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FOOD SAFETY RISK ASSESSMENT OF FUNGAL CONTAMINANTS AND AFLATOXIN B_1 IN MARKETED GROUNDNUT OIL FROM KADUNA METROPOLIS, NIGERIA

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ABSTRACT

Food safety remains a critical global public health concern, particularly with widely consumed products like edible oil. In Nigeria, groundnut oil is a dietary staple, produced on scales ranging from small enterprises to large industries and also imported. However, its safety often receives limited attention. This study evaluated fungal contamination and aflatoxin B_1 (AFB_1) levels in groundnut oil samples from markets in Kaduna Metropolis. Twenty (20) composite samples were randomly collected from five locations. Standard mycological methods were used for fungal isolation, while AFB_1 quantification was performed using Enzyme-Linked Immunosorbent Assay (ELISA). Four fungal genera of *Aspergillus*, *Mucor*, *Fusarium*, and *Rhizopus* spp. were identified. The highest fungal load (9.5×10^3 CFU/mL) was found in Central Market samples, while the lowest (2.1×10^3 CFU/mL) was from Gonin-Gora Market. Fungal prevalence was 20% across all genera, except *Fusarium* spp. (10%). One-way ANOVA indicated significant differences in fungal loads between locations ($P \leqslant 0.05$). AFB_1 was detected in all samples, with highest concentrations from Gonin-Gora (5.8 µg/mL) and Central Market (4.6 µg/mL), both exceeding the European Union's permissible limit of 4.00 µg/mL. Other samples remained within acceptable safety thresholds. AFB_1 levels also varied significantly by location ($P \leqslant 0.05$), likely influenced by environmental and handling factors. The presence of AFB1 above regulatory limits highlights potential health risks associated with prolonged consumption. Thus, routine monitoring and vendor education on hygienic practices in oil packaging and storage are essential to reduce fungal growth and toxin production.

Keywords: Aflatoxin B_1, Fungal contamination, Groundnut oil, Food safety, Kaduna Metropolis

INTRODUCTION

According to Astasie et al. (2009), groundnut (*Arachis hypogaea*), also referred to as peanut, is a member of the *Fabaceae* family and is indigenous to South and Central America. Although botanically classified as legumes, groundnuts are among the most widely consumed "nuts" globally. Groundnut oil, also known as peanut oil or *Arachis* oil, is a light-yellow, transparent edible oil characterized by its clear appearance, pleasant aroma, and palatable flavor. It is easily digestible, making it suitable for human consumption (ABC Machinery, 2019).

Groundnuts are a rich source of essential nutrients with notable health benefits. As reported by Onawo and Adamu (2018), groundnut oil is among the most significant vegetable oils due to its hypocholesterolemic properties and its potential in the prevention of cardiovascular diseases, as further supported by Eshun et al. (2013).

In Nigeria, vegetable oils are widely consumed for domestic use. Babatunde and Bello (2016) identified groundnut and palm oil as the principal sources of edible oils in the country. These oils serve multiple purposes, including their use as cooking oils, salad dressings, and as raw materials in the manufacturing of soaps, margarines, and cosmetic products. Consequently, both the quantity and oxidative stability of these oils are of critical importance to consumers and the food processing industry (Wali et al., 2015).

Oil seeds are susceptible to fungal infections caused by species such as *Aspergillus niger*, *Aspergillus flavus*, *Alternaria*, *Fusarium*, *Rhizopus stolonifer*, and *Penicillium chrysogenum*. These fungi are known to cause seed discoloration, shrinkage, rotting, necrosis, and can also lead to the production of toxic secondary metabolites (Syed, 2013). Notably, many of these fungal species have the capacity to synthesize mycotoxins in both groundnuts and groundnut oil. Aflatoxins, particularly aflatoxin B1, produced predominantly by *Aspergillus flavus* and *Aspergillus parasiticus*, are frequently detected as contaminants in groundnuts and other cereals (Shitu et al., 2021).

A significant proportion of groundnut oil producers still rely on traditional extraction methods and often lack awareness of modern processing techniques or the microbiological risks associated with inadequate sanitary practices and improper storage. These conditions create a conducive environment for contamination by aflatoxins and toxigenic fungi.

Therefore, the current study aims to evaluate the presence of aflatoxin-producing fungi and aflatoxin B_1 in groundnut oil marketed across various locations within Kaduna Metropolis.

MATERIALS AND METHODS

Samples Collection

A total number of twenty (20) composite samples of groundnut oil were collected from five different markets. Four samples from Kasuwan Barchi, Gonin gora, Centra, Kawo and Sabon Tasha markets. All the samples were collected in a well labelled sterile bottles and transported to Laboratory for analyses.

Media Preparation

Saboraud dextrose agar (SDA) medium was prepared according to the manufacturer's instruction. The medium was used for mycological analyses.

Mycological Analyses and Isolation

About 1 ml of the sample was inoculated by pour plate method. The solidified inoculated plates were incubated at 25ºC for 3-5 days. Thereafter, the plates that was examined for growth and distinct colonies were counted and express as cfu/ml, while colonies suspected to be aflatoxigenic moulds were sub cultured in another freshly prepared SDA plate and incubated at 25ºC for 3-5 days for pure isolate.

Identification of Fungal species

The pure isolates obtained from the sub cultured plates were stored in the slants until required.

A drop of lacto phenol cotton blue stain was drop in the center of a clean slide and then a fragment of the fungus was collected with the aid of a wire loop and place in the drop of the stain and teased gently then covered with a cover slip. The cover slip was not pushed down or tap to avoid the dislodging of the conidia from the conidiophores. Then the stained isolates were viewed under the microscope with x10 and x 40 objective lens for its morphological characteristics. The fungi isolates were identified using Atlas based on the morphological appearance.

Sample Preparation for Aflatoxin B_1 Assay

20 mL of the liquid sample was measured into a conical flask and added 100 mL of 70% methanol and for the yam sample, the sample was ground into powder and weighed 20g was weighed into 250 ml conical flask and added 100 mL of 70 % Methanol. The mixtures were shaken in an orbit shaker at 150 rpm for 30 minutes. The mixture was then filtered and the filtrate used for the quantification of aflatoxin B_1.

Aflatoxin B_1 Quantification Procedures

The quantification of aflatoxin B_1 was done using Indirect ELISA technique following the below protocol. 150 µl of the diluted toxin-BSA conjugate was dispensed to each well of ELISA plate and the plate was incubated in an oven at 37ºC for 1 hour while shaking. The toxin after incubation was discarded into a disposal chamber. The plate was washed three times changes of PBS-Tween, allowing 3 min for each wash. About 150 µl of 0.2% BSA was then added to each of the well in the plate and the plate incubated again at 37ºC for 30 minutes in an oven while shaking.

After the incubation period, the plate was removed and the content discarded in a waste sink. The plate was then washed again as above. 150 µl of antiserum solution was added also to each of the well and the plate incubated also for 30 minutes at 37ºC. The plate after incubation was removed, discarded the content and washed three times with PBS-tween at 3 minutes interval after each wash.

Aflatoxin standard was prepared by diluting 1.5 µl of the standard in 0.6 mL mixture of methanol and PBST at a ratio of 1:1. This prepared standard was used to prepare serial solution using 1:1 mixture of methanol and PBST as the diluent. 20 µl of the sample was diluted in 180 µl of 0.2 % BSA and the mixture voxtex very well. 100 µl of both standards and samples were dispensed to the wells of the plates in duplicates and 50 µl of antiserum added. The plate was incubated for 1 hour at 37ºC while shaking. After the incubation period, the plate was removed, discarded the content and washed three times with PBST. 150 µl of goat anti-rabbit was added to the wells of the plate and incubated again for 1 hour at same condition. The plate was then removed, discarded the content and washed three times again. 5 mg of para-nitrophenyl phosphate was dissolved in 10 mL of 10% diethanolamine. 150 µl of the solution was dispensed into the wells and the plate was incubated for 30 minute at same conditions as in above. The absorbance of each standard and sample were measured at 405nm using the ELISA plate reader.

The Aflatoxin Concentrations in the samples were calculated automated using software that came with the ELISA reader.

Statistical Analyses

Data generated were subjected for simple descriptive statistical analysis and mean standard deviation.

RESULTS AND DISCUSSION

In this study, all analyzed groundnut oil samples exhibited fungal contamination; however, the fungal load varied significantly depending on the market location and vendor source. Statistical analysis using one-way ANOVA demonstrated a significant difference in fungal loads among the different sampling sites ($P \leq 0.05$), suggesting that environmental conditions, handling practices, and storage methods at each location may influence microbial proliferation.

The fungal isolates were identified based on a combination of macroscopic (morphological) and microscopic characteristics. Morphological features considered included colony color, texture, and elevation, while microscopic examination focused on the color of aerial and substrate hyphae, type of hyphae (septate or coenocytic), presence of foot cells, and the structures of sporangiophores, conidiophores, and spore heads.

The distribution and percentage occurrence of the fungal isolates are presented in Table 1. The results indicated that *Aspergillus* spp. were the most prevalent fungi, detected in nearly all sampled locations. The identified fungal genera included *Aspergillus* spp., *Fusarium*, *Mucor*, and *Rhizopus* spp. Among these, *Aspergillus* spp. had the highest frequency of occurrence, constituting 20% of the isolates and being present in 4 out of the 10 oil samples. In contrast, *Fusarium* spp. showed the lowest frequency, recorded in only 1 of the 10 samples, representing a 10% occurrence rate. The variation in fungal species distribution was also statistically significant across sample locations ($P \leq 0.05$).

Quantitative analysis of aflatoxin B1 levels using the Enzyme-Linked Immunosorbent Assay (ELISA) method revealed that all oil samples contained detectable amounts of the toxin, though concentrations varied. The highest concentration of aflatoxin B1 (4.2 µg/mL) was recorded in samples obtained from Gonin Gora, while the lowest detectable level (2.1 µg/mL) was found in samples from Sabo Market. Notably, samples from Kasuwan Barchi tested negative for aflatoxin B1, as shown in Table 2. Statistical evaluation confirmed significant variation in aflatoxin B1 levels among samples from different locations ($P \leq 0.05$), reinforcing the need for consistent monitoring of oil quality and storage conditions across markets

Table 1: Distribution and Percentage Occurrence of Fungal Isolate in Groundnut Oil Sold Within Kaduna Metropolis

Location	No of Sample	Fungal Isolates	% Occurrence
Kasuwan barchi	4	*Fusarium*	10
Gonin- gora	4	*Aspergillus flavus*	*20*
Central Market	4	*Aspergillus niger*	*20*
Sabon Tasha	4	*Mucor*	*20*
Kawo Market	4	*Rhizopus*	*20*

Table 2: Mean Aflatoxin B_1 Level in Groundnut Oil from Kaduna Metropolis

Location	No of Samples		Range of AFB_1 µg/ml	Mean of AFB_1 µg/ml
	Analyze	Positive		
Gonin-gora	4	3	3.2-5.8	4.2^a
Central market	4	1	0.0-4.6	2.3^b
Sabo market	4	2	1.05- 3.15	2.1^b
Kasuwan barci	4	1	ND	ND
Kawo	4	3	3.51-4.75	4.13^a

Key: ND = Not detected

Mean values with different superscript across the column are statistically significant, while mean values with the same superscript across the column are not significant, P value ≤ 0.05

Discussion

The findings from this study revealed a significant level of fungal contamination in groundnut oil samples obtained from various markets within Kaduna Metropolis. The presence of multiple fungal genera, including *Aspergillus*, *Mucor*, *Fusarium*, and *Rhizopus* species, suggests that groundnut oil marketed in this region may be subject to unhygienic handling, inadequate storage conditions, or substandard processing techniques. These conditions create a conducive environment for the growth of spoilage fungi and mycotoxin-producing organisms, particularly under the warm and humid climate typical of northern Nigeria.

The fungal species identified in this study closely align with those reported in earlier investigations. For instance, Sylvester and Elijah (2013), as well as Flora et al. (2018), documented similar fungal isolates in edible oil samples collected from different regions. Tobin-West et al. (2018) also reported the isolation of *Aspergillus* spp., *Penicillium*, *Mucor*, *Rhizopus*, and *Fusarium* spp. from groundnut seed oil sold in Port Harcourt Metropolis. These consistent findings across various geographical locations suggest that fungal contamination in groundnut oil is a widespread issue in Nigeria and may be attributed to common traditional production and post-production handling practices.

Of particular concern in the present study is the detection and quantification of aflatoxin B1 in the oil samples, with concentrations in some cases exceeding the maximum allowable limit of 4.00 µg/mL set by the European Union. The detection of aflatoxin B1 in the majority of samples is alarming, especially given the established health risks associated with chronic aflatoxin exposure, including liver cancer, immune suppression, and stunted growth in children. The highest levels were recorded in samples from Gonin-Gora and Central Market, suggesting that certain market environments or storage practices may significantly elevate the risk of toxin accumulation.

The ability of *Aspergillus* spp. to produce aflatoxin B1 in edible oil, as demonstrated in this study, is consistent with previous reports. The Centers for Disease Control and Prevention (CDC, 2006) confirmed that specific strains of *Aspergillus flavus* and *Aspergillus parasiticus* are potent producers of aflatoxins, particularly in oil-rich commodities such as groundnuts and maize. Our findings further corroborate this, as *Aspergillus* spp. were the most frequently isolated fungi and were linked to samples with higher aflatoxin B_1 concentrations. These toxigenic fungi likely originate from the seeds prior to oil extraction, especially when the nuts are inadequately dried, stored in humid environments, or handled improperly during processing.

Moreover, the variation in aflatoxin levels and fungal loads across different sampling sites ($P \leq 0.05$) implies that local environmental factors, including temperature, humidity, sanitation practices, and storage infrastructure, play a crucial role in contamination rates. Markets with poor ventilation, high moisture levels, and limited awareness of food hygiene are more susceptible to harboring mycotoxin-producing fungi.

The study underscores the need for urgent intervention by public health authorities, food regulatory agencies, and local market administrators. Without stringent monitoring, enforcement of safety standards, and training on good manufacturing and storage practices, there is a risk of continued exposure of consumers to contaminated edible oils. Awareness programs are essential to educate local oil producers, traders, and consumers about the dangers of mycotoxins and the importance of maintaining hygienic conditions throughout the oil production chain.

In summary, the present findings add to the growing body of evidence that underscores the public health risk posed by fungal contamination and aflatoxin presence in edible oils, particularly groundnut oil. Enhanced surveillance, stricter regulatory controls, and improved food safety practices are imperative to safeguard consumer health and ensure the microbial and chemical safety of edible oils sold in Nigerian markets.

CONCLUSION

This study highlights significant microbiological and toxicological risks associated with locally processed groundnut oil in Kaduna Metropolis, with widespread fungal contamination, especially *Aspergillus* spp. and aflatoxin B_1 levels in some samples exceeding the EU safety limit (4.0 µg/mL). Significant variation in contamination levels across markets ($P \leq 0.05$) suggests influence from environmental and handling conditions. These findings underscore the urgent need for regulatory intervention, public health awareness, and routine monitoring. Improved hygienic practices, storage, and processing methods among vendors are crucial to mitigating risks. Integrating microbiological assessments into food

safety policies is essential to safeguard public health in regions reliant on traditional oil production methods.

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