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Detoxification of aflatoxin in groundnuts by novel degradation approaches

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Abstract
Aflatoxins are cancerogenic mycotoxins majorly produced by certain species of moulds of the *Aspergillus* genus in different food grains and it is major food safety concern in groundnut. Aflatoxins are highly tolerant to destroy under different conditions; hence it is very common chemical hazard in foods and feeds. Due to its stability and presence in foods trigger various health problems such as liver cancer and thus becoming a more burden to food safety management in global food industry. It is inevitable to develop novel degradation or removal of aflatoxin levels to below the toxic levels in groundnut and practiced for the safeguarding thepeople and livestock health management. Novel degradation methods must appraise the multiple criteria to upscale into commercial degradation processes: (1) ability to degrade or reduce aflatoxins to safer levels; (2) not to produce or leave any toxic residues; (3) no effect on nutrients;(4) preserve the sensory attributes; (5) techno economical feasibility and eco friendly approach; and (5) destroy all kinds of fungal spores. This current review paper emphasized on novel physical detoxification approaches that could bepotentially applied to decontamination of foods and feeds from aflatoxins. However, a detailed and clear discussion of the decontamination methods for aflatoxins in groundnut is still not available. The applications of novel degradation technologies are explained in detail by coveringmerits and limitations of these approaches in this review paper. We put forward that this critical information and our differential opinions could help researchers to understand the decontamination approaches for aflatoxins.

Keywords: Groundnut, aflatoxins, aflatoxin B1, detoxification methods, food safety

1. Introduction
Groundnut (*Arachishypogaea*) is a leguminous crop and is vital affordable source of edible vegetable oil, protein, energy, essential fatty acids such as oleic acid, vitamins, and minerals for human nutrition (Chris *et al.,* 2020) [6]. Ground nut, also known as poor men's food, are affordable and used as a vital ingredient in making of diverse food preparations in most of the countries in the world (Kamika *et al.,* 2014) [26]. Ground nut can be eaten as fresh, boiled, toasted, dried roasted or used as an ingredientwith in regular dishes such as vegetables, porridge, and meat, and spread on bread (Kamika *et al.,* 2011) [27]. The edible oil, fat spreads, bakery shortenings, pea nut butter, hydrolyzed vegetable proteins, protein concentrates or powders are the important processed foods produced from groundnut. Groundnut is placed in eighth position among important nutritional crops and also placed in 6sixth positions in terms of nutrition among oil producing crops (Monyo *et al.,* 2012) [37]. While groundnuts are rich source of vital nutrients and they are also highly susceptible to fungal growthand produces strong carcinogenicaflatoxins. The rich nutrient composition of ground nut makes them an ideal substrate for moulds growth and potential mycotoxin contamination (Innocent *et al.,* 2017) [22]. Aflatoxins are strong carcinogenic and mutagenic fingal toxins. Aflatoxinsreleased by *Aspergillus* species, notably *Aspergillus flavus* and *A. parasiticus*, pose a major threat to world trade of groundnut and limiting the access to overseas commoditytrade and affecting populations that consume it (Chris O *et al.,* 2020) [6]. Aflatoxins are chemically polyketide-derived fungal toxins cause acute hepatotoxicity and immunosuppression (Eaton DL and Groopman JD., 1994; Pier AC *et al.,* 1977) [11, 45] and further aflatoxicosis causes death of human and animals (Azziz *et al.,* 2005) [2]. Aflatoxintoxigenicity in ground nuts is still a serious concern for the food industry and human health. There is not much mycotoxins resistant adoptable commercial cultivar in the world. Several mycotoxins causes food spoilage and there are more than 20 types of aflatoxins, however, the most common aflatoxins are aflatoxin B1, B2, G1, G2, M1 and M2 (AFB1, AFB2, AFG1, AFG2, AFM1 and AFM2). AFM1 and AFM2 are formed from the metabolism of AFB1 and AFB2, respectively

(Thu Nguyen *et al.,* 2020) [54]. Aflatoxin B1, B2, G1 and G2 are classified as human carcinogens (group 1B). Aflatoxin B1 is the highly toxic chemical hazard and is accounts for greater than 75% of all kinds of aflatoxins contamination in food and feed. (My and Sachan, 1997) [39]. However, aflatoxin M1 is less hepatotoxic and immunotoxic than its parent compound, aflatoxin B1, it is not destroyed at milk pasteurization temperature.It is categorized as a possible human carcinogen (group 2B) by the International Agency for Research on Cancer (Kai Zhang and Kaushik Banerjee, 2020) [25]. Many human body organs are affected with the alfatoxins and the functions of liver and kidney are impaired and resulting in liver cancer and links to other types of cancers that are greater in the presence of hepatitis B virus (HBV). High-dose exposure of the risk can result in vomiting, abdominal pain, and even possible death, while small quantities of chronic exposure may lead to liver cancer (Sherif *et al.,* 2009; Patchimaporn *et al.,* 2017) [51, 44]. In addition, long term exposure to aflatoxins may lead to birth defects for children, including stunted growth and immunosuppression (Thu Nguyen *et al.,* 2020) [54]. A high level of aflatoxin intake may cause fatality due to liver damage (WHO/NHM/FOS/RAM/18.1, 2018) [59]. The lethal dose is 20-120 µg/kg body weight per day over 1-3 weeks.Aflatoxins are not only chemical hazards in food and due to its stability in most conditions has been inducing significant health problems and financial loss.

Due to the potent toxicity of aflatoxins, many countries have formulated strict regulations for aflatoxins in food including milk, however, these vary in different countries. For example, in peanuts, dried fruits and cereals regulatory levels range from 2 µg/kg for aflatoxin B1 and 4 µg/kg for total aflatoxins (sum of aflatoxin B1 + B2 + G1 + G2) by the European Union. The USFDA has put the bar for the maximum acceptable limit of 20 µg/ kg for total AFs in all food except milk (FDA, 2011). Similarly, the Food Safety and Standards Authority of India (FSSAI) regulations 2011 has set the limit of total aflatoxins at 10 µg/kg for ready-to-eat nuts, 15 µg/kg for cereal and cereal products, 15 µg/kg for processed nuts. In the US and India the action level of aflatoxin M1 in raw milk is 0.5 µg/kg, while in the EU the maximum level of aflatoxin M1 in milk is 0.05 µg/kg and 0.025 µg/kg in infant formulas and dietary foods for special medical purposes intended specifically for infants.In view of the aflatoxin contamination in food and feed and greater risk to human and animal health as well as to the financial loss, researchers have been searching for techno economical feasible and eco friendly methods for degradation of aflatoxin in foods. The adoption of good agricultural practices (GMP) and the use of systematic controlled storage conditions have minimized the potential for aflatoxin contamination, however, these practices have been shown to be unable to assure removal of aflatoxin producing organism.

In this review, our aim is to provide an updated and comprehensive review of novel physical and chemical aflatoxin detoxification approaches. Different types of aflatoxin risk management approaches exist in literature. Depending on the "type" or mode of application, management of risk has been classified as physical methods, chemical methods and biological detoxification. Nevertheless, a detailed and systematically discussion of the methods of degradation for aflatoxins is still not available. Therefore, in the present review we briefly list out several common strategies, update newly methods and discuss some mechanisms during the degradation period, demonstrating advantages and disadvantages of these methods.

2. Novel detoxification approaches

2.1 Mechanical Sorting

Before pre cleaning of grains and groundnuts may contain sand, dust, husk, weevilled grains and admixtures with damaged grains containing most of the mycotoxin contamination (Johansson *et al.,* 2006) [23]. In general immediately afterharvesting of grains, unit operations like threshing, drying and cleaned through pre-cleaning sorting machine carried out for safe storage and processing. These unit operations are hold up by the fact that mycotoxin contamination favour to have a unbalanced distribution, with the majority of toxin found in a small number of grains or kernels (Kabak *et al*., 2006) [24]. Manual sorting is still a primary practice in many countries to remove aflatoxin infected grains or kernels (Matumba *et al.,* 2015) [33]. Different types of sorting machines have been in use since the late 1800s (Karlovsky *et al.,* 2016) [28] that separated admixture of grains and other foreign materials based on shape, size and density, however technology has significantly advanced since then many studies have shown that mechanical sorting with pre-defined physical characteristics (size, shape, density and colour) of grains is effective (Helina Marshall *et al.,* 2020) [20] in cleaning of grains and rejects most of the unwanted materials from the grains (Fraenkel, 1962) [18]. The sorting of grains by using fluorescence light (based on the BühlerLumovision™) is a modern technology, which performs sorting at a scale which reduces the risk of aflatoxin contamination whilst minimizing the quantity of food waste. The use of ultraviolet (UV) light for detection of aflatoxin is well known. The reaction between plant tissue enzyme peroxidase and kojic acid formed by *A. flavus* or *A. parasiticus*, aflatoxin producing fungi, or the mycotoxin produces Bright Greenish Yellow (BGY) Fluorescence. The Bright Greenish Yellow Fluorescence (BGYF) test is utilized as a presumptive test to identify aflatoxin contamination.

This sorting mechanism utilizes the fluorescent properties related to the kojic acid and combines a camera built and optimised using hyperspectral fluorescence data with an LED-based UV lighting system to identify and remove contaminated grains or kernels at the speed of 15 tonnes per hour, with a decrease in aflatoxin contamination averaging at 85–90% and with the loss of 5% non contaminated grains in tests to date (Bühler, 2018) [4].

2.2 Extrusion cooking

Extrusion cooking technology is widely used for processing of cereals and other composite flour based ready to eat snacks production in food industry. Extrusion is a process that combines several unit operations using mostly high temperature and pressure in a very short period of time to produce puffed snacks. It has been explored that extrusion technology could be used in destruction of some naturally-occurring toxins, for instance deoxynivalenol (DON). Cazzaniga *et al.,* 2001 [5] conducted a study to evaluate the efficiency of extrusion cooking method on degradation of DON and aflatoxins in maize flour and results indicated that extrusion cooking could effectively degrade DON (more than 95%) but had a low affinity with AFB1 even with the addition of sodium metabisulphite 112 (10-25%). Similarly, Elias-Orozco *et al.,* 2002 [12] investigated the effectiveness of extrusion cooking with the addition of lime and hydrogen

peroxide on mycotoxin contamination of grains. They reported that higher elimination efficiency of mycotoxin found together with 0.3% lime and 1.5% hydrogen peroxide (AFB1, 46%; AFM1, 74%; AFB1-dihydrodiol, 85%). Méndez-Albores *et al.,* 2008 [34] evaluated effectiveness of extrusion cookingwith lactic and citric acids on detoxification of B-aflatoxins. They found that higher degradation rates were reported using citric acid compared with lactic acid during extrusion. Consequently, it was concluded that extrusion cooking process alone is not effective to degrade aflatoxin in foods. However, it appear to be effective approach by combination with some additives, we cannot neglect the additive residual problems, following negative effects of those additions on food qualities and even on human and animal health. Extruded corn tortilla snacks taste and aroma was affected by addition of lime and hydrogen peroxide during processing (Elias-Orozco, *et al.,* 2002) [12]. The disadvantages of degradation of aflatoxin through extrusion cooking process are obvious. This technology is high energy consuming and destroys all other vital nutrients present in grains due to cooking at high temperature and pressure. Further research studies should focus on the additives which have least impact on sensory and nutritional attributes of grains or kernals as well protect the human and animal health.

2.3 Degradation of aflatoxins by microwave heating

Microwave heating has been employed in different food processing operations such as drying, heating, cooking and extraction of food components. Few research studies have conducted and results are also postulated on the use of microwave heating in detoxification of aflatoxins in different food grains. P´erez- Flores *et al.,* 2011 [41] assessed the effect of microwave heating during alkaline-cooking of aflatoxin infested corn. The aflatoxin contaminated corn microwave heated at a power output of 1650 W for 5.5 minutes effectively reduced the aflatoxin B1 and aflatoxin B2 by 36 and 58 percent respectively. Mobeen *et al.,* 2011 [36] reported that microwave heating of groundnut and groundnut based products reduced the aflatoxin B1 to 50 to 60% and reduced the aflatoxin B2 to non detectable levels. The microwave heating of contaminated grains have moderate success rate in reducing the aflatoxin content in food grains and kernels. The manufacturers of microwave heating equipments are able to customize the equipment design to meet specific applications and product types. The major disadvantages of microwave heating is non uniform distribution of heat in the food products during microwaving and lesser penetration of temperature may leave cold and hot spots in the treated foods (Menon *et al.,* 2020) [35]. The persistent hot spots during microwave heating may destroy the heat sensitive nutrients and quality deteriorates and other side aflatoxin contamination in cold spots cannot be effectively detoxified. Further, extensive studies need to be conducted to optimize the microwave heating process conditions to enhance the detoxification efficiency along with structure elucidation and safety assessment of the aflatoxin degradation products.

2.4 Degradation of aflatoxins by ultraviolet irradiation

Ultraviolet (UV) irradiation is a well known non thermal physical method for the detoxification of aflatoxins in foods based on the principle of photosensitivity. Use of UV irradiation for aflatoxin detoxification has many advantages such practically possible method, low cost and eco friendly in nature due to no toxic effects and no waste generation (Gay´an *et al.,* 2014) [19]. Aflatoxin B1 absorbs UV rays at wavelength of 222, 265 and 362 nm, with the absorption maximum at 362 nm (Samarajeewa *et al.,* 1990) [49]. The hydroxyl free radicals ($OH^{\bullet}$) produced by UV irradiation could attack on terminal double bond present in aflatoxin B1 structure at C8-C9 position (Liu *et al.,* 2011) [29]. The photo degradation products of aflatoxins have much lower mutagenicity and cytotoxicity when compared to aflatoxin B1 as indicated in the Ames test and cell viability assay (Diao *et al*., 2015; Mao *et al.,* 2016) [8, 32]. The aflatoxin degradation efficiency depends on the UV intensity (wavelength) and duration time of irradiation. Treating of aflatoxin contaminated pea nut oil with the UV irradiation at the intensity of 200 and 400 $\mu w\ cm^{-2}$ reduced the toxins up to 79% and 85% respectively. The aflatoxin B1 in peanut oil was completely removed by treating with UV irradiation at 800 $\mu w\ cm^{-2}$ for 30 minutes (Liu *et al.,* 2011) [29]. The treating of aflatoxin contaminated ground nut with UV irradiation at 254 nm was found to reduce aflatoxin B1 level by 59.7% and 99.1% at exposure time of 2h and 10 h respectively (Yongpeng *et al.,* 2021) [61]. Delorme *et al.,* 2020 [7] demonstrated that the use of moderate doses of UV irradiation does not cause extensive adverse effects on the nutritional and sensory attributes of food products. UV light can easily pass through clear or transparent liquids. Nevertheless, its depth of penetration into solid materials is restricted, which results in low degradation efficiency in food products with high amount of suspended particlesor solids (Fan *et al.,* 2017) [14]. Thus, opaque or granular food products need to be arranged as a thin layer during UV irradiation treatment (Diao *et al.,* 2015) [8].

2.5 Degradation of aflatoxins by irradiation

Gamma rays have been employed as a primary source of irradiation for food processing due to their reactivity and penetration power. The irradiation of food up to a moderate dose of 10 kGy does not produce any toxicological hazards and no affect on nutritional and sensory characteristics of foods (WHO, 1999) [58].

Treating of aflatoxin contaminated foods with gamma irradiation initiates radiolysis of water and produces highly reactive free radicals such radical hydrogen ($H^{\bullet}$), superoxide radical ($O_2^{\bullet-}$) and hydroxyl ion (OH^-). These radicals have the ability to degrade aflatoxins in foods (Rustom, 1997) [47].

The structural examination of radiolysis products of aflatoxin B1 revealed that the double bond of the terminal furan ring was not seen in most of the AFB1 radiolytic products. This may be attributed to the action of free radicals on aflatoxin during gamma irradiation (Wang *et al.,* 2011) [56]. The toxicity of aflatoxin B1 is mainly due to the presence of double bond in the terminal furan ring. In the liver, the oxidation of the double bond in the terminal furan ring of AFB1 by hepatic cytochrome P450 enzymes (CYPs) yields AFB1-exo-8, 9-epoxide, which can react with the N7 atom of guanine to generate pro-mutagenic DNA adducts (Yongpeng *et al.,* 2021; Bbosa *et al.,* 2013) [61]. Thus the treating of contaminated foods with gamma irradiation results in the breakdown of double bond of the terminal furan ring in aflatoxin B1, which led to a significant reduction of its cytotoxicity in Pk15, HepG2 and SH-SY5Y cells (Yongpeng *et al.,* 2021; Domijan *et al.,* 2019) [61, 9]. The degradation efficiency of aflatoxin in foods by gamma irradiation mainly depends on factors like dose of radiation, level of contamination, moisture content in

grains or foods and composition of foods. Many researchers reported that gamma irradiation dose ranging from 5 to 10 kGy could degrade a significant concentration of aflatoxins in food products. The adoption of gamma irradiation for decontamination of foods is increasing as consumers perception changed towards irradiation of foods. As of now more than 55 countries including USA, Japan, China and EU countries have given approval for use of gamma radiation for food processing under specified conditions (Priyadarshini *et al.,* 2019) [46].

2.6 Degradation of aflatoxins by pulsed light

The aflatoxin contamination in food and feed has been detoxifying with another non thermal technology called pulsed light. Pulsed light is an USFDA-permitted novel approach for the rapid and effective surface decontamination of food products with an upper limit fluence of 12 J cm^{-2} (FDA, 2001; Yongpeng *et al.,* 2021) [15, 61]. This non thermal technology produces short, high intensity flashes of broadband emission light (100–1100 nm) including ultraviolet, visible and infrared rays (Yongpeng *et al.,* 2021; Oms-Oliu *et al.,* 2010) [61]. The intensity of the pulsed light is about 20, 000 times more intense than direct sunlight at sea-level (Dunn *et al*., 1995) [10]. The eight flashes of pulsed light (light flux of 1 J cm^{-2} during one 300 ms flash) have the ability to degrade upto 92.7% of aflatoxin B1 in water (Moreau *et al.,* 2013) [38].

The aflatoxin contaminated rice and rice bran were treated with pulsed light at 0.52 J cm^{-1} per pulse for 80 seconds and reduced the aflatoxin B1 and aflatoxin B2 up to 75.0% and 39.2% respectively, while reducing the time to 15 seconds reduced the aflatoxin B1 and aflatoxin B2 up to 90.3 and 86.7% respectively (Wang *et al.,* 2016) [55]. They also further reported that cytotoxicity and mutagenicity of aflatoxin B1 and aflatoxin B2 were inactivated, when they assessed the toxicity with the help of shrimp lethality assay and the Ames fluctuation assay. Abuagela *et al.,* (2018) [1] found that treating of aflatoxin contaminated dehulledgroundnuts with pulsed light of 0.4 J cm^{-1}per pulse have detoxified the aflatoxins up to 91%. Further pulsed light has no effect on chemical properties (peroxide value, fatty acid content and acidity value of oil) of groundnut oil but slight changes in groundnut kernel were observed. However, despite the advances mentioned above, the breakdown products of aflatoxins after Pulsed light treatment are still under investigation. Characterization of the possible photo degradation pathways of aflatoxins under pulsed light treatment will provide deep insight into the degradation mechanism and kinetics of this technology (Yongpeng *et al.,* 2021) [61].

2.7 Degradation of aflatoxins by photocatalysis

Several recent studies in literature reported that the use of UV–visible irradiation in combination with semiconducting photocatalysts can increase the detoxification efficiency of aflatoxins in liquid foods. In photocatalysis method, the photo generated valence band holes (h+), hydroxyl free radicals ($OH^{\bullet}$) and superoxide radical ($O_2^{\bullet -}$) are able to destroy the aflatoxin B1 by oxidation (Sun *et al.,* 2019) [53]. Titanium dioxide (TiO_2) is the most commonly used non toxic highly efficient photocatalyst, which is highly reactive under UV irradiation and has long term photostability (Yong peng *et al.,* 2021) [61]. Sun *et al*., 2019 [53] degraded the aflatoxin B1 up to 95% within 120 minutes by photocatalytic combination of UV–Vis irradiation in the presence of AC/TiO_2 (6 mg mL^{-1}) and 50% reduction in aflatoxin B1 was observed by using UV-visible irradiation alone. Xu *et al*. (2019) [60] designed a glass tube coated with Titanium dioxide immobilized bed photocatalytic reactor for the degradation of aflatoxin B1 in contaminated groundnut oil. The photocatalytic reactor consists of TiO_2 and UV system was able to degrade 60.4% of aflatoxin B1, which was greater than that of UV photolysis alone (35.1%) in peanut oil. Magzoub *et al.,* (2019) [31], reported that with the help of Titanium dioxide immobilized bed photocatalytic reactor completely removed the aflatoxin B1 and aflatoxin B2 in Sudanese groundnut oil. They also reported that use of photocatalysis for degradation of aflatoxin does not affect on physicochemical properties of groundnut oil, including composition of fatty acids, moisture, free fatty acids content, peroxide value, saponification value, acid value, iodine value and volatile matters as well as the refractive index.

2.8 Degradation of aflatoxins by Cold Plasma

Plasma is an ionized quasi-neutral gas composed of free electrons, photons and ions (Pankaj *et al.,* 2014) [42]. It is generated using combinations of different temperature and pressure and categorized as two types thermal andnon-thermal. Thermal plasma is characterized by high temperature and there is anequal distribution between electrons and neutral species in gas (Eliasson and Kogelschatz, 1991; Scholtz *et al.,* 2015) [13, 50]. Non thermal plasma or cold plasma is partially ionized gas, composed of more number of neutral gaseous species and the temperature of which can be closer to room temperature. It is generated under low pressure and power conditions and clearly distinct from thermal plasma. It is believed that the detoxification mechanism of aflatoxin is dependent on the gas used to generate the plasma, thus defining the reactive species produced that go on to interact with the mycotoxin structure (Helina Marshall *et al.,* 2020; Shi *et al.,* 2017) [20, 52]. Park *et al.,* 2007demonstrated the complete degradation of aflatoxin B1 on glass substrate using of microwave argon plasma at atmospheric pressure for 5 seconds. Similarly 90% degradation of aflatoxin B1 on glass substrate was reported by Sakudo *et al.,* 2017 [48] by exposing 15 minutes to nitrogen gas plasma generated using a static induction thyristor as a power supply. Radio frequency plasma at 300 W demonstrated an 88% reduction in aflatoxin B1 after 10 min (Wang *et al.,* 2015; Helina Marshall *et al.,* 2020) [57, 20]. Shi *et al.,* 2017 [52] reported that aflatoxin B1 was degraded into six main products and cold plasma degraded aflatoxin B1 at its furan ring, involving hydrogenation, hydration and oxidation.

Cold plasma has been claimed as a feasible techno-economical approach for aflatoxin detoxification in food and feed. However, this novel physical method is still in the early stages of evaluation (Helina Marshall *et al.,* 2020) [20]. Further studies needs to be conducted to optimize cold plasma process conditions for different food grains and feed materials. Moreover, the potential harmful effects of coldplasma treatment on the nutritional composition and sensory attributes of food products need to be evaluated and regulated at the forefront. Finally, new plasma generating equipment, customized for the food processing industry, that are easy to handle, operative, cost effective compared with conventional approaches, and guarantee safety by adequate insulation, grounding and shielding are needed (Helina Marshall *et al.,* 2020; Hertwig *et al.,* 2018) [20, 21].

3. Conclusions

The food safety issue of aflatoxin hazard will remain a global human and animal health concern and facing diverse problems with the contamination of food and feed with mycotoxins. The changes in climate, world trading of foods and changes in food safety regulatory policies have further aggravated the problem. Researcher's continuously evaluating novel promising detoxification approaches, which integrate jointly a high decontamination efficiency, food safety for human and animal health, eco-friendly solution to find appropriate low cost solution for aflatoxin contamination.

4. Conflict of interests

The authors have declared that no conflict of interest exists

5. References

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