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The Control of Aflatoxin Contamination at Harvest, Drying, Pre-Storage and Storage Periods in Peanut: The New Approach

Isilay Lavkor and Isil Var

Abstract

Aflatoxins produced by *Aspergillus flavus* and *Aspergillus parasiticus* are contaminants of peanut (*Arachis hypogea* L.). Aflatoxin contamination is a serious concern given their hepatotoxic properties and their widespread occurrence during cultivation, harvest, drying and storage. Management of aflatoxin contamination of peanut is very important using cultural practice such as habitat management, soil amendments and pre- and post-harvest managements, using physical control methods, biological control methods and chemical control methods at harvest, drying, pre-storage and storage periods. Some procedures such as upkeep of low temperature and relative humidity (RH) in storage, keeping away the pod- and seed-feeding insects, doing the harvest and post-harvest procedure control, fast post-harvest drying, optimal timing of digging and harvest, providing optimum water to the crop through irrigation, avoiding mechanical damage during cultivation and optimal timing of digging and harvest might prevent the contamination of aflatoxin. In this review, various strategies for control of aflatoxin in peanuts in all periods are discussed.

Keywords: aflatoxin, control, *Aspergillus flavus*, *Aspergillus parasiticus*, peanut

1. Introduction

Peanut (*Arachis hypogaea* L., Family: Fabaceae) is a rich source of fat, proteins and vitamins. Peanuts are a good source of protein and vitamin E and have a good flavour. It is a very popular snack worldwide, and people of all age groups widely consume peanut products [1]. It is widely cultivated in Asia, Africa and the America [2]. Peanuts were found first in Brazil or Peru as early as 950 BC and carried to Africa by early explorers and missionaries. They were brought from

Africa to North America by slave traders in the early colonial days and used as food for slaves on ships [3]. Although there are a few species and kinds which are wild or cultivated, the peanut developed commercially is described as the fruit or pod of A. hypogaea, which belongs to the leguminosae family.

Their bloom grow on the ground, then is fertilized and dried, the stalk extends longitudinally and this ovary is forced underground. The pod holding those seed matures underneath the surface. On maturity, inner lining of the pod darkens, and the seed coat changes from white to reddish-brown. The entire plant, including most of the roots, is removed from the soil during harvesting [3].

As aflatoxin-producing *Aspergillus* species are naturally present in soil, it is difficult to avoid the invasion of these molds. Wounding by insects, mammals, birds and mechanical processes as well as stresses of hot, dry conditions can result in mold infection during the pre-harvesting period. The influence of delayed harvest on contamination is most severe when crops are affected by rain just prior to or during the harvest [4].

Poor agricultural practice and post-harvest treatments of peanuts can lead to an infection by mould fungus *Aspergillus flavus* and *Aspergillus parasiticus* releasing the toxic substance aflatoxins [5]. Infection and contamination of peanuts can occur both in the field (pre-harvest) and during post-harvest drying/curing and in storage facilities (post-harvest). Several species of fungi infect agricultural crops both in the field and during storage. Aflatoxigenic mould growth and aflatoxin contamination may occur in agricultural crops during growth, harvest, transportation and storage [6].

The contamination of food and feed materials with aflatoxins, which have toxic, carcinogenic and mutagenic activity, causes important health problems and economic losses [7]. Among these, aflatoxin B$_1$ (AFB$_1$) is the most naturally occurring compound of toxigenic isolates of *Aspergillus* species and was classified by the International Agency for Research on Cancer of WHO as a group 1A (cancerogenic) agent in 1993 [8].

Toxigenic fungi and aflatoxin contamination in peanuts start at farm level, and contamination occurs in both pre- and post-harvest periods. Lavkor et al. [9] reported that the levels of aflatoxins detected in 74.5% raw peanut samples were in the range of 0.3–1333.42 μg/kg [9]. Williams [10] referred to a study close by African markets indicating that more than 40% of the commodities found there exceed reasonable aflatoxin levels and expected more than 4.5 billion individuals in developing nations are at danger because of uncontrolled or ineffectively control input of aflatoxins [10].

It is recognised that high aflatoxin levels in the circulation system discourage the safe framework, accordingly encouraging tumour and HIV and hindering the development of kids. A cross-sectional review led in Ghana and referred by Williams et al. [11] demonstrates that invulnerable frameworks of as of late HIV-contaminated individuals are fundamentally adjusted if they have above-middle levels of regular exposures to aflatoxins [11]. Alluding to another study, Dr. Williams notes, "Individuals with a high aflatoxin biomarker status in the Gambia and Ghana will probably have dynamic jungle fever". In 2014, the Global Forum for

Innovations in Agriculture (GFIA) assembled an abnormal state meeting in Abu Dhabi, UAE, on reforming worldwide agribusiness through developments. Straight to the point Rijsberman, the CEO of the CGIAR Consortium, in his report in view of a Benin study about on the post-weaning introduction to aflatoxin, presumes that aflatoxins have debilitated development in kids and are costing African agriculturists over $450 million USD every year in lost exports [12]. As indicated by Oladale [13], research has shown that aflatoxins can lead to cause infertility, premature births, and postponement of egg production in birds and sudden calamity in egg generation effectively in ovulation birds. Furthermore, loss of flavour, skin staining and even yellowish colouring on skin can be observed in fish [13]. Kooprasertying et al. [14] found that roasted and ground peanuts and raw samples were contaminated with AFs at 100% and 80%, respectively. They determined the high amounts of AFs in peanuts as 362 ng g^{-1}, which means the highest concentration of AFS in peanuts (68. 22 ng g^{-1}). It has been reported and emphasised by researcher that the average intake of AFs was 0.49, 0.40 and 2.13 ng/kg bw/day for raw, roasted and ground peanuts, correspondingly. In addition, the potential risk for cancer was estimated at 0.01–0.12 cancer/year/100,000 persons. According to the results of the research, the researchers suggest that the current situation of aflatoxins contamination in peanuts and peanut products (especially in ground peanuts) has an adverse effect on the health of the Thai population [14].

In order to eliminate aflatoxins from contaminated peanut materials, numerous physical, chemical and biological methods have been developed. In addition, there are some genetic studies for developing peanut cultivars resistant to a broad spectrum of pathogens that pose a recurring threat to peanut health as well [15]. The work to be done in this context should be considered to have a minimum effect on the nutritional value and chemical composition of the nuts. It is known that peanut contents are very valuable and include 7% water, 25.8 g protein, 16.1 g carbs, 4.7 g sugar, 8.5 g fibre and 49.2 g fat (saturate: 6.28 g; monosaturated: 24.43 g; polysaturated:15.56 g, Omega-3:0 g; Omega 6:15.56 g; trans fat) [16].

An expanding amount of logical research has been given to adapt more about aflatoxin development issues and conceivable arrangements, including utilising hereditarily changed or hybridised seeds detailed for mold resistance or through utilisation of items, for example, AflaSafe, now utilised in Africa. AflaSafe's "biological approach" utilises a firmly related, non-aflatoxin-delivering mold to out-compete the aflatoxin-creating molds. In mild atmospheres, aflatoxin issues have been controlled largely with ventilation amid cooler evenings and through lower winter temperatures [17].

During the past decade, there has been increasing interest in the hypothesis that, the absorption of aflatoxin in consumed food is may be inhibited in the gastrointestinal tract. In recent years, some biological control strategies have been used to reduce aflatoxin contamination in various food materials. Aflatoxins contamination may occur in the field before harvest, during harvesting or during storage and processing; thus, methods for the prevention of aflatoxin contamination can conveniently be divided into pre-harvest, harvest and drying of unshelled peanuts, shelling post-harvest storage strategies. In addition, because of the high occurrence of aflatoxins in crops worldwide, fast and cost-effective analytical methods are required for

the identification of contaminated agricultural commodities before they are processed into final products. In addition, there have been several reports on AFB$_1$ outbreaks, especially in many undeveloped countries. Therefore due to its potential threat in every step of the food production, analytical methods have been developed for the determination of AFB$_1$ in various matrices including liquid chromatography (LC), thin-layer chromatography, TLC), high-performance liquid chromatography (HPLC) immunoaffinity chromatography (IAC), enzyme-linked immunosorbent assay (ELISA), electrochemical immunosensor, etc. [18].

2. Control strategies

The risk of such contamination can be greatly increased because of the poor traditional practices. However, certain treatments have been found to reduce aflatoxin formation in peanuts, and the complete elimination of aflatoxin is not realistically achievable [19].

2.1. Pre-harvest factors influencing aflatoxin contamination of peanuts

2.1.1. Peanut cultivars

In the 1980s, numerous scientists had endeavoured to discover peanut cultivars resistant to *A. flavus* contamination and the production of aflatoxin but they were unsuccesful because of the cultivars exhibiting the complex elements affecting the development and dispersion of the growth and aflatoxin production [20, 21]. As of late, transgenic innovation has been broadly utilised for cultivar change. Transgenic peanuts containing the Bt (*Bacillus thuringiensis*) gene had altogether brought down levels of aflatoxin than non-Bt peanuts in preparatory examination of log-changed information [22]. Guo et al. [23] recognised the resistance-related genes (iso ara h3 and LEA 4) in peanut against *A. parasiticus* disease and resulting aflatoxin contamination, and after that built up a peanut microarray to distinguish hopeful genes that give imperviousness to *A. flavus* contamination [23, 24]. Furthermore, cultivar improvement in expanding the resistance of peanut to ailments can likewise altogether diminish the frequency of fungal contamination contrasted with the unaltered assortments [25].

2.1.2. Soil type

It is outstanding that peanuts can develop in various soil sorts such as light sandy soil and heavier soils. Light sandy soil benefits for the quick multiplication of *A. flavus*, especially under dry conditions in the later development time frame. Despite what might be expected, heavier soil can decrease the level of aflatoxin defilement in peanut grown because of having a higher water-holding limit [26].

Soil preparation is necessary for planting peanut in order to reduce the incidence of aflatoxin contamination. Several chemical control agents have been reported to inhibit aflatoxigenic mold growth and subsequent aflatoxin biosynthesis. Although some studies suggested that pesticides and fungicides might be useful in controlling mycotoxin production under field

conditions, other results have found that pesticides were ineffective in controlling mycotoxin production by *Aspergillus* species [26]. Control of pod-feeding insects through the application of recommended insecticides and use of insect-resistant cultivars should be an integral part of the strategy to eliminate pre-harvest aflatoxin contamination [27]. In order to reduce the aflatoxin contamination in peanut soil rehabilitation with gypsum, cereal crop residue and farmyard fertiliser have been applied either singly or in different combinations at various stages of cropping. However, farmyard manure and gypsum at the sowing time were found to be the most effective in reducing aflatoxin contamination [28]. Biological control of toxigenic *A. flavus* strains can be achieved by the application of atoxigenic *A. flavus* strains to maize, groundnut and cotton fields [29]. Probst et al. [30] reported that A. flavus NRRL-21882 is the atoxigenic active ingredient in AflaGuard(Syngenta, Wilmington, DE) which is used for a biocontrol product currently registered for management of aflatoxins in maize in the United States. In addition, the researchers emphasise that isolate mixtures could compete more effectively than individual isolates in a greater diversity of environmental niches. In Argentina, Alaniz Zanon et al. [31] showed significant reductions of aflatoxin levels in peanut kernels harvested in the peanut core area of the country treated with a biocontrol agent based on the native non-aflatoxigenic *A. flavus* AFCHG2 strain [31]. Another study by Alaniz Zanon et al. [32] characterised native non-aflatoxigenic *A. flavus* strains isolated from the main peanut growing region of Argentina based on phenotypic, physiological and genetic characteristics; and to evaluate selected strains as biological control agents as single or mixed inocula to reduce aflatoxin accumulation in peanuts harvested in Northern Argentina. According to the results of [32], they found that an inoculum comprising a mixture of two nontoxigenic *A. flavus* strains proved to be effective in the reduction of aflatoxin accumulation in peanut kernels. In addition, Lavkor et al. [9] reported that *A. flavus* NRRL21882 (Afla-guard) was applied in three different ways in trial experiment, and it reduced aflatoxin amount varying from 98.4% to 99.8% and suppressed aflatoxin contamination of peanuts [9]. In another research, Power et al. [33] used the method of RNA interference (RNAi) as a promising method to reduce or prevent the accumulation of aflatoxin in peanut seed. In this study, they also performed high-throughput sequencing of small RNA populations in a control line and in two transformed peanut lines that expressed an inverted repeat targeting five genes involved in the aflatoxin biosynthesis pathway and that showed up to 100% less aflatoxin B_1 than the control samples. The researchers stated that the research output would increase their understanding of the effectiveness of RNAi and enable the possible improvement of the RNAi technology for the control of aflatoxins and thus probably it can determine the putative involvement of the small RNA populations in aflatoxin reduction [33].

2.1.3. Species of fungi in soil

Soil is a repository of fluctuated microorganisms including organisms, and peanuts are in direct contact with soil populaces of aflatoxigenic growths [34]. Regular fungal contaminants of peanuts involve *Aspergillus*, *Penicillium*, *Rhizopus* and *Fusarium* species [35, 36]. Numerous literary works detailed that *A. flavus* and *A. parasiticus* are the two firmly related types of organisms that attack peanuts and in this manner prompt to their defilement with aflatoxins B_1, B_2, G_1 and G_2 [25, 37, 38]. The existence of other fungi, for example, *Penicillium* and

Fusarium species, diminishes the aflatoxin generation because of competitive inhibition [39]. Furthermore, different morphological sorts for similar species likewise influence the aflatoxin contamination of peanuts, for example, S-and L-strains, which was kind of *A. flavus*. Although the occurrence of *A. flavus* S-strain has shown to have a relation with the contamination of peanut with aflatoxin, L-strain was not demonstrated and not definitely associated with the aflatoxin levels in peanuts [25].

2.1.4. Climate

Taylor et al. [40] detailed that aflatoxin defilement occurred in most developing zones; however, the most incidence of aflatoxin was in the hotter, more humid developing locales and took after the same geological example. *A. flavus* can be separated from soil in every single climatic zone, and it is separated moderately more as often as possible in warm temperature zones (latitudes 26–35°) than in tropical or cooler temperature zones. It is very uncommon in latitudes over 45° [41, 42]. In this manner, the aflatoxin defilement of peanuts is frequently found in scopes latitudes 35° [43]. In a study by Wu et al. [44], 2494 peanut samples were been collected from four major peanut producing areas in China and were investigated for the occurrence of aflatoxins. As a result, they found a close relationship can be concluded between the aflatoxin presence and the weather a month before harvest. In this survey in China from 2010 to 2013 in peanuts at harvest,they have emphasised that it is essential for taking preventive measures to alleviate pre-harvest contamination of aflatoxin to peanuts [44].

2.1.5. Weather conditions

Sanders et al. [45] documented that aflatoxin contamination is not generally straightforwardly associated with the rate of attack by *A. flavus*. Cole et al. [46] proposed that after the attack of aflatoxigenic fungi occurred, development of the fungi and aflatoxin creation could not occur until a resistance mechanism separated subsequently of natural anxiety (dry season and high temperature). Dry season and temperature stress are basic variables for aflatoxin contamination of peanuts [45, 46]. Cole and co-workers [46] found that drought stress and soil temperature of 29°C for 85–100 days produced the best number of colonised consumable grade peanuts and great aflatoxin levels [46]. End of season drought stress and lifted soil temperature are more advantage for advancing aflatoxin contamination [47, 48]. The reason is that dry season provokes a huge increase in proline in plants, which can improve aflatoxin occurrence [49]. Along these lines, sufficient rainfall can control or decrease aflatoxin generation of peanuts. Moreover, defilement has been observed to be across the board where peanuts are developed under rain-bolstered conditions compared with those developed under irrigation system [50].

2.1.6. Agricultural practices

Inappropriate agricultural pursuits, such as crop revolution, culturing, planting date, fertilisation and irrigation, can likewise expand the occurrence of *A. flavus* and aflatoxin contamination in peanuts [26]. The proceed with development of peanuts on a similar land may bring about

the high disease from fungi and aflatoxin formation [51]. Crop rotation may bring down the rate of between-season survival of various species, particularly if it includes crops that are non-host to *Aspergillus* species [25]. Nevertheless, the impacts of product rotation on aflatoxin rely on upon the planting condition, for instance, in a semi-arid environment, *Aspergillus* occurrence might be high, and crop rotation may have little impact on the fungal action [52].

In non-inoculated, non-insecticide-sprayed territories, thick populace of plants or condensed fertilisation seem to affect the frequency of contamination by aflatoxin [53]. Insects may assume a vital part in the aflatoxin contamination of yields since almost all aflatoxins were found in regions harmed by insects. The insect harms peanut tissue, in this manner making section entrances for the fungus, and after that prompts to the high aflatoxin formation in peanuts [28, 53]. The research of Var and Uçkun [54] showed that the peanuts in the healthy shell have been preserved very well [54]. Numerous authors reported that a few insecticides and fungicides can repress aflatoxigenic fungi development and consequent aflatoxin biosynthesis in field [55, 56]. Bowen and Mack [57] utilised the insecticide to treat peanuts during development and decreased the levels of *A. flavus* disease and aflatoxin contamination. Moreover, early sowing in ideal time, scientific fertilising and solid field administration were required in decreasing the aflatoxin contamination of peanuts [57].

2.1.7. Phytoalexin production

Phytoalexins are described as antimicrobial substances combined by plants that collect quickly at territories of pathogen infection. In spite of the fact that the substance nature of the phytoalexins was not determined, it was demonstrated that peanuts created phytoalexins when tested by a few types of fungi, including *A. flavus* [58]. It was proved just as it was in 1972 that he resistance of immature peanut to fungi was expected to phytoalexins yielded in high amounts in light of fungal infection [59]. It was observed that as long as peanuts had phytoalexin generation they were not contaminated with aflatoxins and in immature peanuts the aflatoxin did not form until phytoalexin generation stopped in dry season stressed plants [60]. It has also been found that the water activity (aw) of the peanut kernels plays a crucial role in controlling the capacity of the nucleus to produce phytoalexins. Therefore, peanuts may produce sufficient phytoalexin in high water activity (> 0.97) to prevent the development of A. flavus and aflatoxin contamination.It was observed as kernel aw diminishes, as a result of elongated drought, the capacity of those kernels to create phytoalexins likewise reduces and in the end is lost (aw < 0.95) [60].

2.2. Post-harvest factors influencing aflatoxin contamination of peanuts

Generally, kernel moisture contents of 10% or higher post-harvest peanuts are prone to generate aflatoxins. Timely drying and keeping at safe moisture level can effectively control aflatoxin contamination of peanuts after harvest [26]. Diener and Davis [61] found that aflatoxin generation can be blocked by quickly drying to or beneath an aw of 0.83 for post-harvest peanuts. Before-storage separating to remove contaminated peanuts is the best approach to decrease aflatoxin generation [62, 63]. To keep an expansion in aflatoxin occurring during capacity and transportation, it is essential to control the dampness content, the temperature in the environment and the hygienic conditions [64]. Unsuitable kernel dampness during

storage can continue from leaky roofs, reduction because of inappropriate ventilation in the warehouse, high-dampness outside material related with put away peanuts and high-dampness peanuts at first going into storage [65]. Thus, the storage and transportation conditions are the most vital reasons controlling aflatoxin defilement of peanuts.

2.2.1. Harvest control strategies

During harvesting, mechanical damage to peanuts must be avoided because it enhances susceptibility to contamination. Moreover, only mature peanuts should be harvested since fungal infection is more likely to occur in shrivelled and cracked kernels [66].

Recently, biosensors based on the use of monoclonal or polyclonal antibodies have seen great development in the field of small molecules analytical determination and specifically in the mycotoxins analyses [67]. Early and reliable precise methods protect health and life by preventing the entry of toxins into food chain. For this reason, it is necessary to transport these fast technologies to commercial products from the research stage using appropriate subsidies [68]. On the other hand, new unthermal preservation methods (Ozone, UV-C, ultrasound and manosound) are used for reducing aflatoxin content on some food and commodities. In addition, some studies try to show that these unthermal preservation methods could be used with hyperspectral imaging methods. Hyperspectral imaging methods could show us about the product or crop composition and distribution of food components [69]. In their research, Kandpal et al. [70] used hyperspectral imaging method for the detection of aflatoxin contamination on corn kernels. They have been reported that corn specimens were inoculated with four different concentrations (10, 100, 500 and 1000 mg/kg) with aflatoxin B1 (AFB1), and control specimens surface was sterilised with a PBs. Both contaminated and control specimens were scanned with an SWIR hyperspectral for the spectral range from 1100 to 1700 nm. The PLS-DA model has been created to arrange control and contaminated kernels and was discovered that most elevated general arrangement exactness yielded of the created model was 96.9% [70]. In their study, Jiang et al. [71] focused to identify the moldy peanuts using near-infrared (NIR) hyperspectral images, and NIR hyperspectral images were obtained at the wavelength ranging from 970 to 2570 nm. In order to select sensitive bands, principle component analysis (PCA) in the spectral dimension was used as well as the spectral vector was employed to identify the moldy information [71]. In another work, utilising a FRET-based method, Sabet et al. [72] have developed a nanobiosensor for detection of AFB_1 in agricultural foods. Aptamer-conjugated quantum dots (QDs) are adsorbed to Au nanoparticles (AuNPs) due to interaction of aptamers with AuNPs leading to quenching effect on QDs fluorescence. Upon the addition of AFB_1, the specific aptamers are attracted to AFB_1, getting distance from AuNPs which result in fluorescence recovery. Under optimised conditions, the detection limit of proposed nanobiosensor was 3.4 nM with linear range of 10–400 nM. Selectivity test demonstrates that the nanobiosensor could be a promising tool for specific evaluation of food stuff. This method was successfully applied for the analysis of AFB_1 in rice and peanut samples [72].

Traditional methods that require intense labour force are currently being used to separate aflatoxinous products. Workers are trying to determine whether there are aflatoxins in the products that pass through the tapes by standing on the UV lamp stands set up in a dark room for

8–12 h a day. Güzel et al. [73] stated that this manual separation technique reduces working efficiency and negatively affects the health of workers exposed to long periods of light. In addition, due to the distraction created by fatigue, the diseased products that need to be separated can escape attention. Therefore, the researchers had developed a UV light-based separator that does not escape toxic products, more rapid sorting and less use of human power. Güzel et al. [73] have believed that many negative conditions will come to an end with their machine.

2.2.2. Drying of unshelled peanut control strategies

The drying stage is all important to reduce attack and damage fungi. Lavkor and Bicici [74] reported that peanut kernels aflatoxin analysis was performed at four distinct periods: harvest, post-harvest, drying and pre-storage, and analysis results showed that aflatoxin contamination was not found on 96 samples sundried on drying sheet at experimental area in 2010 and 2011. According to Cole et al., it seems that post-harvest screening is a chance to decrease or eliminate aflatoxin at defiled seed. Probably, there are generally few, but highly contaminated seeds dispersed in the peanut lots when aflatoxin contamination occurred [65]. Practical methods include manual sorting, seed size and density separation, or electronic colour sorting. Electronic colour sorting has proven to be the most effective aflatoxin management strategy available in the processing phase [75]. Guchi [75] reported that electronic colour sorting is another means that can be used. For example, peanut that has been colonised by aflatoxin-producing fungi is often discoloured. Microwave heating shows great potential for the destruction of aflatoxin in contaminated peanut. Aflatoxin B_1 is sensitive to UV radiation and absorbs UV light at 222, 265 and 362 nm with the maximum absorption occurring at 362 nm. One strategy to reduce the entry of aflatoxin into the peanut chain is the use of chemical treatments such as acetosyringone, syringaldehyde and sinapinic acid and ammonia applications during post-harvest to reduce both fungal growth and toxin production [76]. Ozone due to its safety, environment-friendly, low cost and high efficiency in decomposing aflatoxin B_1 has been widely studied and used in the food industry [1]. Proctor et al. [77] achieved the highest level of degradation for aflatoxin B_1 (77±2%) after ozonation of peanut kernels for 10 min at 75°C [77]. In their study, Chen et al. [78] focused on the optimization of aflatoxin reduction by ozone during air drying of peanuts. They have observed that 5% moisture in peanut provided sensitivity of aflatoxins to ozone and reacted with 6.0 mg/l of ozone at the room temperature for 30 min simply degraded. They also found that the diminution of the total aflatoxins and aflatoxin B_1 (AFB$_1$) was 65.8% and 65.9%, respectively. In this research, they also examined the quality of peanut samples, and it has been observed that no significant differences ($P > 0.05$) were found in the polyphenols, resveratrol, acid value (AV) and peroxide value (PV) between treated and untreated samples. According to the researchers, the results suggested that the ozonation was a promising method for aflatoxin detoxification in peanuts [78].

In another study, Luo et al. [79] examined the ozone treatment effect in reducing aflatoxin B_1 in corn with different moisture content. In this study, the toxicity of the degradation products (DPs) of the ozone-treated aflatoxin B_1 contaminated corn was also evaluated using the human hepatocellular carcinoma cell line (HepG2) as model cells. It was observed that the degradation rate of aflatoxin B_1 in corn increases with ozone concentration and treatment time. It was also observed

that aflatoxin B_1 contaminated corn with 13.47% moisture content was easier to be degraded by ozone than with 20.37% moisture content. In this study, when the safety of ozone used on aflatoxin B_1 contaminated corn was evaluated, the results showed that aflatoxin B_1 contaminated corn had high cell toxicity while the toxicity of ozone-treated aflatoxin B_1 contaminated corn had no significant difference with that of the aflatoxin B_1 free culture solution. The researchers suggested that ozonation can quickly and effectively degrade aflatoxin B_1 in corn and diminish aflatoxin B_1 contaminated corn's toxicity, and therefore, ozonation is expected to be an effective, fast and safe method for aflatoxin B_1 degradation in aflatoxin B_1 contaminated corn [79].

Diao et al.'s [1] study aimed to verify the ozonolysis efficiency of AFB_1 and to evaluate the oral safety of ACPs treated by ozone through a short-term subchronic toxicity study with Wistar rats. As a result of the study, they found that 89.40% of aflatoxin B_1 (AFB_1) in peanuts was decomposed by ozone with a concentration of 50 mg/L and flow rate of 5 L/min for 60 h. In their subchronic toxicity experiment, they declined that all rats did not have unusual changes in behaviour, and no signs of intoxication were observed except for several dead rats due to inappropriate gavage or anaesthesia. The researchers suggested that the deleterious effects of AFB_1 could be highly reduced by ozone, and ozone itself did not show any toxic effects on animals in this processing [1].

2.2.3. Shell extraction

Mechanical harm to food stuff during shell extraction makes them much more susceptible to attack by moulds such as *A. flavus*. Fungal growth may be much faster in the damaged peanuts compared to intact peanuts in any given environmental conditions. Cracks and breaks in peanut shell are mainly caused during shell extraction by use of machines or trampling. The machines used for this purpose are generally manual or motorized shellers. The latter normally use electricity and can be a simple type that can handle small volumes of peanuts or big type that can handle several bags of peanut per hour [80]. The use of ultraviolet light (UV) is well established for surface decontamination. After the application of UV-C for almond and nuts, it was observed that for AFG_1 and AFB_1 degradation result was found to be 100% and 96.5%, respectively [81]. Furthermore, Sharareh et al. [82] evaluated the effect of ultra-violet irradiation on detoxification of aflatoxin total including aflatoxin B_1 (AFB_1), aflatoxin B_2 (AFB_2), aflatoxin G_1 (AFG_1), aflatoxin G_2 (AFG_2) and aflatoxin total (AFT) content in standard solutions and investigated the structural changes using HPLC, GC/MS and FT-IR techniques. For this purpose, standard vials of aflatoxin solutions with concentrations of 1000 µg/kg AFB_1, 200 µg/kg AFB_2, 1000 µg/kg AFG_1, 200 µg/kg AFG_2 and 2400 µg/kg AFT were treated by UV-irradiation at 366 nm wavelength for 10 min in this study. Aflatoxin contents were analysed by high-pressure liquid chromatography (HPLC) method. As a result, in this research, it was observed that the amount of AFB_1, AFB_2, AFG_1, AFG_2 and AFT reduced approximately 98, 99.5, 99.8, 100 and 99.1%, respectively [82].

2.3. Post-harvest storage control strategies

As evidenced by the storage structures, traditional crop storage is not yet improved. The storage conditions should be cool and dry, should be defended from insects, rodents and birds;

should be easy to clean and should be waterproof and protected from flooding. These conditions are indispensable for modern or traditional storage. These suggestions are so important to prevent *A. flavus* contamination and aflatoxin formation in stored products, especially in peanuts. It has been reported that field application not only reduced aflatoxin contamination in the field but also reduced aflatoxin contamination that occurred in storage [83]. Aflatoxin production could be prevented or at least reduced by modification of atmospheric gases in storage silos such as by using carbon dioxide, nitrogen, carbon monoxide and sulphur dioxide. Previous work on peanuts reported that increases in the concentration of CO_2 in storage silo resulted in significant reductions in aflatoxin production within stored peanuts [84].

Globally, there have been increasing incidences relating to foodborne diseases including aflatoxins in both developed and developing countries. Because of the lack of proper hygiene practices and personal sanitation are not applied for food products, significant public health crisis can result from aflatoxins contamination. Studies conducted in these areas indicate that due to the low consciousness and knowledge of food handlers and workers in these subjects, aflatoxin contamination is seen in food and especially in groundnut products. According to some researchers, raising the level of public knowledge by arranging awareness campaigns can diminish the risk of aflatoxin contamination. The important factors to ensure that food handlers are proficient and knowledgeable on the principles of food safety and personal sanitation are advised trainings, food safety education and the developments of food safety certifications [85]. Therefore, Azaman et al. [85] planned a study that was to identify the differences in terms of knowledge, attitude and practices (KAP) of aflatoxins contamination among stakeholders of peanut-based products and to determine factors that mostly influence stakeholders' hygienic practices in peanut-based products. As a result of the study, they strongly emphasised the need for continuous hygiene improvement and training programmes by the stakeholders of peanut-based products. In addition, they stated that relevant strategies such as promotion and motivational models on health education and food safety campaigns would increase awareness and knowledge on food contaminants [85].

It is known that despite all these there remains aflatoxin contamination in the products. Therefore, in order to minimise aflatoxin exposure among consumers, it is essential to prevent highly contaminated kernels from re-entering food chains, and decontamination of such kernels should complement some sorting practices. Schwartzbord and Brown [86], in their study, focussed on to explore a process to transform oil from contaminated peanuts into a safe edible product. Schwartzbord and Brown [86], in their study, focussed on to explore a process to transform oil from contaminated peanuts into a safe edible product. As a result of the study, the researchers found that in extracted oil included aflatoxin concentration was approximately 10% of that of unextracted oil, which means it had a concentration that was only 5% of the original contaminated peanuts.

Therefore, they displayed that without pre-filtration aflatoxin concentration in the final product was 99.5% less than that found in the original peanuts [86].

Extrusion cooking is an important process widely applied in the food industry. The extrusion of AFT in cereals had been studied by different research groups. In one research, it was investigated the extrusion of AFT contaminated corn grits at 105°C and found that the levels of

AFT were reduced by 50%–80% after processing in the extruded corn grits [87]. In their study, Azaman et al. [85] explored the feasibility of degrading aflatoxin B$_1$ (AFB$_1$) in contaminated peanut meal by extrusion cooking. In this study, the effects of barrel temperature, material moisture content, feed rate, and screw speed as well as their interactions on the reduction rate of AFB$_1$ in peanuts meal were evaluated by response surface methodology (RSM) to optimise the extrusion conditions [85]. Zheng et al. [87] emphasised that the study indicated that extrusion cooking was an effective way to remove total AFB$_1$ from contaminated peanut meal. Moreover, the researchers stated that extrusion cooking can be used to treat other cereals. Although extrusion cooking has wide application prospects in food processing industry, but the researchers advised that it is required to perform further research to determine whether certain toxic products are generated during the decomposition of AFB$_1$ [87].

2.4. Analytical methods for the identification of contaminated agricultural commodities

Because of the high occurrence of aflatoxins in crops worldwide, fast and cost-effective analytical methods are required for the identification of contaminated agricultural commodities before they are processed into final products. So far, many aflatoxin detection technologies have been developed for the determination of AFB$_1$ in various matrices including liquid chromatography (LC), thin-layer chromatography (TLC), high-performance liquid chromatography (HPLC) immunoaffinity chromatography (IAC), enzyme-linked immunosorbent assay (ELISA) and electrochemical immunosensor, LC-MS/MS, Fluorescence polarisation immunoassay, capillary electrophoresis, near infrared spectroscopy, hyperspectral imaging electronic nose [88]. Actually, there are some advantages and disadvantages of these aflatoxin detection technologies, and these are still discussed.

Thiel et al. [89], in their study, had described the application of a technique for the determination of aflatoxins by reverse phase HPLC and fluorescence detection incorporating postcolumn derivatisation with iodine. They stated that the procedure proved to be extremely sensitive and reproducible [89]. Researchers suggested new tools for screening aflatoxins in food. For this purpose, one is for aflatoxin B1 and the other for total aflatoxin, they developed two prototypes to be used in the ELISA method. For this reason, they highlighted that seven monoclonal antibodies were produced that were with matchless high sensitivity and at the same time good cross-reactivity properties [90]. However due to limitations associated with these methods, including extensive sample preparation, expensive procedure and unavailability for onsite screening, increasing demand has been emerged especially in developing countries for more simple and cost-effective methods [72]. Utilising a FRET-based method, it has developed a nanobiosensor for detection of AFB$_1$ in agricultural foods. According to Sabet et al. [72] Aptamer-conjugated Quantum dots (QDs) are adsorbed to Au nanoparticles (AuNPs) due to the interaction of aptamers with AuNPs leading to quenching effect on QDs fluorescence. Upon the addition of AFB$_1$, the specific aptamers are attracted to AFB$_1$, obtaining distance from AuNPs, which result in fluorescence recovery [72]. Semiconductor quantum dots (QDs), as a new type of fluorescent probes, have unique optical characteristics such as photostability and high quantum yield originated from "quantum size" effect and have been proven to be of many uses in biosensing application. In their research, Sabet et al. found that

selectivity test demonstrates that the nanobiosensor could be a promising tool for specific evaluation of food stuff. Moreover, they stated that this method was successfully applied for the analysis of AFB_1 in rice and peanut samples. In recent years, with the rapid development of nanostructured materials and nanotechnology in the fields of biotechnology and contaminant detection, magnetic nanoparticles (MNPs) have been receiving considerable attention. In their research, Sun et al. [91] used artificial antigen-modified MNPs employed as immune sensing probes, and antibody functionalised UCNPs were used as signal probes. Besides in this study, the antibodies-functionalised UCNPs were linked to the surface of the MNPs by antibody-antigen affinity [91]. According to Sun and co-workers, rare earth-doped upconversion nanoparticles were used successfully to assemble for sensing Aflatoxins B_1 in actual food samples (peanut oil) [91]. Ezekiel et al. [92] described a reliable and simple analytical method for the determination of aflatoxins (AFB_1, AFB_2, AFG_1 and AFG_2) in cereals, peanuts, vegetable oils and fermented foods such as beer, soybean sauce and soybean paste based on immunoaffinity column (IAC) cleanup coupled with direct competitive enzymelinked immunosorbent assay (dcELISA) detection and confirmed by ultra-high performance liquid chromatography tandem mass spectrometry (UHPLC-MS/MS) in their research. As a conclusion, they suggested this assay could be used as an effective analytical method for the determination of aflatoxins in complex grain foods [92].

3. Conclusion

As we can see above, there are various methods aimed at minimising the aflatoxins in foods, but there still exists Aflatoxin problem in food. Since it is difficult to achieve zero tolerance with AF contamination in commodities, AFs should be minimised in foods as much as possible to prevent the risk of cancer and the other health problems. Thus, legal tolerance limits based on scientific evidence obtained from risk assessment in different countries have been set for AFB_1 and total aflatoxin (AF) in foods and feeds. The limits vary between 4 and 20 parts per billion (ppb) through different countries [93]. The Codex Alimentarius Commission (CAC) has adopted the maximum permissible limits for AFs in unprocessed peanuts and tree nuts, which is 15 ppb as well as10 ppb in ready-to-eat tree nuts. However, European Union (EU) has adopted the level of 4ppb, which is the strictest limit in the world for AFs [93].

In a study, 60 peanut samples were analysed for aflatoxin B_1 using thin layer chromatography. The Democratic Republic of Congo is among African countries listed with high prevalence of liver cancer. As a result, Kamika and Takoy showed that aflatoxin B1 levels increased from the dry season to the rainy season with values ranging from 1.5 to 390 and 12 to 937, respectively. They reported that 70% of the peanut samples from both seasons exceeded the maximum limit of 5 mg/kg prescribed by the World Health Organization (WHO). Therefore, they emphasised continuous research on aflatoxin B_1 should be sought after [94]. In a study in Zambia, another African country, it showed that the high level of AFs in raw peanuts from both open markets and supermarkets samples are a health hazard for the population of the Lusaka region in Zambia. Therefore, the researchers stated that intervention tactics is urgently required to decrease the levels of AF contamination in peanuts [93].

In another Asian country, in Punjab major city of Pakistan, the focused on the assessment of the frequency of aflatoxin contamination in peanut and peanut products (peanut butter, roasted peanut, peanut bran and groundnut nimko) on the market. The researchers reported that the survey is of high importance to create the awareness among consumers, policy makers and law enforcement agencies to establish permissible limits for these toxins. As a result of their study, they told that the level of Aflatoxins in peanut and peanut products is high and poses a significant threat for the health of people [95].

One of the studies about aflatoxins in peanuts comes from Nigeria, which was planned to show the presence of aflatoxigenic *Aspergillus* populations and AFB1 profile in sold peanut cake in Nigeria. In this study in order to measure the awareness of consumers for the incidence of aflatoxin in the snack and public health threats of its steady consumption, was used questionnaire method. As a result *Aspergillus* section *Flavi* populations were recovered from 83% of the peanut cake samples. It was found that all analysed cake samples contained AFB_1 in concentrations exceeding the NAFDAC recommended level for AFB_1 in food and reaching up to 2824 mg/kg [92].

As seen before, most studies have showed us that aflatoxin contamination of peanuts can occur in the field (pre-harvest) when severe late-season drought stress occurs and poor agricultural practice and during storage (post-harvest) when improper conditions of moisture and temperature exist. Moreover, several techniques for aflatoxin controls have been proposed in the scientific literature, but just some are currently used by the peanut producers. So, aflatoxin control strategies are necessary to prevent health risks and economic losses for result from aflatoxin contamination. Besides, the studies and regulations related to Aflatoxins especially in peanuts and the other foods should be improved and carry on.

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