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Aflatoxins Risk: Current situation and biological detoxification

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ABSTRACT

Aflatoxins (AFs) belong to the group of mycotoxins. AFs are a group of highly toxic products of *Aspergillus flavus*, *A. parasiticus* and *A. nomius* and have carcinogenic and teratogenic effects to livestock and human. This mycotoxin can also cause DNA damage, gene mutation, chromosomal anomalies and cell transformation in mammalian cells in vitro. Different studies have been done to detect these toxins and different researches for detoxification. This article reviews the risks, some studies on the current situation of AFs contamination and also some reports on detoxification (including physical and biological methods) of AFs in foods in Iran.

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KEYWORDS

Aflatoxin;
Food safety;
Detoxification

INTRODUCTION

Mycotoxins are secondary metabolites of molds which are associated with certain disorders in animals and humans. In addition to being acutely toxic, some mycotoxins are now linked with the incidence of certain types of cancer and it is this aspect which has evoked global concern over feed and food safety^[1]. Aflatoxins (AFs) belong to the group of mycotoxins^[2]. AFs are a group of highly toxic products of *Aspergillus flavus*, *A. parasiticus* and *A. nomius* and have carcinogenic and teratogenic effects to livestock and human^[3]. *A. flavus* and *A. parasiticus* are ubiquitous fungi, showing particular affinity for oily seeds as a growth source. Main sources of aflatoxins in feeds are peanut, maize and cottonseed meals^[4]. The four major aflatoxins are B1, B2, G1 and G2 based on their fluorescence under UV light (blue or green) and relative chromatographic

mobility during thin layer chromatography (TLC)^[5]. Production of mycotoxins by toxigenic mold species contaminating food and feed depends on several environmental factors, for example temperature, humidity and other storage conditions^[6]. Contamination of agricultural crops with AFs is a worldwide problem not limited to developing countries, where both climatic and technological conditions stimulate aflatoxin formation^[7]. The aim of this review is to introduce the risks and situation of AFs contamination in foods and the biological ways of AFs detoxification.

AFS RISK IN ANIMAL MILK

When animals eat foodstuffs containing AFB1, these toxins will be metabolized and excreted as aflatoxin M1 (AFM1) in milk. There is a general consensus that approximately 1-3% of the AFB1 initially present in the

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animal feed stuff appears as AFM1 in milk^[8,9]. AFM1 is cytotoxic, as demonstrated in human hepatocytes in vitro. This mycotoxin can also cause DNA damage, gene mutation, chromosomal anomalies and cell transformation in mammalian cells in vitro. Different studies are done for AFM₁ contamination in Milk in Iran. Mohamadi sani et al. (2012) detected the amount of AFM₁ in pasteurized milk samples in Mashad in northeast of Iran. For this purpose, 42 milk samples were analyzed by ELISA technique. Results showed presence of AFM1 in 97.6% of the samples by average concentration of 23±16 ppt and contamination level ranging between 6 and 71 ppt^[10]. In another study by Mohamadi sani (2010) the mean AFM₁ content in 96 milk samples was detected 77.92 ppt^[11]. However, AFM1 is less mutagenic and genotoxic than AFB1^[4,12]. Since milk has the greatest demonstrated potential for introducing AFs residues from foods of animal origin into the human diet and is also the main nutrient for infants and children, the occurrence of AFM1 in milk and dairy products is a concern^[13].

DETOXIFICATION METHODS

Numerous strategies for the detoxification or inactivation of aflatoxins contaminated feed-stuffs have been used such as physical separation, thermal inactivation, irradiation, microbial degradation and treatment with a variety of chemicals^[14]. Different studies have been done for the mentioned techniques:

THERMAL TREATMENT

It is very difficult to remove AFT since they are heat resistant and soluble in intermediate polar solvents. So far, detoxification methods are classified as physical, chemical and biological ones. Although AFT are highly stable to dry heat up to temperatures below their thermal decomposition temperature^[15], use of heat to inactivate AFT in contaminated food has been attempted. Some studies have indicated that AFT in contaminated food can be degraded by heat treatment. The extent of destruction achieved depends on the initial level of contamination, heating temperature, moisture content and duration of heating^[16]. In a study by Mohamadi sani et al. (2012) the effect of different cooking methods

was evaluated on the levels of the AFs in domestic and imported rice in Amol (in the north of Iran). For this purpose, 42 rice samples were collected from retail stores. The raw samples were analysed by enzyme-linked immunosorbent assay (ELISA) technique for toxin assessment and then submitted to two different cooking methods including traditional local method and in rice cooker. After treatment, AFT was determined. Results showed that the average concentration of AFT in domestic and imported samples was 1.08 + 0.02 and 1.89 + 0.87 ppb, respectively. The highest AFT reduction (24.8%) was observed when rice samples were cooked by rice cooker but the difference with local method was not statistically significant^[17]. In another study by Mohamadi sani et al. (2013), 32 chickpea samples were collected from retail stores of four cities in Mazandaran province in the north of Iran. Samples were soaked in potable water and cooked, respectively, for 3.5 and 4 h. Then the raw and cooked samples and the soaking water were analyzed for OTA determination by enzyme linked immunosorbent assay (ELISA) technique. Results showed that six raw samples (18.75 per cent) contained detectable amounts of OTA by average concentration of 5.9 which was lower than national standards. Increasing the time of cooking led to slight degradation of OTA but according to statistical analysis and LSD test, only after five hours, thermal treatment caused OTA to degrade significantly^[18].

BIODETOXIFICATION OF AFS

Various physical and chemical methods have been used to detoxify AFs from feed materials. The use of many of the available physical and chemical methods for detoxification of agricultural products contaminated with mycotoxins is restricted due to problems concerning safety issues, possible losses in nutritional quality of treated commodities, coupled with limited efficacy and cost implications. This has led to search for alternative strategies such as biological agents^[3,6]. Bacteria like *lactobacillus* strains have been tested on their ability to inactivate AFs^[19]. Yeasts and lactic acid bacteria cells are known to bind different molecules such as killer toxins and metal ions on complex binding structures on the cell wall surface and binding molecule was identified as the cell wall mannan. Based on some of the studies

reported, it is confirmed that removal of mycotoxins is by adhesion to cell wall components rather than by covalent binding or by metabolism, as the dead cells do not lose binding ability^[20].

In a research by Mohamadi sani et al., (2013), the binding of AFB₁ to *Saccharomyces cerevisiae* in the late exponential and early stationary phases was studied for viable, heat killed and acid killed yeast. AFB₁ at concentrations (5, 10 and 20mg/l) was added to the yeast culture (109 CFU/ml) in yeast mold broth medium and incubated at 25°C for 4, 12 and 24 hrs. The aflatoxin binding capacity of the strain was quantified by the amount of unbound AFB₁ using ELISA technique. Result showed that the detoxification rate for different treatments reported as follows: acid treated cells > heat treated cells > viable cells. Also, the most reduction in AFB₁ concentration happened within the first four hours of incubation with no significant increase in AFB₁ binding on further incubation because of saturation of active sites in yeast cells. According to the results, either forms of *Saccharomyces cerevisiae* (viable or nonviable) was effective in aflatoxin binding from medium and the binding had a physical nature^[21]. In another study by Mohamadi Sani et al. (2013), the interaction of AFB₁ in cottonseed with *Lactobacillus rhamnosus* strain GG was investigated for the first time. AFB₁ at concentrations (5, 10 and 20 µg/l) was added to the cottonseed meal and incubated at 25°C for 4, 12 and 24 hrs. The aflatoxin binding capacity of the strain was quantified by the amount of unbound AFB₁ using ELISA technique. Results showed the binding capacity of viable, heat killed and acid killed bacteria respectively 44, 47 and 49%^[22].

However, question remains on the toxicity of products of enzymatic degradation and undesired effects of fermentation with non-native microorganisms on quality of food^[23].

CONCLUSION

Aflatoxins are naturally occurring toxins produced by many ubiquitous fungi, mainly *Aspergillus flavus* and *Aspergillus parasiticus*. These fungi can grow on foods such as cereals, dried fruits and spices under certain environmental conditions^[24]. Mycotoxin contamination in rice is usually lower as in wheat or

corn. As a result, European Union (EU) food safety legislation on AFT levels is becoming increasingly strict and has established maximum residue limits (MRLs) for these compounds by means of the Regulation (EU) No. 165/2010 of 26 February 2010 (Regulation). For instance, these MRLs are 5 ppb for B1 and 10 ppb for the sum of B1, B2, G2 and G1 for rice. United States has regulated the AFT level in their foods to lower than 20 ppb, and in Korea and Japan the maximum allowed level is 10 ppb^[25]. Currently, the worldwide range of limits for AFB₁ and AFT are 1–20 ppb and 1–35 ppb, respectively^[26]. The MRL for AFT in cereals according to national standard is currently set by Institute of Standard and Industrial Research of I.R. Iran at 30 ppb^[27]. It is very difficult to remove AFT since they are heat resistant and soluble in intermediate polar solvents. So far, detoxification methods are classified as physical, chemical and biological ones. Although AFT are highly stable to dry heat up to temperatures below their thermal decomposition temperature^[15], use of heat to inactivate AFT in contaminated food has been attempted. Some studies have indicated that AFT in contaminated food can be degraded by heat treatment. The extent of destruction achieved depends on the initial level of contamination, heating temperature, moisture content and duration of heating^[16].

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