Comparative Evaluation of Raw Groundnuts from Makurdi Markets in Nigeria Metropolis for Aflatoxin B_1 Secondary Metabolites

Ityo, I. T[1], Nyinoh, I. W[1]*, Kukwa R. E[2], Okoh M. E[1]

[1]Department of Biological Sciences, Benue State University Makurdi, Benue State, Nigeria.
[2]Department of Chemistry, Benue State University Makurdi, Benue State, Nigeria.

ABSTRACT

Aims: The contamination of groundnut by aflatoxins (AF) results in financial losses to farmers' as well as severe food safety and public health challenges globally. This study was carried out to; (i) assess the levels of AFB_1 in husked groundnut seeds in Makurdi (ii) determine the relationship between moisture content and AF levels, and (iii) investigate vendors' knowledge, attitudes and practices (KAP) of AF and their approach towards groundnut storage.
Study design: Quantitative research method was employed in this study.
Place and Duration of Study: This study was conducted at the Department of Microbiology, Benue State University, Makurdi from May – June 2019.
Methodology: Duplicate groundnut samples were collected from ten market locations in Makurdi and analyzed using Enzyme Linked Immunosorbent Assay (ELISA) quantification method.
Results: The moisture content of the groundnuts was determined, and data on Knowledge Attitude and Practice (KAP) relating to groundnut storage were obtained using mini-questionnaires. The results obtained showed that all the sampled groundnuts were contaminated with AFB_1 levels ranging from 17.3 - 35.9 parts per billion (ppb). Furthermore, we found a correlation between high moisture content and high AFB_1 levels and vice-versa. The knowledge of AF among the groundnut retailers was low (<40%), and 40.91 % of the sellers confirmed that groundnuts were stored for ≤one month before sale.
Conclusion: The levels of AFB_1 levels in stored groundnuts are above the permissible limit of 20 ppb for stored groundnut in Nigeria. The data obtained raises concerns for food safety considering that groundnuts are widely consumed in Makurdi. Regular evaluation of AFB_1 levels in food should be conducted in Nigeria.

Keywords: Mycotoxins, Aflatoxin B_1, Awareness, ELISA, Groundnuts, Nigeria

1. INTRODUCTION

Aflatoxins (AFs) are notorious, toxic secondary metabolites produced by black molds, predominantly *Aspergillus flavus*, *A. parasiticus*, and other fungi like *Emericella* sp. (Plascencia-Jatomea et al., 2014; Varga et al., 2009). It has been estimated that approximately 5 billion people in low and middle-income countries (LMICs) risk exposure to AFs (Williams et al., 2004). AF, which was first discovered in the United Kingdom in 1960, caused the sudden death of several turkeys from an unknown turkey "X" disease, and the consumption of *A. flavus* contaminated moldy peanuts was later incriminated (Blount, 1961). Currently, there are approximately 20 known AFs. However, the key ones are - AFB_1, AFB_2, AFG_1, AFG_2, AFM_1, and AFM_2 (Luo et al., 2018). AFs, if present in food and feed products, are likely to pose food safety risks in large populations, and their ingestion has been implicated in severe health challenges like hepatocellular carcinoma (HCC), stunted growth, malnutrition, and immunotoxicity (Kew, 2013; Rushing and Selim, 2019). AF-contaminated foods also result in diminished marketability, lost profit, and product rejection during trading, thereby increasing poverty, and threatening food security globally (Fashube, 2017; Vasan and Bedard, 2019), amongst others.

Aspergillus species, the major AF-causing fungal genus, are commonly soil-inhabiting, and widely distributed in organic debris and decomposing flora (Sellon and Kohn, 2014) in the humid, tropical, and sub-tropical climates of Africa, Asia, and Latin America between the latitude of 40 °N and 40 °S of the equator. Thus, agricultural products are contaminated at any phase, either during pre-harvest or post-harvest, including in storage and/or processing (Kader and Hussein, 2009; Winter and Pereg, 2019). Nigeria, along with China and India, is the world's leading producer of groundnuts, and is a staple diet of Nigerians and many LMICs, but has been reported to be the most common host for aflatoxin contamination globally (Vabi et al., 2018). They are cultivated in many states in Nigeria, with commercial quantities produced in Benue State. In Nigeria, groundnuts are very popular and are widely consumed raw, boiled, or roasted, as a snack, in milk, butter, spices, porridges, soups, cake, oil for food, feed, among other things. Among some ethnic groups, the consumption of raw groundnuts during breastfeeding is encouraged as it has been purported to naturally improve breast milk production.

Studies of AF contamination of food in Sub-Saharan Africa have been reported. In Zambia, the levels of AFB_1 in raw peanuts were 5 - 4.60 (Bumbangi et al., 2016), whereas, in Eastern Democratic Republic of Congo, total AF levels of 1.6 to 2,270 g/kg were detected, but the highest levels were reported in groundnut flour (2,270 g/kg), and roasted groundnut (865 g/kg), which was considered unfit for human consumption based on regulatory limits (Udokmu et al., 2018). A study by Tojo Soler et al. (2010) also evaluated the contamination of peanuts by *Aspergillus flavus* in villages and market granaries in Mali. When compared to globally accepted standards, data obtained from their study indicated a significantly high contamination level of stored peanuts with AFB_1, especially between June - December. In another study, AFs and fumonisins in maize were investigated in South-Western Nigeria (Liverpool-Tasie et al., 2018), and their results indicated that over 50% of the samples had higher AF levels above the regulatory limit in Nigeria. Using high performance liquid chromatography, Ifeji et al. (2014) analyzed raw and roasted peanuts sold in Niger state, Nigeria, and reported AFB_1 levels of 4 -188 µg/kg, while levels of 74.03 - 82.12 $µg\ kg^{-1}$ were determined for groundnuts from the Niger-Delta region in Nigeria (Odoemelam and Osu, 2009), and AFB_1 levels of between 3.09 and 5.11 were detected in shelled groundnut seeds collected from five LGAs in Lagos, Nigeria (Chigoziri and Temitope, 2020). Ekhuemelo and Abu, 2018, sampled raw groundnut seeds stored for one to two years from six Local Government Areas (LGAs) of Benue state and evaluated them for AFB_1 using the ELISA method. They detected levels of 5.92 - 11.02 µg/kg. A study by Ubwa et al. (2014) evaluated the total aflatoxin levels of roasted, hulled, and dehulled groundnuts from markets in Makurdi and observed that dehulled groundnuts had aflatoxin levels of between 1.50 ppb to 20.00 µg/kg. However, Vabi et al. (2020) determined AFB_1 levels in groundnuts and groundnut-based products produced across five Northwestern states and also in Benue state, and observed AFB_1 concentrations of 7.82 and 12.33 µg/kg and 10.15 µg/kg in Benue State respectively. It has been reported that the majority of groundnuts cultivated in Nigeria and products made from them show high AF contamination levels (Vabi et al., 2018). Yet, there is scanty documentary evidence of the levels of AFB_1 in groundnuts and other crops sold in Makurdi markets. The objectives of this study, therefore, are to (i) assess the levels of AFB_1 in husked groundnut seeds sold in various market locations in Makurdi, (ii) determine the relationship between moisture content and AF levels, and (iii) investigate vendors' Knowledge, Attitude, and Practice (KAP) of AF and their approach towards groundnut storage.

2. MATERIAL AND METHODS

2.1 Study Area

The study was conducted in Makurdi, a town situated along the River Benue in Benue state, central Nigeria. Makurdi is located at latitude 7°55 'and' and 7°44 'and' and longitude 8°20 8°38 (Figure 1) and has tropical weather with yearly temperatures of between 21 °C and 38 °C. There are two seasons - the rainy season is observed in April to October and the dry season is from November to March. Benue state is an agrarian state, cultivating a range of crops, mainly tubers, cereals, legumes, fruits, and also vegetables. In Makurdi, the majority of residents work for the government, own farms as a side business or as a second source of income, or own a private business, with only a few being subsistence farmers growing rainfed crops. Groundnuts were investigated because they have been reported to be the most common host for aflatoxin contamination globally (Vabi et al., 2018). In Central Nigeria, Benue state is the major groundnut-producing state in Nigeria. Thus, Makurdi, the state capital, was selected because the majority of the groundnuts produced in the state travel from different villages of the state to Makurdi for consumption and to be conveyed to other states. This study quantified only AFB_1 because it has been reported to be the commonest and most carcinogenic member of the AF family.

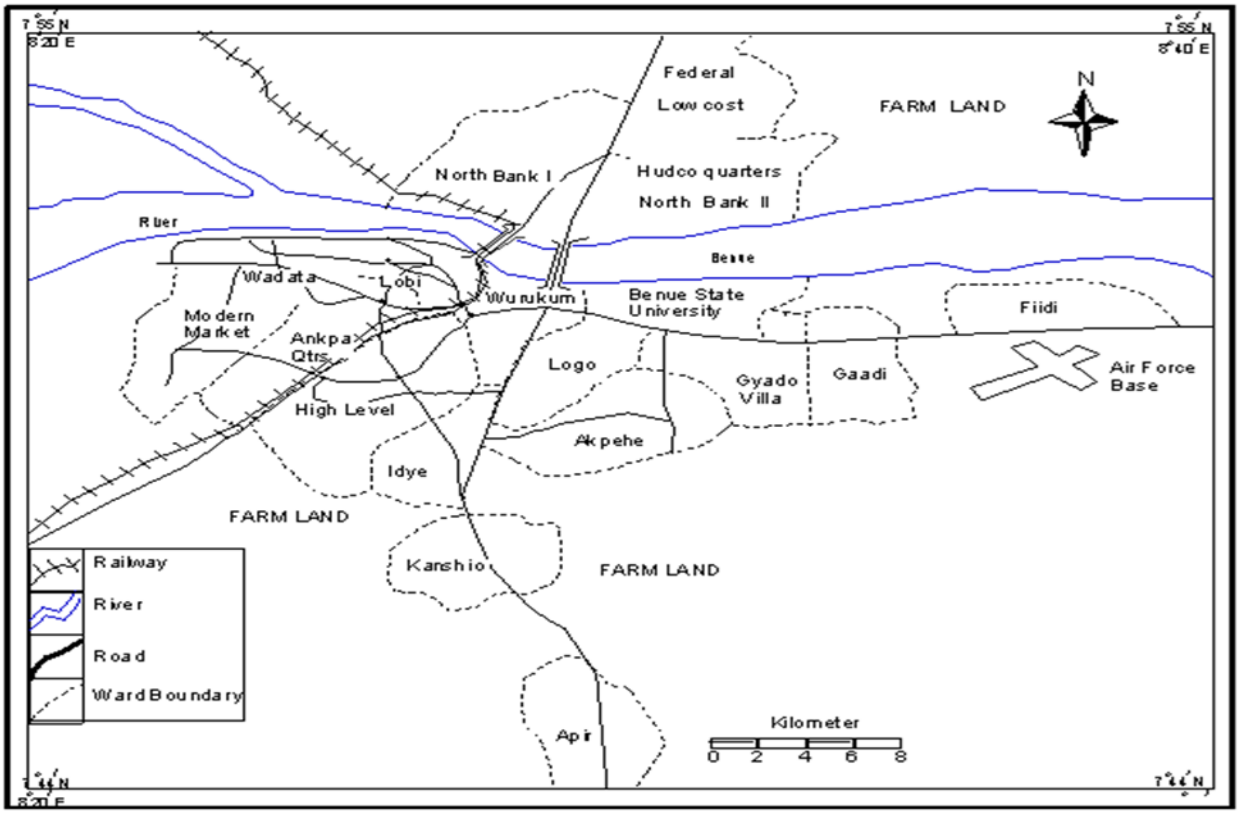

Figure 1: Map of Makurdi Town
Source: Benue State Ministry of Lands and Survey (2013)

2.2 Sample collection and preparation

Twenty (20) local groundnut samples weighing approximately 0.2 kg were collected from market sellers between May - June 2019 in ten designated areas thought to be representative of Makurdi. They include Wadata, UniAgric Road, Wurukum, North bank, International market, Gyado villa, Kanshio, Fiidi, Modern market, and Terwase Agbado (some of the older market locations are shown in Figure 1). The sellers were randomly sampled and received verbal information about the objectives of the study. The word AF was explained to them in their local language (which is mainly Tiv). If the vendors answered yes to knowing AF and AF contamination, they were then each administered a mini-questionnaire to collect data on their knowledge of AF, which includes causes, effects, prevention, and control. They were also about the source of groundnuts (purchased or cultivated), the length of storage of groundnuts, and the type of packaging utilized. The groundnut samples were then transported to the laboratory in clean polythene bags, dehusked by hand under aseptic conditions before further experimentation. Two samples from each lot were analyzed using the method of Ekhuemelo and Abu, 2018, with slight modification. Fifty grams (50 g) of the groundnut samples were weighed and pulverized with the aid of a food blender. A twenty-gram (20 g) portion of the powdered sample was then weighed into a 250 ml conical flask containing a 100 ml portion of 70% methanol. This was placed on an orbital shaker set at a speed of 150 resolutions per minute (rpm) for 30 minutes. The recovered mixture was then filtered with Whatman filter paper 1, and the filtrate was used as the extract for AF level determination.

2.2.1 Estimation of Aflatoxin B_1 levels in groundnut samples

The presence or absence of AFB_1 was determined using groundnut samples obtained from ten market locations. The procedure followed the ELISA protocol of the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT, 2010) and used the Indirect Competitive Enzyme Linked Immunosorbent Assay (cELISA) method as described by Waliyar et al. (2015). This procedure necessitates (i) coating the exterior of an ELISA plate with AFB_1-Bovine serum albumin (BSA) (ii) competing between goat anti-rabbit IgG, labelled with alkaline phosphatase, in PBS Tween containing 0.2% BSA and AFB_1 in the standard. The standard was prepared using 1:10 diluted groundnut extract, and incubated for 1 hour at 37 ^oC to enable toxin-antibody reaction, and (iii) utilizing the enzyme labelled secondary antibodies (p-nitrophenyl phosphate, prepared in 10% diethanolamine buffer at pH 9.8) in the detection of AFB_1 specific antibodies. The AFB_1 level in parts per billion (ppb) was measured by taking the 405 nanometer (nm) absorbance in an ELISA reader (AC3000, Azure Biosystems, Inc.). A regression curve (linear) was plotted for optical density readings and the standard curve was employed in extrapolating AFB_1 concentrations.

***2.1.1.1* Moisture Content Analysis**

Moisture content was determined using the drying and oven method according to the standard procedures by the Association of Analytical Chemists (AOAC, 2010). A Petri dish was weighed using a weighing balance (FX-500I, A & D) and the weight of the container was measured and denoted as W1. Thereafter, five (5) g of the groundnut sample was weighed before drying. This was designated as W2. The groundnut sample was then weighed and measured for 3 hours in a 105 – 110 °C oven (DOL-24A, Gilson Company Inc.). The heated sample was then cooled in a desiccator for approximately 10 minutes. The groundnut samples were re-weighed to obtain the weight of the groundnuts after drying. Finally, the moisture content of the groundnut samples was determined by calculating the percentage of the moisture content on a wet-weight basis using the following formula:

Equation 1: Moisture content (%) = (W2-W3/W2-W1) ×100
Where: W1 = weight of the petri dish
W2 = weight of the petri dish with groundnuts sample before drying
W3 = weight of petri dish with groundnut sample after drying.

3. RESULTS AND DISCUSSION

3.1 Levels of Aflatoxin B_1 of Stored Groundnuts in Makurdi

Literature is scanty on AFB_1 contamination of groundnut in Makurdi, and no study has compared AFB_1 levels in the different markets in Makurdi. This study thus assessed the levels of AFB_1 in raw, husked groundnut seeds at ten market locations in Makurdi. The findings of this study are important because groundnuts are widely consumed in Nigeria, and high levels of AFB_1 if found may pose a threat to health. The mean AFB_1 levels (ppb) in stored groundnut samples from the sampled market locations ranged from 17.3 - 35.9 ppb as presented in Figure 2.

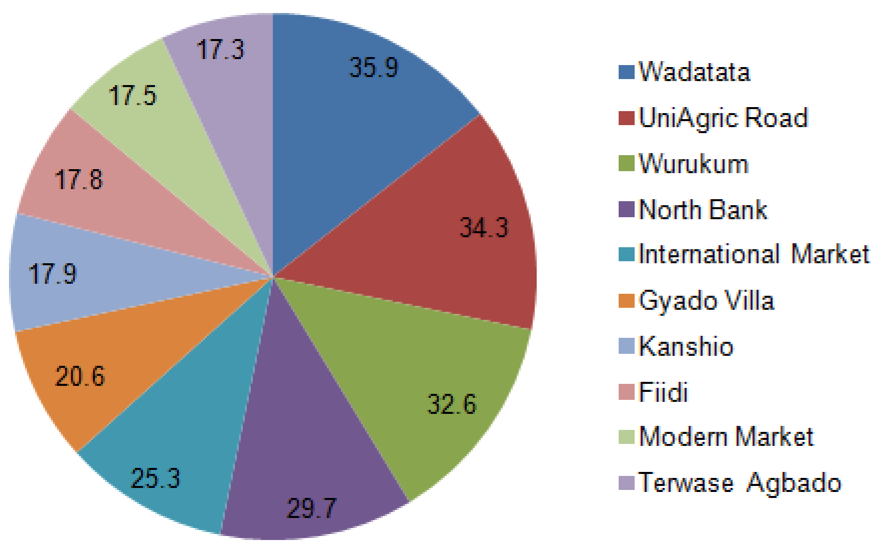

Figure 2: Pie Chart showing the mean AFB_1 levels (ppb) versus market locations sampled.

As shown in the pie chart, AFB_1 was detected in all the groundnut seeds sampled from various locations. At 17.3 ppb, groundnuts collected from Terwase Agbado showed the least AFB_1 level; modern-market (17.5 ppb); Fiidi (17.8 ppb); and Kanshio (17.9 ppb). These AFB_1 levels were below the permissible limit of 20 ppb in unprocessed peanuts as adopted by the Standards Organization of Nigeria (SON, 2006). The variation in levels of exposure between locations as observed in this study is similar to the report of Wartu et al., (2015), where levels of 6.0 - 28.75 ppb were reported in groundnuts sampled in Kaduna, Nigeria. A previous study by Ekhuemelo and Abu, 2018 also detected low AFB_1 levels of 10.41 μg/kg for groundnuts in Makurdi. However, groundnuts sampled from Wadata market showed the highest AFB_1 level (35.9 ppb); University of Agriculture road (34.3 ppb); Wurukum market (32.6 ppb); North Bank Market (29.7 ppb); International market (25.3 ppb) and Gyado villa (20.6 ppb), all above the 20 ppb limit. Research on the levels of AF in Sub-Saharan Africa has also found similar high AF levels. For instance, in a study by Magembe et al. (2016), all the sampled groundnuts were contaminated with AFB_1 at levels ranging from 72.97-195.17 ppb, as, studies by Salau et al. (2016), which measured a contamination rate of 82.5% and AFB_1 levels of between 0.9 - 646.0 ppb on the AF of stored groundnuts in Sokoto State, Nigeria. In a study in Mali, Tojo Soler et al. (2018) reported high AFB_1 levels in groundnuts stored in granaries. Udokmu et al. 2018 also reported levels from 1.6 to 2,270 µg/kg, and the highest levels were found in groundnut flour (2,270 g/kg) and roasted groundnut (865 g/kg), rendering them unfit for human consumption based on global regulatory limits. Another study in Southern Brazil found a lower contamination rate of AFB_1 (14.0%), yet the AFB_1 concentrations were high (24.0 to 87.5 µg/kg) and exceeded the 20.0 µg/kg limit for B1, G1, B2, and G2 in Brazil (Hoeltz, et al., 2012).

3.2 Level of Aflatoxin B_1 in stored Groundnuts in Relation to Moisture Content

The relationship between the moisture content in the groundnuts sampled from various markets in Makurdi and AFB_1 levels was also investigated (Figure 3). As shown, the groundnuts sampled had high moisture content (>50 %), and that high moisture content tends to be associated with high AFB_1 levels and vice versa.

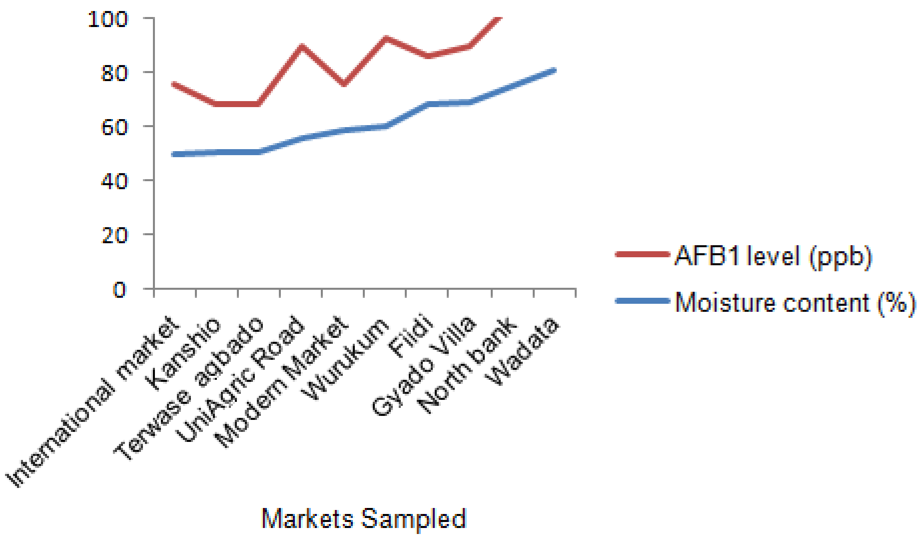

Figure 3: Level of Aflatoxin B_1 (ppb) of stored Groundnuts in Relation to Moisture Content (%)

Fungi, according to Benbrook (2005), can thrive in a variety of environmental conditions and infest crops, resulting in AF production. These factors, temperature, moisture content, relative humidity, and the amount of rainfall, promote fungal growth, infestation, and mycotoxin production in the field and during storage (Darko et al., 2018; Darwish et al., 2014; Farombi, 2003). This statement has been validated by the findings of this study, which shows a gradual rise in AF levels with increasing moisture content (Figure 3). Hence, it implies that contamination in stored groundnut products is dependent on the moisture content of the harvested groundnut before storage. These changes in the moisture content and their impact on AF levels have previously been reported by Salau et al., (2016) and agree with the findings of this study. Similar findings were also reported in studies on nutmeg and chilli spices, which showed a positive correlation between mold, moisture content, and aflatoxin (Pesavento et al., 2016). Another study of wheat also showed relationships between moisture content, fungal growth, and aflatoxin production in wheat flour (Hassane et al., 2017). Comparably, the high humidity that characterizes the Northern Nigerian states provides favourable environmental conditions for AF production and may explain the high AFB_1 levels reported.

3.3 Awareness of Aflatoxins by vendors and their approach towards groundnut storage

Table 1 shows the results of awareness and knowledge of AF by groundnut sellers in Makurdi. From the results obtained, knowledge of AF was low, with only 36.36% of the groundnut sellers admitting that they were aware of AF, while 63.64% were not aware of it. Similarly, 27.27 % acknowledged that they had a sufficient understanding of AFs, such as the etiologic agent, AF risks to human health, preventive measures, and how to control AF, while 72.27% did not. However, the results should be interpreted with caution due to the unequal sample sizes of the recipients.

Table 1: Awareness and Knowledge of Aflatoxins by Groundnut Sellers in Makurdi

Awareness	Respondents (*n* = 22)	Prevalence (%)
Yes	8	36.36
No	14	63.64

Total		100%
Knowledge of Aflatoxins	Respondents (*n* = 22)	Prevalence (%)
Yes	6	27.27
No	16	72.73
Total		100.00

This low level of knowledge of AFs could be a possible reason for the high contamination rate reported in this study, because the lack of knowledge about AFs will result in no deliberate measures to prevent its occurrence from being put in place. A study by Ilesanmi et al. (2011) also reported a low awareness rate of 32.0% in Ibadan, Nigeria. In contrast, in a study conducted in Benin to evaluate groundnut farmers on perception, awareness, and action of AF, 77% of farmers agreed that they were aware of the negative outcomes of AF on human health (Jolly et al., 2016). Similarly, Udomkun et al., 2018 reported that 85% of farmers had knowledge of AFs, however, approximately 41% were unaware of the etiologic agent. Thus, it is imperative to educate farmers, sellers, and the public about AFs. Education is an effective approach for information dissemination. In a study conducted in Western Kenya, it was discovered that education has a favorable and significant impact on farmers' understanding of maize pests (Midega et al. 2016). Similarly, strosnider et al. (2006) suggested that education and awareness are important factors in reducing AF-problems in developing countries, as Udomkun demonstrated in the Democratic Republic of Congo that educated homes were well-informed about AF and suggested creating awareness as a vital approach in the reduction of AF contamination.

3.1.1.2 **Groundnut Vendors approach towards groundnut storage**

Data on the length of time the groundnut vendors (*n* = 29) stored groundnuts before sale, as well as the packaging material (*n* = 22) utilized in storing the seeds, is presented in Table 2. Over 60% of the total number of respondents surveyed stored their groundnuts for approximately one month before selling them, while only 6.90% stored them for up to a year before selling them. When asked about the source of the groundnuts, 68% of the respondents confirmed they bought the groundnuts, whereas 32% cultivated the groundnuts.

Table 2: Length of storage and packaging used by groundnut sellers in Makurdi

Length of Storage for Groundnut	Respondents (*n*=29)	Prevalence (%)
≤1week	10	34.48%
≤1 month	9	31.03%
≤3Months	8	27.59%
Up to 1year	2	6.90%
Total		100%
Packaging type	Respondents (*n*=22)	
Sacks	19	86.36%
Baskets	2	9.09%
Polyethene Bags	1	4.55%
Total		100%

Inadequate drying and poor storage facilities promote the growth of, and toxin production of these fungi (Abbas et al., 2009; Kew, 2013; Klich, 2007; Sarma et al., 2017; Wild and Gong, 2010; Williams et al., 2004). Hygienic practices, as well as the type of packaging employed in peanut storage, and the method of storage, could also be a possible reason for AFB_1 contamination. In a study by Liverpool-Tasie et al. (2018), levels of aflatoxins increased with increasing storage time. Similarly, concerning packaging used to store groundnuts, 86.36% of the respondents stored groundnuts in polypropylene grain bags, 9.09% stored groundnuts in baskets, and 4.54% stored them in polyethene bags. These are often not waterproof, and, as such, lead to increased moisture content and better susceptibility of the groundnuts to fungi attack. All these factors are possible links to the high contamination rate recorded in this study. Countries in LMIC, for instance, Brazil and Nigeria, suffer from poor agricultural practices that favour AF-production. In a study by Darko et al. (2018), the use of zero oxygen hermetic packaging, rather than polypropylene woven sacks, had positive effects on the control of fungal growth and AF and on preserving quality. However, in this study, 86.36% of vendors reportedly stored groundnuts in woven polypropylene sacks, while 9.09% and 4.54% stored groundnuts in rattan baskets and polyethene bags respectively (Table 2). Packaging materials of this type allow air, and there are indications that this may accelerate fungal contamination with subsequent aflatoxin production (Hell et al., 2000).

4. CONCLUSION

Groundnuts are a staple food consumed in most households in Nigeria. Thus, regular monitoring of AF contamination, especially AFB_1 of groundnuts sold in different locations, is important because the regular consumption of AF-contaminated groundnuts could gradually expose consumers to potential health risks. Furthermore, this may allow for proper educative, preventive, and control measures to be put in place. In Nigeria, the Standards Organization of Nigeria (SON) has established the maximum permissible levels for AFs in food to ensure food safety. Yet, this is not enforced, and it is common to find AF-contaminated food and food-products sold across the country. As shown by the results obtained in this study, AFB_1 was detected in groundnuts sampled from all the locations and over 50% of the groundnuts sampled had AFB_1 levels that were above the 20 ppb safety limit in groundnuts in-shell and kernel allowed in Nigeria (SON, 2006). Judging by this regulatory limit, groundnuts sampled from Wadata, UniAgric road, Wurukum, Northbank, International market and Gyado villa at the time of sampling were potentially unsafe for consumption. Furthermore, data obtained from this study also indicates that the level of AFB_1 tends to increase with increased moisture content and vice-versa. The moisture content of all of the sampled grounds was greater than 50%.Individuals, governments, private organizations, and non-governmental organizations could provide proper drying facilities in markets because most farmers currently rely on the sun to dry their products, which may pose a problem with contamination prevention. Similarly, improved storage facilities and better packaging may be provided to farmers at a reduced cost. Finally, the level of knowledge and awareness of AF amongst the groundnut sellers sampled in the various markets was very low. To reduce AF exposure and contamination of groundnuts, it is critical to educate sellers, some of whom cultivate groundnuts, on good agricultural practices, the etiologic agent of AF, how to identify AF-infested food, its prevention, control, and the health effects of AF. By this, it is expected that the knowledge gained will be transformed into action.

COMPETING INTERESTS DISCLAIMER

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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