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Aflatoxins – product contamination and preventive measures

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ABSTRACT

In today's ecologically compromised living conditions, the issue of microbial contamination of food by pathogenic microorganisms and their toxins throughout the entire food chain (from farm to fork) is gaining increasing global importance. This is primarily aimed at protecting the health of humans and animals, safeguarding the economy and trade, and ensuring strict compliance with legal regulations. Over the past decade, the greatest attention has been focused on examining the concentration of aflatoxins in food of both plant and animal origin. The permitted concentration of aflatoxins in food products ranges between 4–30 µg/kg. However, the European Union has established the strictest standard, with AFB1 and total aflatoxins not exceeding 2 µg/kg and 4 µg/kg, respectively, in many products intended for direct human consumption. Through the application of physical, chemical, and biological preventive measures, as well as practices related to cultivation, harvesting, and storage of animal feed and cereals, and measures applied during production, transport, and storage processes, food contamination by mycotoxins can be significantly reduced.

Keywords: *aflatoxins, food contamination, preventive measures.*

INTRODUCTION

In today's ecologically disturbed living conditions, the issue of microbial contamination of food by pathogenic microorganisms and their toxins throughout the entire food chain (from field to table) is gaining increasing global significance. This is aimed at protecting human and animal health, safeguarding the economy and trade, while strictly adhering to legal regulations (Šarkanj et al., 2010).

Mycotoxins are naturally occurring toxic compounds known for their strong carcinogenic effects, produced during the metabolic processes of certain mold species. They enter the food chain either directly through mold growth on food or indirectly via contaminated raw materials and animal feed. Cereals, cereal products, and nuts represent the main sources of mycotoxins in the human diet, but contamination can also occur through animal-derived foods such as eggs, milk, and meat. Among the most widespread and dangerous food contaminants are aflatoxins, toxic compounds produced by molds of the genus *Aspergillus.*

Aflatoxins are secondary metabolites, produced by fungi such as *Aspergillus flavus*, *Aspergillus parasiticus*, and *Aspergillus nomius* (Figures 1, 2 and 3), that grow on agricultural products, including cereals, peanuts, rice, and dried fruit (FAO, 1993). These chemical compounds greatly compromise food safety and pose a potential risk to human health.

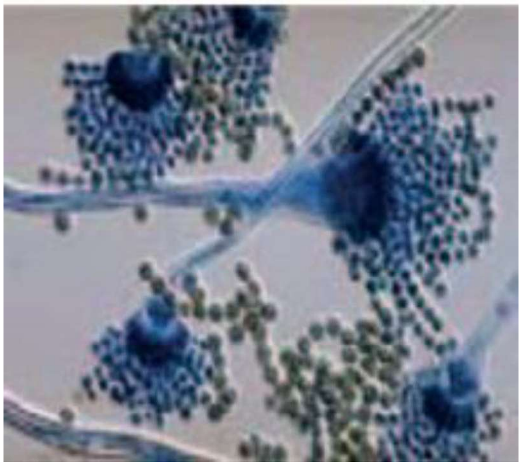

Figure 1. *Aspergillus flavus* (Šarkanj et al., 2010)

Figure 2. *Aspergillus parasiticus Bioimagen (*https://Bioimagen: *Aspergillus parasiticus)*

Figure 3. *Aspergillus nomius* (Chouvenc et al. 2011)

Many climatic factors influence the increase in the abundance of toxin-producing fungi. According to research by Santin (2005), dry conditions and high temperatures during the growth and harvest of corn promote the growth of *Aspergillus* species, and consequently the production of AFB1, with an optimal temperature range between 25 and 42°C. Since tropical and subtropical regions are recognized as areas with a high risk of aflatoxin B1 contamination, special attention is given to monitoring its presence in animal feed such as corn, rice bran, cottonseed, and soybean grain.

Unlike other molds, *Aspergillus flavus* possesses highly developed physiological mechanisms for acclimatization to unfavorable climatic conditions and dominates over other fungal species (Magan, 2007; Nesci et al., 2004).

The greatest danger of these toxins lies in their ability to enter the bodies of humans and animals through the food chain, causing various diseases. For this reason, over the past decade, the main focus has been on examining the concentration of aflatoxins in food of both plant and animal origin. The permitted concentration of aflatoxins in food products ranges from 4 to 30 μg kg^{-1}. However, the European Union has established the strictest standards, limiting AFB1 and total aflatoxins to no more than 2 μg kg^{-1} and 4 μg kg^{-1}, respectively, in many products intended for direct consumption (EC, 2007, 2010).

Contamination of agricultural products with mycotoxins represents a global threat, not only to public health but also to economic stability, as it causes significant financial losses. According to Wu and Guclu (2012), these losses in the United States can reach up to 10,000 US dollars per shipment annually.

Since aflatoxins can pose a serious threat to the health of humans and animals, and consequently cause substantial economic damage, it is crucial to conduct regular monitoring of food and animal feed on a global scale.

To ensure the safety of food products, continuous improvement of methods for detecting aflatoxins and reducing their contamination is essential.

AFLATOXINS

Aflatoxins are a mixture of chemically related compounds, derivatives of difurocoumarin, among which the most important representatives are aflatoxins B1, B2, G1, G2, M1 and M2. The letters B and G denote the color with which aflatoxins fluoresce under specific UV light wavelengths, while M refers to the milk substrate from which they were isolated (Šarkanj et al., 2010).

There are six aflatoxins of analytical interest (Figure 4), four occur in foods and two as metabolites in the milk of animals who have been fed contaminated feed (Perez-Fernandez, Escosura Muniz, 2022). Amongst all the known types of AFs, aflatoxin B1 (AFB1), B2 (AFB2), G1 (AFG1), G2 (AFG2) are the most prevalent naturally occurring toxins found in food and feed crops (Kabak, 2021).

Certain mold species, such as *Aspergillus flavus*, are responsible for producing aflatoxins B1 and B2, while others, like *Aspergillus parasiticus* and *Aspergillus nomius*, produce aflatoxins G1 and G2. Additionally, in ruminant organisms that consume contaminated feed, metabolic conversion of aflatoxins B1 and B2 into aflatoxins M1 and M2 occurs, which are then excreted through milk. Therefore, these secondary forms can be present in milk and dairy products, further emphasizing the importance of controlling aflatoxins in animal feed.

Compared to other countries, the European Union has the most detailed and comprehensive regulations that set the maximum allowable levels of aflatoxins in food for humans and animals. Maximum levels of aflatoxins (aflatoxins B1, B2, G1, G2 and M1) in foodstuffs are laid down in Commission Regulation (EC) No 1881/20064 as amended by Commission Regulation (EU) No 165/20105 and Commission Regulation (EU) No 1058/20126 (European Food Safety Authority, 2013).

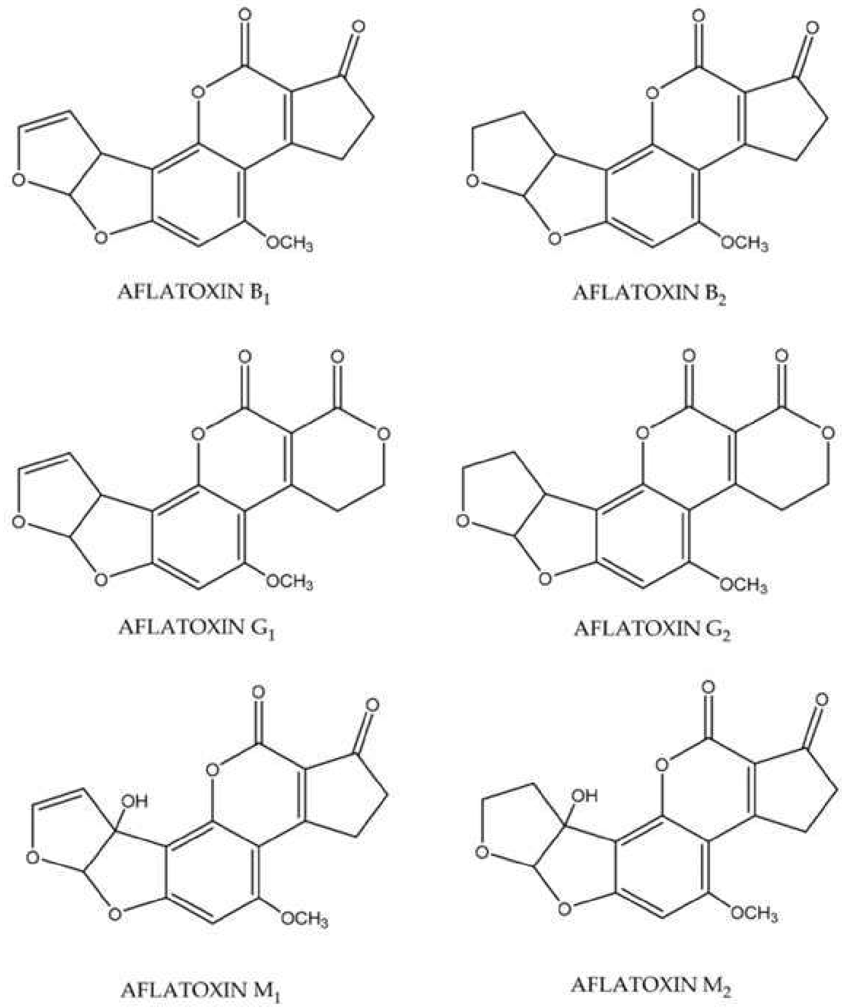

Figure 4. Chemical structures of aflatoxins (Perez-Fernandez, Escosura Muniz, 2022)

ANALYTICAL METHODS FOR AFLATOXIN DETERMINATION

Among the most popular methods for detecting aflatoxins are chromatographic techniques such as thin-layer chromatography, high-performance liquid chromatography, liquid chromatography coupled with mass spectrometry, ELISA tests, and polymerase chain reaction (PCR).

The use of electrochemical biosensors based on nanomaterials for the detection of AFs, represents a vast emerging field of research, metallic nanoparticles and carbon-based nanomaterials, are the main materials used for such purposes, whose role is both to increase the electronic transference and to improve the efficiency of the bioreceptor immobilization, as far as we know, none of the reported electrochemical biosensors for

AFs detection are neither commercially available nor reached the laboratory for routine use yet (Perez-Fernandez, Escosura Muniz, 2022).

PHYSICOCHEMICAL PROPERTIES OF AFLATOXINS

Aflatoxins are highly stable compounds at high temperatures and low pH values, which makes them exceptionally resistant during various food processing methods. Once produced, aflatoxins are chemically stable in food and cannot be easily eliminated from food – even at high temperatures encountered in food processing, such as roasting, baking, and even frying (https://www.sfa.gov.sg/food-safety-tips/food-risk-concerns/risk-at-a-glance/aflatoxins-and-food-safety).

Aflatoxins are carcinogenic compounds that can cause malignant liver diseases after prolonged exposure. Due to their proven carcinogenic effects on animals and humans, aflatoxin B1 has been classified by the International Agency for Research on Cancer (IARC) as a group 1 carcinogen (IARC, 1993). The permitted concentration of aflatoxin M1 in raw milk is up to 50 µg/kg in many countries. According to research by Šarkanj et al. (2010), aflatoxin cannot be completely eliminated from dairy products, but its level can be reduced through processing methods such as drying, pasteurization, and sterilization.

MEASURES FOR PREVENTION AND REDUCTION OF AFLATOXINS

The consequences of aflatoxin contamination highlight the need for appropriate and effective aflatoxin sampling procedures to protect consumers, comply with regulations, and ensure safe food for trade and consumption (Zhang and Banerjee, 2020).

To minimize the health risk from mycotoxins, people are advised to: inspect whole grains (especially corn, sorghum, wheat, rice), dried figs and nuts such as peanuts, pistachio, almond, walnut, coconut, Brazil nuts and hazelnuts which are all regularly contaminated with aflatoxins for evidence of mould, and discard any that look mouldy, discoloured, or shrivelled; avoid damage to grains before and during drying, and in storage, as damaged grain is more prone to invasion of moulds and therefore mycotoxin contamination; buy grains and nuts as fresh as possible; make sure that foods are stored properly – kept free of insects, dry, and not too warm; not keep foods for extended periods of time before being used; and ensure a diverse diet – this not only helps to reduce mycotoxins exposure, but also improves nutrition (WHO, Mycotoxins, 2022).

Innovative food processing methods such as ultraviolet radiation, pulsed light, electrolyzed water, ozone, electron beam, and gamma radiation represent effective means in combating aflatoxins while simultaneously preserving the quality of food and plant-based raw materials. Additionally, inoculation with beneficial microorganisms such as strains of *Pseudomonas, Bacillus*, and *Trichoderma* has proven effective in reducing the presence of pathogenic fungi like *Aspergillus flavus* prior to harvest. The use of various chemical agents aimed at deactivating, binding, or eliminating aflatoxins includes substances such as propionic acid, ammonia, copper sulfate, benzoic acid, citric acid, and other reagents that chemically interact with aflatoxins (Gowda et al., 2004).

CONCLUSION

In today's ecologically compromised living conditions, the issue of microbial contamination of food by pathogenic microorganisms and their toxins throughout the entire food chain (from farm to fork) is gaining increasing global importance. This is primarily aimed at protecting the health of humans and animals, safeguarding the economy and trade, and ensuring strict compliance with legal regulations.

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