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Photodegradation Kinetics of Aflatoxin B_1 in Solvent

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A photodegradation study of aflatoxin B_1 (AFB_1) in acetonitrile solution was performed under UV irradiation at different aflatoxin B_1 initial concentrations, UV irradiation intensities and wavelengths. The effect of UV intensity and wavelengths on the aflatoxin B_1 photodegradation ratio is dominative, comparing to aflatoxin B_1 initial concentration. The photodegradation of aflatoxin B_1 was proved to follow first-order reaction kinetics ($R^2 \geq 0.99$). The aflatoxin B_1 photodegradation rate under various conditions was lower following the order: $v_{(800\ \mu w/cm^2)} > v_{(400\ \mu w/cm^2)} > v_{(200\ \mu w/cm^2)}$ and $v_{(UV\text{-}C)} > v_{(UV\text{-}A)} > v_{(VIS)}$. This study reports the photodegradation behaviour-kinetics in solvent model, providing clues to the study of the photodegradation mechanism of aflatoxin B_1 in real food medium and the UV method applied in practical decontamination of aflatoxin B_1 in food.

Key Words: Aflatoxin B_1, Photodegradation, UV, Kinetics.

INTRODUCTION

Aflatoxins, a group of highly toxic, mutagenic and carcinogenic compounds, are secondary metabolites of *Aspergillus flavus* and *Aspergillus parasiticus*, which are found worldwide in air and soil to infest both living and dead plants and animals[1]. Among the various aflatoxins species, aflatoxin B_1 (AFB_1, Fig. 1) is the most potent teratogen, mutagen and hepatocarcinogen, which is classified as a group 1 carcinogen by the International Agency for Research in Cancer (IARC)[2,3]. Aflatoxins, occur in many countries, especially in tropical and subtropical regions where conditions of temperature and humidity are optimum for growth of the molds and for production of the toxin. Many agricultural commodities and important crops, especially peanuts and peanut-based foods, are susceptible to such contamination. Preventing the contamination of food by the toxigenic fungi, the most rational and economic approach to avoid the potential hazards, is not always possible under certain agronomic storage practices. Therefore, removal or inactivation in salvaging food and feedstuff already contaminated with toxic fungal metabolites is a major concern[1].

Various physical, chemical and biological approaches are available for the detoxification of aflatoxins[1,4,5]. UV irradiation has been discovered for many years as an effective physical method to destroy aflatoxins for its photosensitive property[6], but hardly any report about the photodegradation behaviour, which restrict the development of the photodegradation for the detoxification of aflatoxins. Recently, advanced photochemical transformation has emerged as a powerful method for degrading and transforming the photosensitive materials into harmless substances[7-9], such as in drinking water[10] or pesticide residues in foods[11]. But the exact nature and the degradation behaviour of the photodegradation products become the major apprehensiveness of this advanced photochemical detoxification method.

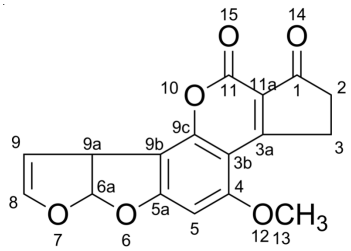

Fig. 1. Molecular structure of aflatoxin B_1

In our earlier work part of the photodegradation products of aflatoxin B_1 in acetonitrile solution were identified[12]. The aim of this study was to study the photodegradation behaviour-kinetics and photodegradation products in solvent model, providing clues to the study of the photodegradation mechanism of aflatoxin B_1 in real food medium and the UV method applied in practical decontamination of aflatoxin B_1 in food.

EXPERIMENTAL

Aflatoxin B_1 (2,3,6α,9α-tetrahydro-4-methoxycyclopenta [c] furo [2,3:4,5] furo [2,3-h]chromene-1,11-dione; purity >

98 %) was obtained from Fermentek (Jerusalem, Israel). HPLC-grade acetonitrile and benzene were purchased from Sigma (St Louis, MO, USA).

For the UPLC-Q-TOF MS studies, deionized water (18-MO cm^{-1} resistivity) was obtained from a Milli-Q SP Reagent Water system (Millipore, Bedford, MA, USA) and prefiltered through a 0.2 μ membrane. Acetonitrile was Optima LC-MS-grade from Fisher Scientific (CA, USA).

Standard stock solutions (200 mg/L) of aflatoxin B_1 were prepared in benzene-acetonitrile 98:2 (v/v) and stored at 4 ± 2 ºC in a refrigerated dark room (stability of stock and standard solutions under these conditions was checked and demonstrated for at least 3 months). Immediately before use, 0.5 mL standard stock solutions were placed in glass tubes and dried under a jet of nitrogen. Working solutions of aflatoxin B_1 with the initial concentration 0.2, 2 and 10 ppm were prepared by adding 500, 50 and 10 mL acetonitrile, respectively.

Photodegradation procedure: For degradation experiments, acetonitrile solutions of pure aflatoxin B_1 were placed in quartz vessels and irradiated at 4 ± 2 ºC under ultraviolet lamp (NatureGene Corp., USA) at different intensities and wavelengths ranging from 220-400 nm (the schematic drawing of the illumination is shown in Fig. 2 and the various UV intensity was made by changing the distance between the lamps and the dark slide which is fixed with the sample box as a single device). The intensity reaching the acetonitrile solutions surface was measured by means of a UV intensity detector and the depth of the solvent is less than 1 cm (Beijing Normal University Photoelectric Instrument Factory, Beijing, China).

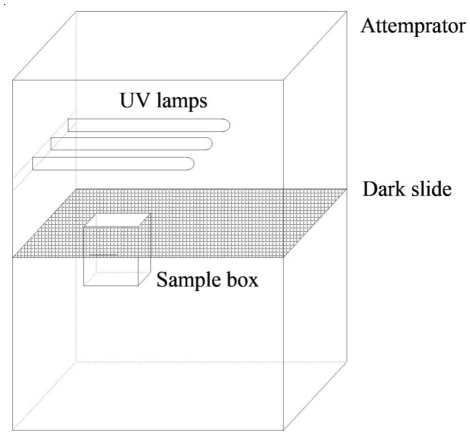

Fig. 2. Schematic drawing of the illumination of photodegradation

Meanwhile, control experiments in the dark (blank experiments) under the same conditions were carried out in parallel for comparison without the application of light on aflatoxin B_1. At selected time intervals, samples were collected and quantitatively analyzed directly by UPLC-Q-TOF MS for the amount of the compound of interest remaining in the solution after irradiation based on external calibration.

Instrumental analysis and quantification: UPLC was performed on a Waters (Milford, MA, USA) Acquity UPLC system equipped with a binary solvent-delivery system and an autosampler. Chromatography was performed on a 10 cm × 2.1 mm, 1.7 μm particle, Waters Acquity C_{18} column. The injection volume was 2 μL. The mobile phase was a gradient prepared from acetonitrile (component A) and 0.1 % formic acid aqueous solution (component B). Elution started with 8 % A for 0.1 min then the proportion of A was increased linearly to 30 % at 10 min and then to 100 % at 15 min and change back to 8 % A at 15.1 min. Total run time, including conditioning of the column prior to the initial conditions, was 17 min. The flow rate was 500 μL min^{-1}.

Mass spectrometry was performed on a Waters Synapt Q-TOF system. Compounds were analyzed in the positive-ion (PI) mode. The optimized conditions were: desolvation gas 500 L/h at a temperature of 420 ºC, cone gas 50 L/h, source temperature 120 ºC and capillary and cone potentials 3000 and 30 V, respectively. The Q-TOF instrument was operated in the wide pass quadrupole (V) mode and data were collected between m/z 50 and 1000, with a scan accumulation time of 0.2 s. The MS-MS experiments were performed using collision energy of 25 or 30 eV, which was optimized for each compound. To ensure accuracy and reproducibility, all analyses were acquired using an independent reference spray *via* the Lock Spay interface; Tyr-Gly-Gly-Phe-Leu was used as lock mass (m/z 556.2771) under positive ion conditions. The Lock Spray frequency was set at 6 s, meaning that every 5 s flow from the Lock Spray was introduced into the mass spectrometer for 1 s, thus giving the software the possibility of performing ongoing correction of the exact mass of the analyte. Data for the reference compound were averaged over ten spectra min^{-1}. The accurate mass and composition for the precursor and fragment ions were calculated using the MassLynx 4.1 software supplied with the instrument. The software has a feature that calculates all possible elemental composition from the accurate mass and then by using previous knowledge, such as low i-FIT (Norm), type and number of atoms, various impossible formulae can be further ruled out. Therefore, Q-TOF system is a powerful tool for forming hypotheses about the identity of an unknown compound. Final identification can then be performed on the basis of accurate measurement of the mass of the parent ions and the fragments obtained in MS-MS experiments.

Calculation methods and reproducibility: The experiments were made at least in triplicate and the analytical methods were applied at least in triplicate. The calculation and statistical methods used are available in the program Origin 8.0.

RESULTS AND DISCUSSION

Effect of initial concentration on degradation of aflatoxin B_1: The effect of initial concentration on the degradation of aflatoxin B_1 by UV irradiation is presented in Fig. 3. The initial concentrations of aflatoxin B_1 were 0.2, 2.0 and 10.0 ppm, respectively and there is no detectable changes shown in blank experiment with different concentration (results exemplified by that pertaining to an initial concentration of 0.2 ppm shown in Fig. 3). Therefore, the declines observed in the degradation curve arise from the photodegradation process. It can be seen that no significant difference in the three degradation curves, indicating that the effect of the initial concentration in the selected range on the aflatoxin B_1 photodegradation is nearly inexistent, which is in agreement with the feature of the first-order kinetics model[13,14].

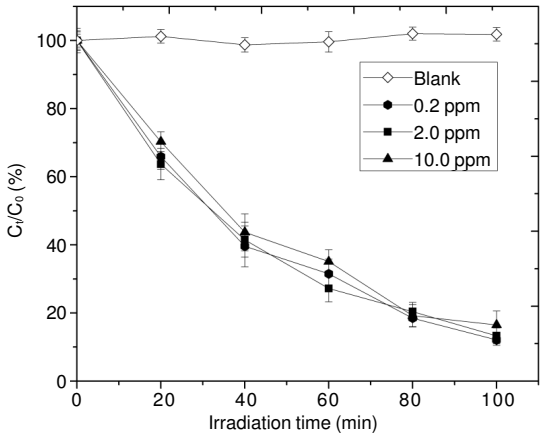

Fig. 3. Photodegradation curve of aflatoxin B_1 at different initial concentration

Effect of UV intensity on degradation of aflatoxin B_1: Significant changes among each degradation curves of aflatoxin B_1 at different UV intensities are presented in Fig. 4. The initial concentration of aflatoxin B_1 was 2 ppm and UV intensities corresponding to the curves were shown in Fig. 4 as an insert. Quantitative recoveries from blank experiments sampled over the entire exposure period of simulated UV irradiation showed that aflatoxin B_1 did not undergo dark reaction, thus the decline observed in the degradation curve should attributed to the photodegradation process. It can be seen that after 100 min UV irradiation, 50, 80 and 97 % aflatoxin B_1 had been degraded nearly at the intensities of 200, 400 and 800 μw/cm^2, respectively. Therefore, photodegradation rate of aflatoxin B_1 is strongly affected by UV intensity.

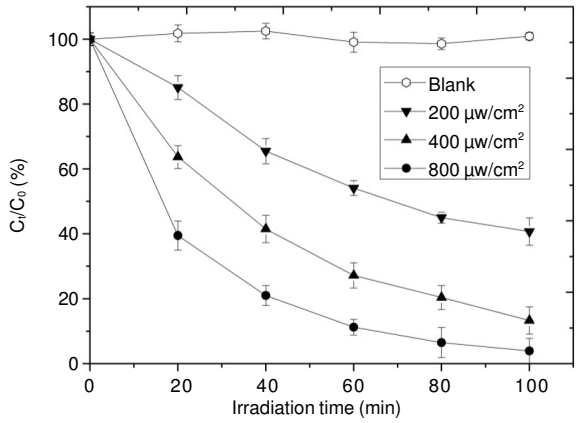

Fig. 4. Photodegradation curve of aflatoxin B_1 at different UV intensities

Effect of UV wavelengths: Fig. 5 shows the photodegradation curves of aflatoxin B_1 with the initial concentration of 2 ppm and exposed to VIS, UV-A and UV-C wavelengths. In white fluorescent light, the toxin degraded slowly and in the 100 min experiment only 28 % lost. In the UV-exposed samples, a significant decrease in the toxin concentration was observed. The total loss of aflatoxin B_1 exposed to UV-A and UV-C after 100 min was 71.1 and 90 %, respectively. It is known that aflatoxin B_1 is an UV sensitive compound and the shorter the UV wavelength is, the stronger the energy is[6,13-15]. For this reason, the photodegradation ratio of aflatoxin B_1 follows the order of $v_{(UV-C)} > v_{(UV-A)} > v_{(VIS)}$, which is in agreement with the study of Mazur-Marzec[16].

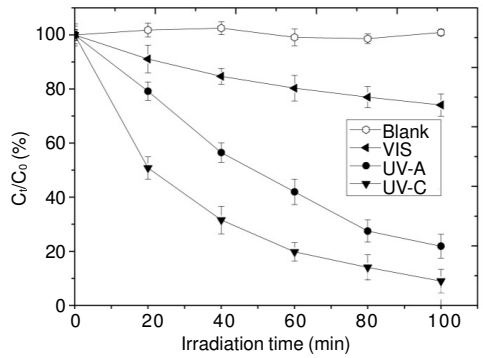

Fig. 5. Photodegradation curve of aflatoxin B_1 at various wavelength of light

Photodegradation kinetic of aflatoxin B_1: The plot of ln (C_t/C_0) *versus* irradiation time for aflatoxin B_1 at different conditions were not shown here, while the deduced parameters were listed in Table-1. The linear relationship between ln (C_t/C_0) and irradiation time indicated that the degradation followed a first-order kinetics well ($R^2 \geq 0.99$), given by the equation: $C_t = C_0e^{-kt}$ where C_0 and C_t are the concentrations at times 0 and t, t is the irradiation time and k is the first-order rate constant. The half-life $t_{1/2}$ of aflatoxin B_1, the time required for its concentration to fall to half its initial value, is related to the rate constant by the equation: $t_{1/2} = \ln 2/k$. It can be seen in Table-1 that the values of k and $t_{1/2}$ are nearly the same of different initial aflatoxin B_1 concentration and they have no connection with the aflatoxin B_1 initial concentration. However, the values of k and $t_{1/2}$ in Table-1 show obvious changes under different UV intensity and wavelength and the aflatoxin B_1 photodegradation rate was lower following the order: $v_{(800\ \mu w/cm^2)} > v_{(400\ \mu w/cm^2)} > v_{(200\ \mu w/cm^2)}$ and $v_{(UV-C)} > v_{(UV-A)} > v_{(VIS)}$, illustrating a strong dependence on the UV intensity and wavelength of UV irradiation.

TABLE-1
KINETIC PARAMETERS OF AFB_1 AT DIFFERENT PHOTODEGRADATION CONDITIONS

Initial concentration (μg/mL)/UV intensity (μw/cm^2)/wavelenths	Equation	R^2	K (min^{-1})	$t_{1/2}$ (min)
0.2 μg/mL	$\ln C_t/C_0 = -0.02095t$	0.99769	-0.02095 ± 0.0004	33.1
2.0 μg/mL	$\ln C_t/C_0 = -0.02051t$	0.99819	-0.02051 ± 0.0004	33.8
10 μg/mL	$\ln C_t/C_0 = -0.01887t$	0.99386	-0.01887 ± 0.0006	36.7
200 μw/cm^2	$\ln C_t/C_0 = -0.00958t$	0.99492	-0.00958 ± 0.0003	72.3
400 μw/cm^2	$\ln C_t/C_0 = -0.02051t$	0.99819	-0.02051 ± 0.0004	33.8
800 μw/cm^2	$\ln C_t/C_0 = -0.03434t$	0.99327	-0.03434 ± 0.0011	20.2
VIS	$\ln C_t/C_0 = -0.0033t$	0.98377	-0.0033 ± 0.0002	210
UVA	$\ln C_t/C_0 = -0.01521t$	0.99671	-0.01521 ± 0.0004	45.6
UVC	$\ln C_t/C_0 = -0.0252t$	0.99332	-0.0252 ± 0.0008	27.5

Conclusion

The photochemical behaviour of aflatoxin B_1 in acetonitrile has been investigated. The effect of UV intensity and the UV wavelength is dominative, when compared with aflatoxin B_1 initial concentration. At the same time, the photodegradation of aflatoxin B_1 was proved to follow the first-order reaction kinetics well ($R^2 > 0.99$) and the photodegradation rate was lower following the order: $v_{(800\ \mu w/cm^2)} > v_{(400\ \mu w/cm^2)} > v_{(200\ \mu w/cm^2)}$ and $v_{(UV\text{-}C)} > v_{(UV\text{-}A)} > v_{(VIS)}$, illustrating a strong dependence on the intensity and wavelength of UV irradiation.

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