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Detoxification of aflatoxin B_1 by irradiation and techniques of detection

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ABSTRACT

Aflatoxins constitute secondary metabolites produced by some Aspergillus species. These chemicals can contaminate a number of crops bound to human consumption. Numerous strategies for the detoxification or inactivation of aflatoxins contaminated feed- stuffs have been used. Detoxification of aflatoxin B_1 ($AFB_{1)}$ by irradiation is one of the economical and practical methods exists. In this review, the effect of different irradiation techniques including UV, gamma and microwave on aflatoxin structure is discussed. Also different quantitative and qualitative techniques for detection of aflatoxin including biological activity test, mass spectrometry, infrared spectrometry, HPLC analysis, thin-layer chromatography and UV spectral analysis are reviewd.

KEYWORDS

AFB_1;
Irradiation;
Detoxification;
Mycotoxin structure.

INTRODUCTION

Aflatoxins are a group of mycotoxins with mutagenic, carcinogenic and immunosuppressive properties[4]. Aflatoxins constitute secondary metabolites produced by *Aspergillus flavus, Aspergillus parasiticus* and *Aspergillus nomius*[7]. These chemicals can contaminate a number of crops bound to human consumption, for example, corn, peanut, sorghum, rice, wheat, and nut[8].

The most toxic compound is aflatoxin B_1 (AFB_1)[23]. When AFB_1 and AFB_2 contaminated food or feed is consumed, the toxins are metabolized to AFM_1 and AFM_2 and excreted into the tissues, biological fluids, and milk of lactating animals, including breast milk[9].

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. Duck and Voorde (1980) observed that autoclaving at 120°C for up to 1h did not destroy this mycotoxin, and even after sterilization in an acid or alkaline medium, slight mutagenic activity is still detectable[19]. Viroben et al. (1976) inactivated aflatoxin by treatment of gaseous ammonia under pressure of 2 to 3 bars[10]. Mukendi et al. (1991) observed that AFB_1 was transformed by treatment of sodium sulfite into a more prominent toxic metabolite AFB_1 epoxide and subsequently into a detoxified trihydroxy AFB_1 derivative[15]. Goncalez et al. (2003) observed that AFB_1 was inactivated by chlorine gas treatment. The chlorine gas treatment of 4 ml corresponding to 15 mg of pure chlorine gas was resulted into 90 % loss of AFB_1 and B_2 present in various alcoholic extracts of *P.sandhifolia* leaves using Vero cell lines[7].

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IRRADIATION

Radiation is classified into two categories: ionizing and non-ionizing. In ionizing radiation (X-rays, gamma ray, ultraviolet ray) potential changes may occur in molecules of the irradiated mycotoxin. These molecular changes might be quite harmful to living organisms exposed to large doses of ionizing radiation. Non-ionizing radiation (radio waves, microwaves, infrared waves, visible light) in sufficient intensity leads to a rise in temperature usually accompanied by molecular changes that are not usually of hazardous nature to man. Despite the debate on safety of irradiated foods in connection with human health the food irradiation is becoming a technique of potential application on a commercial scale to render food products sterile[6].

UV irradiation

Visible light and UV light are also able to stop biological activity, but these sources of radiation have a low penetration capacity in solids and liquids. Shiva and Gawade (2010) used UV radiation for destruction of AFB_1[13]. Aflatoxins are sensitive to UV radiation. AFBl absorbs UV light at 222, 265 and 362 nm with maximum absorption occurring at 362 nm which may lead to the formation of up to 12 photodegraded products[24]. AFB_1 and AFG_1 underwent photochemically driven series of reactions when exposed to UV light (365 nm, 1h) on silica gel TLC plates. The photodegradation products were less toxic to chick embryos than the parent toxins[17].

Gamma irradiation

Gamma irradiation has been established as a safe and effective physical means for microbial decontamination, disinfestation, shelf-life extension and improvement of quality attributes of raw and processed agricultural commodities. Ionizing radiations have also been proved effective in improving the overall nutritional attributes, including some desired changes in functional properties of seed flours. However, application of this treatment may lead to change in the physical and chemical properties of grains[1]. Umesh et al., 1989, inactivated AFB_1 by using the synergistic effect of hydrogen peroxide and gamma radiation[5]. Van dyck et al.(1982) used gamma irradioation for destruction of AFB_1[12]. The toxicity of a peanut meal contaminated with AFBl was reduced by 75% and 100% after irradiation with gamma rays at a dose of 1 and 10 kGy[18]. Doses higher than 10 kGy inhibited the seed germination and increased the peroxide value of the oil in gamma-irradiated peanuts[21].

Microwave irradiation

Microwave heating reduced the aflatoxin content considerably in contaminated material[20]. There has been considerable controversy over non-thermal effect of microwave radiation. Non-thermal effect of microwave radiation was suggested to have an important role in the inactivation of microorganisms in suspension[16, 25].

At present, no economical and practical method exists for removing aflatoxin from foodstuffs for human consumption. For animal feed, ammonia vapors at high temperatures have been used for destruction of aflatoxin[2], and extraction by means of methoxy methane is also efficient for this purpose, but both methods are expensive. Therefore, gamma irradiation may be more attractive for mycotoxin decontamination of feed and foodstuffs.

Techniques for detection of AFB_1 transformation

Modern technologies used for the detection of mycotoxins include mass spectrometry-based assays, ambient ionization mass spectrometry, electrochemical immunoassays, piezoelectric sensors, enzyme inhibition assays, biosensor arrays, and fluorescence polarization immunoassays. However, it is impossible to use one single method for analysis of mycotoxins due to the variety of chemical structures. Therefore, many analytical methods have been developed and validated. The demand for a fast, simultaneous and accurate determination of multiple mycotoxins, along with the heterogeneity of food matrices, creates extreme challenges for routine analysis[11].

Biological activity test

Ames microsome mutagenicity test is used for demonstrating the disappearance of biological activity. This test is generally done on *Salmonella typhimurium* as the test strain by plate incorporation assay method.

Infrared spectroscopy

Molecular analyses with Fourier transform infrared

spectroscopy (FT/IR) technique with attenuated total reflectance (ATR) and chemometrics enable to detect structural features on a molecular basis. To investigate differences in aflatoxin molecular composition and functional group spectral intensities after any intervention and decomposition technique, we can use this technique which shows any probable transformation. Infrared absorption spectroscopy, broadly applied to analyse polymer structures (within the spectral wavenumber range of 400-4000 cm-1), is also employed as a method of studying AFB_1 structure and it's changes.

Mass spectrometry

Although the selectivity of mass spectrometry is unchallenged if compared to common GC and LC detection methods, accuracy, precision, and sensitivity may be extremely variable concerning the different mycotoxins, matrices, and instruments. The sensitivity issue may be a real problem in the case of LC/MS, where the response can be very different for the different ionization techniques (ESI, APCI, APPI). Therefore, when other detection methods (such as fluorescence or UV absorbance) can be used for the quantitative determination, LC/MS appears to be only an outstanding confirmatory technique. In contrast, when the toxins are not volatile and do not bear suitable chromophores or fluorophores, LC/MS appears to be the unique method to perform quantitative and qualitative analyses without requiring any derivatization procedure. The problem of exact quantitative determination in GC/MS and LC/MS methods is particularly important for mycotoxin determination in food, given the high variability of the matrices, and can be solved only by the use of isotopically labeled internal standards or by the use of ionization interfaces able to lower matrix effects and ion suppressions. When the problems linked to inconstant ionization and matrix effects will be solved, only MS detectors will allow to simplify more and more the sample preparation procedures and to avoid clean-up procedures, making feasible low-cost, high-throughput determination of mycotoxins in many different food matrices[22].

Thin-layer chromatography analysis

Although TLC is a reference method, it is often used as a mycotoxin screening assay. TLC is a very powerful tool to determine the presence of one or more mycotoxins in a sample, but does not permit critical quantitation. Typically, TLC involves the spotting of extracts, individually, near one end of a glass or aluminium plate on which a thin layer of silica gel or similar matrix has been placed. Suitable standards are also spotted on the plate for comparison after the plate has been developed. During development, the edge of the plate nearest the location of the spotted extracts and standards is placed in a specified solvent preparation covering the bottom of a tank that allows the plate to stand nearly vertical. The solvent is adsorbed by the silica or similar matrix and travels up the plate through the spotted extracts and standards. As this occurs, the various compounds in an extract spot are separated, depending on their adsorption to the matrix and solubility. Because these properties vary, the compounds are deposited at different heights on the plate. The plate can be removed from the tank when the solvent front nears the top of the plate, dried, and the spots can be visualized. So the effect of destruction of AFB_1 would be clearly visible on thin-layer chromatography plates. The chromatogram obtained from silica gel-coated plates shows AFB_1 if broken down into unidentified components which remain still fluorescent[3].

HPLC analysis

AFB_1 can be identified by confirming its retention time with standard AFB_1 by high-performance liquid chromatography (HPLC) technique. HPLC is the most frequently and widely used method of mycotoxin analysis. HPLC reference methods that are quite sensitive and have reasonably low levels of detection have been developed for most of the major mycotoxins; thus, these are good quantitative methods[3].

UV spectral analysis

Optical density measurements at 200 to 400 nm of control and photooxidised samples of aflatoxins can be performed spectrophotometrically by comparison of UV profile for changes in the absorbance and wave length.

CONCLUSION

At present, the only economical and practical method for removing aflatoxin from foodstuffs is irra-

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diation. Ionizing radiation can alter aflatoxin structure which can be detected by different techniques. Chromatographic analysis including HPLC and TLC can be used for quantitative detection of aflatoxin. By using infrared spectroscopy and mass spectrometry the structural features on a molecular basis can be determined.

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