A Review on Aflatoxins Reduction in Food

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ABSTRACT

Aflatoxins (AFs) are cancerous secondary metabolites produced primarily by Aspergillus flavus and Aspergillus parasiticus in agricultural foodstuff such as peanuts, maize grains, cereals, and animal feeds. Food and Agricultural organization (FAO) estimated that as much as 25% of the world's agricultural commodities are contaminated with mycotoxins, leading to significant economic losses. Moreover, AFs are highly toxic, mutagenic, teratogenic and carcinogenic. Therefore AFs reduction in food and feedstuffs is a major global concern. This review aims to bring up to date the detoxification methods applied for reduction of aflatoxins by physical (cleaning, heating, irradiation, adsorption), chemical (chemical compound, ozonization) and biological (applying bacteria, yeast and nontoxigenic Aspergillus strains) methods in different foods from 2000 to 2015. Papers related to aflatoxin reduction by managing aflatoxins risks, using resistant crops varieties, and good agricultural practices and papers related to other aflatoxins (M1, M2) were excluded.

Key words: Aflatoxin, Reduction, Physical method, Chemical method, Biological method

INTRODUCTION

Food and Agricultural organization (FAO) estimated that as much as 25% of the world's agricultural commodities are contaminated with mycotoxins, leading to significant economic losses [1]. Moreover the mycotoxins can cause a variety of toxic effects such as chronic in human and animal, therefore, they are one of the most relevant and worrisome problem about food safety [2]. Among the 400 known mycotoxins, Aflatoxins B1 (AFB1), B2 (AFB2), G1 (AFG1) and G2 (AFG2) are the most significant mycotoxins in foods and feeds. They are dangerous to human health because of their highly toxic, carcinogenic, teratogenic, hepatotoxic and mutagenic characteristics. There is a high risk of Hepatitis B and Hepatitis C carriers developing liver cancer when they are exposed to aflatoxin [3]. Due to the toxic effects of AFB1, it has been classified as group 1, as a human carcinogen by the International Agency for Research on Cancer [4].

Aflatoxins (AFs) are difuranocoumarins composed from two furans and a coumarin ring. The structure of four major compounds of aflatoxin B1, B2, G1 and G2) is shown (Fig. 1).

AFs are produced primarily by Aspergillus flavus and Aspergillus parasiticus in agricultural foodstuff such as peanuts, maize, grains, cereals, and animal feeds [5]. AFs production normally occurs in the field, particularly when stimulated by drought, stress, and high temperature or during prolonged drying [6].

Due to the harmful effects of aflatoxins most research effort has concentrated on the means for prevention of AFs formation. Preventive policies including good agricultural practices in the field and good manufacturing practices in storage are known as the best way of reducing Aflatoxin content in food stuff. However, regard to the fact that AFs prevention is not always possible, recently, decontamination methods have gained attention as alternative way of reducing Aflatoxin uptake through food chain [7]. In general, process to degrade the toxin to safe levels should meet the following requirements: 1) inactivate, destroy, or remove the toxin, 2) not produce or leave toxic residues i

n the food/feed, 3) retain the nutritive value of the food/feed, 4) not alter the acceptability or the technological properties of the product, and, if possible, 5) destroy fungal spores [8]. So far, detoxification of AFs is achieved by removal or elimination of contaminated commodities or by inactivation of the toxins present in these commodities by physical, chemical, or biological methods [9]. The current paper reviews recent development from 2000 to 2015 on this topic.

A total of 102 papers from 2000 to 2015 were studied. The collected papers had focused on reduction of aflatoxins (B1, B2, G1, G2) by physical (cleaning, heating, irradiation, adsorption), chemical (chemical compound, ozonization) and biological (applying bacteria, yeast and nontoxigenic

Aspergillus strains) methods in different food. Papers related to aflatoxin reduction by managing aflatoxins risks, using resistant crops varieties, and good agricultural practices and papers related to other aflatoxins (M1, M2) were excluded.

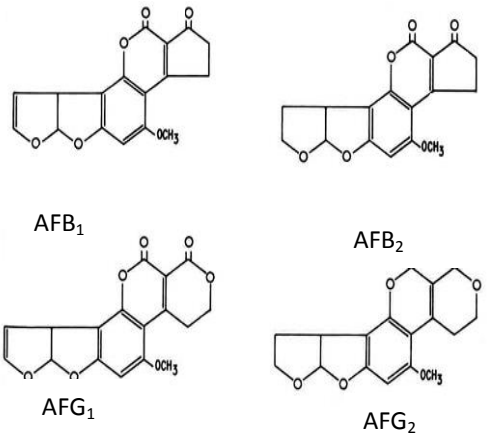

Fig.1: Structure of aflatoxins B_1, B_2, G_1 and G_2 [4]

PHYSICAL METHODS

Main Physical approaches applied to decrease aflatoxin can be classified as cleaning, heating, irradiation and adsorption from solution.

Cleaning

Cleaning is a multi step process such as removing dust, husks and products colonized by molds, mechanical sorting and washing. Hulling of some products such as coffee can reduce mycotoxins. Coffee, cocoa, some cereals and some spices are subjected to a dehulling step, which has to be done as efficiently as possible since it has been demonstrated that the husks are very susceptible to mycotoxin contamination [10-11].

Approximately 80% of aflatoxin contaminations can be attributed to small, shrivelled seeds mouldy and stained seeds [12, 13], and damaged seeds. Contaminated foods do not have the same color or density of safe foods. Hence, sorting of kernels to remove discoloured pods (according to appearance or density) is often recommended to minimise aflatoxin levels [1]. When mycotoxin contamination is heterogeneous, sorting the noncontaminated portion may reduce the level of mycotoxin in the final product [14].

Due to the low solubility of AFs in water, it is generally hard to remove AFs by washing. However, in a study conducted by Hwang [15], about 40% of AFB1 was removed from contaminated wheat, by washing. Fandohan reported that since AFs are usually attached on surface of wheat, it's possible to remove them by washing. But, it is very difficult to remove aflatoxin bonded or attached strongly to the inner texture of food [12]. Some examples of aflatoxins reduction by cleaning are stated in Table 1.

Heating

AFs have high decomposition temperatures ranging from 237 °C to 306 °C. Solid AFBl is quite stable to dry heating at temperatures below its thermal decomposition temperature of 267 °C. However it has been reported all heat treatment (boiling, roasting, baking and steaming) still provides a feasible mechanism for reducing the AFs concentration in foodstuffs (Table 1). The effects of household processing on AFs content of maize products (boiled maize, porridge, roti, biscuits, muffins and idli) was studied. All processing methods (boiling, roasting, baking and steaming) destroyed AFs to a considerable extent. The percentage destruction ranged from 50-70% [16]. The efficacy and extent of reduction method is depends on several factors, including AFs concentration, the extent of binding between AFs and food constituents, heat penetration, moisture content, pH, ionic strength, processing conditions [15] and source of contamination (naturally or artificially) [17].

The relationship between moisture content of foods and reduction of AFs has been demonstrated several times [18-19]. According to these reports, by increased moisture content the destruction of AFs is increased during cooking or baking. Kabak and co-workers also reported that the moisture content is a critical factor in AFs reduction and in presence of water decontamination of food by heating is easier and more effective. They suggested that the presence of water helps in opening the lactone ring in AFBl (by the addition of a water molecule to the ring) to form a terminal carboxylic acid. The terminal acid group thereafter undergoes heat-induced decarboxylation [1].

However, in contrast with this idea, Mendez Albores [18] reported that higher reductions in AFs levels were achieved during the toasting process and only a moderate extra-reduction occurred during the boiling. Moreover Hussain and coworkers [17] reported that roasting resulted in a significant decrease in the AFs content of nuts, corn and oilseed meals. Degradation of aflatoxins by roasting was both time and temperature dependent. Roasting at 150 ?C for 120 min degraded more than 95% of AFB1 in peanuts. The author also reported that Aflatoxins in form of naturally occurrence were more resistant to degradation with heat compared to artificially contaminated samples [17]. In a related study a mean reduction of 66.5% was obtained by roasting, but the reduction seems to be heterogeneous [20].

In several model assays it has been shown that the degradation of mycotoxins is improved by the existence of certain matrix compounds [21]. It seems that different samples showed different behavior under heat treatment and more research must be done to evaluate the effect of heat treatment on AFs.

Irradiation

In general radiation can be classified into two categories: ionizing and non-ionizing. Ionizing radiation (e.g. X-rays, gamma rays and ultraviolet rays) may produce potential changes in molecules of the irradiated object with little or without temperature increasing and producing hazardous molecular changes. But non-ionizing radiation (e.g. radio waves, microwaves, infrared waves, visible light) in sufficient intensity leads to a rise in temperature and usually molecular changes that are not hazardous to man. Gamma radiation, considered a cold temperature process, has been applied by many researchers to extend the storage life of certain foods by reducing microbial populations. The use of gamma radiation to inactivate AFs has been investigated by many researchers and conflicting results have been reported (Table 1). Some researchers believe that the gamma ray is not effective on reduction of AFs [22] and others reported different level of decontamination in different food by gamma irradiation [23, 24]. Effectiveness of gamma radiation in mycotoxin destruction, significantly is dependent on radiation dose. Ghanem and co-workers [25] showed that degradation of AFB1 in food crops (peanut, peeled pistachio, unpeeled pistachio, rice, and corn) and feed (barley, bran, corn) was positively correlated with increasing in the applied dose of gamma ray. Jalili showed that there was no reduction in the AFs content at doses less than 10 kGy in black and white pepper [26]. However, Ahsan [23] reported that after treatment with gamma ray at 6 kGy, more than 95% reduction in AFB1 was observed in the rice samples contaminated with high concentrations of AFB1.

The presence of water has an important role in the destruction of AFs by gamma radiation since radiolysis of water leads to the formation of highly reactive free radicals. These free radicals can readily attack AFs at the terminal furan ring and yield products of lower biological activity.

Of the different types of aflatoxins, AFB1 and AFG1 seem to be more sensitive to gamma radiation as compared to AFB2 and AFG2 [26]. This finding may be related to the 8,9 double bound present in AFB1 and G1, which undergoes a reaction induced by the gamma ray.

Some researches indicated that irradiation is a promising method for mold inhibition and therefore reduces the aflatoxins occurrence indirectly. For example, Prado reported that decontamination of molds by irradiation, before production of AFB1, is the most acceptable method in the preservation of peanut [22]. In a related study, Aziz showed that irradiation of fruit at dose of 1.5 and 3.5 kGy decreased significantly the total of fungal count compared with non-irradiated samples [27]. It is therefore concluded that the decontamination of mycotoxins by irradiation is necessary prior to their production from moulds [28].

adsorption

Adsorption, a very common treatment of mycotoxin reduction, involves binding the toxin to absorbent compound during the digestive process in the gastrointestinal tract. The absorption of AFs requires polarity and suitable position of functional groups. Some more common aflatoxin absorbents include active carbon, diatomaceous earth, alumino (clay, bentonite, montmorillonite, sodium and calcium aluminum silicates mainly zeolite, phyllosilicates and hydrated sodium calcium aluminosilicate (HSCAS)), complex carbohydrates (cellulose and olysaccharides) present at cellular wall of yeasts and bacteria (such as glucomannans, peptidoglycans), and synthetic polymers (such as cholestyramine, polyvinyl pyrrolidone, and its derivatives).

Hasheminya and Dehghannya believe that use of aflatoxin absorbents in infected feed is a promising way of reducing AFs in livestock feed. Through binding to absorbents, AFs present in feed inhibits from toxic reactions in livestock body as well as from absorption into digestive tract [29].

In agreement with this idea, Bentonite has been shown to remove up to nearly 100% of AFs from liquid solution by binding AFs in ingested feed and eliminate the toxicity [30]. Bentonite deposits are found throughout the world and mostly consist of expandable smectite minerals. Surfaces of smectite minerals can be treated with organic compounds to create surface-modified clay that more readily bind some contaminants than the untreated clay [31].

Recently, modified zeolites have been shown to be the most powerful adsorbent materials as they have shown good results in foodstuff decontamination [32-33]. In a research conducted by Jebali and coworkers Zeolite was used for reducing Afs in fruit juices. Results showed that the Aflatoxin was reduced after passing through the zeolite column related to zeolite's quantity and passing time. The authors showed that zeolite could act as an Aflatoxin absorbent and can be used in fruit juices factories [34]. The effectiveness of yeast, zeolite and active charcoal as aflatoxin absorbents in broiler diets was evaluated by Khadem [35]. Results of the study indicated that the mixtures

of the tested absorbents were more effective for reducing the signs of AFB1toxicities in growing broiler.

Nanocomposite $MgO\text{-}SiO_2$ was used for aflatoxin adsorption in wheat flour samples. Results showed that nanocomposite $MgO\text{-}SiO_2$ was an effective adsorbing agent for aflatoxin ranged from 80 to 100%, related to aflatoxin concentration [36].

Table1. Examples of aflatoxins reduction by physical (cleaning, irradiation and heating) methods (2000- 2015).

Method	Condition / treatment	Sample	Toxin	Reduction (%)	Ref.
	Washing	Korean wheat	AFs	41.6-60	[15]
	Washing	Black pepper	B1 B2 G1 G2	15.3±2.9 14.3±2.1 17.8±4.8 14.5±2.5	[37]
	Washing	Black pepper	B1 B2 G1 G2	14.7±2.9 13.5±2.1 19.8±2.9 18.0±2.5	[37]
	Sorting	Corn	AFs	81	[38]
	Sorting	Peanut	AFs	27.8- 33.8	[39]
Irradiation	10 kGy	Peanut	B1	55-74	[22]
	10 kGy	Maize Rice Barley Bran Corn Peeled pistachio un Peeled pistachio peanut	B1	81.1 87.8 86 84 81.1 68.8 84.6 58.6	[25]
	20 kGy	Yellow corn and peanut	B1	100	[44]
	2 kGy	Maize	B1 B2	68.9 97.6	[45]
	5 kGy	Maize	B1 B2	46 94	[45]
	4 kGy	Maize Wheat Rice	B1	15.54 22.25 27.46	[46]
	6 kGy	Maize Wheat Rice	B1	32.39 43.84 56.38	[46]
	8 kGy	Maize Wheat Rice	B1	60.26 64.24 64.68	[46]
	15 kGy	Almond	B1 B2 G1 G2	19.25 10.99 21.11 16.62	[28]
	Microwave	Peanut	B1+ B2	50-60	[47]
Heating	Microwave	Poultry feed	B1	32.3	[48]

Roasting	Coffee bean	AFs	42.2-55.9	[49]
Roasting (90-150 °C)	Peanut meal	B1 B2 G1 G2	78.4 57.3 73.9 25.2	[50]
Roasting (150°C)	Peanut seed	B1 G1	70 79.8	[51]
Roasting (140°C)	Peanut seed	B1 G1	58.8 64.5	[51]
Roasting (90-150°C)	pistachio nuts	AFs B1	17-63 95	[52]
Roasting (150°C)	Peanut	B1	95	[17]
Hot air oven drying	Feed	B1	57.6	[53]
Heating (≥ 180°C)		B1	100	[21]
Heating (150-200°C)	Dry wheat	AFs	50-90	[15]
Pressure cooking	Rice	B1	78-88	[8]
Ordinary cooking	Rice	B1	31-36	[8]
Ordinary cooking	Polished rice	B1	34	[54]
Ordinary and pressured cooking	Meat	B1 B2	15 30	[55]
Ordinary cooking	Whole meal	AFs	0	[56]
Heating (180°C)	Ginger Curry powder	B1 B1	62.5 40	[57]

AFs: Total aflatoxins

CHEMICAL METHODS

Chemical Compounds

A large number of chemicals include acids, bases and oxidising agents can react with AFs and convert them to non-toxic or less toxic compounds. some chemical compounds have been brought to test their effectiveness on detoxification of AFs and other mycotoxins including hydrochloric acid [58], citric acid [59], lactic acid [60], ammonium persulphate [61], calcium hydroxide [62], sodium bicarbonate and potassium carbonate [40] formaldehyde, hydrogen peroxide [41], sodium bisulfite [42], ozone gas (O3) [43], sodium hydroxide and sodium hypochlorite [37].

Under alkaline and acidic treatment, the lactone rings of AFs may be opened and the AFs are transformed to a compound named beta-keto acid, a water-soluble compound, can be easily removed from the sample by washing with water. Moreover by hydrolysis of lactone ring, beta-keto acid may converted to AFD1, a nonfluorescent compound, which exhibits phenolic properties and lacks the lactone group (derived from the decarboxylation of the lactone ring-opened form of AFB1); and to a lesser extent, a second compound (a nonfluorescent phenol, commonly known as AFD2), which retains the difurane moiety but lacks both the lactone carbonyl and the cyclopentenone ring, characteristic of the AFB1 molecule (60). The probable degradation mechanism of AFB1 has been shown (Fig. 2).

The possibility of removing AFs by treatment of the sample with dilute alkali or other chemicals has been the subject of much discussion. The effect of 18 different chemicals, included acidic compounds (sulfuric acid, chloridric acid, phosphoric acid, benzoic acid, citric acid, acetic acid), alkaline compounds (ammonia, sodium bicarbonate, sodium hydroxide, potassium hydroxide, calcium hydroxide), salts (acetate ammonium, sodium bisulfite, sodium hydrosulfite, sodium chloride, sodium sulfate) and oxidising agents (hydrogen peroxide, sodium hypochlorite), on the reduction of aflatoxins was investigated in black and white pepper during washing step at 2% concentration. Almost all of the

applied chemicals showed a significant degree of reduction on mycotoxins. The lowest and highest reduction of AFB1 was 20.5%±2.7% using benzoic acid and 54.5%±2.7% using sodium hydroxide. However undesirable changes such as discoloration of white pepper and loss of the outer layer of black pepper were occurred by applying bases and acids [37]. More AFs reduction was reported when food and feed were treated with more concentrated citric acid and other chemicals. Aflatoxins (AFB1 and AFB2) were reduced (96.7%) by means of 1N aqueous citric acid in maize grain [15]. In a related study, 86% reduction has occurred in commercial AFB1 contaminated feed by using 1N aqueous citric acid [59].

Food and feed treatment with bases also reduced the AFs. Currently, ammoniation and treatment with sodium bi-sulfite are the major industrial processes widely used to decrease AFs in peanut meal, maize and cottonseed destined for animal feeding. Applying ammonia (under appropriate conditions) leads good results in reduction in the level of AFs in contaminated food and feed. Treatment of contaminated maize with 1.0% ammonia resulted in destruction of 98% of all four types of aflatoxins [63].

Large-scale feeding studies to further evaluate the safety of ammonia-decontaminated corn were initiated by USDA in 1975 at the recommendation of the Food and Drug Administration (FDA).

With respect to FDA standards, use of ammonia for reducing AFs in livestock feed is permitted in US. About 95% of aflatoxin in feed has been alleviated with gaseous or liquid ammonia. Ammonia may convert AFB1 to non-toxic compound of aflatoxin D1 through hydrolyzation of AFB1 and its decarboxylation [63].

Most of the chemical processes that have been investigated are impractical (carried out under drastic conditions of temperature and pressure), unsafe (form toxic residues) and unfavorable (degrade the nutritional, sensory and functional properties of the product). Moreover, although acidic compounds are able to destroy mycotoxins but the obtained degraded products are not stable therefore by removing the acidic condition, the degraded products may convert to their parent products. Therefore, it seems that applying chemicals with other methods such as high pressure or heat, leads more reduction of AFs and better food quality. Nyandieka [64] reported that ammoniation treatment under high pressure is more destructive to aflatoxins than treatment under atmospheric or low pressure. In a related study, the inactivation of AFB1 during the extrusion process using calcium hydroxide together with hydrogen peroxide showed higher detoxification of AFB1 than treatment with calcium hydroxide or hydrogen peroxide alone [39]. Some of the chemical compounds (alone or in combination with other methods), applied for reducing aflatoxins in foods are summarized in Table 2.

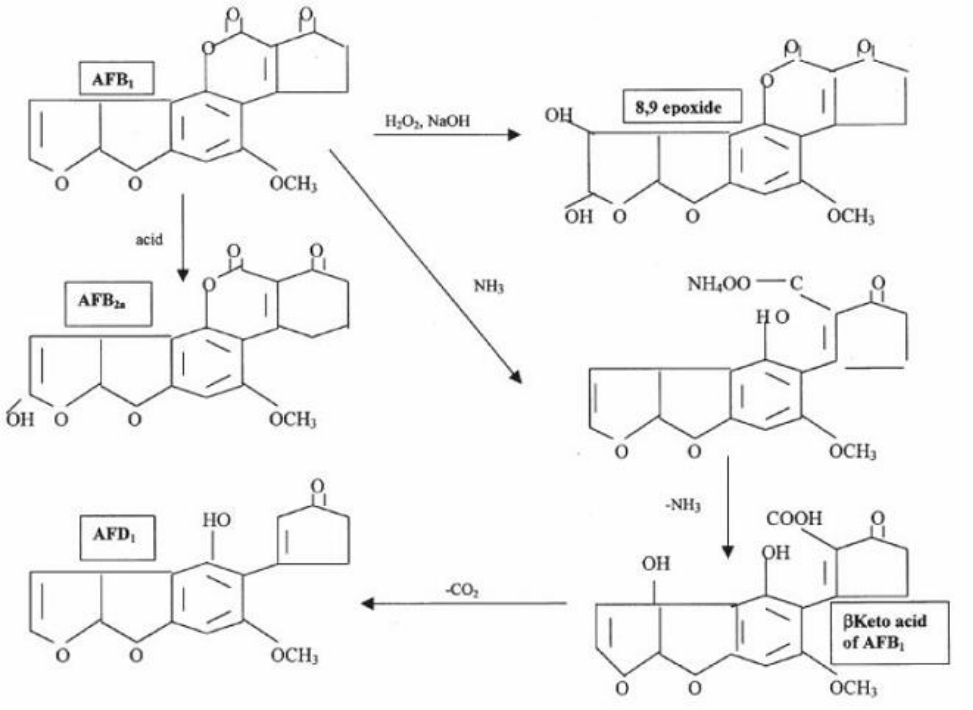

Fig. 2: Probable mechanism of degradation of AFB_1 [65]

Table 2. Examples of aflatoxins reduction by chemical compound (2000- 2015)

Chemical treatment	Sample	Toxin	Reduction (%)	Ref.
Citric acid	Maize	B1+B2	96.7	[60]
Citric acid	Barely	B1	86	[59]
Nixtamalization	Maize	B1	94	[62]
Ammoniation (2%)	Maize	B1	88 ± 1	[64]
		B2	85 ± 1.3	
		G1	96 ± 0.6	
		G2	93 ± 0.8	
Ammoniation (2%) + pressure	Maize	B1	98 ± 0.3	[64]
		B2	98 ± 0.3	
		G1	99 ± 0.2	
		G2	99 ± 0.2	
Extrusion + lime (0.3%)	Corn tortilla	B1	74	[62]
Extrusion + lime (0.5%)	Corn tortilla	B1	85	[62]
Nixtamalization	Corn tortilla	B1	94	[62]
Extrusion + citric acid	Sorghum	B1 + B2	17-92	[59]
Heating (50-98°C)+ alkaline ph (10)	Dried fig	B1	97±1	[66]
		B2	87± 1	
		G1	100	
alkaline solution+ Heating (98°C)	Tortilla	AFs	30	[19]
Sodium hydrosulfite +boiling	Black pepper	B1	64.8	[67]
		B2	43.4	
		G1	83	
		G2	69.6	
Sodium hydrosulfite + heating at pressure	Black pepper	B1	96.1	[67]
		B2	77.7	
		G1	100	
		G2	100	
Heating (30-70°C)+ CaOH2 (1%) + H_2O_2(1-3%)	White pepper	B1	94	[68]
		B2	68.9	
		G1	100	
		G2	77	

Ozonization

Although there are not many reports on the use of ozone against filamentous fungi or their mycotoxins, promising results have been reported. With a short half-time, at neutral pH and ambient temperature, ozone is able to inactivate microorganisms and decompose their toxic metabolites, leaving no traces of ozone in the treated commodity [69]. Ozone, a powerful oxidant, reacts across the 8, 9 double bond of the furan ring of aflatoxin through electrophilic attack, causing the formation of primary ozonides followed by rearrangement into monozonide derivatives such as aldehydes, ketones and organic acids. Inan reported that reductions of content of AFB1 in flaked and chopped red peppers were 80% and 93% after exposures to 33 mg/l ozone and 66 mg/l ozone for 60 min, respectively [43]. The reduction percentages of AFB1 in artificially

contaminated wheat ranged from 84.1 to 99.66% after exposures 20 and 40 ppm ozone for 20 min [70]. Luo and coworkers indicated that ozonization can be quickly and effectively degrade AFB1 in corn and diminish aflatoxin toxicity, and therefore, ozonation is expected to be an effective, fast, and safe method for AFB1 degradation in corn [71]. In agreement with this idea, De-Alencar [72] reported that ozone is an important alternative for peanut detoxification since it is effective in controlling potentially aflatoxigenic fungi and also acts in the reduction of aflatoxin levels in kernels.

BIOLOGICAL METHODS

Biological methods are based on the action of microorganisms on mycotoxins and their mechanism of action is based on competition by nutrients and space, interactions, and antibiosis, among others [73]. Biological control of mycotoxin is a promising approach for reducing both pre harvest and post harvest mycotoxin contamination in food crops [9]. Different organisms, including bacteria specially, probiotics and dairy strains of lactic acid bacteria (LAB), yeasts strains of Saccharomyces cerevisiae and nontoxigenic Aspergillus fungi, have been tested for their ability in the control of AFs contamination [74].

Bacteria

Several bacterial species, such as *Bacillus subtilis, Lactobacilli spp., Pseudomonas spp., Ralstonia spp. and Burkholderia spp*., have shown the ability to inhibit fungal growth and production of AFs by Aspergillus spp.

Lactic acid bacteria (LAB) are a large group of genetically different bacteria that show antibiosis ability. They are able to inhibit the development of undesirable microorganisms that may spoil the product or be hazardous to human health. One of the effects of the LAB is protection against toxins produced in foods, such as heterocyclic amines, polycyclic aromatic hydrocarbons, reactive oxygen species, and mycotoxins [75]. Many studies have demonstrated that LAB has the ability to inhibit aflatoxin biosynthesis, or to remove mycotoxins from the medium (Table 3). Lactic acid bacteria *(Lactobacillus casei, Lactobacillus plantarum,* and *Lactobacillus fermentum*) isolated from traditional Iranian sourdough and dairy products were capable of removal of AFB1, ranged from 25 to 61%. The L. casei was a stronger binder of AFB1 compared with the other bacteria [74]. In a related study, five different cultures consisting of *Lactobacillus acidophilus, L. brevis, L. casei, L. delbruekii*, and *L. plantarum* were used to inoculate the AFB1 contaminated maize. Pronounced reduction (44.5%) was observed in maize contaminated at 50 ng/g, while maize contaminated at 500 ng/g was the least reduced (29.9%). The *L. plantarum* was the most efficient organism in degrading AFB1 [76].

Reduction of mycelial growth of A. parasiticus as a result of co-inoculation of the four bacteria (*Leuconostoc mesenteroides, Lactobacillus plantarum Lactobacillus casei,* and *Bacillus subtilis*) was observed to range between 20.9 to 86.2% while reduction of aflatoxin production ranged from 21.6 to 70.4%. The great reduction was found when the mold was co-inoculated with *B. subtilis*, then with Leu. Mesenteroides, then with L. casei, and the least reduction with *L. plantarum* [77]. Several strains of B. subtilis and P. solanacearum isolated from the non-rhizophere of maize soil were also able to inhibit aflatoxin accumulation [78]. A soil bacterium, designated strain No. 27, was found to produce aflatoxin-production inhibitors [79]. Palumbo [80] reported that in a laboratory experiment, a number of Bacillus, Pseudomonas, Ralstonia and Burkholderia strains isolated from California almond samples could completely inhibit A. flavus growth.Oluwafemi [76] reported that inclusion of culturally appropriate fermented foods and incorporating lactic acid bacteria or probiotics into the diet might be a feasible method of partially reducing aflatoxin risk. Therefore use of lactic acid bacteria, has generally regarded as safe (GRAS) status, should be encouraged for use as a bio-detoxification agent for AFs.

In contrast with these results, Dorner reported that in most cases, although these strains were highly effective against aflatoxin production and fungal growth under laboratory conditions, they do not give good efficacies in fields because it is difficult to bring the bacterial cells to the Aspergillus infection sites on commodities under field conditions [81].

Yeast

Some saprophytic yeast species (such as Candida krusei and Pichia anomala) have shown promise as biocontrol agents against A. flavus. Similar to bacterial agents, these yeast strains were able to inhibit Aspergillus growth greatly in laboratory conditions [82]. However, binding of aflatoxins by yeast strains is also a fast and reversible process, their binding ability is generally lower than bacterial strains. lt is strain specific and varies largely among different strains. AFB1 binding by S. cerevisiae was a rapid process in liquid medium and it involved the formation of a reversible complex between the toxin and yeast cell wall surface [83]. To date, a number of studies have demonstrated that the structure and components of the cell wall are responsible for microbial binding of aflatoxins, though the mechanism of binding by a specific strain is still

unclear. The esterified glucomannan (EGM) and mannanoligosaccharide (MOS) have been proposed to be responsible in yeast cell wall. While in LAB, cell wall peptidoglycans and polysaccharides have been proposed to be the most crucial elements responsible for AFB1 binding [84].

Saccharomyces cerevisiae showed aflatoxin surface binding ability for about 40 percent in its exponential. After the addition of S. cerevisiae, AFB1 contamination in peanuts was reduced by 74.4 and 55.9% after 7 and 15 days, respectively [85]. In a related study the effect of three types of commercially available yeast including active dry yeast, instant dry yeast and compressed yeast was studied during bread making. All types of yeast showed promising effect on AFs reduction. The order of AFs reduction was AFB1>AFB2>AFG1. Furthermore, the results indicated that the instant dry yeast was the most effective yeast [86].

Fermentation in combination with other methods also was studied. Motawe indicated the effect of probiotic plus yeast as a potential protective agent against aflatoxin toxicity which decrease the risk of occurrence of liver and kidney dysfunction [87]. Maximum amount of reduction (70%) was observed by the combined action of fermentation and steaming [16].

Nontoxigenic Aspergillus Strains

In general, nontoxigenic Aspergillus strains (A. niger, A. parasiticus), Trichoderma viride, Mucor ambiguus and few other fungi have been reported to show significant AFB1 degradation abilities. Application of competitive nontoxigenic strains of Aspergillus showed the greatest successes to date in biological control of aflatoxin contamination in both pre- and post-harvest crops in many field experiments, particularly with peanut and cotton. Recently, two products of nontoxigenic strains have received U.S. Environmental Protection Agency (EPA) registration as biopesticides to control aflatoxin contamination in cotton and peanuts in several states of USA (81). In general, the strategy is based on the application of nontoxigenic strains to competitively exclude naturally toxigenic strains in the same niche and compete for foodstuff substrates. Thus, for competitive exclusion to be effective, the biocontrol nontoxigenic strains must be predominant in the agricultural environments when the foodstuff is susceptible to be infected by the toxigenic strains [74]. The success of this method is depending on some factors such as, formulation (the combination of competitive strain and carrier or substrate), inoculum rate, Herbicide application and soil temperature. Application of nontoxigenic strains to soil should be delayed until soil temperature reaches at least $20^{\circ C}$ [88 Rajani et al., 2012].

Some studies demonstrated different range of reductions in aflatoxin contamination (Table 3).

A two-year study was conducted to evaluate the efficacy of nontoxigenic strains of A. flavus and parasiticus to reduce pre harvest AFs contamination of peanuts. In the first year, the percentage of kernels infected by wild-type A. flavus and A. parasiticus was significantly reduced in plots treated with rice and corn flour granules. AFs concentration in peanuts was significantly reduced in second year by all formulation treatments with an average reduction of 92% [89]. Tehnkeng in Africa showed that non-toxigenic strains of A. flavus reduce aflatoxin concentrations in both laboratory and field trials by 70 to 99% [90]. A similar study, conducted in Australia, showed application of nontoxigenic strains could reduce aflatoxin formation in peanuts by 95% [91]. In China, one highly competitive strain AF051, screened from more than 30 nontoxigenic strains of A. flavus, reduced naturally Aspergillus populations by up to 99% in the soil of peanut fields [74].

Although biological methods considered being potential biocontrol agents for management of aflatoxins, further field experiments are necessary to test their efficacies in reducing AFs contamination under field conditions.

COMPARISON PHYSICAL, HEMICAL AND BIOLOGICAL MEHODS

Tripathi studied the efficacy of various physical (UV irradiation, heating, microwave); chemical (oxidation, bleaching, ammoniation, sulphitation) and biological treatments methods for detoxification AFB1 in red chili powder. Amongst the physical methods, direct oven heating (at $120^{\circ C}$) produced maximum (83.32%) reduction of AFB1. With the exception of oxidation with H_2O_2 which produced 58.32% degradation, other selected chemical compounds were ineffective on AFB1. Biological detoxification of 66.2% was achieved by treating spiked chili powder with purified peroxidase. The author reported that the physical methods were more efficient over other methods in degrading AFB1, but produced significant ($p \leq 0.05$) nutritional losses [14].

In general, the success in detoxification of aflatoxins with physical, chemical and biological methods is depend on many factors such as, aflatoxins concentration, composition and physicochemical properties of food sample (moisture content, fat content, acidity, texture and so on), and source of contamination (natural or artificial). Therefore selecting the proper approach is too much

complicated. For example, despite the fact that irradiation may be a proper method for removing contamination from spices but it's not a promising method for food with high moisture content such as fruit, vegetables and meats. As another example, however, roasting showed good results in decontamination of peanuts, it is not convenient for cereal. That's why it cannot be stated with certainty that which method is more effective in reducing aflatoxins.

Table 3: Examples of aflatoxins reduction by biological (bacteria, yeast and nontoxigenic strains) methods (2000- 2015).

Biological method	Bacteria	Sample	Toxin	Reduction (%)	Ref.
Bacteria	*Lactobacillus rhamnosus* GG (LBGG), *Lactobacillus rhamnosus* (LC-705)	Liquid medium	B1	80	[92]
	L. rhamnosus GG, *Propionibacterium freudenreichii* ssp., *Sherman*	chicken duodenum	B1	74 63 37	[93]
	Lactobacillus and *Propionibacterium* strains	chicken duodenum PBS solution	B1	57-66 25	[94]
	Lactobacillus, Lactococcus, BifKiobacterium sp	PBS solution	B1	5.6-59.7	[95]
	Lactobacillus fermentum, Lactobacillus easel, Lactobacillus plantarum	Liquid media	B1	25-61	[73]
	Enterococcus faecium M74 and EF031 AFB1 19.3-37.5	Liquid media	B1	19.3-37.5	[96]
	Lactobacillus plantarum, Lactobacillus fermentum, Lactobacillus spp., *Selangorensis, Pediococcus acidilactici* and *Weisse/la confusa*	Liquid media	B1	15-60	[97]
Yeast	Saccharomyces cerevisiae	PBS solution	B1	40	[83]
	Saccharomyces and *Candida* strains	PBS solution	B1	15-60	[97]
	Saccharomyces cerevlsiae cell wall component esterified glucomannan (EGM)	Contaminated feed	B1	81.6	[98]
	S. cerevisiae strains	PBS solution	B1	10-40	[99]
Nontoxigenic strains	K94	Maize	AFs	83-98	[100]
	Afla-guard	Maize	AFs	9-75	[101]
	Afla-guard	Maize	AFs	85-88	[102]
	Afla-guard	Peanut	AFs	89-96	[89]
	AFCHG2	Peanut	AFs	75	[103]

Moreover, almost all of the methods have considerable limitations. Physical methods are usually more expensive. Although, AFs adsorbant showed promising results in the laboratory conditions, the use of these substances in livestock body is different and method is time consuming. In addition, some factors such as livestock species, age and genus influence results of the experiments [35]. Since aflatoxins are heat resistance, applying high degrees of temperature may produce undesirable changes in foods and sometimes it is impossible to heat foods at over 100 $^{\circ}C$ to reduce AFs level.

Despite promising results of a chemical compound on reduction aflatoxins, they usually produce undesirable toxic residues and cause changes in nutritional, sensory (the texture, taste, aroma, color) and functional properties of food [59].

In the terms of biological degradation strategies, some limitations such as long degradation time (lasting more than 72h), incomplete degradation,

non-adaptation to typical food fermentations, and culture pigmentation are the main factors that reduce the potential of biological methods for use in the food industry. Moreover, some of these strains with degradation potential may also produce AFB1 under varying conditions [71].

CONCLUSION

This review furnishes the following conclusions:

1) The efficiency of a physical, chemical and biological method to reduce AFs depends, to a great extent, on the nature of the foods and its physicochemical properties, level of contamination and degree of association of aflatoxins with the food constituents. Therefore establishment of a unique detoxification method for all foods and feedstuffs is impossible.

2) Using a combination of methods (such as heat and chemical, fermentation and steaming and so on) to reduce Afs is more effective than each method alone. Therefore current review paper suggests a combination of moderate two or more treatments.

3) Further research is still needed especially on naturally contaminated food to develop these processes further for practical application.

4) The most desirable approach to control the presence of aflatoxins in feeds and foods is to Prevent their formation during pre-harvest, harvest and post-harvest.

ETHICAL ISSUES

Ethical issues have been completely observed by the authors.

COMPETING OF INTEREST

The author declares that she has no competing interests.

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