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Quality and safety evaluations of commonly consumed foods stuff from Dawanau market, Kano State, Nigeria

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ABSTRACT

Ensuring food quality and safety is central to achieving sustainable global food security. This study investigated the quality and safety of cowpea, maize, yam chips, groundnut, melon and smoke-dried fishes from Dawanau market in Kano State. Total aflatoxin (TA), proximate compositions, microbial analysis, physical and entomological status were conducted following the official protocols of Association of Analytical Chemists. Impurity level ranged from 0.12 to 2.80% accounting for smoked dried fish and groundnut respectively. While the highest bacteria load (2.7×10^6 cfu/g) in smoked dried fish and the highest fungi load (7.2×10^5 cfu/g) in groundnut, the commonly isolated and identified bacteria and fungi were respectively *E. coli* and *Aspergillus* species. Total aflatoxin contaminations was highest (278.08 $\mu\text{g}/\text{kg}$ and 7.99 $\mu\text{g}/\text{kg}$) respectively for groundnut and melon; however, both had <10% moisture contents. After sorting, washing, and drying, a significant reduction in total aflatoxin contamination was observed in all food samples. The highest impact of sorting (62.54%) and wash-dried (73.33%) was recorded for maize, however, groundnut TA contamination remained in the three-digit range despite sorting, washing, and drying measures. It is therefore, important to enforce routine checks on foodstuffs in markets, while also, sorting, washing and drying should be encouraged as a way forward to minimize the level of total aflatoxin contaminations.

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1. Introduction

Aflatoxins are secondary metabolites produced by filamentous fungi especially the species of *Aspergillus*

and other related fungi" can be used for better precision. (1). These fungal species are often prevalent in humid tropical climates and their prevalence is exacerbated by drought, pests, delayed harvest, insufficient drying and poor postharvest handling (2).

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Agricultural products especially grains play major hosts to aflatoxins contaminations from production practices, harvest practices and storage practices which could result in trade restrictions across countries borders (3). Aflatoxins, like other mycotoxins, can be found at very low concentrations in various foods and exert multiple toxicological effects, including hepatotoxicity, nephrotoxicity, vomitoxin effects, neuro-musculotoxicity, carcinogenicity, immunosuppression, or mutagenicity, depending on the case (4,2). The complexity of food chemical composition such as water, protein, carbohydrates, lipids among others have made several technological breakthroughs to detect aflatoxins in variety of matrices even as low doses as they could be (RASFF) (http://ec.europa.eu/food/safety/rasff/index_en.htm). Total aflatoxin comprises of the B1, B2, G1, and G2 are found in plant based food, while M1 (metabolite of B1) and M2 are found in foods of animal origin (4).

Six samples including maize, cowpea, melon, groundnut, yam chips and smoked dried fish were considered in the market survey for the estimation of aflatoxin contamination levels. Maize is one of the most important food crops in the world and serve as staple food for majority of sub-Saharan African countries. It is largely susceptible to mycotoxins contamination especially aflatoxins causing high risk of human and animal health. Maize and rice at European level for instance, to be subjected to sorting and other physical treatment before consumption, a limit of 5 µg/kg (AFB1) and 10 µg/kg for aflatoxins total has been established (5). To prevent and reduce contaminations of mycotoxins in cereals and other commodities, several codes of practice and guidelines have been published (6). These include Good Agricultural

Practices (GAP) and Good Storage and Manufacturing Practices (GSP and GMP). All these are aimed at preventing fungal growth and mycotoxins production both in the field and during storage. Groundnut (*Arachis hypogaea* L.) is a leguminous oilseed crop cultivated in the semi-arid and subtropical regions of the world (7). Groundnut production in Nigeria has suffered major setbacks from the groundnut rosette epidemics and foliar diseases, aflatoxin contamination and lack of sufficient and consistent supply of seed of improved varieties. This has significantly affected productivity and thus production and subsequently led to lose its share in the domestic, regional and international markets (5,7). A large decline in international markets for groundnut is constraint due to aflatoxins contaminations which is a potential carcinogen. Melon (*Colocynthis citrillus* L.) is a highly nutritious oil seed consumed in Nigeria and across west Africa. Melon seeds and their products are highly susceptible to infection by Aflatoxigenic fungi due to ambient and relative humidity during storage (8). Aflatoxin contamination of melon seed restrict its trade at international market, hence, contributing to relative decline in nation's economic growth. Cowpea (*Vigna unguiculata* L.) remains an important native African legume crop. It is the cheapest source of protein in Nigeria and sub-Saharan Africa for its affordability and availability. Cowpea is one among many grains which could be contaminated by filamentous fungi either in the field or at postharvest (8). The major problem of cowpea is at storage since maintaining an ambient humidity levels below the range of mold development is essential to prevent fungal colonization. The presence of aflatoxins alters not only the quality of contaminated foods but also, the health of its consumers (9). Yam

chips (*Dioscorea* spp.) is third most consumed crop in sub-Saharan region, especially in West Africa. Fresh tubers are perhaps very perishable due to microbe induce rotting. To minimize huge losses, fresh yams are processed into dried chips, flakes and flour (10). During processing or storage of dried yam chips, Aflatoxigenic molds contamination could occur and lead to aflatoxins contaminations. Smoked dried fish is unarguable an important source of nutrient especially protein all over the world (11). There is high percentage of losses of fishes and quality deterioration which could predispose the health of its consumers to dangers (12). Smoked and dried fishes may be contaminated with pathogenic microorganism such as fungi species that might produce secondary metabolites (mycotoxins). Aflatoxins production on smoked fishes is endangers the nation's economy and impose food insecurity (11). This study is aimed at exploring the need for routine checks on market food quality and possible contaminations of total aflatoxins.

2. Material and Methods

2.1. Sample preparation and handling

Two hundred fifty grams sample each of maize, groundnut, melon, cowpea, yam chips and smoked dried fish samples were purchased from Dawanau market in Kano State and brought to respective laboratory for physical and chemical analysis.

2.2. Physical and entomological observation of samples

Each grain sample and smoked-dried fish were physically examined for the presence of insects and eggs under a microscope in the entomology laboratory.

2.3. Determination of bacterial load and fungi identification

About 1.0 g of each sample was serially diluted up to 10^{-4} in 9ml of sterile distilled water, 1ml of the aliquot at 10^{-4} were plated on nutrient agar and potato dextrose agar, incubated at 37°C for 18-24 h and up to 7 days at room temperature respectively. Colonies were counted manually to determine bacteria load. The fungal growths were identified after 7 days of incubation at room temperature.

2.4. Estimation of proximate composition

Proximate composition was determined for each sample to estimate percentage moisture, crude fiber, ash, protein, oil according to the methods of Association of Official Analytical Chemists (AOAC) (13) and carbohydrate content was expressed as difference of 100% and the sum of percentage compositions of proximate components.

2.5. Determination of Total Aflatoxins

Aflatoxin total (AF_{total}) was determined according to protocols of Enzyme Linked Immunosorbent Assays (ELISA). 50 µl each of standard and prepared samples (2 g in 70% methanol) was respectively added into separate wells created as standards and samples and this was followed by 50 µl of conjugate into each well. 50 µl of antibody was added to each respective well and then the mix were gently shaken and incubated for 30 min at room temperature. The liquid was thoroughly removed by turning upside down the microwell holder against absorbent paper for about three times. Then for two consecutive times, the wells were later filled with 250 µl of wash buffer and again the wells were emptied. 100 µl of substrate and chromogen was added to each well, gently shaken and incubated for 15 min at room

temperature (20-25°C). A 100 µl of stop solution was added to each well, shaken gently and read photometrically at wave length 450 nm within 30 min.

2.6. Data analysis

Experimental analysis was carried out in triplicate and expressed as means ± standard deviation. Data were analyzed using a one-way analysis of variance using SPSS 16.0 version 2007 and Tukey's test at 95% confidence level ($p < 0.05$).

3. Results

The entomological indices ranging from impurities to standard weights examined and expressed in percentages for each of the sampled food stuff are depicted in Table 1 below.

Table 1. Physical and entomological status of selected foodstuffs from Dawanau Market

| Sample | IMP(%) | INSC(%) | IEH(%) | EGG(%) | GP(%) | STW(%) |
|-----------|--------|---------|--------|--------|-------|--------|
| Cowpea | 0.70 | 0.00 | 0.00 | 5.00 | 35.00 | 5.40 |
| Groundnut | 2.80 | 0.00 | 0.00 | 0.00 | 65.00 | 8.60 |
| Yam-chip | 1.20 | 0.00 | 0.00 | 0.00 | 0.00 | 3.90 |
| Melon | 1.90 | 0.00 | 0.00 | 0.00 | 0.00 | 4.90 |
| Maize | 1.10 | 0.00 | 1.50 | 1.00 | 90.00 | 6.00 |
| SDF | 0.12 | 0.00 | 0.00 | 0.00 | 0.00 | 93.50 |

IMP=Level of Impurity, INSC= Insect count, IEH= Insect Emergence Hole, EGG= Egg, GP= Germination potential, STW= Standard Weight.

SDF= Smoke-dried fish.

Table 2. Bacterial counts and identification from selected foodstuff from Dawanau Market

| Food sample | Bacterial Count (cfu/g) | Identified Bacterial Specie (s) |
|-------------|----------------------------|---|
| Groundnut | 4.4x10 ⁵ | <i>Escherichia coli</i> , <i>Klebsiella pneumoniae</i> , <i>Staphylococcus aureus</i> |
| Cowpea | 1.2x10 ⁴ | <i>Escherichia coli</i> , <i>Staphylococcus aureus</i> |
| Yam chip | 1.6x10 ⁴ | <i>Proteus mirabilis</i> , <i>Escherichia coli</i> |
| Melon | 2.3x10 ⁴ | <i>Staphylococcus aureus</i> , <i>Klebsiella pneumonia</i> |
| Maize | 1.1x10 ³ | <i>Bacillus species</i> , <i>Escherichia coli</i> |
| SDF | 2.7x10 ⁶ | <i>Staphylococcus aureus</i> , <i>Escherichia coli</i> , <i>Klebsiella pneumoniae</i> |

cfu/g=Colony forming unit per gram. SDF= Smoke-Dried Fish

Table 3. Fungi counts and identification from selected foodstuff from Dawanau Market

| Food sample | Fungal Count (mcg ⁻¹) | Identified Fungi Species |
|-------------|--------------------------------------|---|
| Groundnut | 7.2 x10 ⁵ | <i>Aspergillus niger</i> , <i>Aspergillus flavus</i> , <i>Penicillium chrysogenum</i> . |
| Cowpea | 2.4x 10 ⁵ | <i>Aspergillus niger</i> , <i>Penicillium chrysogenum</i> . |
| Yam chip | 1.27x 10 ⁵ | <i>Aspergillus niger</i> |
| Melon | 5.2 x 10 ⁴ | <i>Aspergillus fumigatus</i> , <i>Penicillium species</i> . |
| Maize | 3.8 x10 ³ | <i>Aspergillus flavus</i> , <i>Aspergillus niger</i> , <i>Fusarium species</i> . |
| SDF | 8.4 x 10 ⁴ | <i>Fusarium species</i> . |

Mcg⁻¹ =mould Count per gram. SDF= Smoke-Dried Fish

Tables 2 and 3 displayed the results obtained for bacterial and fungal counts as well as the identified species.

The total aflatoxin levels expressed in microgram per kilogram of the selected food stuff sample are depicted in Table 4 below.

Table 4. Total Aflatoxin level of some selected food samples from Dawanau Market

| Sample | Total Aflatoxin (µg/kg) | | % AF _{total} Reduction | Washed/dried | % AF _{total} Reduction |
|-----------|--------------------------|--------------------------|------------------------------------|--------------------------|------------------------------------|
| | Unsorted | Sorted | | | |
| Groundnut | 278.08±3.02 ^a | 239.76±3.01 ^b | 13.78 | 127.91±5.04 ^c | 46.65 |
| Melon | 7.99± 1.01 ^b | 5.99±1.20 ^c | 37.52 | 5.81±1.09 ^d | 3.13 |
| Maize | 2.40± 0.15 ^e | 0.90±0.04 ^f | 62.54 | 0.24±0.04 ^g | 73.33 |
| Cowpea | 2.91 ±0.55 ^f | 2.11±0.07 ^d | 27.71 | 1.41±0.12 ^e | 33.39 |
| Yam chip | 3.96 ± 1.00 ^e | 2.11±0.06 ^d | 46.72 | 2.05±0.40 ^b | 2.78 |
| SDF | 4.67 ± 1.12 ^f | 3.51±0.02 ^e | 24.76 | 3.42±0.27 ^g | 2.62 |

Values are means ± SD of duplicate experiments. Values on the same row with different superscripts are significantly different (p<0.05). SDF Smoke-Dried Fish.

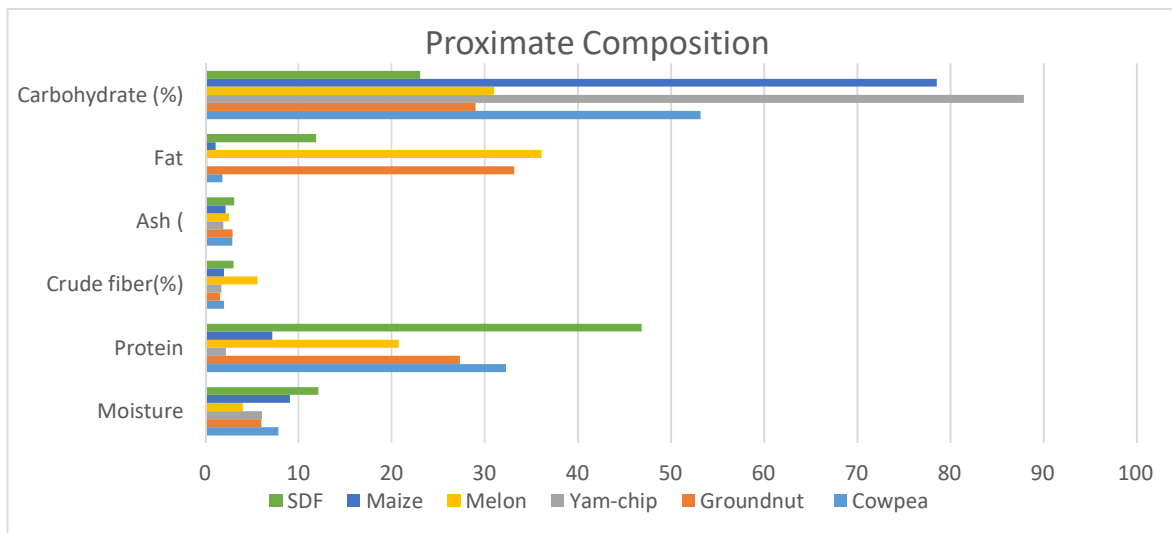


Figure 1. Proximate analysis of some selected foodstuffs from Dawanau Market

Fig. 1 is a representative chart of the proximate compositions of each of the foodstuff sampled from the Dawanau market.

4. Discussion

Food safety is one of the most momentous public health issues globally but, predominantly in emerging and developing countries (14). So, evaluating the proximate composition, microbial, entomological and aflatoxin levels of many agricultural produces at different levels of postharvest handling will provide substantive nutritional and safety information. Table 1 above showed some entomological indices of samples conducted prior to the proximate, microbial and total aflatoxin analysis. The level of impurities, insect, hole emergence, egg, germination potential and standard weight were targeted for each food sample. The level of impurities was significantly ($p < 0.05$) different among the samples. Impurity level was highest (2.80%) in groundnut and lowest (0.10%) in maize. The average level of impurity of exportable grains above 0.5% is not acceptable according to the grading standard for Canadian Western Amber Durum (CWAD) wheat milling grade (15). This means that there is need for improvement in postharvest grain handling of grains in major markets to minimize the level of impurity to global standards. The result also showed that cowpea had 5% egg emergence while maize had 1% and 1.5% egg and insects' emergence respectively. On account of this research, it implies that the randomly selected cowpea falls short of exportable standards of insect egg free of $< 2.5\%$ set by the Malaysian Cocoa Board (16). Among the germinal crops, cowpea was observed to be the samples with the least (35 %) level of average germination potential as at the period of this research. Also, the table showed the average seeds germination

in maize and groundnut samples was high (90 % and 65 % respectively) which fall within the acceptable standards for export (17,18).

Table 2 displayed the respective bacteria counts and identifications of each food sample collected for this study. It was observed that *Escherichia coli* was the commonly identified bacteria amongst the food samples followed by *Staphylococcus aureus*. Total bacteria count ranged from 1.1×10^3 (maize) to 2.7×10^6 (smoke-dried fish) cfu/g. *Escherichia coli* could be entero-toxicogenic, which may cause diarrhea, vomiting and stomach cramp when not properly controlled through proper heat treatments (19). Table 3 displayed results of fungal isolates and their respective counts. Groundnut had the highest counts (7.20×10^5) while lowest counts (3.80×10^3) was observed with maize. The numerical disposition of species of *Aspergillus* and *Penicillium* as common isolates gives the more reasons high total aflatoxin contaminations of the respective food stuffs (20).

In Table 4 above, groundnut recorded relatively high level of total aflatoxins contaminations exceeding the Codex standards of between 4-15 $\mu\text{g}/\text{kg}$. To protect humans and animals from the harmful effects of mycotoxins, especially aflatoxin, the European Commission (EC) and international communities have proposed maximum allowable limits for aflatoxins in foods and feeds. It usually between 4-30 ppb, depending on the country, for example, it 20 ppb (20 $\mu\text{g}/\text{kg}$) in the USA (21,22). Washing and drying of food stuffs greatly reduce the level of mycotoxin contaminations (23). After sorting, washing and drying, significant differences ($p < 0.5$) in terms of reduction in level of aflatoxin total was observed for each food samples under this study. Reduction ranged from 13.78%

to 62.54% after sorting and between 3.13 to 73.33% after washing and drying. Melon, cowpea, yam chips, maize and smoke-dried fishes recorded aflatoxin contamination level within the tolerable level (4-30 µg /kg) while groundnut remained highest (239.76 and 127.91 µg /kg) beyond the tolerable level. The incidence of report in this study portray groundnut to have higher total aflatoxin contaminations than in the report of (24) and far lower compare to collated report of Rustom (25) between 1982-1994 with record high of about 2,88820 µg/kg. The level of aflatoxins food contaminations cannot really be compared due to non-homogeneity of climatic differences and agricultural practices among others (26, 29).

The proximate composition for the selected foodstuffs is shown in figure 1 above. The moisture contents from among the six samples ranged between 4.03% - 12.11%

accounting for smoked dried fish and melon respectively. The primary factors influencing fungal growth in stored food products is moisture content i.e. water activity (27, 28). However, this does not correlate with the aflatoxin contamination levels of smoke-dried fish compared to groundnut, which may have been influenced more by its oil content. Oil contents were highest (36.07 and 33.16) % for melon and groundnut respectively. The oil content is important in diets as it promotes fat-soluble vitamin absorption. It is a high-energy nutrient and does not add to the bulk of the diet. They characteristically contain high levels of oil and are regarded as high oil-yielding seeds more prone to aflatoxin contaminations than others regarded as non-oil seeds or grains (19).

5. Conclusion

Six different samples of foodstuffs randomly purchased from the Dawanau market were analyzed for nutritional, microbial, and level of total aflatoxins contaminations. It was discovered that all samples had aflatoxin contaminations but only groundnuts had total aflatoxin contaminations beyond the tolerable values even after the measures of sorting, washing, and drying were adopted.

Recommendations

Based on the intent of this research and the results obtained, it is highly recommended that routine checks for food quality and possible contaminations with total aflatoxins be enforced.

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Authors' Contributions

Ibrahim Bala conceptualized the research work and greatly took part in the proximate and total aflatoxin analyses and involved in the writing and editing of the manuscript, Bamishaiye Eunice supervised the fund acquisition and projection supervision, Habeeb Saka was actively involved in the microbial assays, the total aflatoxins analysis, and involved in the writing and editing of the manuscript. Uzoma Donatus was involved in the laboratory examinations of the physical parameters of the samples and manuscript writing and editing, Farida Ahmad contributed to the microbial evaluations and manuscript editing.

Declaration of competing interest

The authors unanimously declared no conflict of interest in the research input from whoever and wherever.

Data availability

Data will be available on demand.

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