

Assessment of aflatoxin contamination in peanuts of selected markets in Kampala. A cross-sectional study.

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Abstract

Background

Aflatoxin is a mycotoxin that is frequently known to contaminate peanuts. Aflatoxins are secondary toxic metabolites primarily produced by *A. flavus* and *A. parasiticus*. This study was hence aimed at evaluating the level of aflatoxin contamination in peanuts from selected markets in Kampala.

Methodology

Twelve (12) samples were collected by a simple random technique from Nakawa, Banda, Kireka, and Bweyogerere markets. The samples were collected from different sales points, and three samples were collected per market. These samples were prepared and extracted using a suitable solvent to achieve an effective extract. The solvent used was 70% methanol. Thin layers plated were activated, spotted, and developed under a chloroform/acetone mobile phase system. The plates were analyzed for aflatoxin using a thin-layer chromatography mobile phone-based technique.

Results

The results obtained from the analysis of samples collected from the selected markets of Kampala show that five out of the twelve samples had bright spots when viewed under UV light. For the Nakawa market, sample N1 had 0 (Ppb), N2 had 0 (Ppb), and N3 had 88(Ppb). Banda market, B1 had 0(Ppb), B2 had 0(Ppb), B3 had 0(Ppb). Kireka market, K1 had 101(Ppb), K2 had 101.5(Ppb), and K3 had 88(Ppb). Bweyogerere market, BW1 had 0(Ppb), BW2 had 0(Ppb), and BW3 had 81(Ppb). 42% of the total samples were contaminated with aflatoxins, and these were found to be above the permissible levels by UNBS.

Conclusion

The level of contamination of 42% of the total samples was highly above the permissible levels as stipulated by UNBS and FDA.

Recommendation

Therefore, peanuts sold in the different Markets need to be continually evaluated to ensure food safety and public health concerns.

Keywords: Aflatoxin Contamination, Peanut, Selected Markets, Kampala.

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Background of the study

Peanuts are a subtropical legume that grows best in warm conditions well well-distributed rainfall ranging from 500mm to 600mm. Peanut and peanut products are known to be susceptible to contamination by fungi that produce secondary metabolites such as aflatoxins (John D Groopman, 1988). Peanut consumption is predominant in Eastern and Southern Africa. In Uganda, peanut consumption is mainly in the form of butter. Uganda

comprises the fertile areas surrounding Lake Victoria, stretching to the central plateau north of Kampala, encompassing both sides of the Nile, the Busoga region, northern Uganda, and the highland regions of Kigezi and the Ruwenzori Mountains. These areas are favored by the high rainfall amounts received throughout the year.

Aflatoxin is a potent mycotoxin naturally produced as a secondary metabolite by several fungal Aspergilli species, primarily *Aspergillus flavus* and *parasiticus* (Wacoo et al.,

2014). Aflatoxin contaminates a variety of food and crop commodities such as corn, peanuts, spices, tree nuts, honey, fruit juices, wine, and cotton (C. Ian Johnston et al., 2012). It is estimated that 25% of the world's crops are affected by mycotoxins, according to the evaluation of the Food and Agriculture Organization (Rudolf Krska 2020). The four main aflatoxins frequently encountered are AFB1, AFB2, AFG1, and AFG2. Aflatoxin B1 is labeled as the most potent naturally produced class 1A human carcinogen. (Carlos A et al, 2009). They also show the greatest toxigenic potential. *A. flavus* and *A. parasiticus* are saprobic fungi ubiquitous in nature, commonly found living off decaying debris in the soil and on crops (2012). Aflatoxins were observed to be harmful in different parts of the world where people consume large amounts of foodstuffs that are prone to contamination. Such countries include Asia and Africa, particularly Uganda.

Aflatoxins have been reported in Uganda on peanuts. Uganda is a tropical country, and the hot and humid conditions lead to the growth of fungal species that infect the peanut in the field and after harvest during storage.

Children are particularly affected by aflatoxin exposure, which leads to stunted growth, delayed development, liver damage, and liver cancer (Chu 1991). Even adults are at a higher tolerance to exposure, but are also at risk. No animal species is expected to be immune to the toxins.

Aflatoxins are more toxic, mutagenic, and carcinogenic even in minute quantities (IARC 1995). Once the aflatoxins are in the human body, they are metabolized by the liver to reactive epoxide intermediates or hydrolyzed to become the less harmful aflatoxins M1 (Al-Ayoubi et al. 2022). Therefore, this study aimed to evaluate the level of aflatoxin contamination in peanuts from selected markets in Kampala.

Methodology

Research design

It was a descriptive cross-sectional study in the selected markets in Kampala. The peanut samples were collected randomly from the different markets within Kampala. Three samples were collected from different outlets for each market. These were the Nakawa, Banda, Kireka, and Bweyogerere markets.

Study setting

The analysis of aflatoxin levels in peanut samples was carried out at the Kyambogo University Chemistry Laboratory, located within the Department of Chemistry, Faculty of Science, at Kyambogo University, Kampala, Uganda. The laboratory is well-equipped for chemical and biochemical analyses and offers a controlled environment

suitable for precise and safe analytical procedures. It contains essential instrumentation and safety infrastructure, including fume hoods, drying ovens, balances, pipettes, sample storage facilities, and equipment necessary for aflatoxin detection, such as High-Performance Liquid Chromatography (HPLC) or Enzyme-Linked Immunosorbent Assay (ELISA) kits, depending on the method employed in the study. The analysis of samples commenced immediately after collection and was conducted over a span of approximately six months, from 21st January 2024 to July 2024. During this period, laboratory activities included sample preparation, aflatoxin extraction, quantification, data recording, and verification.

EQUIPMENT AND REAGENTS

Equipment.

Weighing balance
A blender
Test tubes
Beakers
Automatic Oven
A spatula
Measuring cylinders
A pencil
A ruler
A developing tank
A coated plate
Micropipettes
UV lamps

Reagents

Chloroform, Acetone, Methanol, and Sodium chloride [common salt]

Solvent preparation

A solvent solution of 70% methanol was prepared. 120ml of 70% methanol was prepared by measuring 84ml of methanol and 36ml of deionized water.

Extraction

Weighed 5.0 grams of the peanut sample into the centrifuge tubes.

Weighed 2.0 grams of sodium chloride, and it was added to the sample and mixed thoroughly.

Measured 10 mL of 70% methanol and added it to the sample.

The mixture was shaken thoroughly, and the slurry filtered off. The extract was left to settle, waiting for analysis.

After drying off the extraction solvent, the spotted thin layer plate was carefully placed into the developing tank. The mobile phase moves by capillarity, and the plates are removed before reaching the solvent front. The plates were allowed to dry before visualization under UV light.

VISUALIZATION

The developed thin layer chromatography plates were visualized in mobile phone equipment using ultraviolet lamps of 360nm, with the distance between the phone camera and the plates 11.5cm. The TLC plates, which produced positive results, were taken for quantification using software.

SPORTING AND DEVELOPMENT

Spotting

The coated thin layer plates were activated in the oven at a temperature of 105 °C for 30 minutes.

The activated thin layer plates were marked using a pencil and a ruler.

10ml of the extract for each sample was pipetted using a micro pipette and it was carefully spotted.

The plates were left to dry off to allow the solvent to evaporate before placing them in the developing tank.

Developing the plate

The mobile phase solvent was prepared and placed into the developing tank. The mobile phase solvent was prepared by measuring 18 mL of chloroform and 2 mL of acetone.

Pictorial of the laboratory activities



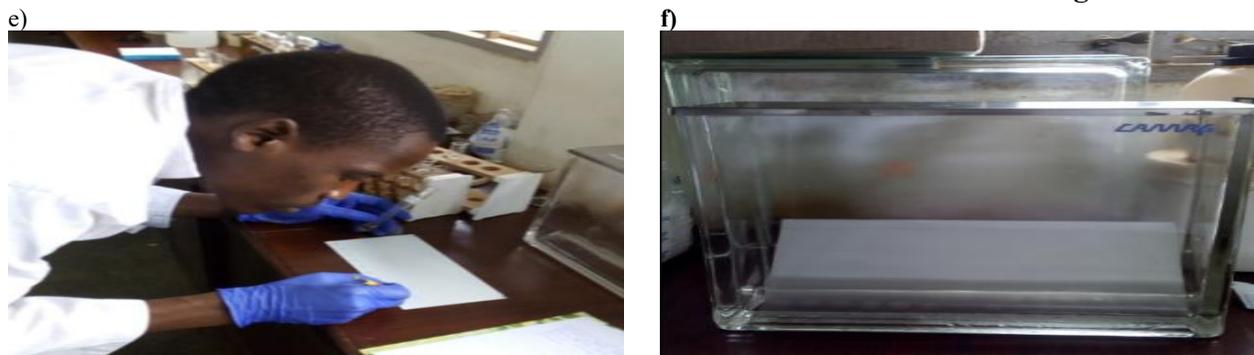


Figure 4 : (a) shows samples in centrifugal tubes during extraction, (b) shows sample extracts awaiting analysis, (c) shows geofrey spotting using a micro pipette, (d) shows geofrey drawing the solvent front on the TLC plate, (e) shows filtration of the extract after extraction, and (f) shows TLC plate immersed in a mobile phase during development

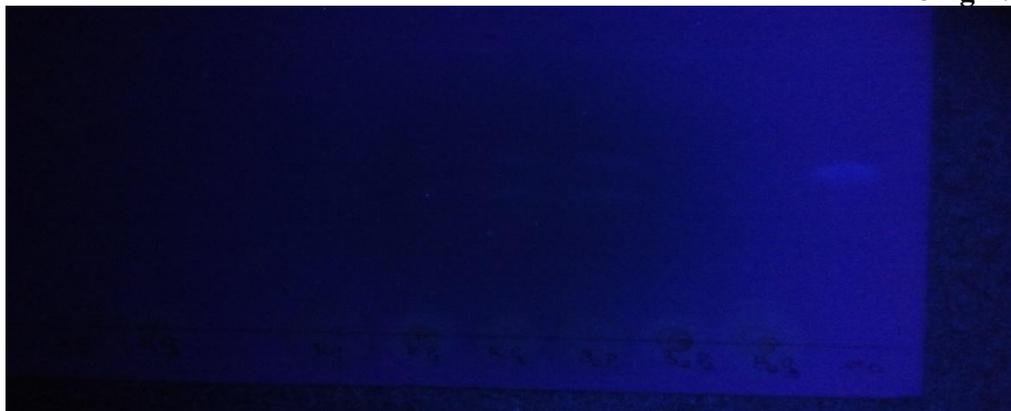
Results

The analysis of the twelve samples collected from different markets within Kampala showed that only five samples were positive. These were analyzed under UV light and are shown in the figures.

Figure 1 shows a TLC plate viewed under UV light, showing a bright spot for sample N₃.



Figure 2 shows a TLC plate viewed under UV light, showing bright spots for samples K₁, K₂, K₃, and BW₁.



The bright spots observed on the TLC plates under UV light show the presence of aflatoxin. The concentration of the bright spots was quantified using the TLC integrated mobile software.

Table 1 shows results obtained from the analysis of samples collected from Nakawa Market.

Market	Nakawa	
Samples Collected.	Sample ID	Concentration In Parts Per Billion (Ppb)
	N ₁	0
	N ₂	0
	N ₃	88

Table 2 shows results obtained from the analysis of samples collected from Banda Market.

Market	Banda	
Samples Collected.	Sample ID	Concentration In Parts Per Billion (Ppb)
	B ₁	0
	B ₂	0
	B ₃	0

Table 3 shows results obtained from the analysis of samples collected from Kireka Market.

Market	Kireka	
Samples Collected.	Sample ID	Concentration In Parts Per Billion (Ppb)
	K ₁	101
	K ₂	101.5
	K ₃	88

Table 4 shows results obtained from the analysis of samples collected from Bweyogerere Market.

Market	Bweyogerere	
Samples Collected.	Sample ID	Concentration In Parts Per Billion (Ppb)
	BW ₁	0
	BW ₂	0
	BW ₃	81

Interpretation And Data Analysis

The results obtained from the analysis of samples collected from the selected markets of Kampala show that five out of the twelve samples had bright spots when viewed under UV light. The bright spots indicate the presence of aflatoxins in the analyzed sample. The intense bright spot on the right side of Figure 3 is the standard.

Nakawa Market Sample IDs N1, N2, and N3 indicate respective sales points 1, 2, and 3 selected randomly. Banda Market sample IDs B1, B2, and B3 indicate respective sales points 1, 2, and 3. Kireka Market sample IDs K1, K2, and K3 indicate respective sales points 1, 2, and 3 selected randomly. Finally, Bweyogerere Market sample IDs BW1, BW2, and BW3 indicate respective sales points 1, 2, and 3 collected on a random basis.

Samples N1, N2, B1, B2, B2, BW1, and BW2 had concentration of 0ppb. This shows that these samples did not have bright spots when viewed under UV light. Hence were free from aflatoxin contamination.

Sample ID N3 had a concentration of 88ppb compared to sales points N1 and N2 with 0ppb. Samples from Banda Market had concentrations of 0 ppb. Samples from Kireka K1 101ppb, K2 101.5ppb and K3 88ppb. And finally, samples from Bweyogerere BW1 and BW2 had 0ppb compared to BW3 with 81ppb.

Discussion.

The results obtained from the analysis of samples collected from the selected markets of Kampala showed that five out of the twelve samples had bright spots when viewed under UV light. These bright spots were observed from samples collected from Nakawa samples ID N3, Kireka samples ID K1, K2, and K3, and Bweyogerere sample ID BW3. Aflatoxins B and G fluoresce blue and green, respectively, under UV light (Felicia et al, 2013). Hence, a bright spot under UV light indicates the presence of aflatoxins in the sample under analysis. Similarly, a study analyzing Gambian peanuts (2011–2018) found that 42% of 1,168 samples had aflatoxin levels above Codex maximum limits (Al Tamim et al. 2024). 42% of the contaminated samples were identified from the different markets, with Kireka leading with 100% of the collected samples contaminated. Nakawa and Bweyogerere Markets have one sample contaminated.

However, all the contaminated samples were observed to have concentrations above the regulatory limit (20 ppb) (FDA 2000). Kireka market had concentration values of 101ppb, 101.5ppb, and 88ppb for sales points 1, 2, and 3, respectively. Nakawa market had only one sales point with

a concentration of 88ppb. And Bweyogerere had only one sample contaminated with a concentration of 81ppb.

The high level of contamination of peanuts in the Kireka market is anticipated to be due to poor agricultural practices practiced by their suppliers. Such activities are practiced during planting, harvesting, drying, transportation, and storage of the product. This is where conditions may be favorable for the growth of fungal species *A. flavus* and *A. parasiticus*. Hence, favors fungal contamination and growth, and aflatoxin production.

Samples collected from the Banda market were analyzed to be free from aflatoxin contamination. Similarly, in a study testing 178 peanut and peanut-product samples, no aflatoxins were detected in any samples obtained from importers, while those from retailers and manufacturers did show contamination (Norlia et al. 2018). This is anticipated to be due to proper monitoring of the storage conditions, thorough sorting of the peanuts, hygienic transportation, and probably better agricultural practices done by suppliers.

Generalizability

The generalizability of the study is limited due to the small sample size, narrow geographic focus, and single-time-point data collection. While the results highlight significant aflatoxin contamination in specific markets like Kireka, they cannot be confidently extended to all markets in Kampala or Uganda. Differences in storage practices, supplier quality, and environmental conditions mean that the findings are best seen as indicative rather than representative. Broader, more comprehensive studies are needed for general conclusions.

Conclusion

From the findings of my study, 42% of the peanut samples, which were collected randomly from Nakawa, Banda, Kireka, and Bweyogerere Markets, were contaminated with aflatoxins. All the samples from Kireka Market were contaminated as compared to those from Banda Market, which were free from contamination.

The level of contamination of 42% of total samples was highly above the permissible levels as stipulated by UNBS and the FDA. This is already bad news, and hence aflatoxins remain a stubborn chemical hazard to food safety, endangering the lives of consumers.

Therefore, peanuts sold in the different Markets need to be continually evaluated to ensure food safety and public health concerns.

Limitations

Limited Geographic Scope, the study was confined to selected markets within Kampala, Uganda's capital city.

While this urban setting offers access to high-volume markets and diverse supply chains, it does not reflect aflatoxin contamination trends in rural or less-commercialized regions.

Temporal Limitation, aflatoxin contamination is highly sensitive to climatic conditions such as temperature and humidity, both of which can fluctuate seasonally. Because the study was conducted during a specific period, it provides only a snapshot of contamination levels at that time. For instance, higher contamination might occur during the rainy season due to increased moisture levels in storage environments. Without data across multiple seasons, the study cannot fully account for temporal variability or establish long-term trends in aflatoxin prevalence.

Recommendations

Peanut aflatoxin contamination can be minimized by ensuring proper sorting of the peanuts. This can be achieved by removing brownish-colored and broken pieces of peanuts.

Public awareness of the dangers of aflatoxin accumulation in sufficient amounts in the body, and hence identify the preventive measures against aflatoxin contamination.

Ensure proper agricultural practices that minimize the rate of pre-harvest invasion of pod and seed by fungal species *A. flavus*.

Proper monitoring of the storage conditions and maintaining a high degree of cleanliness during transportation

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List of Abbreviations

AFB1: Aflatoxin B1

AFB2: Aflatoxin B2

AFG1: Aflatoxin G1

AFG2: Aflatoxin G2

UV: Ultraviolet

ELISA: Enzyme-linked immunosorbent assay

Ppb: Parts per billion.

RNA: Ribonucleic acid

DNA: Deoxyribonucleic acid

TLC: thin layer chromatography.

ml: Millilitres.

IARC: International Agency for Research on Cancer.

UNBS: Uganda National Bureau of Standards

FDA: Food and Drug Administration.

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Conflict of interest

The author declares no conflict of interest.

Author contributions

GS, DSK, and BK conceptualized the study and wrote the manuscript; DSK and DM carried out the data analysis.

Data availability

Data is available upon request.

Informed consent

All the participants consented to the study.

Author Biography

GS, SDK, and BK hold a bachelor's degree in Chemistry from Kyambogo University.

DM holds a Bachelor's degree in Science in Public Health from Lira University.

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