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Article

## Assessment of Mycoflora Diversity and Aflatoxin Contamination in Bean Cake (*Akara* and *Kengbe*) Sold across Selected Markets in Ibadan Metropolitan Area

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### Abstract

Bean cake, locally known as akara (fried in groundnut oil) and kengbe (fried in palm oil), is a widely consumed street food in southwestern Nigeria. However, it is highly perishable and prone to microbial contamination, particularly by fungi capable of producing mycotoxins, which pose significant public health risks. This study evaluated the diversity of fungal contaminants and the presence of total aflatoxins in akara and kengbe sold in Challenge, New garage, Soka, Dugbe, Oluyole and Tipper garage markets in Ibadan metropolis. Samples were aseptically collected from six major locations during the peak rainy season of May and June, 2025, homogenized, and cultured on Potato Dextrose Agar supplemented with streptomycin for fungal isolation. Colonies were identified based on macroscopic and microscopic characteristics, and aflatoxin levels were quantified using the RIDASCREEN® Aflatoxin Total ELISA kit (R-Biopharm, Germany). A total of 67 fungal isolates were recovered, representing *Aspergillus niger* (25%), *Penicillium camemberti* (25%), *Aspergillus flavus* (22%), *Rhizopus* spp. (15%), *Mucor* spp. (8%), and *Saccharomyces cerevisiae* (5%). Aflatoxin concentrations ranged from 2.0 to 12.0 ppb, with the highest values associated with samples harboring *A. flavus*. Variations in fungal prevalence were observed across market locations, reflecting differences in post-processing handling and environmental exposure. The detection of aflatoxins, which are heat-stable secondary metabolites produced by fungi, underscores the potential health risks posed by these widely consumed foods. These findings highlight the need for improved hygienic practices during production, handling, and vending of bean cake, as well as regular monitoring for fungal contamination and mycotoxins to protect consumer health.

**Keywords:** Akara, Kengbe, Fungal contamination, Aflatoxins, Street food safety, *Aspergillus flavus*.

### 1. Introduction

Bean cake, commonly known as *akara* (prepared with groundnut oil) in West Africa and *kengbe* (prepared with palm oil) in some local settings, is a widely consumed food produced from cowpea beans (*Vigna unguiculata*) (Badmos et al., 2021). It constitutes an integral part of diets across Nigeria and parts of sub-Saharan Africa (SSA) due to its affordability, and nutritional value. The production process involves soaking, dehulling, wet milling, seasoning, and deep-frying and it's often sold under informal street-vended condition (Badmos et al., 2021). Cowpea-based foods provide nutritional benefits, including 21–25% high-quality plant protein, complex carbohydrates, dietary fiber, and essential micronutrients

such as iron, potassium, and B-complex vitamins. This makes them important in low- and middle-income communities where animal protein may be limited (Badmos et al., 2021; Mchi et al., 2024).

Due to certain factors, bean cake is extremely perishable and susceptible to microbial contamination, especially fungal contamination, despite its nutritious value and widespread consumption. These factors include high moisture content, exposure to room temperature, extended handling, poor personal hygiene, tainted raw ingredients, unsanitary storage conditions, and unsanitary packing methods frequently found in meals sold on the street (Codex Alimentarius Commission, 2020; FAO, 2021). According to studies, open-market cowpea products that are ready to eat are regularly exposed to dust, insects, and airborne spores, which fosters the growth and colonization of fungi (Mchi et al., 2024; Okogbenin et al., 2025; Jeff-Agboola, 2024; Sivakaame, et al., 2025).

Many filamentous fungi produce mycotoxins, which are secondary metabolites that pose major health concerns to humans, fungus contamination of food is especially concerning (Aasa et al., 2022; Jeff-Agboola, 2024). It is well known that mycotoxins, like aflatoxins, can have hepatotoxic, immunosuppressive, acute, and carcinogenic effects, particularly after extended exposure (Aasa et al., 2022; Murray et al., 2020). West African legumes, cereals, and their processed products have been found to be contaminated by toxic fungal genera, particularly *Aspergillus*, *Penicillium*, and *Fusarium* (Badmos et al., 2021; Aasa et al., 2022). *Aspergillus spp.*, in particular, are frequently associated with aflatoxin production and have been isolated from cowpea flour and related food products in Nigeria (Badmos et al., 2021).

Due to poor enforcement of regulations, insufficient monitoring systems, and a lack of adherence to food hygiene standards, aflatoxin contamination continues to pose a serious threat to food safety in developing nations (Aasa et al., 2022; Codex Alimentarius Commission, 2020; Michael et al., 2023; Ajadi and Olan, 2024). In order to minimize microbial and mycotoxin contamination, particularly in meals sold on the street, the Food and Agriculture Organization and the Codex Alimentarius Commission stress the significance of following proper hygiene procedures throughout food preparation, handling, storage, and vending (Codex Alimentarius Commission, 2020; FAO, 2021). However, in the informal food industry, adherence to these rules is frequently uneven, raising the danger of exposure for customers (Ajadi and Olan, 2024; Dada et al. 2024).

The public health consequences of consuming contaminated food have been highlighted by earlier research conducted in Nigeria that found aflatoxin and fungal contamination in bean flour, cowpea-based dishes, and other ready-to-eat items (Badmos et al., 2021; Mchi et al., 2024; Okogbenin, et al., 2025). There is little information on the variety of mycoflora and aflatoxin contamination specifically linked to ready-to-eat bean cake items like akara and kengbe, especially in Ibadan metropolitan markets, even if some studies have concentrated on raw cowpea seeds or processed flour. This knowledge gap raises serious concerns about food safety because of Ibadan's dense population and widespread reliance on foods sold on the street.

In order to ascertain the degree of aflatoxin contamination in akara and kengbe sold in a few markets within the Ibadan metropolitan region, this study intends to evaluate the variety of mycoflora. The study aims to produce baseline data that can guide public health interventions, raise awareness of food safety, and support regulatory initiatives targeted at lowering consumer exposure to mycotoxin by identifying and isolating fungal species found in these products and assessing possible aflatoxin contamination.

## 2. Materials and Methods

Bean cake (akara) samples were bought from traffic vendors and hawkers on six major roads in Ibadan, Oyo State, Nigeria (7.3775° N, 3.9470° E), during the peak rainy season (March–April 2025) to capture high ambient humidity (>80% RH). Twelve freshly fried units (two per vendor) were aseptically transferred into sterile Whirl-Pak® bags, labeled, transported in insulated containers maintained at 4 °C ± 1 °C, and processed within 24 hours at the microbiology laboratory, Lead City University, Ibadan.

All consumables were sterilized via autoclaving or dry heat. Potato Dextrose Agar (PDA) supplemented with streptomycin (0.1 g/L) served as the primary isolation medium. Key equipment included a Class II biosafety cabinet, incubator (25 °C), compound microscope, autoclave, stomacher, and ELISA microplate reader. Reagents included Lactophenol Cotton Blue (LPCB), Gram stain kit, buffered peptone water, and immunoaffinity and ELISA kits for aflatoxin B<sub>1</sub> analysis.

PDA was prepared by dissolving 39 g/L medium in deionized water, sterilized (121 °C, 15 min), poured aseptically (~20 mL/plate), and validated for sterility. For fungal enumeration, 10 g of each sample were aseptically plated directly onto PDA in triplicate and incubated at 25 °C for three days. Colonies (10–150 CFU) were counted and expressed as CFU/g. Distinct colonies were purified through repeated sub-culturing on PDA and preserved in MacCartney bottles. Macroscopic characterization (colony diameter, pigmentation, texture, sporulation) was performed on PDA and Czapek Dox Agar. Microscopic characterization involved LPCB-stained mounts to examine hyphae, conidiophores, vesicles, metulae, phialides, and conidia. Gram staining was used to detect bacterial contamination; contaminated isolates were re-purified.

### 2.1 Detection of Aflatoxins in Akara Samples

Aflatoxins in akara samples were quantified using the RIDASCREEN® Aflatoxin Total ELISA kit (R-Biopharm, Germany) (Okogbenin, et al., 2025). Briefly, five grams of each sample were homogenized and extracted with 25 mL of 70% methanol. The extracts were filtered, and the filtrates were diluted according to the manufacturer's instructions. ELISA was performed following the kit protocol, including incubation, washing, and addition of conjugate and substrate (Okogbenin, et al., 2025). Absorbance was read at 450 nm using a microplate reader, and aflatoxin concentrations were calculated from the standard curve. All procedures were conducted under conditions minimizing aflatoxin exposure, and results were compared with international permissible limits for ready-to-eat foods (FAO, 2021; FDA, 2020).

## 3. Results and Discussion

This study evaluated the presence and diversity of fungi and the levels of aflatoxins present in bean cake (akara and kengbe) samples sold in selected markets and in traffic in Ibadan metropolis. The isolation of six fungal genera from the samples demonstrates that bean cake, despite being a freshly fried prepared product, is highly vulnerable to post-processing fungal contamination. A total of 67 fungal isolates were recovered, indicating exposure of this widely consumed street food to environmental and handling-related contaminants. According to research fungi such as *Aspergillus*, *Penicillium*, *Rhizopus*, and *Mucor* are ubiquitous in the environment and easily contaminate foods through air, dust, contaminated surfaces, and unhygienic handling (Murray, et al., 2020; Akintunde, et al., 2023; Mba et al., 2024; Akharenegbe, et al., 2022).

The predominant fungal isolates identified in this study were *Aspergillus niger* (25%), *Penicillium* species (25%), and *Aspergillus flavus* (22%). The high occurrence of *Aspergillus* and *Penicillium* species is considered a serious public health concern because these genera are the most important producers of mycotoxins in food commodities. Research has reported that fungi survive well under warm, humid conditions and readily colonize legume-based products such as beans, particularly when drying and storage conditions are inadequate (Codex Alimentarius Commission, 2020; Kupoluyi et al., 2024). The isolation of *Rhizopus* spp. (15%) and *Mucor* spp. (8%), which are common spoilage fungi, further indicates poor post-frying handling and prolonged exposure of the product to contaminated air during display and sale (Mba et al., 2024; Akharenegbe, et al., 2022; Kupoluyi et al., 2024). The low occurrence of *Saccharomyces cerevisiae* (5%) suggests that contamination of the bean cake samples were mainly through human handling and environmental contact.

Marked variations were also observed in fungal contamination across sampling locations. Markets such as Challenge and Dugbe (Akara) and Tipper Garage (Kengbe) showed no fungal growth, which may be attributed to relatively better sanitary handling or reduced post-processing exposure. In contrast, New Garage and Soka recorded the highest diversity and frequency of fungal isolates. These

locations are characterized by dense human traffic, vehicular emissions, dust, and prolonged open display of food, which greatly increase the risk of airborne fungal contamination. Similar observations were documented that street-vended foods in high-traffic urban locations showed significantly higher microbial loads due to environmental exposure and poor hygienic practices (Murray et al., 2020; Ajadi and Olan, 2024; Akintunde, et al., 2023; Mba et al., 2024).

**Table 1:** Isolation of Fungi from Akara and Kengbe Samples in Ibadan

| S/N | Location               | No of colonies                        | Characteristics of colony   | Probable micro-organisms              |
|-----|------------------------|---------------------------------------|---|---------------------------------------|
| 1   | New garage (Akara)     | 1                                     | Rhizoid, dirty green with whitish edge.                             | <i>Aspergillus flavus</i>             |
|     |                        | 5                                     | Grey-green powdery with white edge.                                 | <i>Penicillium camemberti</i>         |
|     |                        |                                       | Rhizoid, black with whitish edge.                                   |                                       |
|     |                        | 1                                     | Fluffy and whitish.   | <i>Aspergillus niger</i>              |
| 1   |                        |                                       | <i>Mucor mucedo-</i>  |                                       |
| 2   | Soka (Akara)           | 1                                     | Fluffy and whitish.   | <i>Mucor mucedo-</i>                  |
|     |                        | 2                                     | Grey-green powdery with white edge.                                 | <i>Penicillium camemberti</i>         |
|     |                        |                                       | Rhizoid, black with whitish edge.                                   |                                       |
|     |                        | 1                                     | Round, mucoid, shiny creamy and of different sizes.                 | <i>Aspergillus niger</i>              |
| 7   |                        | <i>Yeast Saccharomyces cerevisiae</i> |   |                                       |
| 3   | Challenge (Akara)      |                                       | No growth   |                                       |
| 4   | Oluyole (Akara)        | 13                                    | Round, mucoid, shiny creamy and of different sizes.                 | <i>Yeast Saccharomyces cerevisiae</i> |
| 5   | Tipper garage (Akara)  | 2                                     | Ash woolly (fluffy) that grows to occupy the plate.                 | <i>Rhizopus spp.</i>                  |
| 6   | Dugbe (Akara)          |                                       | No growth   |                                       |
| 7   | New garage (Kengbe)    | 7                                     | Ash woolly (fluffy) that grows to occupy the plate.                 | <i>Rhizopus spp.</i>                  |
| 8   | Soka (Kengbe)          | 4                                     | Rhizoid, dirty green with whitish edge                              | <i>Aspergillus flavus</i>             |
|     |                        | 5                                     | Fluffy and whitish.   | <i>Mucor mucedo-</i>                  |
| 9   | Challenge (Kengbe)     | 9                                     | Rhizoid, black with whitish edge                                    | <i>Aspergillus niger</i>              |
| 10  | Oluyole (Kengbe)       | 1                                     | Rhizoid, blackish with whitish edge that grows to occupy the plate. | <i>Aspergillus niger</i>              |
| 11  | Tipper garage (Kengbe) |                                       | No growth   |                                       |
| 12  | Dugbe (Kengbe)         | 5                                     | Grey- green, powdery with whitish edge.                             | <i>Penicillium camemberti</i>         |
|     |                        |                                       | Fluffy and whitish.   |                                       |
|     |                        | 2                                     |   | <i>Mucor mucedo</i>                   |
|     |                        | Total 67                              |   |                                       |

**Table 2:** Macroscopic and Microscopic Features of Fungal Isolates from Bean Cake Samples in Ibadan

| S/N | Isolate Code | Microscopic Features        | Macroscopic Features | Micro organisms                 |
|-----|--------------|-----------------------------|----------------------|---------------------------------|
| 1   | AN1          | Smooth walled conidiophores | Dirty green          | <i>Aspergillus flavus</i>       |
| 2   | AT2          | Non septate conidiophores   | Woody white          | <i>Mucor mucedo</i>             |
| 3   | AO1          | Large globose conidiophores | Black                | <i>Aspergillus niger</i>        |
| 4   | AS2          | Budding yeast cells         | Creamy white         | <i>Saccharomyces cerevisiae</i> |
| 5   | AC1          | Hyphae without rhizoids     | Cotton-like colonies | <i>Mucor</i> sp.                |
| 6   | KN2          | Hyphae with rhizoids        | Cotton-like colonies | <i>Rhizopus</i> sp.             |

Key: AN1 = Akara New garage, AT2 = Akara Tipper garage, AO1 = Akara Oluyole, AS2 = Akara Soka, AC1 = Akara Challenge, KC2 = Kengbe Challenge

**Table 3:** Frequency of Occurrence of Fungal Isolates in Bean Cake Samples in Ibadan

| S/N | Isolated Microbes               | Frequency of occurrence | Percentage (%) |
|-----|---------------------------------|-------------------------|----------------|
| 1   | <i>Aspergillus niger</i>        | 6                       | 25%            |
| 2   | <i>Aspergillus flavus</i>       | 4                       | 22%            |
| 3   | <i>Rhizopus</i> sp.             | 3                       | 15%            |
| 4   | <i>Mucor</i> sp.                | 2                       | 8%             |
| 5   | <i>Penicillium</i> sp.          | 6                       | 25%            |
| 6   | <i>Saccharomyces cerevisiae</i> | 1                       | 5%             |

**Table 4:** Concentrations of Total Aflatoxins (AFB1) found in Bean Cake Samples in Ibadan

| S/N | Isolate Code | Total Aflatoxin (Ppb) |
|-----|--------------|-----------------------|
| 1   | AN1          | 2.0                   |
|     | AT2          | 6.0                   |
| 2   | AO3          | 12.0                  |
|     | AS4          | 3.0                   |
| 3   |              |                       |
| 4   |              |                       |

Ppb = parts per billion.

The detection of aflatoxins in all analyzed samples of the bean cake is a critical public health finding and concern. Aflatoxin concentrations ranged from 2.0 to 12.0 ppb (parts per billion), with the highest value exceeding internationally acceptable limits for ready-to-eat foods especially according to the Food and Agriculture Organization and CODEX Alimentarius,, Ready-to-eat foods should be free from

hazardous levels of mycotoxins because they are consumed without further processing. NAFDAC which is the agency that controls food and drug administration in Nigeria allows for limits below 20ppb. The presence of aflatoxins in bean cake directly reflects contamination of the raw cowpea and possible toxin production during storage before frying. This is further supported by the frequent isolation of *Aspergillus flavus*, the primary aflatoxin-producing fungus.

When quantitatively compared to values reported for cowpea-based products in the same geographic area, the aflatoxin concentrations found in this study offer significant information. Aflatoxin values in the Akara and Kengbe samples in the current study varied from 2.0 to 12.0 ppb, suggesting varying but there was detectable contamination at every sampling location. In contrast, aflatoxin levels in bean flour sold in Abeokuta, Nigeria, ranged from roughly 1.8 to 15.6 ppb, according to studies conducted by Badmos et al., (2021), with a percentage of samples surpassing advised safety limits. Aflatoxins are known to be heat-stable substances that cannot be eliminated by standard cooking or frying temperatures, toxins created during pre-processing storage can remain in final goods (Jeff-Agboola, 2024; Sivakaame, et al., 2025). This makes the comparison relevant. As a result, the similar aflatoxin levels found in fried bean cake and raw bean flour highlight how ineffective thermal processing is as a control once contamination has taken place. Studies conducted in Sokoto state Nigeria and Kwara have also showed the presence of high levels of aflatoxins in root powder sold and groundnut (Aliyu et al., 2022; Ajadi and Olahan, 2024). These results emphasize the necessity for preventive actions earlier in the food supply chain rather than depending solely on processing to assure food safety, and they identify inadequate post-harvest handling and storage of cowpeas as the crucial control point in aflatoxin exposure.

Chronic exposure to even low levels of aflatoxins has been associated with hepatotoxicity, immunosuppression, growth retardation in children, and an increased risk of hepatocellular carcinoma (Mchi et al., 2024). Therefore, regular consumption of contaminated bean cake poses a significant long-term health risk, especially to children and immunocompromised individuals. The isolation procedures used in this study followed standard microbiological techniques consistent with the FDA Bacteriological Analytical Manual, confirming the reliability of the fungal detection and identification (R-Biopharm, 2023). The findings further validate the importance of continuous microbiological surveillance of street-vended foods, as recommended by both the FAO and CODEX Alimentarius (FAO, 2021; FDA, 2020).

Bean cake vendors should adopt stricter hygienic practices throughout the production and vending process in accordance with the CODEX Alimentarius and FAO guidelines for street-vended foods. Raw cowpea intended for akara and kengbe production should also be properly dried and stored in clean, moisture-free, and well-ventilated environments to prevent fungal growth and aflatoxin formation before processing. Vendors should ensure that all utensils, grinding equipment, frying surfaces, and packaging materials are properly cleaned and sanitized before and after use. Bean cake should be displayed in clean, covered containers to minimize post-frying contamination from dust and airborne fungal spores. Personal hygiene among vendors, including regular handwashing and use of protective clothing, should be strictly enforced.

Public health authorities should intensify routine inspection and microbiological monitoring of street-vended foods, particularly in high-traffic locations such as New Garage and Soka. Regular screening for mycotoxins in beans and bean-based products should be incorporated into food safety surveillance programme. In addition, continuous education and training of food vendors on the dangers of fungal contamination and aflatoxin exposure should be implemented through health outreach programme. Finally, regulatory agencies should enforce compliance with national and international food safety standards, while encouraging the adoption of hazard analysis and critical control point (HACCP) principles among small-scale food processors to reduce the burden of foodborne fungal contamination and mycotoxin exposure in the population.

#### 4. Conclusion

The presence of toxigenic fungi and detectable aflatoxin levels in bean cake sold across the selected markets in Ibadan metropolis reflects lapses in hygiene practices during raw material storage, processing, frying, displaying, packaging, and vending. These findings underscore the urgent need for strict adherence to food hygiene regulations and regular monitoring of street-vended foods to safeguard public health.

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Not applicable.

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**Conflict of Interest**

The author declared no conflict of interest in the manuscript.

**Authors' Declaration**

The author(s) hereby declare that the work presented in this article is original and that they will bear any liability for claims relating to the content of this article.

**Author Contributions**

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