

## Review Article

# A Comprehensive Review of Aflatoxin in Groundnut and Maize Products in Africa: Prevalence, Detection and Mitigation Strategies

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Aflatoxins are a toxic secondary metabolite, mainly produced by the fungi *Aspergillus flavus* and *A. parasiticus*. Aflatoxin contamination of food is a global concern, as they are carcinogenic, mutagenic and teratogenic. Groundnuts and maize products are highly susceptible to aflatoxin contamination at both pre- and postharvest stages; this leads to a great risk for those countries that rely on these products for food and nutrition security as well as income. Groundnut and maize products have contributed a substantial amount of aflatoxin exposure to human and animal health risks, especially in countries that experience tropical climate and recurrent drought, favouring mould developments. Due to the strange health impacts of aflatoxin in agricultural commodities, different countries have set the acceptable limits for groundnut and maize products, whereas most of the countries use the same limit for both commodities. Detection and quantification of aflatoxins in groundnut and maize products are mainly through enzyme-linked immunoaffinity assay (ELISA) and high-performance liquid chromatography (HPLC), among others. However, currently rapid, accurate and cost-effective techniques are emerging to quickly monitor and enforce the regulation limits. Among the widely applied strategies for aflatoxin mitigation are biological control including atoxigenic *Aspergillus* strains, plant extracts, and chemical and physical methods of detoxification and decontamination. Aflatoxin decontamination using plant extracts is promising for most countries in sub-Saharan Africa owing to the availability, ease of access and affordability; however, there is a need for further screening to isolate the bioactive ingredients. This review could provide insight into the researchers, stakeholders and consumers on the prevalence of aflatoxin in groundnut and maize products as well as mitigation strategies to improve food safety.

**Keywords:** aflatoxin; *Aspergillus*; carcinogenic; decontamination; food safety; health risk

## 1. Introduction

Aflatoxins are a group of mycotoxins (secondary metabolites) produced by *Aspergillus* species that contaminate a variety of food and agricultural products with a special preference for groundnut and maize among nuts and cereals, respectively. *Aspergillus* species, mainly the section *Flavi*, *A. flavus* and *A. parasiticus*, produce aflatoxins [1]. Fungal growth and consequent aflatoxin production could occur at any stage of the crop production and value chain, depending on environmental factors and farm management practices [2–4]. Aflatoxin synthesis in agricultural products is by plant immunocompromising variables such as drought stress, damage, pest infestation and inadequate fertilizer [5–7]. According to Eskola et al. [8], 60%–80% of the world's food crops (cereal and nuts) are contaminated with mycotoxins. This estimate is above the known 25% estimate by the United Nations' Food and Agricultural Organization (FAO); the high occurrence in the later study was attributed to improved methods of mycotoxin detection and the impact of climate change, which is leading to the detection of mycotoxins in nontropical regions of the world.

Groundnut (*Arachis hypogaea* L.) and maize (*Zea mays* L.) are among the economically important crops grown worldwide [6, 9, 10]. These crops are a major source of food and income for both smallholder and commercial farmers in the countries where they are grown, contributing significantly to food and nutrition security as well as income generation. Due to the volume of global production and consumption, groundnut (also known as peanut), maize and their products are among the most commonly implicated crops in human aflatoxin exposure [11–13]. Moreover, these crops are common food sources in areas where the climate is conducive to fungal development and aflatoxin production; therefore, groundnut and maize product-induced human and animal aflatoxin exposure continues to be a serious food safety concern [14–16].

Aflatoxin contamination of groundnut and maize products has consequences for both developed and developing countries that go beyond public health concerns to commerce. Wu [17] has reported that aflatoxin-related damages cause maize farmers in the United States of America an annual loss of \$160 million, while Gbashi et al. [18] have indicated that losses amounting to \$450 million were registered in African nations, which is about 38% of the global agricultural loss.

Currently, there are 18 recognized analogues of aflatoxin groups, and three are of particular importance in terms of food safety. These are the B-group consisting of aflatoxin B<sub>1</sub> and B<sub>2</sub> (AFB<sub>1</sub> and AFB<sub>2</sub>), G-group aflatoxin G<sub>1</sub> and G<sub>2</sub> (AFG<sub>1</sub> and AFG<sub>2</sub>) and M-group aflatoxin M<sub>1</sub> and M<sub>2</sub> (AFM<sub>1</sub> and AFM<sub>2</sub>) [19–21]. The B and G nomenclature is derived from the fluorescence colours under UV light; the aflatoxin B-group is a pentanone derivative that shows strong blue fluorescence under UV light, whereas the G-series AFs are six-membered lactones that fluoresce yellow-green under UV light [22]. However, AFM<sub>1</sub> and AFM<sub>2</sub> are metabolites of AFB and AFG which show blue-violet fluorescence, often detected in products of animals that have consumed

contaminated feeds [23, 24]. AFB<sub>1</sub> is classified as Group 1 carcinogen according to the International Agency for Research on Cancer (IARC), owing to its toxic, mutagenic, immunotoxic, teratogenic and carcinogenic effects on humans and animals [25]. AFB<sub>1</sub> affects organs such as the liver and kidney in both humans and animals [26].

AFB<sub>1</sub>, AFB<sub>2</sub>, AFG<sub>1</sub> and AFG<sub>2</sub> are major contaminants of products of cereals (maize, rice, sorghum, millet and wheat), nuts (peanut, walnuts, cashew nuts and hazelnuts) and oil seeds, root tubers, spices, tea and coffee [27]. On the other hand, AFM<sub>1</sub> and AFM<sub>2</sub> are detected in milk and milk products, infant formula and breastmilk [28]. When aflatoxin-contaminated feed is consumed by a lactating animal, the AFB<sub>1</sub> and AFB<sub>2</sub> are metabolized in the liver causing the milk to be contaminated with AFM<sub>1</sub> and AFM<sub>2</sub>, which are hydroxylated derivatives of AFB<sub>1</sub> and AFB<sub>2</sub>, respectively [29].

Carryover of AFB<sub>1</sub> from feed to milk is reported to vary among animal species, with the rates being higher during the early stages of lactation [30]. M<sub>1</sub> is categorized as a Group 2B human carcinogen by the IARC [31], hence the need for its regulation in agricultural products in many countries [32].

Aflatoxin carcinogenicity is a result of genotoxicity involving metabolite activation to a genotoxic epoxide metabolite, formation of DNA adducts and modification of the TP53 gene [19, 31, 33]. Prolonged exposure to aflatoxin is associated with stunting and other congenital diseases [34]. On the other hand, aflatoxicosis, a condition that occurs as a result of acute exposure to high doses of aflatoxin, can be fatal. Acute aflatoxin exposure following consumption of contaminated groundnut and maize products has been reported in Kenya and Tanzania, which are countries experiencing a tropical climate [11, 35].

Several countries have enacted strict regulations for food and feed to avert the adverse effect of aflatoxin on human and animal health as well as on trade [36]. Additionally, strategies for the prevention of contamination of agricultural crops, both at pre- and postharvest, are being implemented [37–39]. Other innovations are implemented for degradation and decontamination of aflatoxins in affected food products [40, 41]. Several studies have shown that good agricultural practices (GAPs) and good manufacturing practices (GMPs) as aflatoxin-preventive measures are effective when accompanied by proper postharvest practices [1, 42, 43]. Total aflatoxin elimination is not envisaged in a near future. Therefore, the strength of this review is in focussing on the prevalence of aflatoxins in groundnut and maize products and their regulation limits in different countries, as well as the aflatoxin mitigation strategies especially the decontamination of aflatoxins in groundnut and maize products as a way of ensuring food safety. The review provides additional information to the investigation by Meijer et al. [44] on the aflatoxin situation in Africa.

## 2. Materials and Methods

The study was conducted by reviewing literature on aflatoxin contamination of groundnut and maize products, particularly on prevalence in African countries. Subsequently,

a search on the method of aflatoxin detection and quantification was explored to enhance the understanding of prevalence. Literature search on the regulations of aflatoxin and mitigation was not restricted to Africa, as groundnuts and maize products are commodities that are traded globally. Online databases that were used in the review included Google Scholar (<https://scholar.google.com/>), Scopus (<https://www.scopus.com/>), PubMed (<https://pubmed.ncbi.nlm.nih.gov/>) and Web of Science (<https://apps.webofknowledge.com>). These were searched using the following keywords: 'aflatoxin prevalence in groundnut and maize', 'aflatoxin exposure', 'aflatoxin detection and quantification in food', 'aflatoxin regulation', 'aflatoxin mitigation' and 'aflatoxin decontamination in food'. Other relevant scientific studies were also obtained through a cross-referencing approach. The abstracts of the generated abstracts were first screened to determine whether they fit the review and to eliminate duplicate articles. Thereafter, the full versions of these articles were retrieved, read and judged according to established inclusion criteria. For aflatoxin prevalence, the following inclusion criteria were applied: Studies conducted on groundnuts and maize products obtained from African countries, stating the country where the samples were collected; the type of groundnut or maize product analysed and number of samples examined; number/percentage of the aflatoxin-contaminated samples; and the mean or range of aflatoxin contamination, type of aflatoxin and method of detection. The studies had to be published in English in a peer-reviewed journal or book between the years 2013 and 2023. Screening was done for groundnuts and maize products separately.

Screening of articles reporting comprehensively on the methods of aflatoxin detection and quantification was focused on the methods used in studies that reported on the prevalence of aflatoxin contamination in groundnut and maize products. Literature on aflatoxin regulations that are applied in different countries, specifically on groundnut and maize products, was identified to compare the maximum acceptable limits of aflatoxins among countries in Africa and those in other continents, as regulations impact on both local and international trade. Strategies for aflatoxin contamination were considered at a global scale, particularly those that are applicable to the groundnut and maize value chain, with special recommendations for those that are feasible in Africa.

### 3. Aflatoxin Contamination in Groundnut and Groundnut Products

The summary of recent studies on the level of aflatoxin contamination in groundnut and its products in various countries are presented in Table 1. According to a study conducted in Nigeria, aflatoxins were found in more than 80% of groundnut samples, including raw, roasted, processed-coated and processed de-coated samples. Of the raw and roasted samples, about 25% had AFB<sub>1</sub> concentrations above the 20 µg/kg limit set by the National Agency for Food and Drug Administration (FDA) and Control [63]. The raw and coated samples had much higher levels of aflatoxin than the roasted and uncoated nuts [14].

Aflatoxin levels in groundnut products sold locally in Malawi were noticeably higher than those in samples of groundnut marked for export [12]. In Zimbabwe, analysis of groundnut from the formal and informal markets revealed that AFB<sub>1</sub> was the most prevalent, with the commercial groundnut butter samples having higher aflatoxin levels [47]. On the other hand, Masaka et al. [48] analysed raw peanut and peanut butter from an informal market in Zimbabwe and reported that AFB<sub>1</sub> was the most prevalent in both raw peanuts (40%) and peanut butter (95%) samples, exceeding the maximum limits (15 µg/kg) of AFB<sub>1</sub> set by Zimbabwe legislation. In Zambia, the majority of raw groundnuts traded in the Lusaka district markets were contaminated with aflatoxin, but the concentration levels were generally low (0.014–48.67 µg/kg) [50]. However, eight brands of groundnut butter were repeatedly tested for aflatoxin contamination during a three-year period from 2012 to 2014, and the results revealed that none of the brands consistently averaged ≤ 20 µg/kg. This indicates that high levels of AFB<sub>1</sub> are predominantly encountered in peanut butter in Zambia [49].

In Uganda, there were significantly higher concentrations of aflatoxins in market-processed than in home-processed peanut samples [51]. All the groundnut paste samples from major markets within Kampala city had aflatoxin levels above 20 µg/kg [52]. Similarly, 41.8% of groundnut samples collected from northern and eastern Uganda had aflatoxin levels above 20 µg/kg, with groundnut paste and flour being the most contaminated compared to the roasted, raw-shelled and unshelled groundnuts [15].

On the other hand, aflatoxin levels in the majority of samples of different groundnut varieties grown in the Busia and Kisii districts in Kenya were within the Kenya Bureau of Standards (KEBS) and the EU's regulatory limits for total aflatoxins [54]. Ndung'u et al. [55] observed that the mean aflatoxin level was greater for raw groundnut samples from Nairobi than from Nyanza areas of Kenya and that there was a positive correlation between defective nuts and the aflatoxin levels of resultant products. Boni et al. [9] revealed that groundnut grain samples from 9 districts in Tanzania were contaminated with aflatoxins, with 30% exceeding the EU limit of 20 µg/kg. Mohammed et al. [58] reported various aflatoxin contamination levels for groundnut seed/grains and groundnut cake from different locations in Ethiopia. In addition, the difference in contamination levels on stored and market groundnut sample in eastern Ethiopia was reported [57, 59]. Analysis of processed products of groundnut from DRC and Burundi showed that groundnut flour and roasted nuts had higher levels of aflatoxin contamination when compared to unprocessed grain [60].

In Mali, Burkina Faso and Niger, the mean aflatoxin concentrations in groundnut samples collected from farmers' fields or stores (1–2 of harvesting) were 115, 277 and 628 µg/kg, respectively; several samples were extremely unsafe, exceeding the acceptable regulation limits of many countries [61]. A study in three districts of Mali indicated low aflatoxin concentration in preharvest groundnut samples taken from the farmers' fields, with the majority falling within the category of 0–4 µg/kg; however, aflatoxin concentration increased from the point of harvesting up to 3 months of storage in granaries [62].

TABLE 1: Recent publications (2013–2023) on aflatoxin contamination in groundnut/peanut and products in Africa.

Country	Type of product	N* (%)	Type of aflatoxin	Concentration (mean/range) $\mu\text{g/kg}$	Detection methods	Reference
Nigeria	Raw groundnut	22/26 (84.6)	AFB <sub>1</sub>	104.1	HPTLC	[14]
	Raw groundnut	22/26 (84.6)	Total AFs	197.9	HPTLC	[14]
	Roasted groundnut	21/22 (95.5)	AFB <sub>1</sub>	14.1	HPTLC	[14]
	Roasted groundnut	21/22 (95.5)	Total AFs	23.9	HPTLC	[14]
	Processed coated	25/30 (83.3)	AFB <sub>1</sub>	93.3	HPTLC	[14]
	Processed coated	25/30 (83.3)	Total AFs	176.8	HPTLC	[14]
Ghana	Processed de-coated	18/18 (100)	AFB <sub>1</sub>	14.2	HPTLC	[14]
	Processed decoated	18/18 (100)	Total AFs	24.2	HPTLC	[14]
Malawi	Stored groundnut	63.6/120 (53.3)	Total AFs	25	HPLC	[45]
	Raw peanut (local)	64/69 (92.7)	Total AFs	122.3	HPLC	[46]
	Raw peanut (export)	16/27 (59.3)	Total AFs	2.6	HPLC	[46]
	Peanut butter (local)	14/14 (100)	Total AFs	72	HPLC	[46]
	Peanut butter (imported)	8/11 (72.7)	Total AFs	2.7	HPLC	[46]
	Peanut butter	10/11 (91)	Total AFs	75.66	HPLC	[47]
Zimbabwe	Raw peanut	7/10 (70)	AFB <sub>1</sub>	1.9–90.8	LCMS	[48]
	Raw peanut	2/10 (20)	AFB <sub>2</sub>	21.9–66.7	LCMS	[48]
	Raw peanut	4/10 (40)	AFG <sub>1</sub>	18.6–335.6	LCMS	[48]
	Raw peanut	0/10 (0)	AFG <sub>2</sub>	ND	LCMS	[48]
	Raw peanut	8/10 (80)	Total AFs	1.2–426.4	LCMS	[48]
—	Peanut butter	20/20 (100)	AFB <sub>1</sub>	4.7–382.9	LCMS	[48]
	Peanut butter	5/20 (25)	AFB <sub>2</sub>	2.7–42.8	LCMS	[48]
	Peanut butter	6/20 (30)	AFG <sub>1</sub>	9.1–162.9	LCMS	[48]
	Peanut butter	3/20 (15)	AFG <sub>2</sub>	39.9–74.8	LCMS	[48]
	Peanut butter	20/20 (100)	Total AFs	4.7–435.9	LCMS	[48]
	Peanut butter (local—2012)	70/70 (100)	AFB <sub>1</sub>	1–263 (24)*	ELISA	[49]
	Peanut butter (imported—2012)	26/26 (100)	AFB <sub>1</sub>	1–47 (10)*	ELISA	[49]
	Peanut butter (local—2013)	170/170 (100)	AFB <sub>1</sub>	1–4375 (130)*	ELISA	[49]
	Peanut butter (imported—2013)	80/80 (100)	AFB <sub>1</sub>	1–10,740 (55)*	ELISA	[49]
	Peanut butter (local—2014)	406/406 (100)	AFB <sub>1</sub>	1–3000 (35)*	ELISA	[49]
Zambia	Peanut butter (imported—2014)	200/200 (100)	AFB <sub>1</sub>	1–600 (6)*	ELISA	[49]
	Raw peanut	41/92 (44.6)	AFB <sub>1</sub>	0.015–46.60	HPLC	[50]
Zambia	Raw peanut	41/92 (44.6)	AFB <sub>2</sub>	0.006–13.17	HPLC	[50]
	Raw peanut	21/92 (22.8)	AFG <sub>1</sub>	0.005–0.51	HPLC	[50]
	Raw peanut	7/92 (7.6)	AFG <sub>2</sub>	0.006–0.04	HPLC	[50]
	Raw peanut	51 (55.4)	Total AFs	0.014–48.67	HPLC	[50]

TABLE 1: Continued.

Country	Type of product	N* (%)	Type of aflatoxin	Concentration (mean/range) µg/kg	Detection methods	Reference
Uganda	Peanut paste	(63)	AFB <sub>1</sub>	103.1	LC/MS/MS	[51]
	Peanut paste	(45)	AFB <sub>2</sub>	25.1	LC/MS/MS	[51]
	Peanut paste	(61)	AFG <sub>1</sub>	41	LC/MS/MS	[51]
	Peanut paste	(68)	AFG <sub>2</sub>	11.4	LC/MS/MS	[51]
	Peanut paste	(82)	Total AFs	180.7	LC/MS/MS	[51]
	Groundnut paste	5/5 (100)	Total AFs	99–725	ELISA	[52]
	Raw groundnut	2/5 (40)	Total AFs	17–940	ELISA	[52]
	Groundnut flour	11/11 (100)	Total AFs	96.46	ELISA	[53]
	Groundnut paste	13/13 (100)	Total AFs	196.52	ELISA	[15]
	Groundnut flour	12/12 (100)	Total AFs	187.90	ELISA	[15]
	Roasted groundnut	9/9 (100)	Total AFs	13.25	ELISA	[15]
	Shelled groundnut	17/17 (100)	Total AFs	31.77	ELISA	[15]
	Unshelled groundnut	16/16 (100)	Total AFs	90.01	ELISA	[15]
	Raw peanut	201/204 (98.5)	AFB <sub>1</sub>	0.0–510	HPLC	[54]
	Raw peanut	Na	AFB <sub>2</sub>	0.08–48.27	HPLC	[54]
Kenya	Raw peanut	Na	AFG <sub>1</sub>	0.0–43.98	HPLC	[54]
	Raw peanuts	Na	Total AFs	18.3	ELISA	[55]
	Roasted peanut	Na	Total AFs	54.8	ELISA	[55]
	Peanut butter	Na	Total AFs	318.3	ELISA	[55]
	Unsorted peanut	Na	Total AFs	0.0–364.7	ELISA	[55]
	Unsorted peanut	Na	Total AFs	0.0–82.4	ELISA	[55]
	Raw peanut	Na	Total AFs	146.8	ELISA	[56]
	Roasted coated	Na	Total AFs	56.5	ELISA	[56]
	Roasted decoated	Na	Total AFs	19.9	ELISA	[56]

TABLE 1: Continued.

Country	Type of product	N* (%)	Type of aflatoxin	Concentration (mean/range) µg/kg	Detection methods	Reference
Tanzania	Groundnut grain	173/180 (96.1)	Total AFs	6.37	HPLC	[9]
	Groundnut (stored)	93/120 (77.5)	Total AFs	15–11,900	ELISA	[57]
	Groundnut (market)	93/120 (77.5)	Total AFs	15–10,100	ELISA	[57]
	Groundnut cake (Babile town)	(70)	AFB <sub>1</sub>	0.7–39.1	HPLC	[58]
	Groundnut cake (Babile town)	(70)	AFB <sub>2</sub>	0.2–4.6	HPLC	[58]
	Groundnut cake (Dire Dawa city)	(80)	AFB <sub>1</sub>	1.5–158.1	HPLC	[58]
	Groundnut cake (Dire Dawa city)	(80)	AFB <sub>2</sub>	0.2–15.3	HPLC	[58]
	Groundnut seed (Babile)	Na	AFB <sub>1</sub>	4.45–1624.5	HPLC	[59]
	Groundnut seed (Babile)	Na	AFB <sub>2</sub>	0.2–88.25	HPLC	[59]
	Groundnut cake (Jijiga city)	Na	AFB <sub>1</sub>	2.8–9.4	HPLC	[59]
Burundi	Groundnut roasted	10/10 (100)	Total AFs	220.3	ELISA	[60]
	Groundnut flour	10/10 (100)	Total AFs	824.0	ELISA	[60]
DRC	Groundnut grain	9/9 (100)	Total AFs	3.4	ELISA	[60]
	Groundnut roasted	11/11 (100)	Total AFs	4.0	ELISA	[60]
Mali	Groundnut seed	80/	Total AFs	33.8–124.1	TLC	[61]
	Groundnut unshelled (Kayes)	90/90 (100)	AFB <sub>1</sub>	34–163.5	ELISA	[62]
	Groundnut unshelled (Kita)	90/90 (100)	AFB <sub>1</sub>	75.9–310.9	ELISA	[62]
	Groundnut unshelled (Kolokani)	90/90 (100)	AFB <sub>1</sub>	172.1–270.3	ELISA	[62]
Burkina Faso	Groundnut seed	53	Total AFs	13.7–47.7	TLC	[61]
Niger	Groundnut seed	159	Total AFs	89.9–702.6	TLC	[61]

Note: NB. The information presented in the table is a summary of studies which is further discussed in the text. AFs = aflatoxin values in the bracket are the percentage of samples with detectable levels of aflatoxins. ()\* = arithmetic mean of aflatoxin contamination. AFB<sub>1</sub>, AFB<sub>2</sub>, AFG<sub>1</sub>, AFG<sub>2</sub> = aflatoxin B<sub>1</sub>, B<sub>2</sub>, G<sub>1</sub>, G<sub>2</sub>; N\* = number of samples contaminated with aflatoxins. Abbreviation: Na = not available.

#### 4. Aflatoxin Contamination in Maize and Maize Products

The summary of contamination levels of maize and maize products in different countries reported by the recent research is presented in Table 2. Kortei et al. [64] analysed aflatoxins on 90 maize (72 white and 18 coloured) samples from markets across all the regions of Ghana and reported that 72 (80%) tested positive for AFB<sub>1</sub> in the range of  $0.78 \pm 0.04$  to  $339.3 \pm 8.6$  µg/kg, while AF total ranged between  $0.78 \pm 0.04$  and  $445.01 \pm 8.9$  µg/kg. A total of 33 (41.25%) samples were above the limits of AFB<sub>1</sub> and total aflatoxins for the Ghana Standards Authority (GSA) (5 and 10 µg/kg) and the European Food Safety Authority (EFSA) (2 and 4 µg/kg). In another study, samples of maize grains collected from seven out of the eight farms and five out of the eight communities had AFB<sub>1</sub> levels above 20 µg/kg [65]. Evaluation of the aflatoxin contamination in three maize varieties stored using different storage methods (hermetic bags, woven polypropylene sacks and local crib) in Ghana revealed that maize samples stored in the polypropylene sack exhibited significantly higher ( $p < 0.05$ ) total aflatoxin levels compared to hermetic bags and local crib after storage for 6 months [66].

In Nigeria, Ekpakpale et al. [67] reported that aflatoxins were prevalent in 73.8% of the 42 regular food samples, including maize grain, and that 41.9% of these aflatoxins were above the Nigerian standard of 10 µg/kg for total aflatoxins. In Zambia, the proportions of maize samples unsafe for human consumption due to aflatoxin contamination differed significantly across agroecologies, with more contamination in the warmest and the least in the cool, wet agroecologies. Furthermore, poor storage conditions (31.1°C, 100% RH, 1 week) increased aflatoxin in safe maize grains by over 1000-fold [68]. Similarly, Mwalwayo and Thole [69] analysed samples of maize produced, stored and consumed in rural households in Malawi and showed that about 20% of maize samples exceeded the tolerable maximum limit for aflatoxins in Malawi.

Aflatoxin levels in maize grain from 10 districts in Tanzania were observed to be between 0.92 and 44.11 µg/kg, with 11.5% above the EU limit [9]. In a similar study, 30% of the maize samples ( $n = 91$ ) were contaminated with aflatoxins (mean = 13 µg/kg). Maize stored in polyethylene bags (uncontrolled) for 180 days showed an increase in aflatoxin levels along with the storage period, with a mean of 19.06 µg/kg [71]. Aflatoxin levels in ready-for-harvest maize cob samples from farmers in Babati district, Northern Tanzania, on the other hand, were all within the East African Community (EAC) standard of 10 µg/kg for total aflatoxin [70].

In Kenya, according to Mahuku et al. [72], 153 (55.8%) of maize samples from eastern and 102 (43.8%) from south-western Kenya exceeded the maximum acceptable level of AFB<sub>1</sub> (5 µg/kg) set for maize intended for human consumption in Kenya, whereas aflatoxin was reported in 45% and 35% of maize kernels and maize meal from western Kenya, with concentrations ranging from 18 to 480 and 6–30 µg/kg, respectively [73]. Nabwire et al. [74] observed that AFs were detected in 100% of maize kernels and flour

samples drawn from Makueni and Siaya Counties of Kenya in the range of 2.14–411 µg/kg.

In Uganda, maize flour samples from six major markets in Kampala city had a mean total aflatoxin concentration of  $7.6 \pm 2.3$  µg/kg with about 20% of the samples having higher than 10 µg/kg, as the maximum acceptable level in East Africa. At the household level, about 45% of maize samples contained total aflatoxin levels higher than the acceptable limit [75]. Osuret et al. [52] reported that maize samples from markets in the same location were lower than 20 µg/kg. In the eastern and northern parts of Uganda, 62.8% of maize products had aflatoxin levels higher than 20 µg/kg with maize on cobs having the highest levels (126.4 µg/kg). On the other hand, the aflatoxin content of preharvest maize samples ( $n = 256$ ) collected from 23 major maize-growing districts in eight agroecological zones (AEZ) of Uganda ranged from 0 to 3760 µg/kg, with about 5% and 16% of samples containing aflatoxins above the Ugandan and EU tolerance thresholds [76].

In DRC, 32% of the maize samples at preharvest had aflatoxin levels ranging from 1.5 to 51.23 and 3.1 to 103.89 µg/kg for AFB<sub>1</sub> and total aflatoxin, respectively. However, as the supply chain advanced, the contamination of maize samples also increased, with 100% of the maize samples found to have aflatoxins at levels 300 times greater than the World Health Organization (WHO) set a maximum limit of 10 µg/kg for total aflatoxin [77]. The mean levels of aflatoxins in all the maize meals collected in the 3 cities (Beni, Goma and Butembo) in the DRC were above 10 µg/kg [78]. Matendo et al. [79] analysed freshly harvested, stored (3 months  $\pm$  1.5 months) and market maize samples in the DRC; aflatoxin was found in 100% of the samples, ranging from 0.3 to 18.5, 1.16 to 841.5 and 2.05 to 905.1 µg/kg in freshly harvested, stored and market maize, respectively. Similarly, maize flour samples from DRC and Burundi local markets had higher levels of aflatoxin compared to maize grain [60]. In Rwanda, aflatoxin levels in maize samples collected from 15 districts most suitable for maize production in five provinces ranged between 0 and 100.9 g/kg, with 90.4% of samples scoring below the East Africa/Kenya limit of 10 µg/kg of aflatoxin in food for human consumption [80].

Chauhan et al. [5] revealed that all samples of maize flour, fruit and maize kernels collected from the Gedeo zone in South Ethiopia contained aflatoxin levels above the FDA and European Union safety levels for aflatoxins. Abera et al. [81] reported that incidences of aflatoxin were 100% in stored maize samples collected from five major maize-growing districts with long-term storage practices in Ethiopia, ranging between 6.3 and 150 µg/kg. Incidences of safe levels of AFT were 94.6%, 11.3% and 0% when evaluated by the maximum tolerable level (MTL) of the FDA, EAC and the EU, respectively. A study in the central delta provinces of Egypt revealed that AFB<sub>1</sub> was more predominant than AFG<sub>1</sub> in maize samples ranging from 280 to 720 and 360–440 µg/kg, respectively, while AFB<sub>2</sub> was not detected [83].

Hanvi et al. [82] reported a 76% prevalence of aflatoxins in maize dough samples from households in the southern rural region of Togo; the levels ranged from 1.1 to 75.9 µg/kg and AFB<sub>1</sub> was the most prevalent. Aflatoxins in maize

TABLE 2: Recent research on aflatoxin contamination in maize and maize products.

Country	Product	N* (%)	Aflatoxin type	Concentration (mean/range µg/kg)	Detection methods	Reference
Ghana	Maize grain (market)	72/90 (80)	AFB <sub>1</sub>	0.78–339.3	HPLC	[64]
	Maize grain (market)	14/70 (15.5)	AFG <sub>2</sub>	1.09–5.51	HPLC	[64]
	Maize grain (market)	72/90 (80)	Total AFs	0.78–445.01	HPLC	[64]
	Maize grain (farm)	7/8 (87.5)	AFB <sub>1</sub>	20–60	ELISA	[65]
	Maize grain (community)	5/8 (62.5)	AFB <sub>1</sub>	20	ELISA	[65]
	Maize (stored –local crib)	Na	Total AFs	48.9	ELISA	[66]
	Maize (stored –Hermetic)	Na	Total AFs	49.0	ELISA	[66]
Nigeria	Maize (stored—polypropylene)	Na	Total AFs	82.9	ELISA	[66]
	Maize grain	12/12 (100)	Total AFs	101	ELISA	[67]
Zambia	Maize	Na	Total AFs	16	ELISA	[68]
Malawi	Maize	Na	Total AFs	1.5–22.5	ELISA	[69]
	Maize grain	99/200 (49.5)	Total AFs	12.48	HPLC	[9]
Tanzania	Maize	84/440 (19)	Total AFs	2.94	LC/MS	[70]
	Maize (30 days in store)	7/23 (30)	Total AFs	13.12	ELISA	[71]
	Maize (90 days in store)	13/32 (41)	Total AFs	14.75	ELISA	[71]
	Maize (180 days in store)	24/36 (67)	Total AFs	19.39	ELISA	[71]
	Maize grain	507/789 (64.3)	AFB <sub>1</sub>	22.3	ELISA	[72]
Kenya	Maize grain	(45)	Total AFs	53	HPLC	[73]
	Maize meal	(35)	Total AFs	6	HPLC	[73]
	Maize kernel and flour	Na	AFB <sub>1</sub>	76.2	HPLC	[74]
	Maize kernel and flour	Na	AFB <sub>2</sub>	0.66	HPLC	[74]
	Maize kernel and flour	Na	AFG <sub>1</sub>	0.99	HPLC	[74]
	Maize kernel and flour	Na	AFG <sub>2</sub>	0.47	HPLC	[74]
	Maize kernel and flour	Na	Total AFs	77.9	HPLC	[74]
	Maize grain	5/5 (100)	Total AFs	3–12	ELISA	[52]
	Maize grain	11	Total AFs	109.44	ELISA	[15]
	Maize on cob	5	Total AFs	126.44	ELISA	[15]
Uganda	Maize flour	17	Total AFs	7.83	ELISA	[15]
	Maize (hulled)	(83.3)	Total AFs	24.2	ELISA/HPLC	[75]
	Maize (dehulled)	(15)	Total AFs	5.6	ELISA/HPLC	[75]
	Maize (market sample)	21/60 (35)	Total AFs	7.6	ELISA/HPLC	[75]
	Maize (household)	53/72 (74)	Total AFs	22.2	ELISA/HPLC	[75]
	Maize (preharvest)	Na	Total AFs	23.5	ELISA/HPLC	[76]



TABLE 2: Continued.

Country	Product	N* (%)	Aflatoxin type	Concentration (mean/range µg/kg)	Detection methods	Reference
DRC	Maize	16/50 (32)	AFB <sub>1</sub>	10.33	HPLC	[77]
	Maize	16/50 (32)	Total AFs	20.64	HPLC	[77]
	Maize (grilled)	Na	Total AFs	17.44	ELISA	[78]
	Maize (ungrilled)	Na	Total AFs	18.34	ELISA	[78]
	Maize (freshly harvested)	(100)	Total AFs	3.2	ELISA	[79]
	Maize grain (stored)	(100)	Total AFs	97.9	ELISA	[79]
	Maize flour (stored)	(100)	Total AFs	148.9	ELISA	[79]
	Maize grain (market)	(100)	Total AFs	95.1	ELISA	[79]
	Maize flour (market)	(100)	Total AFs	415.8	ELISA	[79]
	Maize grain	9/9 (100)	Total AFs	10.7	ELISA	[60]
	Maize flour	9/9 (100)	Total AFs	47.9	ELISA	[60]
	Maize grain	10/10 (100)	Total AFs	38.7	ELISA	[39]
	Maize flour	10/10 (100)	Total AFs	41.9	ELISA	[39]
Burundi	Maize	Na	Total AFs	6.69	ELISA	[80]
Ethiopia	Maize flour	65 (100)	Total Fs	53.89 (54.86)	ELISA/TLC	[5]
	Corn fruit	54 (100)	Total AFs	52.47 (50.87)	ELISA/TLC	[5]
	Maize kernels	31 (100)	Total AFs	49.79 (48.29)	ELISA/TLC	[5]
	Maize	150 (100)	Total AFs	14.7	ELISA	[81]
Togo	Maize dough	53/76 (74.7)	AFB <sub>1</sub>	1.14–68.9	HPLC	[82]
Egypt	Maize	7/13 (53.8)	AFB <sub>1</sub>	440	TLC	[83]
Mali	Maize (at harvest)	112	Total AFs	27.7–156.3	TLC	[61]
Burkina Faso	Maize (at harvest)	62	Total AFs	7.7–54.3	TLC	[61]
Niger	Maize (at harvest)	123	Total AFs	99.5–658.9	TLC	[61]
Côte d'Ivoire	Maize flour	51/51 (100)	Total AFs	128.7	LC-ESI-MS/MS	[84]
	Maize flour	51/51 (100)	AFB <sub>1</sub>	107.9	LC-ESI-MS/MS	[84]
	Maize flour	38/51 (74.5)	AFB <sub>2</sub>	8.19	LC-ESI-MS/MS	[84]
	Maize flour	18/51 (33.3)	AFG <sub>1</sub>	8.05	LC-ESI-MS/MS	[84]
	Maize flour	13/51 (25.5)	AFG <sub>2</sub>	5.63	LC-ESI-MS/MS	[84]

Note: NB. The information presented in the table is a summary from studies which is further discussed in the text. Source: [86]. AFs = aflatoxin values in the bracket are the percentage of samples with detectable level of aflatoxins, ( ) = arithmetic mean of aflatoxin contamination, AFB<sub>1</sub>, AFB<sub>2</sub>, AFG<sub>1</sub>, AFG<sub>2</sub> = aflatoxin B<sub>1</sub>, B<sub>2</sub>, G<sub>1</sub>, G<sub>2</sub>; N\* = number of samples contaminated with aflatoxins. Abbreviation: Na = not available.

collected at harvest or from farmers' stores within two weeks of harvest from Mali, Burkina Faso and Niger showed that the mean aflatoxin concentrations in maize were high, 128, 517 and 659  $\mu\text{g/kg}$  in Mali, Burkina Faso and Niger, respectively.

## 5. Regulation of Aflatoxin in Groundnut and Maize Products

Regulation of aflatoxin in food and feed dates back to the late 1960s, and globally, about 120 countries have already enacted regulatory limits on allowable aflatoxin levels in human food and animal feed [85]. Figure 1 shows an overview of the aflatoxin regulation limits in groundnuts and maize products in some countries in Africa (A), compared to the regulation of the European Union, the United States and countries in Asia. The decision-making process for establishing the regulation limit considers the known toxic effects, reliable data on the occurrence, distribution of the concentration in products and the socioeconomic balance [86].

Regulation of aflatoxins is undertaken by national and multinational organizations. Internationally, the Codex Alimentarius Commission (CAC), the US FDA and the European Union (EU) regulation have all been recognized as the standards for determining the maximum permissible regulatory limit for aflatoxins. The WHO and the Food and Agriculture Organization (FAO) together formed Codex in 1963 with the goal of establishing the Codex standards, guidelines and Code of Practice for safeguarding consumer health and ensuring ethical conduct in the world food trade [29]. The aflatoxin regulatory limit at the national level is influenced by variations in countries' risk perception, data availability, methodology and risk assessment models [28]. In some countries, different limits are applied to the four different types of aflatoxin: B<sub>1</sub>, B<sub>2</sub>, G<sub>1</sub> and G<sub>2</sub>. For instance, the maximum limit for the total aflatoxins is 20  $\mu\text{g/kg}$  in the US standards and 10  $\mu\text{g/kg}$  in Kenyan regulations, respectively. In contrast, the EU has maximum levels of 2 and 4  $\mu\text{g/kg}$  for AFB<sub>1</sub> and total aflatoxins in maize and peanuts, respectively, although having varied limits for other aflatoxin–food combinations [87].

The high aflatoxin incidence and exposure levels prevalent in Africa call for strict monitoring and enforcement of regulations. However, Matumba et al. [88] reported seldom enforcement of available regulations due to socioeconomic concerns such as food scarcity, inadequate infrastructure, lack of expertise and other factors [89]. In order to keep aflatoxin levels in food products at the lowest possible levels, routine monitoring and stronger food safety systems are required due to the relatively high prevalence of aflatoxin food contamination. The development and implementation of regulations is a crucial component of the entire institutional framework designed to prevent human exposure to aflatoxin [28].

## 6. Sampling, Extraction, Detection and Quantification of Aflatoxins in Foods

Aflatoxin detection procedure consists of several stages that include right sampling, extraction protocols, purification

and cleaning-up, enrichment, analysis and interpretation of the data obtained from analysis [90]. In food samples, aflatoxin is usually unevenly distributed in the food matrix, and proper sampling is required to avoid false positives and negatives. Sampling contributes a significant source of error in the food sample [28].

**6.1. Sampling for Aflatoxin.** The European Union has published a guideline on the sampling procedure for aflatoxin and other mycotoxin analysis in various food products (Commission Regulation) [91]. According to the guidelines, the weight of the aggregate sample, which represents the sum of all incremental samples taken from the lot or subplot, is required to be greater for foods with larger particle sizes (like grains and nuts) than in the case of batches of foods with smaller particle sizes (such as flour, paste and powders). This is necessary to obtain a representative sample from the batches of food products, as the distribution of mycotoxins in processed products is less heterogeneous than in the unprocessed cereal products [90]. The incremental sample (a portion of material taken from a single location inside a lot or subplot) will be 100 g in each case, but the incremental sample weight for samples obtained from smaller packages will depend on the weight of the pack. It is further recommended that samples in the form of grain and nuts must first be comminuted prior to collection of the laboratory sample for aflatoxin and other mycotoxin analysis [28]. The recent findings revealed that indirect sampling of grain dust may be a more effective alternative technique for identifying mycotoxins and resolving the issue of their variability in various food products [92]. Every time grain is moved or transported, particle abrasion causes dust to be produced, which can be easily collected for analysis, hence reducing the cost and labour intensity required in sampling.

**6.2. Aflatoxin Extraction.** One of the critical processes in the identification of aflatoxins is sample preparation. Most methods of aflatoxin detection and quantification require the toxin to be extracted and cleaned up to reduce the interferences of other substances like protein and produce an accurate detection. The commonly used extraction and cleaning-up techniques include liquid–liquid extraction (LLE), liquid–solid extraction (LSE), ultrasonic extraction, pressurized liquid extraction (PLE), supercritical fluid extraction (SFE), solid-phase extraction (SPE), immunoaffinity chromatography and quick, easy, cheap, effective, rugged and safe (QuEChERS) methods [93]. Relatively, the LLE is used in many studies because it is cheaper. The method is based on the principle of solubility of aflatoxin in organic solvents (acetone, hexane, chloroform and methanol) or their combination [90]. It has also been reported that an aqueous combination of the organic solvent improves the extraction efficiency [9, 50]. However, some studies have used methanol–water (80/20 v/v) [51, 94]. In a previous research, AFB<sub>1</sub>, AFB<sub>2</sub>, AFG<sub>1</sub>, AFG<sub>2</sub> and AFM<sub>1</sub> in breast milk were tested with LLE extraction followed by high-performance liquid chromatography (HPLC) with photochemical derivatization (PHRED) and fluorescence

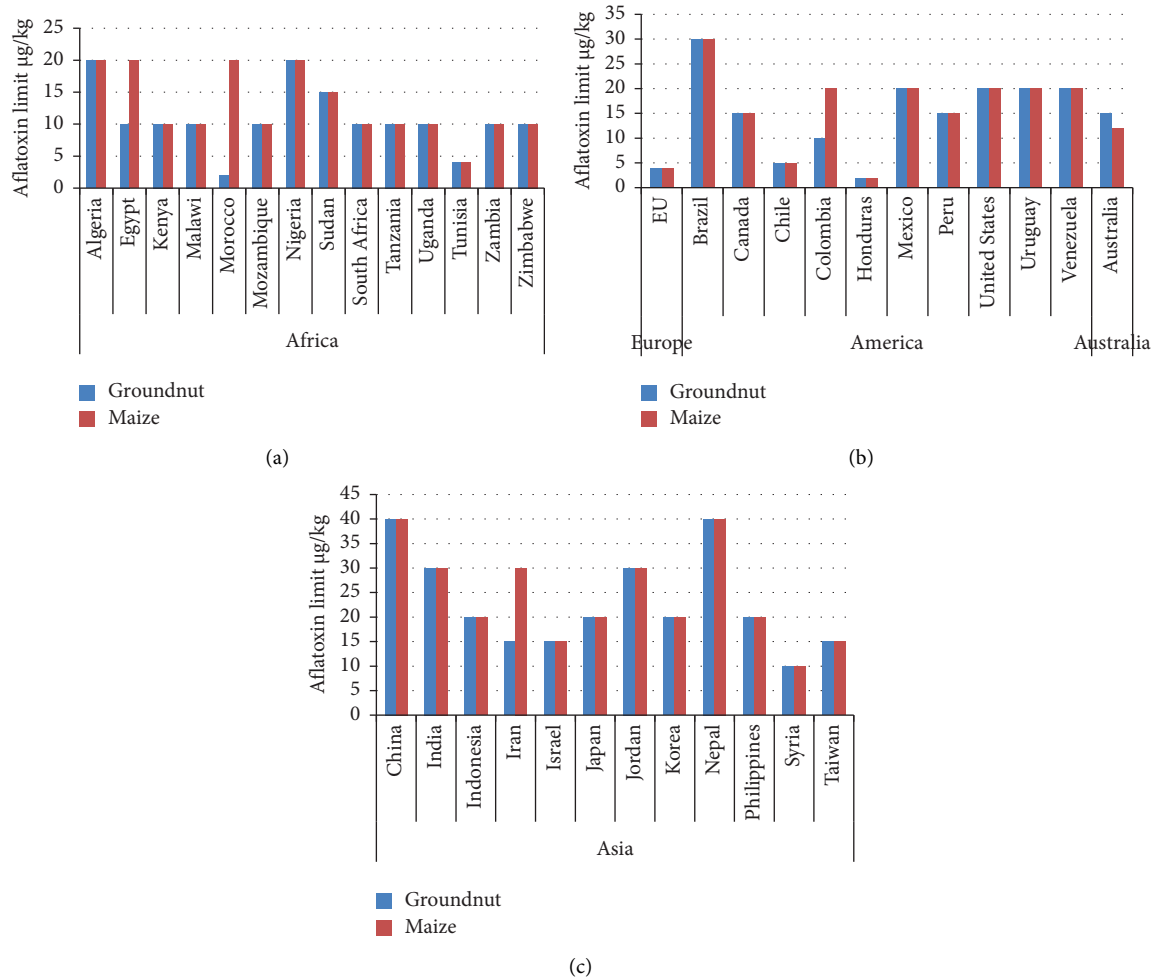


FIGURE 1: Aflatoxin regulatory limit in African countries (a) compared to countries in Europe, America, Australia (b) and Asia (c).

detection (FLD). The limits of the quantification (LOQs) were between 0.005 and 0.03 mg/kg [95]. LOQ of 15 ng/mL was reported in a skimmed milk matrix extracted with LLE using sodium chloride and ethyl acetate extraction agents and quantified using high-performance liquid chromatography (HPLC)/MS-MS measurement. The mean overall recovery ( $n = 24$ ) was 95% with a confidence interval of 1.9% and a CV% of 4.5% [96].

On the other hand, the LSE is a simple method for the extraction of aflatoxins from solid matrices of different consistency. The extraction steps include the weighing of homogenized sample of the appropriate particle size, adding the suitable extraction agent and then disintegrating the mixture by shaking, blending or vortexing to extract the component of interest. The extracts are then filtered and subjected to further clean up, where necessary. The most commonly used extraction agents are mixtures of acetonitrile/water or methanol/water in different ratios [97]. The efficiency of extraction is greatly influenced by the sample/solvent ratio, the composition of the extraction agent and the time of extraction. Kong et al. [98] reported that 80% of methanol/water mixture proved to be the most optimal for the extraction of aflatoxins in the case of nutmeg samples.

Ultrasound-assisted solid-liquid extraction and immunoaffinity column clean-up coupled with high-performance liquid chromatography (HPLC) and online postcolumn PHRED-FLD was performed. This method showed limits of detection (LODs) (from 0.02 to 0.25  $\mu\text{g/kg}$ ) and LOQs (from 0.06 to 0.8  $\mu\text{g/kg}$ ).

**6.3. Aflatoxin Detection and Quantification.** Methods used in the detection and quantification of aflatoxins in food samples can be categorized into three broad categories: chromatographic, immunochemical and spectroscopic methods. The chromatographic techniques are among the oldest and the most common methods of aflatoxin detection, and it is the reference methods for the determination of aflatoxins in the food samples [28]. The most common methods used for aflatoxin detection and quantification in maize and groundnut products in this review are shown in Tables 1 and 2. Overall, the methods included chromatographic methods such as thin-layer chromatography (TLC), HPLC, high-performance thin-layer chromatography (HPTLC), UPLC and the state-of-the-art liquid chromatography-tandem mass spectrometry (LCMS/MS), followed by enzyme-linked

immunosorbent assays (ELISA). According to Omara et al. [13], the aflatoxin analysis instrumentation has undergone a remarkable improvement, transitioning from the non-differential TLC in 1973 to the differential UHPLC with triple quadruple mass spectrometry (UHPLC-TTQS) evidenced in most studies between 2017 and 2020. The chromatographic techniques rely on the partitioning of the sample solute between the stationary and mobile phase. The mobile phase is often liquid passing on the stationary bed which is either liquid or solid [94].

Techniques such as LCMS/MS enable simultaneous multiple detection of aflatoxins and other mycotoxins present in minute quantities within the food sample [99]. Despite their high sensitivity, accuracy and reliability, chromatographic methods have several drawbacks. They tend to be expensive because of laboratory equipment and supplies, involve lengthy sample preparation and trained personnel to deliver accurate results [100, 101].

ELISA was the most commonly used rapid method for detecting and quantifying aflatoxin contamination in groundnut and maize products. This could have been facilitated by the fact that the process is made easier with the use of ELISA kits that only require simple sample preparation [13, 102]. The technique depends on the principle that a particular antibody can identify the three-dimensional structure of a particular mycotoxin [93, 103]. A variety of antibodies are imprinted on a microplate or a column before use. When an analyte is introduced into the plate or column, the antibodies attach to it and create a complex; this complex then reacts with a chromogenic material to provide a readable signal [90, 101].

ELISA technique is highly specific and sensitive in detecting aflatoxin contamination in food, even in the presence of other contaminants [102]. There are different types of ELISA; however, indirect ELISA is the most often used for mycotoxin analysis [104]. Development and optimization of immune-based assay to improve its sensitivity and cross-reactivity [65, 69, 105]. Chen et al. [106] and Yu et al. [107] reported that a kit developed based on lateral flow immunoassay can be used to test corn and peanut samples for mycotoxins, including AFB<sub>1</sub>. This method provided results comparable to other instrumental analyses within 5–15 min and was recommended for field inspection. Electrochemical immunosensors and radioimmunoassays are other types of immune chemical alternatives for the detection of aflatoxins in food. Wacoo et al. [75] reported the use of an electrochemical immunosensor device for on-site detection of aflatoxin in maize flour from different markets and households in Kampala–Uganda. The results indicated a linear range of  $0.7 \pm 0.1$  to  $11 \pm 0.3$   $\mu\text{g/kg}$  and a LOD of  $0.7$   $\mu\text{g/kg}$ . It had correlation coefficients of 0.94 and 0.98 with the ELISA and HPLC assays for AFB<sub>1</sub>, respectively. In another study, Azri et al. [108] developed an ultrasensitive immunosensor for the detection of AFB<sub>1</sub> with a working range of 0.0001–10 ng/L, and analysis of AFB<sub>1</sub> in spiked peanut samples showed recoveries ranging from 80% to 127%.

Spectroscopic techniques like near-infrared (NIR), Raman, fluorescence and hyperspectral imaging (HSI) have been used to nondestructively evaluate food and agricultural

product quality and safety attributes such as mycotoxin detection [109–111]. Spectroscopic techniques have been utilized to detect aflatoxin in different food matrices by studying the behaviour of light (absorption, emission and scattering) when it interacts with a specimen across a wide wavelength range. Chu et al. [112] used short-wave infrared (SWIR) HSI to detect AFB<sub>1</sub> in single maize kernels; while Kimuli et al. [113] used a SWIR HSI system combined with chemometric data analysis to detect AFB<sub>1</sub> on surface maize kernels.

## 7. Aflatoxin Mitigation Strategies

Aflatoxin mitigations can be categorized into field and postharvest methods. The field/preharvest activities, postharvest methods and strict regulations for each product are key strategies for aflatoxin mitigation [27, 28]. Aflatoxin contamination control strategies have been well documented, with their benefits and drawbacks, and their levels of effectiveness have been extensively discussed by different researchers and practitioners. These include the use of genetic engineering, physical methods, chemical methods, GAPs, biological control, postharvest precautions and breeding for resistance [1, 28, 105, 114]. Despite numerous recommendations on aflatoxin management abundant in the literature, aflatoxin contamination of important crops such as groundnut and maize remains high in sub-Saharan regions as a result of insufficient knowledge on the recommendations, the time and labour intensiveness of some technologies, insufficient know-how on using technologies and ethical aspects, as well as climate change that favours aflatoxin accumulation even when mitigation strategies are available. One of the aflatoxin contamination mitigation technologies that is efficient in preventing contamination during pre- and postharvest phases of contamination in susceptible crops in a cost-effective manner is the use of beneficial fungi [115].

**7.1. Aflasafe® Products and Technology.** In Africa, IITA, USDA-ARS and other development partners have collaborated to successfully adapt the biocontrol technology for use on groundnut and maize in a number of African countries as well as developing a number of biocontrol products under the trade name Aflasafe [115, 116]. The concept of ‘competitive exclusion’, which states that when two species compete for the same limited resources in an ecosystem, one of them will eventually outcompete and overtake the other, underlies Aflasafe® and other biocontrol techniques for aflatoxins [117]. Aflasafe® involves the utilization of carefully selected atoxigenic strains of *A. flavus* that outcompete the toxin-producing strains. Twelve safe and effective atoxigenic strains were identified, and four were further tested and eventually developed into the Aflasafe® product.

To date, Aflasafe products have been registered for use in five countries in Africa (Table 3). Further research is still ongoing to produce country-specific and secure registration of Aflasafe® in Burkina Faso, Burundi, Ghana, Malawi,

TABLE 3: Status of Aflasafe® in different countries in Africa.

Country	Product	Year	Status
Nigeria	Aflasafe™	2014	Registered and commercialization in process
Kenya	KE01	2015	Registered and commercialization in process
Senegal	SN01	2016	Registered and commercialization in process
The Gambia	SN01	2016	Registered and commercialization in process
Burkina Faso	Aflasafe BF01	2016	Product ready for registration
Ghana	GH01/GH02	2016	Product under testing in farmers' fields
Malawi	Aflasafe MWMZ01	2020	Unpublished registration documents
Zambia	Aflasafe ZM01/ZM02	2020	Unpublished registration documents
Mozambique	Aflasafe MZ02/MWMZ01	2020	Unpublished registration documents
Tanzania	Aflasafe TZ01/TZ02	2020	Unpublished registration documents
Uganda, Burundi and Rwanda		2020	Development of strains

Note: Source: [118–124].

Mozambique, Rwanda, Tanzania, Uganda and Zambia [119]. To date, all Aflasafe products registered and under experimental use reduced aflatoxin concentrations in treated crops by > 80%, in comparison with untreated crops in both field and storage conditions. In Nigeria, results indicate that states where Aflasafe was promoted as a management intervention for aflatoxin had very high levels of aflatoxin awareness. Since Aflasafe® launched in 2010, there has been a steady rise in usage in Kaduna state, the area with the longest intervention. Additionally, farmers were more likely to continue using Aflasafe® if they bought it packaged (mixed) with other inputs [120]. In Kenya, a study in four counties classified as aflatoxin hotspots revealed that farmers were willing to pay for Aflasafe KE01 in the range of Kenya Shillings (Ksh) 113 to 152/kg [121]. In Ghana, application of either Aflasafe® product resulted in significantly ( $p < 0.05$ ) less aflatoxin content (< 95%) in grains from treated fields compared to grains from nontreated fields [125]. In Senegal and Gambia, an aflatoxin biocontrol product containing four atoxigenic isolates of *A. flavus*, Aflasafe SN01, has been registered and is approved for commercial use in groundnut and maize. The product was tested in 129 maize and groundnut fields and compared with corresponding untreated fields cropped by smallholder farmers in the Gambia. Treated crops contained up to 100% less aflatoxins than untreated crops.

## 8. Decontamination of Aflatoxin in Maize and Groundnut Products

**8.1. Biological Decontamination.** This involves the use of microorganisms or their metabolites for the removal of aflatoxin in food products [42]. Their mechanism of action involves binding of the aflatoxins, thereby inhibiting their bioavailability, surface adsorption and degradation into nontoxic compounds [7]. In a previous study, Jackson and Pryor [126] used the fungal strain white-rot fungus *Pleurotus ostreatus* (Oyster mushrooms) to degrade 94% of AFB<sub>1</sub> in naturally contaminated maize, with minimal reversion of the breakdown products to the parent compound. Branà et al. [127] investigated the ability of the white-rot fungus *Pleurotus eryngii* (king oyster mushroom) to degrade AFB<sub>1</sub> both in vitro and in a laboratory-scale mushroom cultivation; in

a growth medium containing 25% (w/w) of maize spiked with AFB<sub>1</sub> to the final content of 128 µg/kg, *P. eryngii* degraded up to 86% of the AFB<sub>1</sub> in 28 days, with no significant reduction of either biological efficiency or mushroom yield. In an in vitro study, Salati et al. [128] observed that batch anaerobic fermentation of corn grains resulted in AFB<sub>1</sub> degradation ranging from 69% to 87% of the total initial AFB<sub>1</sub> content. It is suggested that pH, temperature and incubation time were essential factors in the fungal degradation of aflatoxins [42, 129].

Guo et al. [130] reported that aflatoxins could be degraded by laccases, peroxidases, oxidases and reductases. Aflatoxin degradation potential of laccase in an in vitro study and on contaminated corn was tested, the results showed that the AFB<sub>1</sub> was completely removed in the in vitro study, whereas a reduction of 26% was observed in the contaminated corn [23]. Another strategy to aflatoxin control is the use of aflatoxin-binding agents in foods. The basic concept is that aflatoxins, which have contaminated foods, can be bonded to an agent to reduce the health concerns caused by ingesting aflatoxins [37]. Bacterial cells, yeast, proteins and clays are some of the examples of binders. Clays have been specifically studied for usage in animal feeds. Mutua et al. [24] reported the use of nine different types of binders of various compositions being used by farmers in the urban and periurban areas in Kenya for mixing of livestock feeds. According to the principle, less absorption and thus less toxin harm would occur as the binding agent and the bound toxin passed through the digestive tract [131]. Although biological methods are believed to be less aggressive, more specific and environment-friendly, other factors may limit their implementation in large-scale applications such as the cost of purifying organisms or metabolites such as enzymes [129]; little data on the toxicity of the secondary metabolites produced after enzymatic aflatoxin degradation [132].

**8.2. Physical Decontamination.** Sorting, segregation, sieving, washing, dehulling, floating, milling, heat treatment and other physical techniques are used to decontaminate aflatoxin in food. Hand sorting and segregation of grains based on their physical characteristics have been established as effective in lowering aflatoxin in agricultural products,

despite being time-consuming and ineffective for wide-scale application. Matumba et al. [133] evaluated the effects of hand-sorting, flotation and dehulling on the decontamination of white maize contaminated with mycotoxin and found that hand-sorting was effective as aflatoxin was reduced by 94% in hand-sorted maize samples. The authors added that the level of aflatoxins was least affected by flotation. Xu et al. [134] reported a 96.7% reduction in hand-sorted peanut grains.

The effect of commercial milling procedures on aflatoxin and distribution in milled fractions of corn was assessed [135]. The authors observed a fourfold reduction in aflatoxin levels in the final product of the processed maize and a significant increase of aflatoxin in the by-products (germs and bran). The use of UV light illumination to segregate aflatoxin-affected grains to facilitate hand sorting is recorded [136], while other studies have focused on the use of ultraviolet-visible-near-infrared (UV-Vis-NIR) spectroscopy to segregate aflatoxin-contaminated corn kernels [137].

Due to aflatoxin photosensitivity, UV irradiation has long been recognized as an efficient physical technique for their eradication. UV irradiation is a nonthermal food decontamination technique that has the benefits of being practical, affordable and environmentally benign because it produces no waste and has no hazardous side effects [130]. The efficiency of UV irradiation to decompose AFB<sub>1</sub> in peanut oil was studied; the results showed that aflatoxin was reduced by 86% after 10 min of the reaction [138]. UV intensity and duration of irradiation are the important factors affecting aflatoxin elimination efficiency. Liu et al. [139] reported that UV irradiation at 800  $\mu\text{W}/\text{cm}^2$  for 30 min completely removed AFB<sub>1</sub> in peanut oil, with a reduction of about 79% and 85% at the intensity of 200 and 400  $\mu\text{W}/\text{cm}^2$ , respectively. AFB<sub>1</sub> is known to absorb UV rays at 222, 265 and 362 nm, with peak absorption at 362 nm; their mechanism of action is attributed to the formation of the hydroxyl free radicals (OH•) initiated by UV irradiation, and this radical attacks the terminal double bond at the C8-C9 position of AFB<sub>1</sub> leading to the formation of other compounds with less toxicity [130]. Notably, UV light can easily penetrate clear or transparent liquids, but its ability to penetrate solid materials is limited. This leads to low decontamination efficiency in food products with high levels of suspended solids [140]. More sophisticated and novel physical measures, including microwave heating, gamma irradiation and cold plasma, are among other new technologies that are being studied the decontamination of aflatoxins in a variety of food samples.

**8.3. Chemical Degradation.** The capacity of several chemical compounds to degrade and detoxify aflatoxin has been explored. These comprise of oxidizing (ozone and hydrogen peroxide) and reducing agents, acids (acetic, citric and propionic), alkalis (ammonia, sodium hydroxide and calcium hydroxide) and other compounds such as sodium sulphite and sodium hydrogen sulphate [130, 141]. According to the FAO, any detoxification procedure used on human food must be able to inactivate, destroy or remove aflatoxin; not produce or leave toxic or carcinogenic and mutagenic residues on the

treated substrate; retain the nutritional, sensory or other quality characteristics of the food product; and be able to remove any remaining fungal spores or mycelium that could multiply and produce new toxins [28].

Ammonification has been considered the most economical, safe and successful method of detoxifying aflatoxin from feedstuff since the 1990s. The characteristics of the substrate, both intrinsic and extrinsic, significantly affect the rate of deterioration. Gomaa et al. [142] have demonstrated that during ammonia treatment of contaminated yellow corn, the aflatoxin detoxification was faster and more efficient under conditions of high pressure and high temperature compared to experiments conducted under atmospheric pressure and ambient temperature. Nixtamalization is another strategy used to degrade aflatoxin; it involves heating of cereal for 8–16 h before the solution is decanted. The grain is thoroughly washed to leave the grain ready for milling to obtain the maize dough for making the tortillas. In an earlier study which used traditional nixtamalization (using lime and hydrogen peroxide) for making dough for corn tortillas, the process reduced the levels of AFB<sub>1</sub> up to 94%. Sodium hydroxide, sodium sulphite and sodium hydrogen sulphate are other bases that have exhibited varying degrees of efficacy in facilitating the decomposition of aflatoxin [143].

Organic acids, such as citric, lactic, acetic, formic and propionic, have been used in food substances to detoxify aflatoxins [20, 130, 144]. Decontamination of aflatoxins in different nuts using organic acids (citric, lactic and propionic acids) at various concentrations has been reported by [40]. The results showed that the treatment of peanuts ( $10 \pm 3\%$  moisture content) for 15 min using citric, lactic and propionic acid decreased the aflatoxins by about 96.07%; treatment with citric and lactic acids resulted in the conversion of AFB<sub>1</sub> into less toxic products, and citric acid was reported as the most efficient organic acid in degrading the aflatoxins. Aflatoxin-contaminated agricultural foods have been effectively treated with chemicals using a variety of settings, process parameters and food products. This method has been effective and, to a certain extent, is recognized as safe. However, environmental hazards, food safety and food quality remain unresolved [131, 141].

Among the oxidizing agents, ozone has been recognized as the most effective in degrading aflatoxins [1]. Additionally, ozone treatment of food substances has generally recognized as safe (GRAS) by the FDA since 2001. The mode of action of ozone is by an electrophilic attack on the C8 to C9 double of the furan ring of aflatoxin, forming the primary ozonides which are later broken down to lower weight molecular compounds such as ketones, organic acids and aldehydes which are less toxic [145, 146]. In a study by Proctor et al. [147], peanut kernels and flour were subjected to gaseous ozonation under varying temperatures (25, 50 and 75°C) and time (5, 10 and 15 min). The efficiency of ozonation was observed under increased temperature and longtime exposures, with higher degradation levels being observed in peanut kernels compared to the flour. Diao et al. [148] reported a reduction of 89.40% of AFB<sub>1</sub> in peanuts after ozone treatment of 50 mg/L at a flow rate of 5 L/min for 60 h. In a study by Luo et al. [149], ozone treatment of 90 mg/

L for 20 min and 40 min decreased the concentration of AFB<sub>1</sub> in contaminated corn with 13.47% moisture content from 83 µg/kg to 18.12 µg/kg and 9.9 µg/kg, respectively.

Plant extracts are currently being reviewed as a potential solution for degrading aflatoxins in food products. This follows their ancient use as food additives for their antimicrobial and antioxidant properties without any adverse effect on food safety [150–153]. An investigation into the ability of aqueous extracts of 31 medicinal plants to detoxify aflatoxin revealed that among the plant extracts, the leaf of *Adhatoda vasica* Nees showed the highest activity, degrading up to 98% of AFB<sub>1</sub> when incubated for 24 h at 37°C [154]. In another study, aqueous leave extracts of *Rosmarinus officinalis* exhibited a time-dependent degradation of AFB<sub>1</sub>, with the maximum reduction (60.3%) recorded after incubation for 48 h [41]. Iram et al. [155] compared the ability of two plant extracts *Ocimum basilicum* and *Cassia fistula* to detoxify AFB<sub>1</sub> and AFB<sub>2</sub> in contaminated corn; the authors reported that the leaves of *O. basilicum* were highly portable in degrading AFB<sub>1</sub> (86.9%) and AFB<sub>2</sub> (83.5%) in spiked maize samples, compared to *C. fistula* 43.1% and 49.6%, respectively. The bioactives in plant extract responsible for detoxification of aflatoxins have not been sufficiently studied. It is postulated that the breakdown of aflatoxin by plant extracts could be the effect of several components interacting in a multistep process [130]. Further studies are therefore necessary to document the mechanisms of plant extracts to detoxify aflatoxins in the food matrices.

## 9. Conclusion

Aflatoxin contamination of groundnut and maize products remains a food safety challenge at a global level. The most affected population is in the developing countries, as most studies in these regions reveal contamination levels above the acceptable limits, where the regulations exist; in some cases, the countries do not have regulation limits. This is indicative of the health risks and economic losses incurred in those countries. The chromatographic, immunochemical and spectroscopic methods used to detect and quantify aflatoxins are effective, but improved methods are required for fast, cost-effective and accurate detection to enhance aflatoxin regulation. Several physical, chemical and biological approaches are used for managing aflatoxin in maize and groundnut products. Researchers are currently focussing on the use of atoxigenic agents to outcompete the toxin-producing agent, but the application is not yet widespread. Utilization of plant extracts and other strategies for decontaminating aflatoxin in food and feed is another upcoming strategy; however, further research is required on the safety of the aflatoxin-decomposed products. Additionally, the high prevalence of aflatoxins in groundnut and maize products calls for systematic sensitization among stakeholders in the value chain to ensure good handling and hygienic practices at every stage.

## Data Availability Statement

All data are included in the manuscript.

## Conflicts of Interest

The authors declare no conflicts of interest.

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