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RESEARCH ARTICLE

Approaches in determining Aflatoxin B_1 in food materials using a range of analytical methods

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Abstract

Aflatoxins are the secondary metabolites produced by fungi under stress which suppress the primary metabolites inside the cell. *Aspergillus flavus* is the main factor for the production of Aflatoxin in common food which leads to the spoilage and contamination of foods, specifically in developing countries like Asia and Africa. Several methods for aflatoxin determination have been developed including thin layer chromatography (TLC), UV-Spectrometry, high performance liquid chromatography (HPLC), Fourier transform infrared (FT-IR) and high performance liquid chromatography-mass spectrometry (HPLC-MS). This review deals about each one of the techniques advantages and disadvantages.

Introduction

Mycotoxins are the secondary metabolites produced by important saprophytic and spoilage fungi that are associated with severe toxic effects to vertebrates. They are Aspergillus, Penicillium, Fusarium and Alternaria species these are associated with severe toxic effects to vertebrates. Approximately 400 compounds are recognized as mycotoxins of which only a few are addressed by food legislation. In 1960, ten thousand young turkeys on poultry farms in England died due to "Turkey X disease". This is mainly because of the consumption of contaminated peanut meal and it was found that this peanut meal was highly toxic to poultry and ducklings. The nature of the toxin suggested that it might be of fungal origin. Later, the toxin producing fungus was identified as Aspergillus. The toxins are groups of polyketide-derived furanocoumarins. There are at least 16 characterized structurally related aflatoxins, but for now, there are only four major aflatoxins, B_1, B_2, G_1, and G_2 (AFB_1, AFG_1, AFB_2, and AFG_2). *Aspergillus flavus* produces AFB_1 and AFB_2 whereas *Aspergillus parasiticus* produces AFB_1, AFG_1, AFB_2, and AFG_2. Some other species that produce aflatoxins are *Aspergillus nomius*, *Aspergillus pseudotamarii*, *Aspergillus bombycis*, *Aspergillus ochraceoroseus*, *Aspergillus nominus* [1]. Aflatoxins are the most toxic and carcinogenic compounds among the existing mycotoxins. The growth of aflatoxin producing Aspergillus species depends on a substrate and environmental factors, such as water activity, temperature, pH and microbial competition. As a result, *A. flavus* and *A. Parasiticus* are considered as xerophilic since they can grow at low water activities (aw 0.75-0.8). Both these fungi can grow in a temperature range from 12ºC to 48ºC, the best conditions for aflatoxin growth is around 25ºC. The produced aflatoxins can be found in a diverse range of products either in the field of pre-harvesting, storage or post harvest. However, higher level of aflatoxin contamination is mainly associated with the post - harvest growth of Aspergillus molds in poorly stored commodities [2]. Aflatoxin concentrations in the mg/kg range have been detected. The food items that have been reported to contain aflatoxins are cereals such as corn, barley and oats, dried fruits such as figs, nuts and oilseeds such as peanuts and cotton seeds as well as spices such as pepper, paprika or chili. However, corn and peanuts are the most commonly contaminated food items worldwide. There are several reviews on the occurrence of aflatoxins. Which clearly show that the occurrence of

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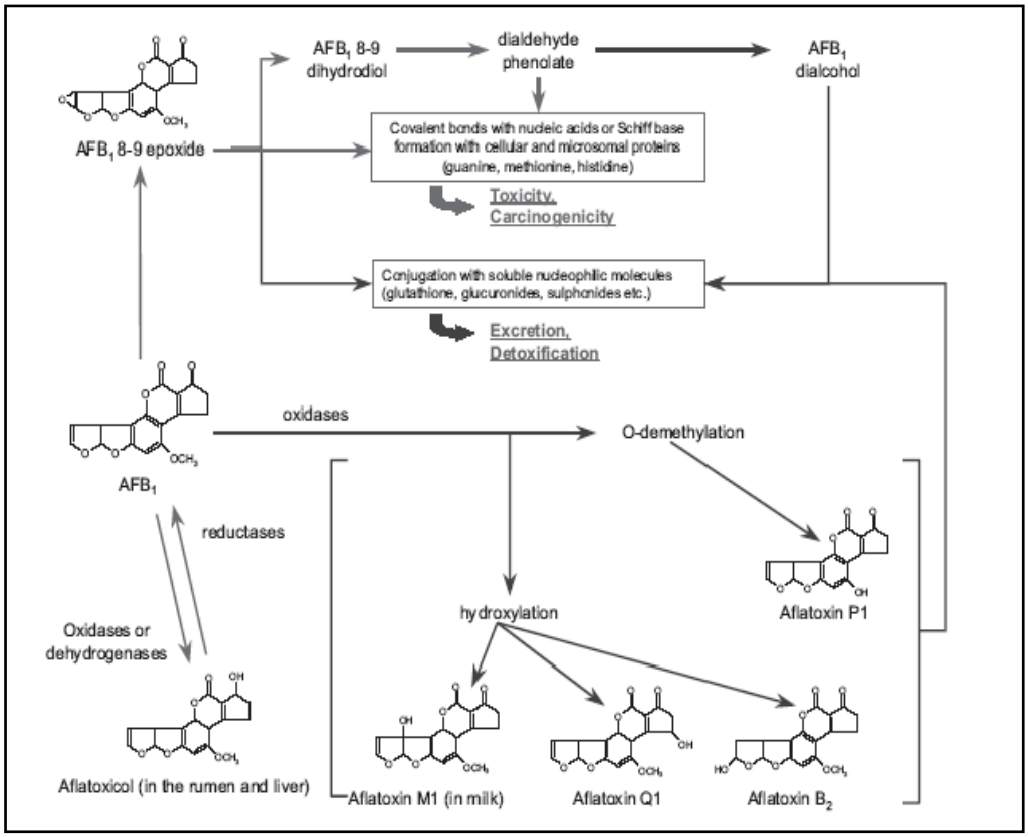
aflatoxins in food and feed is still a relevant issue in food safety [3-4].

Aflatoxin B_1 is considered as more toxic by the International Agency for Research on Cancer (IARC). Chemistry of the aflatoxins: Aflatoxins are di-Furano coumarins with AFB_2 and AFG_2 as individual derivatives of AFB_1 and AFG_1. The biosynthesis pathway of aflatoxins has been postulated as follows: norsolorinic acid → averufin → versiconalacetate → versicolorin-A → sterigmatocystin→ AFB_1 → AFG_1. The toxicity of the aflatoxins decreases from AFB_1 →AFG_1 → AFB_2 → AFG_2, which is an indicator that the double bond at the 8, 9-position of the terminal furan ring is a key factor for the toxicity of the toxin [5]. Aflatoxins are heat stable compounds and normally do not easily degrade during common food or feed processing [6-8]. Many researchers have studied the pathway of aflatoxin biosynthesis and they have devised strategies to intervene its production so that its contamination can be reduced. Here in this work we aim to provide an overview and improve the different methodologies to detect and quantify aflatoxin B_1 in the food analysis field.

Formation of DNA adduct the aflatoxins are among the most potent genotoxic agents among the existing mycotoxins known. AFB1 induces chromosomal aberrations, micronuclei, sister chromatid exchange, unscheduled DNA synthesis, chromosomal strand breaks, and forms adducts in human cells [9-12]. Aflatoxin containing an unsaturated terminal furan ring, which binds covalently with the DNA and leads to the formation of epoxide. The specific role of cytochrome P450 is, it metabolises aflatoxin to 8,9-exo-epoxide and 8,9-endo-epoxide respectively. The reaction of AFB1–8,9-exo-epoxide with DNA strongly induces adduct formation. AFB1–8,9-exo-epoxide appears to be the only product of AFB1 involved in reaction with DNA in the N7 guanine position. The imidazole portion of the formed AFB1–N7-Guanine adduct gives rise to a ring opened formamidopyrimidine (AFB1–FAPY) and the other minor N7-guanyl adducts can happen through enzymatic oxidation of AFP1 and AFM1[12-14]. The exception is AFQ1, which is comparatively poor substrate for epoxidation to occur and presenting very low potency for DNA binding (Croy, R.G and Wogan, G.N., 1981; Yu, M.W et al., 1997 and Theo, K. B et al., 2000).



Aspergillus species are the common fungi isolated in this study. The prevalence of *Aspergillus* species in these stored food samples is the major factor for the high level of aflatoxin detected in the food sample. AFB_1 is a carcinogenic secondary metabolite of fungi produced by *A. flavus* and *A. parasiticus*, found in a wide range of agricultural commodities. There are quite a few methods used for its detection like thin layer chromatography, liquid chromatography, gas chromatography, high performance liquid chromatography, etc. These instrumentations have limitations in terms of sensitivity and time duration of the test. At the beginning the only separative method was GC, nevertheless, it is restricted to a small set of molecules.

The analyzed results of the sugar cane samples for mycotoxins, showed the prevalence AFB_1in the sugar cane infected with the fungus. The amount of AFB_1 detected in the sugar cane sample was quite higher (5µg/g). These results showed the levels of AFB_1 contamination in the fungal infected sugarcane sample exceeded the maximum AFB_1 residue limit of 30µg/kg permitted in Indian foods. The maximum AFB_1 concentrations allowed for human consumption ranged from 5 to 50 ppb. This level varies from country to country [13]. The ample moisture content allows the toxigenic fungi to produce high level of AFB_1 in the food sample. The results obtained is similar to that of Adebayo-Tayo et al. (2006)

The chloroform extract from sugar cane sample showed the presence of AFB_1 spot on TLC. The presence of AFB_1 was also confirmed by comparing with standard AFB_1 on HPLC along with the sample. Thin layer chromatography is used to analyze agricultural products and plants. It has advantages as: simplicity of operation, detection, confirmation with standard, able to repeat detection and quantification and cost effectiveness analysis, because many samples can be analyzed on a single plate with low solvent usage, and the time that TLC employs to analyze the sample is less that LC method. Because of the advantages of this method, researches have been focused to develop new techniques to improve the methodologies for quantification of aflatoxins for food analysis and quality control. Applications of TLC have been reported in areas of food composition, additives, adulterants, contaminants, etc. HPLC, it is one of the most common methods to detect and quantify of aflatoxins in food. It is coupled with the UV absorption, fluorescence, mass spectrometry and amperometric detectors. Elizalde-González et al. (1998) analyzed AFB_1, AFB_2, AFG_1 and AFG_2 based on HPLC and amperometric detection, and reported that it is possible to detect 5 ng of all four aflatoxins. It has been reported that aflatoxins has a maximum absorption at 360 NM with a molar absorptivity ranging 20,000 cm^2/mol. There are several techniques that use chromatography for aflatoxin analysis in food. Commonly the quantification of the aflatoxins is made by a fluorescence detector that takes advantage of the fluorescence properties of aflatoxins under determined wavelength. As a result, researchers are focused on improving these fluorescence properties to develop more sensitive methods than the commonly used so far. Currently techniques such as pre-column derivatization and post column derivatization are commonly used to improve aflatoxins fluorescence properties. They also have a cleanup stage to obtain a more pure sample, permitting a better quantification. Some of the common methods used in the cleanup stage are immunoaffinity column and solid phase extraction [15]. But, even if AFB_1 could be detected by UV absorption, the sensitivity is not so sufficient to detect compounds at parts per billion (ppb) levels required for food analysis [16]. The detection limit of UV detector can reach only up to the micro molar range [17]. To overcome this kind of limitation, UV detectors have to be coupled with the high performance liquid chromatography.

Conclusion

Determination of aflatoxins has been carried out using TLC, HPLC, LC–MS and immunological methods. Each one of the techniques has advantages and disadvantages. The search for sample preparation methods that allow fast extraction, good accuracy and precision, low extraction of interferences, low consumption of solvents will continue together with the increase in detection techniques with higher accuracy and sensibility. So, the determination of aflatoxins in foods will continue to be developed and improved.

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