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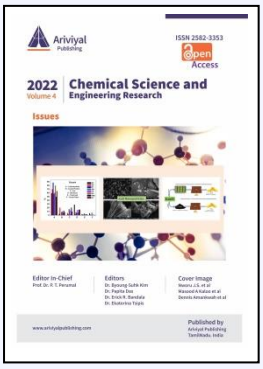

Detoxification of Aflatoxins in Food Commodities by Various Methods

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Abstract: Aflatoxins are secondary metabolites which are produced by fungi belonging to species *Aspergillus flavus* and *Aspergillus parasiticus*. These are found in difference food commodities such rice, corn, maize, cereals, chilli pepper, sesame seeds. As these are harmful for human health, so to detoxify the aflatoxin from food stuff, different methods and treatments are applied. These detoxification methods included physical (cleaning, heating, roasting, irradiation and adsorption) chemical (chlorination, sodium bisulfite, formaldehyde, ammonia and different sorbents) and biological (*Bacillus licheniformis*). All these methods are helpful for the detoxification of aflatoxin.

Keywords: Aflatoxin; Detoxification; Physical; Chemical; Biological methods

1. Introduction

Aflatoxins are cancer-causing metabolites that are essentially created by two contagious species which are: *Aspergillus flavus* and *Aspergillus parasiticus*. These are principally delivered by these species in horticultural food stuff like maize, corn, rice, cereals and creature takes care of. The other aflatoxins delivering species *incorporate A. bombycids, A. ochraceoroseus, A. pseudotamari, A. tamari, Emericella astellata* and *Emericella venezuelensis*, which are scant in nature and sometimes found in farming contrasted with *A. flavus* and *A. parasitica*. ID of aflatoxin was connected to a groundnut feast polluted with *A. flavus* prompting baffling sickness "Turkey X infection" that slaughtered in excess of 1,00,000 Turkey poultry birds in Britain in 1960's. Out of 18, 6 sorts of aflatoxin are vital and are designated as B1, B2, G1, G2, M1 and M2 (Bennett et al., 2003).[1] These aflatoxins bunch showed sub-atomic differences.

For model, the B-bunch aflatoxins (B1 and B2) have acyclopentane ring while the G-bunch (G1 and G2) contains the lactone ring. Though the B-bunch aflatoxins display blue fluorescence, the G-bunch shows yellow-green fluorescence under bright (UV) light, in this manner utilizing fluorescence significant in distinguishing and separating between the B and G gatherings (Dors et ., 2011).[2]

Aflatoxin B_1 is the most well-known and the most far and wide on the planet and records for 75% of all aflatoxins defilement of food and feeds. Aflatoxins M1 and M2 are hydroxylase results of aflatoxins B1 and B2, individually, and are related with cow endless supply of B1 and B2 aflatoxins' defiled feed. Also once shaped from B1 and B2 structures, aflatoxins M1 and M2 stay stable during milk preparing (Gourama et al., 1995).[3]

Various conditions for example, high temperature and high humidity are favorable for the development of the aflatoxins. The environmental conditions favorable for aflatoxin growth are high dampness during harvest, lacking drying and capacity of yields. Postharvest conditions like transportation, stockpiling (abundance warmth and dampness, bother related harm, extensive stretches of capacity) and food handling impact the formation of aflatoxins. Aflatoxins are harmful to human and creature wellbeing. They cause liver and kidney harm, cause immunosuppressive, cancer-causing and mutagenic impacts. People are presented to aflatoxins straightforwardly from utilization of sullied food or by implication, from food stuffs starting from creatures recently presented to aflatoxins in takes care of (Gong et al., 2002).[4]

Since aflatoxins are intensely harmful, cancer-causing, mutagenic, teratogenic and immunosuppressive to most mammalian species, their essence in food products significantly impacts the food and feed ventures (Shelver et al., 2019).[5] The aflatoxins are amazingly tough under most states of capacity, dealing with and

handling of food sources or feeds. Thus forestalling the pollution of food by *A. flavus* and *A. parasiticus* is the most level-headed and monetary way to deal with stay away from potential wellbeing perils.

2. Physical and chemical properties of aflatoxin

Aflatoxins are colorless to light yellow, reflecting fluorescence under UV light. They dissolve slowly in water (10-20 µg/ml) and dissolve freely in moderate solvents such as chloroform, menthol and dimethyl sulfoxide (Delmulle *et al*., 2005).[6] They are not stable in UV light where there is oxygen, not very stable p H (<3 or> 10). The lactone ring opens under alkaline conditions and aflatoxins are destroyed, but this reaction is restored to acidification. The manifestation of the results in the opening of the lactone ring at high temperatures, triggers decarboxylation of aflatoxins and this reaction cannot be reversed.

3. Occurrence and exposure of aflatoxin

Aflatoxins were first identified in 1961 in the United Kingdom in 100,000 turkey died. Aflatoxins appear in plants before harvest and are considered wild mycotoxins compared to other mycotoxins, detected after crop harvesting. Human exposure and organic stock in aflatoxins are foods such as nuts, treenuts, corn, rice, figs and other dried foods, spices and raw vegetable oils and cocoa beans, which are contaminated with fungi before and after harvest. The use of aflatoxin-contaminated feed by animals results in contamination of milk, eggs and meat (Masoomi *et al.,* 2013).[7]

The most dangerous substances for aflatoxin are maize, nuts and cotton seeds. Aflatoxin M1 in dairy products and dairy products, including skim dry milk, cheese, ice cream and yogurts, are the result of Aflatoxin contaminated maize and cotton-based foods in the dairy diet. Even in butter, at the time of its production due to its lipid-rich chemicals, the collection and concentration of any Aflatoxin M1 present in milk is usually collected in butter during production due to its high lipid density (Sapsford *et.al* 2006).[8]

4. Toxicity and the mode of action of aflatoxin

Aflatoxin B_1 is considered to be the cause of both toxicity and carcinogenicity. It was classified as a carcinogen by the International Agency for Research on Cancer (IARC). Diseases caused by the use of aflatoxins are known as aflatoxicosis (Papp *et al.,* 2002).[9] Chronic aflatoxicosis leads to cancer, depression and other, slow-moving illnesses, while acute aclatoxicosis leads to death. The liver is the most targeted organ and the damage to the liver occurs when chickens, fish, mice and non-human animals are fed food contaminated with aflatoxin B_1 (Li *el al.,* 2011).[10] As the liver is a lipophilic organ, it retains and absorbs all the chemicals carried by the bloodstream, namely drugs, impurities, mycotoxins etc., in the hepatocytes and over a long period of exposure, can turn them into a cancer cell.

Aflatoxins are converted to an active form of epoxide (also known as aflatoxin-2,3 epoxide) which is able to bind both DNA and proteins to the cytochrome P450 enzymes. The active aflatoxin epoxide binds to the N7 position of guanines. In addition, aflatoxin B1-DNA adducts can lead to the conversion of GC to TA. The active system of glutathione S-transferase found in cytosol and microsomes promotes the synthesis of active aflatoxins with reduced glutathione, leading to the release of aflatoxins. The LD50 values for aflatoxin B1 and M1 are ≤18 and 12-16 mg/kg bodyweight respectively (Santini and Ritieni, 2013).[11]

5. Detoxification methods for aflatoxin

Various methods used to eliminate pollution / detoxification aflatoxins include physiological, chemical or biological methods. Physical, chemical, and natural processes must ensure that the dehydration process maintains a healthy diet, does not lead to the introduction of new toxic or carcinogenic-mutagenic substances, and the process must also kill Aspergillus and mycelia seeds, preventing the growth and production of new toxins under ideal conditions. Aflatoxins have low solubility in water. Washing wheat seeds in water has removed 40% of AFB1 (Li *et al.,* 2005).[12]

5.1. Physical method of detoxification

Physical processes involve the separation of the contaminated fractions, removal or inactivation of aflatoxins by physical means, such as cleaning, heat, cooking, roasting, and irradiation.

5.1.1. Cleaning

Cleaning is a multistep process that removes dust, husks and mold-based products through mechanical engineering and washing. Separation of seeds / colored seeds also reduces aflatoxin contamination. Aflatoxins have low solubility in water. So washing may not remove the aflatoxins from the feed. However, a 40% reduction in aflatoxin in wheat contaminated by washing was reported (Shi *et al.,* 2014).[13]

5.1.2. Heating

Heat is another way of destroying aflatoxins. Aflatoxins have a high degree of decay temperature from 237°C - 306°C. Various heat treatments such as heat, roasting, baking and steaming provide an effective way to reduce the concentration of aflatoxins in food. All processing methods like boiling, roasting and baking in maize products (boiled corn, porridge, roti, biscuits and muffins) destroyed aflatoxins to some extent i.e. 50-70%. The sensitivity of aflatoxins to heat is governed by natural conditions (Ndagijimana et al., 2020).[14] On the other hand, the presence of moisture in food can improve spoilage by hydrolyzing the lactone ring in a sensitive area of moisture and heat. On the other hand, aflatoxins can be protected from food, in part, by “binding” them or mixing them with proteins and other nutrients. Experimental evidence for AFB degradation in food shows that temperatures above 100°C are required to obtain at least excessive dehydration.

Typical conditions for food preparation and preparation appear to cause, on average, a 60% reduction in laboratory conditions. Edible oil (coconut, nuts, and olives), temperatures above 200°C are required for the reduction of AFB. In the presence of moisture, AFB is

believed to be activated by the hydrolytic ring of lactone to form carboxylic acid, which is heated by decarboxylation (Samarajeewa et al., 1990).[15]

5.1.3. Roasting

Microwave treatment at high energy levels shows a significant decrease in aflatoxin (Farag *et al.*, 1996).[16] A 95% reduction in peanut pain was achieved by roasting a contaminated microwave sample at 6 kilowatt (kw) for 4 minutes. Similar results were obtained when contaminated peanuts were treated at 1.6kw in 16 minutes or 3.2kw in 5 minutes. A temperature of 150°C or higher was required to achieve a 95% reduction in total aflatoxin. Peanut butter at a low energy level of 0.7kw for 8.5 minutes using the microwave home oven was ineffective; 61% of aflatoxin B_1 and 40% of aflatoxin G1 in naturally polluted nuts were destroyed.

5.1.4. Irradiation

Ionizing radiation is currently recommended and used to eliminate pathogenic microorganisms in foods. Gamma radiation penetrates effectively through both liquid and solid media. Ionizing radiation such a gamma radiation had little effect when used directly in detoxifying the aflatoxins (Morehouse, 2002).[17] It indirectly decontaminates the aflatoxins by radiolysis of water, which generates free radicals. Microorganisms inactivation depends on the dosage of gamma radiation, at low dosage (0.1 MRad) was reported to stimulate aflatoxin production in bread and other foods, whereas gamma radiation at 0.3-0.4 MRad dosage, suppressed mold growth as well as aflatoxin production (Pankaj *et al.,* 2018).[18] The available experimental evidence describing the radiation sensitivity of AFB, in a pure form and in contaminated foods does not indicate the potential usefulness for the technique within the permitted dosage level of 1 KGy (0.1 Mrad). Although 1 Mrad doses contained deionizing radiation are currently recommended and used to eliminate pathogenic microorganisms in food. Gamma rays penetrate both liquids and solids. Ionizing Radiation such gamma rays has little effect when used directly to eliminate aflatoxin toxins. Indirectly it eliminates aflatoxins by releasing radioactive fluids, which produce free radicals (Rowe-Taitt *et al.,* 2012).[19] Inorganic microorganisms are dependent on gamma rays, low doses (0.1 MRad) and are reported to promote the production of aflatoxin in bread and other foods, such as gamma rays at 0.3-0.4 MRad dosage, suppress mold growth and produce aflatoxin. The available experimental evidence describing the sensitivity of AFB radiation, pure and contaminated food does not indicate a potential benefit of this process within the permissible dose range of 1 KGy (0.1 MRad). Although 1 dose of MRad has shown some promise in reducing aflatoxin it is a potent solution showing some promise in reducing aflatoxin.

5.1.5. Adsorption

Adsorption is another way to reduce aflatoxin. It involves the binding of a toxin compound to an adsorbent compound during digestion in the intestinal tract. The active ingredient is active carbon, diatomaceous earth, alumino (clay, bentonite, montmorillonite, sodium and calcium aluminum silicates especially zeolite, phyllosilicates and hydrated sodium calcium aluminosilicate (HSCAS), heavy carbohydrates (cellulose and polysaccharides) present in cell wall yeast and bacteria (galactomannans, peptidoglycans), and synthetic polymers (cholestyramine, polyvinyl pyrrolidone, and its availability). Khadem *et al.,* 2012[20] studied the effectiveness of yeast, active charcoal and zeolite can be alone or combined as aflatoxin compounds in broiler food. They found that the acidic mixture was effective in reducing aflatoxin toxicity in growing mice.

5.2. Chemical methods of detoxification

There are different chemicals techniques, methods are used to detoxify the foods such as cereals, corn, maize etc. from aflatoxins. Different chemicals are also used to detoxify the food commodities from aflatoxins. Some chemical treatments are applied such as treatment with ammonia, treatment with sodium bisulfite, treatment with calcium hydroxide, treatment with formaldehyde and addition of sorbents.

5.2.1. Chlorination

Liquid chlorine is used in the food industry to clean processing equipment and to wash various dishes including fruit, nuts, fish and meat before processing. Gas chlorine acts as a bleaching agent and oxidizing agent in the flour industry with no signs of danger. Sodium hypochlorite was first recommended for removing aflatoxins from contaminated areas with glass. It has also been found to be effective in reducing aflatoxins in the diet. Chlorine and sodium hypochlorite at concentrations of 0.2%, 1%, 5% or 11%, and 3% perchloric acid, or containing chlorine gas at 10% or concentrations of 15 mg chlorine gas per 100 mg of pure AFB almost completely reduces AFB, either in its pure form or in spicy foods; The only result of this effect was nutritious peanut butter, of which only 50% of inactivity (Samarjeewa *et al*., 1990).[15]

5.2.2. Sodium bisulfite

Food industry needs to make aflatoxins inactive in various food commodities. Bisulfite at low concentrations (0.5 and 1%) works well than ammonia or sodium hydroxide that does not work in AFB in corn (Jalili *et al.,* 2011).[21] The inactivity response was shown following the first order of kinetics with respect to AFB, at pH 5.5; the influence of heat and energy exposure to aflatoxin detoxification has also been investigated. Aflatoxin inactivation with sodium bisulfite was first suggested to involve the process of free radicals; methanol and citric acid have been shown to slow the degradation of aflatoxin by bisulfite. Sodium bisulfite can react in two active areas of AFB, resulting in cracking of the lactone ring or addition to the last ring of furan, or both. The formation of sodium sulfonate has been proven to be the most significant response to dysfunction. Although sodium bisulphite-treated maize did not show significant changes in color and behavior management, concerns about low bisulfite activation for AFB2 and AFG2, as well as decreased awareness of possible reactivation of AFB1 or active toxin organization (particularly epoxide) during sample metabolism managed to reduce progress in the industrial use of this type of treatment.

5.2.3. Formaldehyde

Formaldehyde is a moderately effective compound in attacking and acquiring AFB, a molecule, even if no data for its responses are available. Studies have shown that it has improved performance in combination with ammonia and calcium hydroxide. In contaminated milk samples the application of 0.5% formaldehyde reduced 1.1 µg AFM, to 0.05 pg. (Samarjeewa *et al*., 1990).[15]

5.2.4. Ammonia

Treatment with ammonia in the gas phase, in solution, or by means of extracts, has had excellent results in eliminating the toxins of nuts, cotton, and corn (Ellis *et al.,* 1991).[22] It is legal in some North American states (Arizona, California, Georgia, and Alabama) and the French technology used to make medicinal plants in Senegal and more recently in France. The mechanism of action of ammonia in the aflatoxins B, (AFB,) molecule has been extensively studied and appears to be specific. It was found that for AFB, the molecular structure of cells is altered irreversibly if the presence of ammonia remains long enough.

Conversely, if exposure does not release enough time, the molecule may return to its original state. In fact, another study aimed at confirming the transport of milk-fed dairy-fed cows revealed a percentage of AFB, the elimination of milk metabolite (i.e. aflatoxin M, or AFM,) between 10-20 % compared to AFB, included. These percentages usually vary from 1-3%. This is explained by examining the response to the release of toxins from the body. The first (variable) step consists of the release of aflatoxin cyclic lactone in an acidic environment (such as in the stomach) the balance can be converted to the original products by subsequent conversion of AFB. In this case the animal would have absorbed more toxins compared to the figure presented in the analysis, explains the higher percentage of AFM, in milk (Samarjeewa *et al*., 1990).[15]

5.2.5. Addition of sorbents

One of the most important measures aimed at reducing the risk of aflatoxins or limiting the decline in animal activity and metabolite of milk, meat and eggs, the use of clay in contaminated feeds to reduce the absorption of aflatoxin in the intestine. Other in vitro experiments have shown that different absorbers divided into as aluminas, silica's and alum inosilicates are able to bind aflatoxin solution (Abdel-Wahhab *et al.,* 1999).[23] The same authors found that hydrated sodium calcium alum inosilicates (HSCAS) were more effective at binding aflatoxin. Descriptive details in the AFB molecule, remedial measures cannot be given at this time, even if chemo-physical adsorption events may occur. AFB, the molecule can easily remain attached inside a standard structure with HSCAS cables. However, the stability of these advertising especially pH conditions, such as those in the stomach, should be ensured.

However, many experimental studies have shown the legitimacy of this binding act. Experiments performed on chickens, turkeys and pigs that feed AFB, contaminated feeds and additional HSCAS revealed the absence or reduction of common intoxication, e.g. weight loss of vital organs, weight loss or weight gain and dietary modification, bones-brittle and metabolite infiltration (Piva *et al.,* 1995).[24] Some authors found effective results by adding HSCAS to the feed of contaminated growing lambs. The same authors performed toxicity testing by including HSCAS for AFB-fed dairy cows, low levels of contamination (200µg AFB/Kg) to find significant reductions in continuing to ensure the effectiveness of treatment even at low levels of frequent contaminants in operation (Samarjeewa *et al*., 1990).[15]

5.3. Biological methods for detoxification of aflatoxins

Aflatoxin B_1 is the most harmful among the mycotoxins commonly present in food and feed, and it may lead to hepatocellular carcinoma in humans and animals (Chhonker et al., 2018).[25] Therefore, limiting its exposure to humans and livestock is very much essential. Some biological methods are used for detoxify aflatoxins from food and feed.

Micro-organisms such as soil or water bacteria, fungi, and protozoa as well as certain enzymes isolated from microbial systems can degrade members of the aflatoxin group by the various functions of sub- or non-toxic products. Some aflatoxin-producing fungi from the Aspergillus species have the ability to lower my synthetic mycotoxins. Yeast and lactic acid bacteria act as biological adsorbents that prevent the transmission of aflatoxin in the intestines of humans and animals (Luo *et al.,* 2020).[26] Aflatoxin B_1 injected into the body can be repaired in very different ways. It leads to the production of non-toxic compounds on the one hand, or on active toxic forms on the other.

5.3.1. By Bacillus licheniformis CFR1

The current study aims to differentiate and differentiate Aflatoxin B_1 from viral repellent from various sources, to create a safe and environmentally friendly strategy for the administration of Aflatoxin B_1 (He *et al.,* 2017).[27] Fifty-six strains were isolated using coumarin-modified media as a single carbon source. 7 genes have shown a more than 70% reduction in AFB1 in cultural media.

Among them, split CFR1 reduced Aflatoxin B_1 by 94.7%, and was selected for further studies. CFR1 has been identified as *Bacillus licheniformis* CFR1, by chemical synthesis and genetic sequence of 16S rRNA. A supernatant with no B cells *licheniformis* CFR1 is able to degrade AFB1 better than cell lysate. AFB1 damage was assessed using High-Performance Thin Layer Chromatography (HPTLC), High-Performance Liquid Chromatography (HPLC) and Electron Spray Ionization-Mass Spectrometry (ESI-MS) (Rao *et al.*, 2017).[28] The total temperature, duration, and pH value of the high-density Aflatoxin B_1 was found to be 37°C, 24 h and 7, respectively. In addition, Ames tests for mutagenicity showed that when treated with *B. licheniformis* CFR1 is a cell component associated with the loss of Aflatoxin B_1 mutagenicity. To the best of our knowledge, this is the first study showing a 90% reduction in AFB1 by *B. licheniformis*. Therefore, *B. licheniformis* CFR1 may be an excellent component of bioremediation and detoxification of Aflatoxin B_1 from the field and dietary matrix (Taheur *et al*., 2017).[29]

6. Conclusions

In conclusion, methods for the detoxification of aflatoxin in difference food commodities such as rice, corn, maize, cereals, chilli

pepper, and sesame seeds are discussed. Three major detoxification methods, physical (cleaning, heating, roasting, irradiation and adsorption) chemical (chlorination, sodium bisulfite, formaldehyde, ammonia and different sorbents) and biological (*Bacillus licheniformis*), are focused.

Conflicts of Interest

The authors declare no conflict of interest.

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