DIFFERENT MYCOTOXIN INACTIVATION APPLICATIONS AND THEIR INACTIVATION MECHANISMS

ABSTRACT: Control of mycotoxins is the need of the hour, since their occurrence in foods and feeds is continuously posing threats to both health and economics all over the world.

Besides the post-harvest preventive measures, it is important that suitable detoxification methods must be developed for inactivating or removing mycotoxins from the contaminated commodities, as the toxins are also produced by Aspergillus flavus and A. parasiticus even during pre-harvest stages of crop production.

Several physical and chemical detoxification methods developed so far have been critically discussed in different reviews for their advantages and limitations based on certain adopted strategies and specific criteria.

Understanding of mechanisms of mycotoxins detoxification by physical, chemical and microbiological methods will enable establishment of combined treatment procedures to effectively decontaminate, contaminated foods and feeds. Such treatment methods are expected to be cost effective and minimally deleterious to food constituents.

KEY WORDS: mycotoxins, food, feed, methods, inactivation, detoxification

INTRODUCTION

Mycotoxins are toxic mould metabolites produced by toxigenic strains of different mould species. They have an important role in the occurrence of some human diseases such as liver cancer, chronic hepatitis and cirrhosis. When animals eat foodstuffs containing aflatoxin B1, these toxins are metabolized and excreted as aflatoxin M1 in their milk. Aflatoxin M1 is resistant to thermal inactivation and is not destroyed completely by pasteurization, autoclaving or other food processing procedures. The control of aflatoxins is the need of the hour, since their occurrence in foods and feeds is continuously posing threats to both health and economics all over the world. Since aflatoxin contamination is unavoidable, numerous strategies for its detoxification have been proposed. These include physical methods of separation, thermal inactivation, irradiation, solvent extraction, adsorption from solution, microbial inactivation and fermentation. Chemical methods of detoxification are also practiced.
as a major strategy for effective detoxification. Besides the post-harvest preventive measures, suitable detoxification methods are developed for inactivating or removing aflatoxins from the contaminated commodities, since toxins are also produced by *Aspergillus flavus* and *A. parasiticus* even during the pre-harvest stages of crop production. Understanding of mechanisms of aflatoxin detoxification by physical, chemical and microbiological methods will enable the establishment of combined treatment procedures to effectively decontaminate contaminated foods and feeds. Such treatment methods are expected to be cost effective and minimally deleterious to food constituents.

**Inactivation strategies**

Several physical and chemical detoxification methods developed so far have been critically discussed in different reviews for their advantages and limitations based on certain adopted strategies and specific criteria. Detoxification by microbiological means is also reviewed with respect to the status on potential microorganisms and their enzymes that can degrade aflatoxins to less toxic or innocuous end products. Understanding the mechanisms of aflatoxin detoxification by physical, chemical and microbiological methods will enable the establishment of combined treatment procedures to effectively decontaminate contaminated foods and feeds. Such treatment methods are expected to be cost effective and minimally deleterious to food constituents (Basaappa and Shanta, 1996).

**Application of ammonia**

Ammoniation of corn, peanuts, cottonseed and meals for the alteration of toxic and carcinogenic effects of mycotoxin contamination has been the subject of intense research effort by scientists in various government agencies and universities worldwide. Engineers have devised workable systems of treatment of whole seeds, kernels or meals; chemists have identified and characterized the products formed from the reaction of aflatoxin B1 with ammonia, with and without meal matrix; biochemists have studied the biological effects of these compounds in model systems; and nutritionists have studied animal responses to rations containing ammoniated and non-ammoniated components. The results of aflatoxin/ammonia decontamination research demonstrate the efficiency and safety of ammoniation as a practical solution to aflatoxin detoxification in foods and animal feeds (Park, 1993).

Corn is, all over the world, frequently contaminated with the fungus *Fusarium moniliforme* that produces toxic fumonisins. However, ammonia detoxifies effectively aflatoxins in corn and cottonseed. Since corn can be contaminated by both fumonisins and aflatoxins, application of ammoniation of corn cultured with, or naturally contaminated by *F. moniliforme*, showed that Fumonisin B1 levels in the culture material and in naturally contaminated corn were reduced by 30 and about 45%, respectively, by the treatment. Despite the
apparent reduction in fumonisin content, the toxicity of the culture material in rats was not altered by ammoniation. Reduced weight gains, elevated serum enzyme levels and histopathological lesions, typical of *F. moniliforme* toxicity, occurred in rats fed with either ammoniated or non-ammoniated culture material. Atmospheric ammoniation of corn does not appear to be an effective method for the detoxification of *F. moniliforme*-contaminated corn (Norr ed et al., 1991).

Although there was no significant change in dietary intake, body weight gain, and feed conversion ratio in chickens fed with ammonia treated aflatoxin contaminated maize, these parameters were suppressed in birds fed with aflatoxin-containing diet. These data suggest that replacement of aflatoxin-containing maize with ammoniated grains can significantly suppress aflatoxicosis, leading to an improvement in production parameters in broilers (Allameh et al., 2005).

Rice, a widespread cereal used for human and animal nutrition, is susceptible to aflatoxin contamination in the field and during storage. Therefore, the goal of the research was the evaluation of the efficacy and permanence of the ammoniation process through high pressure/high temperature (HP/HT) and atmospheric pressure/moderate temperature (AP/MT) conditions applied to rice samples artificially contaminated with aflatoxin B1. For this purpose, a 2(k) design was drawn up with temperature, rice moisture and the process time as its variables. Under both sets of conditions, aflatoxin B1 concentration was reduced in a range of 90—100%. In conclusion, the process efficacy and permanence were achieved through the use of high temperature and long process time for both sets of conditions (HP/HT and AP/MT), respectively (Trujillo and Yépez, 2003).

**Chlorine dioxide**

The efficacy of chlorine dioxide (ClO₂) in the detoxification of trichothecene mycotoxins verrucarin A and roridin A, was evaluated. In the first experiment, verrucarin A (1, 5 or 10 μg) and roridin A (5 or 10 μg) were each inoculated onto square-inch sections of glass, paper, and cloth, and exposed to 1000 ppm of ClO₂ for either 24 or 72 h at room temperature. In the second experiment, verrucarin A and roridin A (1 or 2 ppm in water) were treated with 200, 500 or 1000 ppm ClO₂ for up to 116 h at room temperature. The results of the first experiment showed that ClO₂ treatment had no detectable effect on either toxin. In the second experiment, both toxins were completely inactivated at all tested concentrations in less than 2 h after treatment with 1000 ppm ClO₂. For verrucarin A, the effect was seen at 500 ppm level, but this effect was not as strong as that observed at 1000 ppm level. Roridin A toxicity was decreased after treatment with 200 and 500 ppm ClO₂, but this was not significant until the 24-hour exposure time was reached. These data show that ClO₂ (in solution) can be effective for detoxification of roridin A or verrucarin A at selected concentrations and exposure times (Wilson et al., 2005).
Citric acid

Chemical inactivation of aflatoxin B1 (AFB1) and aflatoxin B2 (AFB2) in maize grain by means of 1 N aqueous citric acid was confirmed by the AFLA-TEST™ immunoaffinity column method, high performance liquid chromatography (HPLC), and the Ames test (Salmonella-microsomal screening system). The AFLATEST™ assay showed that aflatoxins in the maize grain, with an initial concentration of 29 ng/g, were completely degraded, and 96.7% degradation occurred in maize contaminated with 93 ng/g when treated with the aqueous citric acid. Aflatoxin fluorescence strength of acidified samples was much weaker than the untreated samples, when observed in HPLC chromatograms (Mendez et al., 2005).

Sulfhydryl compounds

Most food toxicants have specific groups responsible for their deleterious effects. Modifying such sites with specific a-acids, peptides, and proteins lessens their toxicity. Sulfhydryl (thiol) compounds, such as cysteine, N-acetylcysteine, reduced glutathione, and mercaptopropionylglycine interact with disulfide bonds of plant protease inhibitors and lectins via sulfhydryl-disulfide interchange and oxidation-reduction reactions. Such interactions with inhibitors from soybeans and lectins from lima beans facilitate heat inactivation of the potentially toxic compounds, resulting in beneficial nutritional effects. Related transformations of protease inhibitors in soy flour are also beneficial. Since thiols are potent nucleophiles, they have a strong affinity for unsaturated electrophilic centers of several dietary toxicants, including aflatoxins, sesquiterpene lactones, such as elephantropin and parthenin, urethane, carbonyl compounds, quinones, and halogen compounds. Such interactions may be used in vitro to lower the toxic potential of the diet, and in vivo for prophylactic and therapeutic effects against oxidative damage. A number of examples are cited to illustrate the concepts and mechanisms of using sulfur amino acids to reduce the antinutritional and toxic manifestations of food ingredients (Friedman, 1994).

Feed additives

The possible protective effect of four feed additives against the toxic effects of T-2 toxin in growing broiler chickens was investigated in randomized trial consisting of six dietary treatments (control with no T-2 toxin or feed additive added, 2 ppm T-2 toxin alone, 2 ppm T-2 toxin plus 2.0 g/kg Mycofix, 2 ppm T-2 toxin plus 2.0 g/kg Mycosorb, 2 ppm T-2 toxin plus 2.5 g/kg MycoAd, and 2 ppm T-2 toxin plus 3.0 g/kg Zeolex). When no feed additive was included, 2 ppm dietary T-2 toxin significantly decreased BW and increased feed: gain ratio. When 2.0 g/kg Mycofix were added to the diet, the feed additive protected against the adverse effects of T-2 toxin on BW, BW gain, and feed: gain ratio. However, no protection against the adverse effects of T-2...
toxin on final BW and BW gain were obtained by the supplementation of any of the other 3 feed additives. The results of trial indicate that the only feed additive capable of counteracting the adverse effects on performance caused by the dietary administration of 2 ppm T-2 toxin was the additive based on the enzymatic inactivation of the 12,13-epoxide ring of the trichothecenes (Mycotix). This study also confirms the previous reports showing that aluminosilicates are not effective against trichothecene mycotoxins (Diaz et al., 2005).

Aqueous extract of ajowan seeds was found to contain an aflatoxin inactivation factor (IF). Approximately 80% reduction in total aflatoxin content over the controls was observed. This observed phenomenon of reduction in total toxin was referred to as toxin inactivation. It was discovered that the temperature influenced the rate of toxin inactivation. At 45°C, toxin inactivation was rapid during the initial 5 hours, after which it decreased. The IF was found to retain considerable activity even after boiling and autoclaving, indicating partial heat stability. Toxin decontamination in spiked corn samples could be achieved using IF. This study emphasizes the potential of ajowan IF in aflatoxin removal from contaminated food commodities. However, the biological toxicity, if any, of the IF inactivated aflatoxins needs to be confirmed (Hajare et al., 2005).

**Biological detoxification**

Some toxin-producing fungi are able to degrade or transform their own products under suitable conditions. Pure cultures of bacteria and fungi which detoxify mycotoxins were isolated from complex microbial populations by screening and enrichment culture techniques. Genes responsible for some of the detoxification activities were cloned and expressed in heterologous hosts. The detoxification of aflatoxins, cercosporin, fumonisins, fusaric acid, ochratoxin A, oxalic acid, patulin, trichothecenes, and zearalenone by pure cultures were also reported (Karlovsky, 1999).

**Extrusion process**

Cottonseed is an economical source of protein and is commonly used in balancing livestock rations. However, its use is typically limited by protein, fat, gossypol, and aflatoxin contents. The extrusion temperature study showed that aflatoxin levels were reduced by an additional 33% when the cottonseed was extruded at 160°C as compared to 104°C. Furthermore, the multiple-pass extrusion study indicated that aflatoxin levels were reduced by an additional 55% when the cottonseed was extruded four times as compared to one time. Total estimated reductions of 55% (three stages of processing at 104°C), 50% (two stages of processing at 132°C), and 47% (one stage of processing at 160°C) were obtained from the combined equations. If the extreme conditions (four stages of processing at 160°C) of the evaluation studies are applied to
the combined temperature and processing equation, the resulting aflatoxin re-
duction would be 76% (Michael et al., 2002).

Traditional nixtamalization and an extrusion method for making dough
(masa) for corn tortillas, which requires the use of lime and hydrogen pero-
oxide, were evaluated for the detoxification of aflatoxins. The traditional nixta-
malization process reduced levels of aflatoxin B1 (AFB (1)) by 94%, aflatoxin
M1 (AFM (1)) by 90% and aflatoxin B1-8,9-dihydrodiol (AFB (1)-dihydro-
diol) by 93%. The extrusion process reduced levels of AFB(1) by 46%,
AFM(1) by 20% and AFB(1)-dihydrodiol by 53%. Extrusion treatments with
0,0.3 and 0.5% lime reduced AFB (1) levels by 46,74 and 85%, respectively.
The inactivation of AFB (1), AFM (1), and AFB (1)-dihydrodiol in the
extrusion process using lime together with hydrogen peroxide showed higher
elimination of AFB(1) than the treatments with lime or hydrogen peroxide
alone. The extrusion process with 0.3% lime and 1.5% hydrogen peroxide was
the most effective process of aflatoxin detoxification in corn tortillas, but the
high level of those reagents negatively affected the taste and aroma of the corn
tortilla as compared with tortillas elaborated by the traditional nixtamalization
process (Elias et al., 2002).

Samples of corn flour experimentally contaminated with aflatoxin B1
(AFB1) (50 ppb) and deoxynivalenol (DON) (5 ppm) were extruded. The ef-
ects of three extrusion variables (flour moisture, extrusion temperature and so-
dium metabisulphite addition) were analyzed according to a two-level factorial
design. The process was effective for the reduction of DON content (higher
than 95%) under all the conditions assessed, but was only partially successful
(10—25%) for the decontamination of AFB1.

The results show that extrusion cooking is effective for the inactivation of
DON, but is of a limited value for AFB1, even when metabisulphite is added.
More severe extrusion conditions are needed for the detoxification of AFB1.
As DON contamination occurs mainly in the field, prior to harvesting, and that
of AFB1 is normally produced during grain storage, maize is often contami-
nated with DON and not with AFB1. Under these conditions, the described
extrusion process can be used for the detoxification of DON. The addition of
sodium metabisulphite did not significantly affect the inactivation of AFB1.
Extrusion cooking is therefore an appropriate treatment for vomitoxin-contami-
nated maize in the countries where, due to the prevailing conditions, these are
the only toxins present (Cazzaniga et al., 2001).

Miscellaneous

Aflatoxins are also sensitive to UV light and gamma radiation. Exposure
of artificially contaminated milk to UV light inactivated 3.6—100% of AFM1,
depending on the exposure time. In the case of dried figs artificially contami-
nated with AFB1, the toxin level was reduced by 45.7%. Toxicity of a peanut
meal contaminated with AFB1 was reduced by 75 and 100% after the irradiation
by gamma rays at dose of 1 and 10 kg, respectively.
Solar energy is also widely used in the decrease of the amount of aflatoxins from 30—80% in peanut cakes, flakes peanut oil, and olive oils in different parts of the world. High hydrostatic pressure application is another method of inactivating mycotoxins present in food, but the pressure exciting 500 MPa has detrimental effects on the food itself.

Fig. 1 — Proposed mechanism for the acidification of aflatoxin B₁ to produce aflatoxin D₁

REFERENCES


Фарук Бозоглу

Стратегије контровере и механизми инактивације миکотоксина

Контрола миکотоксина је ургентна потреба данашњице јер њихово присуство у храни и сточној храни угрожава здравље и економију у светским размерама. Поред примене последњих превентивних мера, неопходно је развијати и одговарајуће методе детоксификације које служе за инактивацију и уклањање миکотоксина из контаминираних храни, с обзиром на чињеницу да се токсини стално производе, чак и у току предхрестих фаза у производњи усева, нарочито од стране родова Aspergillus flavus и A. parasiticus.

Неки данас познати, методи физичке и хемијске детоксификације критички су разматрани у више прегледних радова, са становишта њихових предности и ограничења а на основу одређених увојених стратегија и неколико специфичних критеријума.

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Разумевање механизама детоксификације микотоксина физичким, хемијским и микробиолошким методима ће омогућити успостављање процедура комбинованих третмана који могу ефикасно да деконтиминирају угрожену храну и сточну храну. Очекује се да ови третмани буду економични и да минимально утичу на састојке хране.