

# Determination of total aflatoxin and aflatoxin B1 in groundnuts and cereal-based street vended foods sold in selected locations in Thika Town, Kenya

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## ABSTRACT

**Objective:** Food contamination with mycotoxins such as aflatoxin in street vended foods is a public health concern in Kenya. The objective of this study was to determine the level of aflatoxins in street vended foods sold in six locations within Thika Town, Kenya.

**Methods:** A total of 36 samples consisting of cereal and cereal based foods and roasted groundnuts were randomly collected for analysis. Screening for aflatoxins was done using NEOGEN's Reveal® Q+ for Aflatoxin test kit while quantification of aflatoxin B1 and total aflatoxin was done through a competitive direct enzyme-linked immunosorbent assay (ELISA).

**Results:** Total aflatoxin and aflatoxin B1 in street vended roasted groundnuts averaged between 4.420 - 14.241 µg/kg and 3.431-11.289 µg/kg, respectively. Thika town center had significantly ( $p=0.0012$ ) higher total aflatoxin and aflatoxin B1 level in roasted groundnuts. On average 16.7% and 44.4% of roasted groundnut samples had total aflatoxin and aflatoxin B1 levels above the regulatory limits of 10 µg/kg and 5 µg/kg established by the Kenya Bureau of Standards as limits for total aflatoxin and aflatoxin B1 in groundnuts, respectively. Compared with the 4 µg/kg and 2 µg/kg limit set by the European Union for total aflatoxin and aflatoxin B1, 72.2% and 100% of the samples were above these limits, respectively.

**Conclusion:** The high levels of aflatoxins, especially aflatoxin B1, highlights the need for Kenya to take the necessary steps to effectively manage aflatoxins in ready-to-eat street vended groundnuts.

**Key words:** Total aflatoxin; Aflatoxin B1; Enzyme-linked immunosorbent assay; Street vended foods; Food safety

## Introduction

Mycotoxins are secondary metabolites of a variety of mold species that produce toxins with adverse health effects on humans, resulting mostly in acute and chronic illnesses called mycotoxicoses [1, 2]. Among the mycotoxins of the greatest health and economic importance are aflatoxins [1, 3]. Aflatoxins are produced by some strains of *Aspergillus*

*flavus*, *Aspergillus parasiticus*, and *Aspergillus nomius*. There are four main aflatoxin types ( $B_1$ ,  $B_2$ ,  $G_1$ ,  $G_2$ ) and two more ( $M_1$ ,  $M_2$ ) that are produced by animals through milk after metabolizing the main four types [4].

A wide range of food commodities including cereals especially maize and maize products, rice, sorghum, millet, cassava, yams, beer, and animal products; dairy products, dried fish, meat, and eggs have been implicated in myco-

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toxin contamination [2, 5-9]. In street vended foods, aflatoxins were reported in corn, groundnut, coconut, tiger nut, walnut, cassava-based foods and wheat snacks among other street vended products in Nigeria and Congo [9-12]. In Kenya, there is limited research on mycotoxin contamination in street-vended ready-to-eat foodstuffs. Nevertheless, aflatoxins and aflatoxicosis incidences have been reported involving foodstuffs [13-15]. In Kenya, at least 500 acute human illnesses as well as 200 deaths due to aflatoxins have been reported [6]. Recently, aflatoxin was reported in ready-to-eat peanut butter in Kenya [16].

Cereals as well as groundnuts form a substantial part of street vended foods [17]. Many researchers have reported the presence of mycotoxigenic fungi and the presence of mycotoxins in especially cereals and nuts [5, 18, 19]. Food contamination with mycotoxins such as aflatoxin may occur at various points along the food chain if conditions such as temperature and moisture are suitable for mycotoxin production [20]. This can be during the pre-harvest, harvest, drying, or storage period for the raw materials used in making ready-to-eat food [20-22]. Contamination may also result from the use of additives in food processing. For instance, spices may be contaminated with aflatoxins [23] which may end up in processed foods.

Consumption of food that contain mycotoxins may cause serious health complications in humans. Mycotoxins such as aflatoxins are known to be hepatotoxic, carcinogenic, genotoxic, nephrotoxic, teratogenic, and immunosuppressive. They are also capable of causing bile-duct hyperplasia as well as hemorrhage in the intestinal tract and the kidneys [4, 6, 21]. Aflatoxin exposure among school children in Kenya, who form a substantial part of street food consumers has been reported as a potential cause of chronic hepatomegaly [24].

The risks associated with mycotoxins have a huge economic impact which strongly supports the need for further research in this area [2, 20]. Thus, there is a need for the analysis of street vended foods to identify contamination levels, especially those produced by microorganisms. This study aimed to determine the presence of aflatoxin in street vended foods sold in selected locations in Thika town.

## Materials and methods

### Study location

This study was carried out in six street food selling locations including Thika Level 5 Hospital area, Juakali area, Kiandutu area, Makongeni area, Ngoigwa area, and Thika Town Center in Thika town.

### Sampling

A total of 36 street vended food samples consisting of cereal-based foods (18) and roasted groundnuts (18) were randomly collected from the six study locations. Approximately 200 grams were collected from each street food vendor and bagged in a sterile sample bag. Samples were immediately transferred into a cooler box (4°C) and transported to the laboratory for analysis.

### Determination of moisture content of street vended food samples

Determination of moisture content was done using the standard oven drying method. Approximately 5 g of each sample was weighed after grinding and placed in a drying oven at 105°C for 3 hours. The sample was then cooled in a desiccator and weighed again taking care not to expose the sample to the atmosphere. Moisture content was calculated and reported as the percentage of the wet sample.

### Screening for aflatoxins in cereals and roasted groundnuts using NEOGEN's Reveal® Q+ for Aflatoxin test kit

The screening was carried out using NEOGEN's Reveal® Q+ for the Aflatoxin test kit following the manufacturer's instructions. Dried samples were ground using an MRC laboratory grinder (Model SM-450). Ten grams of the ground material were weighed into an extraction cup and 125 mL of 65% ethanol solution was added. The mixture was vigorously shaken by hand for 3 min and allowed to settle. Filtration followed using Whatman No. 4 filter paper to collect 3 mL filtrate into a sample collection tube. A new Reveal Q+ for the Aflatoxin test strip was inserted into the sample cup and allowed to stand for 6 min. The test strip was withdrawn from the sample cup and immediately read using the AccuScan reader.

### Determination of aflatoxin B1 and total aflatoxin through competitive direct enzyme-linked immunosorbent assay

#### Sample extraction and analysis

Determinations were carried out using enzyme-linked immunosorbent assay (ELISA) testing kits (Helica Biosystems, USA) for total aflatoxin and aflatoxin

B1 levels, following the manufacturer's instructions. Preparation and analysis of the samples were carried out at room temperature. A 20 g ground (capable of passing through a 20 mesh screen) portion of the food sample was accurately weighed into a test tube into which 100 mL of 70% methanol in distilled water was added for the extraction process. This mixture was shaken for 2 min and then filtered through a Whatman No.1 filter paper to obtain 5 – 10 mL of the filtrate. The extract obtained was used in the testing process.

The appropriate number of mixing wells for all the samples and standards were placed into a microtiter wells holder. In addition, two antibody-coated wells were set in place for each sample or standard. Test wells were correctly labeled for standards and samples to be tested. Then, 200  $\mu$ L of the enzyme conjugate was pipetted into each mixing well and 100  $\mu$ L of standards and samples were added to the respective mixing wells. For each addition, mixing was achieved by priming the pipettor at least 3 times. Using a new pipette tip for each sample or standard, 100  $\mu$ L of contents from each mixing well was transferred to a corresponding labeled antibody-coated well after which incubation was done for 15 min. Afterward, the contents of the antibody-coated wells were appropriately discarded. Then, the antibody-coated wells were filled with the manufacturer's wash buffer solution, and the contents were also discarded. The washing process using the wash buffer was then repeated four more times. After the final wash, the antibody-coated wells were carefully inverted and tapped onto absorbent paper to remove the remaining wash buffer solution. Afterward, 100  $\mu$ L of the substrate was transferred into each antibody-coated well, covered to avoid direct light, and incubation was carried out at room temperature for 5 min. Finally, 100  $\mu$ L of stop solution was transferred into each test well in the same sequence and pace as the substrate. Then optical densities of each antibody-coated well were read at 450 nm using a microtiter plate reader.

## Data analysis

Data were analyzed using Statistical Analysis System (SAS) version 9.4 to perform analysis of variance and means separated using Tukey's HSD (honestly significant difference) test. Significance was established at a  $P < 0.05$ . Results were reported as mean  $\pm$  standard deviation for all samples.

## Results and discussion

### Moisture level in street vended roasted groundnuts and cereal-based street foods

Moisture levels in stored roasted groundnuts has been reported as a risk factor for the production of aflatoxins [25, 26]. However, in this study, no link could be established between moisture level in roasted groundnuts and the level of aflatoxin present in the samples. Roasted groundnuts in this study had moisture levels between 0.8 to 3.7% for the six study locations (Table 3.1).

There was no significant linear correlation between the moisture level in roasted groundnuts and the total aflatoxin ( $r = 0.026$ ,  $p = 0.92$ ) or aflatoxin B1 ( $r = -0.035$ ,  $p = 0.89$ ) levels reported. Similar findings were reported by Hlashwayo [27] who found no relationship between high levels of aflatoxin B1 ( $\chi^2 = 0.04$ ,  $p = 0.85$ ) in roasted groundnuts and the moisture level of the roasted groundnuts. Although many fungal strains have the capability of producing mycotoxins, they may not do so until many conditions are met. Despite the availability of moisture, other physical, chemical and biological factors may also affect toxin production [20]. Thus, higher moisture levels may not always be indicative of high aflatoxin levels in food samples.

**Table 3.1:** The moisture level in street vended cereal-based foods and roasted groundnuts.

Location	Cereal-based foods (%)	Ground nuts (%)
Thika level 5 hospital area	66.293 $\pm$ 2.823 <sup>a</sup>	0.877 $\pm$ 0.160 <sup>a</sup>
Juakali area	64.910 $\pm$ 0.130 <sup>a</sup>	1.497 $\pm$ 0.463 <sup>a</sup>
Kiandutu slums	64.670 $\pm$ 4.964 <sup>a</sup>	3.758 $\pm$ 2.854 <sup>a</sup>
Makongeni area	68.630 $\pm$ 1.011 <sup>a</sup>	1.270 $\pm$ 0.632 <sup>a</sup>
Ngoigwa area	71.397 $\pm$ 1.689 <sup>a</sup>	1.162 $\pm$ 0.461 <sup>a</sup>
Thika town center	65.763 $\pm$ 5.406 <sup>a</sup>	1.702 $\pm$ 0.456 <sup>a</sup>

Values are means  $\pm$  standard deviation. Values in each column with different superscript letters are significantly different ( $p < 0.05$ ).

## Screening for aflatoxins in cereal-based foods and roasted groundnuts

The results for screening cereal-based foods and roasted groundnuts for aflatoxins are shown in Table 3.2. All cereal-based food samples had aflatoxin levels below the detectable level (limit of detection = 0.002  $\mu\text{g/g}$ ) while 83.3% of all roasted groundnuts had aflatoxin levels above the detection level according to the NEOGEN®'s Reveal® Q+ for Aflatoxin test kit. All samples showing the presence of aflatoxins were subjected to the competitive direct enzyme-linked immunosorbent assay (ELISA) test for quantification.

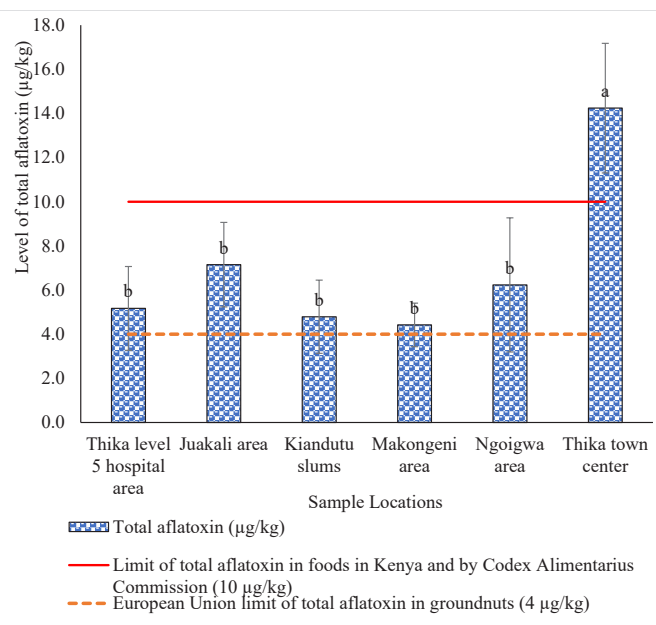
**Table 3.2:** Proportion of samples contaminated with aflatoxin in street vended cereal-based foods and roasted groundnuts.

Location	Cereal-based foods*	Roasted groundnuts (%)
Makongeni	Not detected	100.0
Thika town center	Not detected	100.0
Kiandutu	Not detected	66.6
Thika level 5 Hospital area	Not detected	100.0
Ngoigwa	Not detected	100.0
Juakali	Not detected	100.0

\*Cereals-based foods included maize mixed with legumes such as beans and pigeon peas. Not detected = <2  $\mu\text{g/kg}$ .

## Total aflatoxin and aflatoxin B1 in roasted groundnuts

Figure 3.1 shows the total aflatoxin levels reported in roasted groundnuts from selected locations in Thika Town. Total aflatoxin in street vended roasted groundnuts averaged between 4.4 - 14.2  $\mu\text{g/kg}$ . There was no significant difference in the total aflatoxin level in roasted groundnuts for all locations except Thika town center which had significantly ( $p=0.0012$ ) higher total aflatoxin level. The range of total aflatoxin in this study in roasted groundnuts was comparable but lower than those reported in a study on the safety of chilled cereal beverages sold as street food in some open markets in Ghana [28], where the total aflatoxins content in samples ranged from 7.0 to 22.2  $\mu\text{g/kg}$ . Higher contamination levels were also reported by Sombie, Ezekiel [1] in Sierra Leone where they found roasted street-vended nuts to contain aflatoxins averaging 487.8  $\mu\text{g/kg}$ .

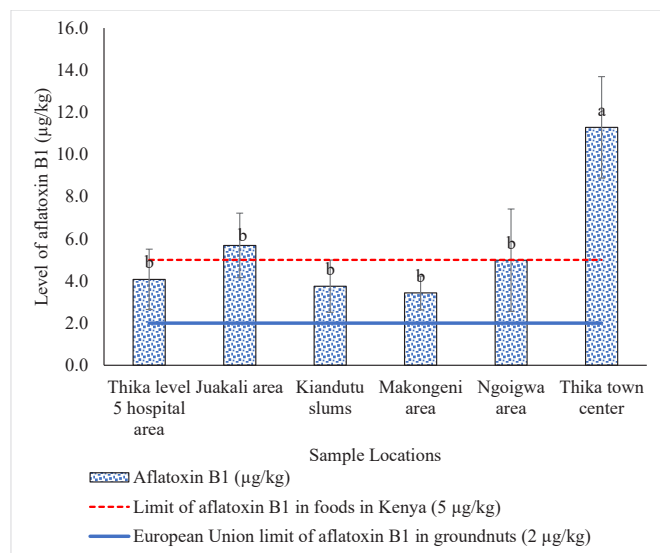


**Fig. 3.1:** The mean total aflatoxin level in roasted groundnuts from selected locations in Thika Town.

The Kenyan regulatory limit for total aflatoxin in food products is 10  $\mu\text{g/kg}$  as set by the Kenya Bureau of Standards (KEBs) [25, 29]. On average 83.3% of groundnut samples had aflatoxin levels below this regulatory limit while only 16.7% of samples had aflatoxin levels above the limits. Compared to the 4  $\mu\text{g/kg}$  limit set by the European Union [5, 30] for total aflatoxin, 72.2% of the samples were above the limit. However, on average, none of the study locations had mean total aflatoxin levels below the European Union established limit. Higher contamination levels were reported by Mutegi and others [25] in a study on incidence of aflatoxin in roasted groundnuts from various markets in Kenya where they observed that aflatoxin levels in 37% of the samples exceeded the regulatory limit for aflatoxin levels (10  $\mu\text{g/kg}$ ) set by the KEBs.

Aflatoxin B1 level in street vended roasted groundnuts averaged between 3.4-11.3  $\mu\text{g/kg}$  as shown in Figure 3.2. There was no significant difference in aflatoxin B1 level in roasted groundnuts for all locations except Thika town center which had significantly higher aflatoxin level ( $p = 0.001$ ). Sombie, Ezekiel [1] in Sierra Leone reported even higher contamination levels in roasted street-vended nuts which contained aflatoxins levels ranging between 0.6-1,387.0  $\mu\text{g/kg}$  while Vabi, Eche [31] found aflatoxin B1 contamination levels ranging from 3.8 - 12.3  $\mu\text{g/kg}$  in groundnut kernels and 12.3 - 99.4  $\mu\text{g/kg}$  in groundnut-based products in Northwestern Nigeria. In addition, Hlashedway [27] found groundnut contamination of 0.0 - 72.9  $\mu\text{g/kg}$  with an average of 2.7  $\mu\text{g/kg}$  while studying the

level of aflatoxin B1 contamination in raw peanuts sold in Maputo City, Mozambique.



**Fig. 3.2:** The mean aflatoxin B1 level in roasted groundnuts from selected locations in Thika Town.

The Kenyan regulatory limit for aflatoxin B1 in food products is 5.0 µg/kg [29]. On average 55.6% of groundnut samples had aflatoxin B1 levels below the regulatory limit while 44.4% of samples had aflatoxin B1 levels above the allowable limits. Compared to the 2 µg/kg limit set by the European Union [30] for aflatoxin B1, all the individual samples from all study locations were above the established limits. Similar to this study, levels of aflatoxin B1 exceeding the maximum allowable limit set by the European Commission [30] were found in 91% of groundnut and groundnut-based products in Northwestern Nigeria [31].

The high level of total aflatoxins and aflatoxin B1 reported in Thika town center as compared to the other locations could be attributed to the possible differences in handling practices and storage among street food vendors as well as the sources of raw materials. It is possible that despite the possible poor handling practices such as exposing groundnuts to moist air resulting in growth and toxin production by *Arspergillus* spp., vendors in Thika town center may have bought their raw materials from the same source which may have had high contamination levels in groundnuts and hence the high contamination levels reported in the street vended product. Nonetheless, aflatoxin production has been reported to vary greatly depending on many physical, chemical, and biological factors such as moisture content of the food product, temperature of storage, the location of the street food vendors, the storage practices, and personal hygiene [20].

The high levels of total aflatoxins and aflatoxin B1 reported in this study could be attributed to many factors.

This is because aflatoxin contamination of foods may occur at different points in the food chain depending on the time of molds invasion. It may be produced during the pre-harvest, harvest, drying, or storage period of the groundnuts although they ultimately depend on the handling method, packaging, or transport conditions of the food materials [20]. All these factors may be different for different street food vendors from different regions. Insufficient drying and humid storage environmental conditions may result in high mold invasion and concurrent aflatoxin contamination in foods [20, 32]. For instance, poor packaging and storage methods that are mostly applied by street food vendors may contribute to the contamination of groundnuts with aflatoxins. According to Mutegi and others [25], packaging materials used in groundnuts significantly influenced the levels of aflatoxin in the product, with 68% of groundnuts that were stored in plastic containers having more than 10 µg/kg of aflatoxin. The majority of street vendors use plastic containers for packaging or storage of groundnuts [33]. The plastic containers may retain moisture and trap heat and retain them inside thus providing a conducive environment for mold growth and eventual toxin production. Similar findings were reported by Hlashwayo [27] who reported that the highest levels of aflatoxin B1 contamination were found in groundnuts sold in plastic containers.

These high levels of aflatoxin particularly aflatoxin B1 in groundnuts are worrying considering that aflatoxins are hepatotoxic, carcinogenic, genotoxic, nephrotoxic, teratogenic, and immunosuppressive [6]. Aflatoxin B1 has been reported as being the most potent of the aflatoxin compounds capable of causing the development of hepatocellular carcinoma in humans as well as many other complications including malnutrition, immunomodulation, and growth impairment [34, 35]. These high levels of aflatoxins, especially aflatoxin B1, highlights the need for Kenya to take the necessary steps to effectively manage aflatoxins in ready-to-eat foods.

## Conclusion

Although aflatoxin was absent in cereal-based foods, the results revealed the presence of aflatoxin B1 in 44.4% of groundnut samples above the recommended limits (5 µg/kg) set by the Kenya Bureau of Standards (KEBs). In addition, all groundnut samples had contamination levels exceeding the EU set limits of 2 µg/kg which may pose serious food safety concerns. Aflatoxin B1 has been proven to be carcinogenic and thus, there is a great need for interventions to reduce contamination in street vended groundnuts. These high levels of aflatoxins, especially

aflatoxin B1, highlights the need for Kenya to take the necessary steps to effectively manage aflatoxins in ready-to-eat foods. Mapping the groundnut value chain in Kenya may assist in identifying avenues of contamination and developing strategies to reduce contamination.

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## Conflict of Interests

The authors declare that they have no conflict of interest.

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