is creamish-yellow. The head radiates and becomes loosely columnar with age. The stipe is long, verrucose and hyaline. It has a small metule. Phialides borne directly on the vesicle. Conidia globose to subglobose.	*Aspergillus flavus*
2	Blackish-brown often with yellow mycelium. Reverse greenish - yellow to yellow -orange. Its head globose, splitting with age. Its metulae are long closely packed and brownish, which is often septate. Conidia globose to subglobose.	*Aspergillus niger*
3	Conidia heads mostly radiate or split into fine columns or rarely globose, small, primuline yellow, or wax yellow citrine.	*Aspergillus parasiticus*
4	The colony is rusty -brown when viewed, but pale brown on the reverse. The stripe is long and rough. The head is partly globular, the conidia are thick and strongly roughened. Orange-yellow.	*Aspergillus tamarii*
5	Floccose in texture, whitish-cream and deep rose-red to burgundy in reverse. Chlamydospores are abundant in chains or clusters, rough golden yellow.	*Fusarium compacticum*
6	The stipe is small and smooth. Greenish grey colony while reverse side is pale to brown. The penicillin is biverticillate, philades ampliforms. It has a medium -sized collula. The conidia are spherical, smooth and greenish. Conidia sub-globose to ellipsoidal.	*Penicillium chrysogenum*
7	The texture is velutimous, sporulation very heavy. Colony is greenish-grey while the reverse is pale yellow. The stipes are long and smooth. The penicillium is asymmetrically biverticulate, metulae closely appressed, phialides acerose, collula very short. The conidia are ellipsoidal, large, smooth, pale green.	*Penicillium oxcalicum*

Of the bacterial species identified, *Corynebacterium* spp was predominantly present with 5 different species representing 38.46 % of the bacterial species isolated and 25 % of the total microorganisms isolated (i.e. including the moulds). However, two (2) species each of *Staphylococcus* and *Micrococcus* species were isolated which represent 15.39 % of bacteria isolated and 10% of the total microbial species. Only one (1) species each of *Bacillus* (*B. cereus* var. *mycoides*), *Listeria* (*L. monocytogenes*), *Salmonella* (*S. choleraesuis* subsp. *Choleraesuis* serovar *gallinarum*) and *Streptococcus* (*S. salivarius*) was isolated (Table 4). Previous studies on kulikuli and donkwa have reported the presence of *Staphylococcus aureus*, *Bacillus cereus*, *Streptococcus faecalis*, *Micrococcus* spp., *Enterobacter* and *Salmonella* among other bacterial species (Adebesin *et al.,* 2001; Ezekiel *et al*., 2011; Adjou *et al*., 2012; Oko *et al*., 2015). However, this study reported the presence of *Listeria monocytogenes* which has not been previously reported. *Listeria monocytogenes* is a Gram positive bacterium that is widespread in nature and is a transient flora excreted by 1-10 % of healthy humans. It is pathogenic to both animals and man and is reported to cause abortion in pregnant women and meningitis in newborn infants and immunocompromised adults. Also, the presence of *Staphylococcus aureus* reported in this study may be of public health importance since this bacterium produces a heat-stable enterotoxin which causes gastroenteritis (Frazier and Westhoff, 1998; Jay *et al.* 2005).

Although other enterobacteriaceae often associated with faecal contamination were not detected, the presence of Salmonella in the samples is of public health importance since the intestinal tracts of humans and animals are their primary habitats. Most *Salmonella* species have also been reported to be pathogenic or opportunistic, being capable of causing several diseases in animals and man including typhoid fever, septicemia and gastroenteritis, when taken into the body in sufficient numbers (Jay *et al*., 2005). The presence of human pathogens may be linked to the unhygienic and manual processing methods of hand mixing, pressing and moulding employed during production of kulikuli and donkwa. Hence, there is need for mechanization of the production of these snacks to reduce direct human contact and contamination of the snacks which may cause foodborne disease outbreaks among the consumers.

Seven (7) fungal species were identified including 4 *Aspergillus*, 2 *Penicillium* and 1 *Fusarium* species. *Aspergillus* predominated the fungal species representing 57.14 % of the moulds and 20% of the

total microorganisms isolated from the samples. Among these were *A. flavus* and *A. parasiticus* which have been reported as the major producers of aflatoxin B_1 (a potent human carcinogen) in groundnut and groundnut-based products (Bankole and Adebanjo, 2003). Also, *Aspergillus niger* synthesizes ochratoxin A which is nephrotoxic, genotoxic and teratogenic; hence the presence of these moulds in these samples may be of immense public health importance. However, Ezekiel *et al*. (2012) reported that Aspergillus *tamarii* isolated from groundnut-based snacks did not produce aflatoxin. Their presence may be due to unhygienic environmental conditions, unwholesome raw materials which are already contaminated with moulds and poor handling of the products during processing and distribution. Also, the exposure of the snacks to air may result in their contamination with mould spores since these are often present in the air. Previous studies have reported the presence of *Aspergillus, Penicillium* and *Fusarium* in kulikuli, donkwa and other peanut-based snacks, with *Aspergillus* being predominant (Adebesin *et al.*, 2001; Ezekiel *et al.,* 2011; 2012).

Table 4: Percentage Predominance of Bacteria And Moulds Isolated from Kulikuli and Donkwa Vended on Three Highways in Southwest Nigeria

Genera of organisms isolated	**Species detected in the peanut based snacks**	**Percentage predominance (%) of each genus with respect to total number of bacterial/fungal species isolated from the peanut -based snacks**	**Percentage predominance (%) of each genus with respect to total number o f microbial species isolated from the peanut -based snacks**
Bacteria			
Bacillus	*Bacillus cereus* var. *mycoides*	7.7	5
Corynebacterium	*Corynebacterium cystitidis*, *Corynebacterium glutamicum*, *Corynebacterium minutissimum*, *Corynebacterium ulcerans*, *Corynebacterium xerosis*	38.46	25
Listeria	*Listeria monocytogenes*	7.7	5
Micrococcus	*Micrococcus halobius* , *Micrococcus lylae*	15.39	10
Salmonella	*Salmonella choleraesuis* subsp. *Choleraesuis* serovar *gallinarum*	7.7	5
Staphylococcus	*Staphylococcus aureus* subsp. aureus, Staphylococcus *carnosus*	15.39	10
Streptococcus	*Streptococcus salivarius*	7.7	5
Total number of bacterial species isolated from the snacks = 13			
Moulds			
Aspergillus	*Aspergillus flavus*, *Aspergillus niger*, *Aspergillus parasiticus* , *Aspergillus tamari*	57.14	20
Fusarium	*Fusarium compacticum*	14.29	5
Penicillium	*Penicillium chrysogenum*, *Penicillium oxcalicum*	28.57	10
Total number of mould species isolated from the snacks = 7			
Total number of all microbial species isolated from the snacks = 20			

Incidence and Percentage Occurrence of the Microorganisms, and Potential Exposure Level to Pathogenic Microorganisms in the Peanut-based Snacks

Table 5 presents the incidence of the occurrence in the snacks, their percentage occurrence and the exposure level of the consumers of these snacks to known pathogenic bacteria and mycotoxigenic moulds. Results obtained showed that while *Bacillus cereus* var *mycoides*, *Corynebacterium glutamicum*, *Aspergillus tamarii* and *Penicillium chrysogenum* had a 16.67 % occurrence, having occurred in only one (1) out of the six (6) samples, organisms like *Corynebacterium ulcerans*, *Micrococcus halobius* and *Staphylococcus carnosus* had a 66.67 % occurrence being isolated from 4 out of the 6 samples. Of particular interest are the pathogenic organisms (including *Staphylococcus aureus* subsp. *aureus*, *Aspergillus flavus* and *Aspergillus parasiticus*) which all had very high percentage occurrence of 83.33 %, being present in 5

out of the 6 samples, while *Listeria monocytogenes* and *Salmonella choleraesuis* subsp. *Choleraesuis* serovar g*allinarum* both occurred in 4 out of the 6 samples giving a percentage occurrence of 66.67 %. This calls for serious concerns and is a high indication that most of these street-vended foods, especially those that are ready-to-eat may be heavily contaminated with pathogenic organisms which may result in disease outbreaks and thus unsafe for direct human consumption.

The potential exposure for consumers of these snacks to pathogenic bacteria and mycotoxigenic moulds estimated for *Bacillus cereus* var. *mycoides*, *Listeria monocytogenes*, *Staphylococcus aureus* subsp. *aureus* and *Salmonella choleraesuis* subsp. *Choleraesuis* serovar g*allinarum*, *Aspergillus flavus*, *Aspergillus parasiticus, Aspergillus niger* and *Fusarium oxysporium* showed that only *Bacillus cereus* var. *mycoides* and *Aspergillus niger* had low exposure levels of 5.56 % and 7.69 %, respectively. However, high exposure levels ≥50 % were observed for organisms like *Listeria monocytogenes* (55.56 %), *Staphylococcus aureus* subsp. *aureus* (50 %) and *Aspergillus parasiticus* (66.67 %) hence showing that the probability of being infected or poisoned with any of these organisms or their toxins on consumption of these snacks is high (above 50 %). On the other hand, *Salmonella choleraesuis* subsp. *Choleraesuis* serovar g*allinarum*, *Aspergillus flavus* and *Penicillium oxcalicum* had exposure levels of 33.33, 38.89 and 38.89 %, respectively. Although these values are below 50 %, this may be a source of concern for consumers whose immune systems are already compromised. Also, the low exposure levels may mean that the probability of getting infected or poisoned with these organisms or their toxins may be low provided the samples do not contain sufficient number of these organisms or their toxins large enough to cause a food infection or an intoxication (infectious dose). Moreover, the high holding temperatures and unhygienic handling of these snacks by the vendors will most often result in increased numbers and support toxin production. Notwithstanding these low values or whether the numbers increase, the presence of these moulds in food has negative health implication for consumers of these snacks because when aflatoxins produced by these moulds are consumed in large doses, they are lethal and cause acute hemorrhagic syndromes. However, long term consumption of sublethal (small) doses of these mycotoxins cause liver tumors since these mycotoxins are potent carcinogens (Jay *et al.* 2005).

Table 5: Incidence and percentage occurrence of each isolate in the peanut-based snack samples and exposure levels to pathogenic bacteria/mycotoxigenic mould

Microbial isolates	Akure-Owo		Akure-Ado-Ekiti		Ibadan-Ogbomoso		Total number of times each isolate was detected in the samples (as seen in the triplicate experiment)	Number of samples in which each isolate occurred as compared to total number of samples	Percentage occurrence of each isolate in the samples (%)	Estimated Potential to Exposure of pathogenic bacteria/mycotoxigenic mould (%)
Bacteria	Donkwa	Kulikuli	Donkwa	Kulikuli	Donkwa	Kulikuli				
Bacillus cereus var. *mycoides*	1	0	0	0	0	0	1/18	1/6	16.67	5.56
Listeria monocytogenes	2	2	0	3	3	0	10/18	4/6	66.67	55.56
Corynebacterium cystitidis	1	1	0	2	2	1	7/18	5/6	83.33	
Corynebacterium xerosis	1	1	0	2	2	1	7/18	5/6	83.33	
Corynebacterium minutissimum	3	1	3	1	2	1	11/18	6/6	100	
Corynebacterium ulcerans	1	0	0	1	1	1	4/18	4/6	66.67	
Corynebacterium glutamicum	0	1	0	0	0	0	1/18	2/6	16.67	
Micrococcus halobius	0	1	1	0	1	1	4/18	4/6	66.67	
Micrococcus lylae	0	1	0	2	0	1	4/18	3/6	50	
Streptococcus salivarius	0	0	1	0	1	0	2/18	2/6	33.33	
Staphylococcus aureus subsp. *aureus*	0	1	2	2	3	1	9/18	5/6	83.33	50.0
Staphylococcus carnosus	0	1	2	0	1	1	5/18	4/6	66.67	
Salmonella choleraesuis subsp. *Choleraesuis* serovar *gallinarum*	0	1	2	0	2	1	6 /18	4/6	66.67	33.33
Moulds										

Key: 0 represents absence of the isolate in each sample; 1-11 – represents the number of times each isolate was found in each sample**Aflatoxin contamination**

Results presented in Table 6 showed that most of the donkwa and kulikuli samples had high contents of aflatoxin with values ranging from 3.0-46.0 µg/kg. Values reported here are comparable with values of 0-50 µg/kg reported in previous studies and may be attributed to the high susceptibility of corn and groundnut to infestation by aflatoxigenic fungi as compared to other crops (Vaamonde *et al*., 2003; Bandyopadhyay *et al*., 2007; Ezekiel *et al.,* 2012; Adetunji *et al*., 2018). AFB1 was the most prevalent aflatoxin in the samples, with values ranging from 17.5-46.0 µg/kg and closely followed by AFB2 (values ranged between 11-26 µg/kg). On the other hand, AFG1 and AFG2 had lower values of 4.5-10.0 µg/kg and 3.0-7.5 µg/kg, respectively. The results also showed that donkwa samples had higher aflatoxin contents than kulilkuli samples. This may be due to the presence of maize which is one of the major ingredients for preparation of donkwa. These higher counts are in agreement with previous studies where maize-based ready-to-eat foods have been reported to have higher aflatoxin contents as compared to other foods (Bandyopadhyay *et al*. 2007; Ezekiel *et al.*, 2012). Most of the values, particularly AFB1 for donkwa and kulikuli and AFB2 for donkwa samples from all the locations exceed the Maximum Allowable Limits (MAL) of 15 µg/kg recommended by NAFDAC (Nigeria). This poses serious health risks to the consumers of these snacks who are mostly children, the middle aged and younger persons, especially putting into consideration the hepatotoxic and carcinogenic nature of aflatoxins. Furthermore, these snacks have been reported to serve as cheap sources of available and affordable protein, providing essential nutrients for a large number of urban population due to its rich content of protein and crude fat similar to the raw material peanut from which it is made (Aletor and Ojelabi, 2007; Kolapo *et al*, 2012; Ejoh and Ketiku, 2013; Boli et al., 2014). Several authors have also reported the presence of large amounts of aflatoxins in kulukuli and donkwa samples from different parts of Nigeria and these snacks having being classified as ready-to-eat do not go through any form of heat processing before they are consumed. Hence, they may be said to serve as potent sources of large amounts of aflatoxins to humans.

Table 6: Aflatoxigenic status of kulikuli and donkwa vended on three highways in Southwest Nigeria

Samples	AFB1 (µg/kg)	AFB2 (µg/kg)	AFG1 (µg/kg)	AFG2 (µg/kg)	Maximum Allowable Limits (MAL) of Aflatoxin for different countries (µg/kg)	
DA	45.00^{b}±0.05	21.00^{b}±0,54	7.50^{b}±0,20	5.00^{b}±0.21	Country	MAL **(µg/kg)**
DE	34.00^{c}±0.13	17.00^{c}±0,31	7.50^{b}±0.01	5.00^{b}±0.02	China	10
DI	46.00^{a}±0.07	26.00^{a}±0.71	10.00^{a}±0.41	7.50^{a}±0.13	Japan	10
KA	21.50^{e}±0.80	14.00^{d}±0.21	6.00^{c}±0.04	4.00^{c}±0.10	Thailand	10
KE	17.50^{f}±0.17	11.00^{f}±0.02	4.50^{e}±0.12	3.50^{d}±0.11	Indonesia	15
KI	23.00^{d}±0.21	13.50^{e}±0.32	5.50^{d}±0.01	3.00^{e}±0.01	Malaysia	15
					EU	*4/**15
					NAFDAC (Nigeria)	15.0

Values are means (n=3) ±SE. Means with different superscript letters on the same column are significantly different at $p < 0.05$.

Conclusion

This study investigated the microbiological quality, aflatoxigenic status and exposure level of consumers to pathogenic bacteria and mycotoxigenic moulds of peanut cake (kulikuli) and spiced maize-peanut (donkwa) snacks vended at different locations from 3 Southwestern highways. The presence of different pathogens including Staphylococcus aureus subsp. aureus, *Salmonella choleraesuis* subsp. *Choleraesuis* serovar g*allinarum*, *Listeria monocytogenes*, and mycotoxigenic moulds including *Aspergillus flavus*, *Aspergillus parasiticus*, *Aspergillus niger*, *Fusarium compacticum* and *Penicillium oxcalicum* in the samples may be an indication of contaminated raw materials and unhygienic handling during processing, packaging and distribution. Aflatoxin contents above the Maximum Allowable Limits as recommended by NAFDAC were also obtained. These constitute a food safety issue and may pose public health risk for the consumers. In order to reduce these potential risks, control measures may include application of stringent regulatory measures by food regulatory agencies like NAFDAC in the monitoring and inspection of raw materials and regulations of the processing of these snacks. Also, educating processors on the use of wholesome raw materials and the need for strict adherence to Good Manufacturing Practices (GMP) and other safety measures so as to reduce potential food poisoning thereby safeguarding the lives of the consumers. Vendor education may be necessary to educate vendors on Good Hygienic Practices (GHP) in the handling and packaging of the snacks. Although mechanization of the processing of these snacks may result in higher production costs and invariably increase cost of the snacks, it will help to reduce direct human contact with the snacks, thereby reducing human pathogens often associated with these snacks.

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