Available online at www.sciencedirect.com
ScienceDirect
Surface & Coatings Technology 201 (2007) 5733–5737
SURFACE & COATINGS TECHNOLOGY
www.elsevier.com/locate/surfcoat

Degradation of mycotoxins using microwave-induced argon plasma at atmospheric pressure

Bong Joo Park [a,b], Kosuke Takatori [a,b], Yoshiko Sugita-Konishi [b], Ik-Hwi Kim [c], Mi-Hee Lee [d,e], Dong-Wook Han [d,e], Kie-Hyung Chung [f], Soon O. Hyun [f], Jong-Chul Park [d,e,*]

[a] The United Graduate School of Veterinary Sciences, Gifu University, 1-1 Yanagido, Gifu, Gifu 501-1193, Japan
[b] Division of Microbiology, National Institute of Health Sciences, 1-18-1 Kamiyoga, Setagaya, Tokyo 158-8501, Japan
[c] Division of Pharmacognosy and Phytochemistry, National Institute of Health Sciences, 1-18-1 Kamiyoga, Setagaya, Tokyo 158-8501, Japan
[d] Department of Medical Engineering, Yonsei University College of Medicine, 134 Shinchon-dong, Seodaemun-gu, Seoul 120-752, South Korea
[e] Brain Korea 21 Project for Medical Science, Yonsei University College of Medicine, 134 Shinchon-dong, Seodaemun-gu, Seoul 120-752, South Korea
[f] Physico-Technology Laboratory, Korea Accelerator and Plasma Research Association, Jadung-ri, Seo-myun, Cheorwon-gun, Gangwon-do, South Korea

Available online 22 August 2006

Abstract

Mycotoxins are toxic secondary metabolites of fungi causing health problems in humans, animals and agricultural products. Therefore, the inactivation or degradation of mycotoxins in contaminated foods and feedstuffs is a major global concern. This study was designed to investigate the degradation of three different mycotoxins, aflatoxin B1 (AFB1), deoxynivalenol (DON) and nivalenol (NIV) by using our self-designed microwave-induced argon plasma system at atmospheric pressure. After plasma treatment, the remnants of mycotoxins were analyzed by thin-layer chromatography and high performance liquid chromatography and their cytotoxicity was assessed using mouse macrophage RAW264.7 cells. The mycotoxins, AFB1, DON, and NIV were completely removed after 5 s of plasma treatment. Moreover, the cytotoxicity of mycotoxins was significantly reduced with the progress in the treatment time. These results suggest that this plasma system may have strong potentials to degrade mycotoxins and can be effectively used in the process of foods and feedstuffs.
© 2006 Elsevier B.V. All rights reserved.

PACS: 52.50 Sw; 52.70-m
Keywords: Mycotoxin; Aflatoxin B1; Deoxynivalenol; Nivalenol; Microwave-induced argon plasma

1. Introduction

Mycotoxins, such as aflatoxins, zearalenone, deoxynivalenol (DON, vomitoxin) and fumonisins, are secondary metabolites of fungi. They exhibit properties of acute, sub-acute or chronic toxicities in animals and/or human, also being carcinogenic, capable of causing mutations in susceptible organisms and teratogenic, capable of causing deformities in developing embryos. Moreover, they cause loss of viability of the seeds and reduce the quality and acceptability of all type of products, limit the storability and decrease the nutritional quality of the foods. Therefore, they are world-wide serious problem for the public health, agriculture and economics [1–4].

Although the prevention of mycotoxin contamination in the field is the main goal of agricultural and food industries, the contaminations of various commodities by fungal species including *Fusarium*, *Aspergillus*, *Alternaria* and *Penicillium* and mycotoxins are unavoidable under certain environmental conditions [2]. Therefore, decontamination/detoxification procedures are essential in order to recuperate mycotoxin-contaminated commodities. Several strategies are available for the detoxification of mycotoxins. These can be classified as physical, chemical and biological approaches [5]. The physical and chemical methods made different degrees of success. Microbes

* Corresponding author. Department of Medical Engineering, Yonsei University College of Medicine, 134 Shinchon-dong, Seodaemun-gu, Seoul 120-752, South Korea. Tel.: +82 2 2228 1917; fax: +82 2 363 9923.
E-mail address: parkjc@yumc.yonsei.ac.kr (J.-C. Park).

0257-8972/$ - see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.surfcoat.2006.07.092

or their enzymes could be applied to mycotoxin detoxification; such biological approaches are now widely studied [6,7]. However, all of these methods have their own disadvantages as they undergo undesirable changes during the process for the detoxification of mycotoxins in the food and feedstuffs [6,7]. These situations led to the development of new techniques that are at least as effective as the established ones, and in some part have superior characteristics, such as short processing times, non-toxicity to its operator and medium preservation.

Recently, much attention has been paid to plasma treatment among the methods of removing or inactivating the dangerous materials. It has been used as a well-established technique in a number of processes, e.g., plasma cleaning, etching, coating and sterilizing [8–11]. This paper describes the efficiency of our self-designed microwave-induced argon plasma system at atmospheric pressure for the degradation of mycotoxins, such as afltoxin B1 (AFB1), DON and nivalenol (NIV), the most common to contaminate foods and feedstuffs.

2. Experimental details

2.1. Materials

All reagents, including three mycotoxins, AFB1, DON (3α,7α,15-trihydroxy-12,13-epoxytrichothec-9-en-8-one, vomitoxin), NIV (3α,4β,7α,15-tetrahydroxy-12,13-epoxytrichothec-9-en-8-one), were purchased from Sigma (St. Louis, MO). Silicagel plate (60 F254, 20 cm × 20 cm) for thin-layer chromatography (TLC) was purchased from Merck and Company (Whitehouse Station, NJ). For cytotoxicity test, cell counting kit-8 containing a highly water soluble tetrazolium salt [WST-8, 2-(2-methoxy-4-nitrophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2H-tetrazolium, monosodium salt], reduced to a yellow color formazan dye by mitochondrial dehydrogenase of viable cells, was purchased from Dojindo Laboratories (Kumamoto, Japan).

2.2. Microwave-induced argon plasma system

As previously described [9–11], a microwave-induced argon plasma system to generate plasma at atmospheric pressure was used in this study. Briefly, this system consists of a 2.45 GHz, waveguide-based, 1 kW magnetron power supply commonly used in a microwave oven, an applicator including a tuning section, which is required to reduce the reflected power, and the nozzle section made of quartz. The plasma generated at the end of a nozzle was formed by an interaction between the high electrical field, which is generated by the microwave power, the waveguide aperture and the gas nozzle. Argon was used as a working gas for this plasma system, which was chosen because of its inertness, and the gas flow rate was approximately 100 l/min at 8 kgf/cm^2. The intensity of UV light generated by plasma was measured indirectly by using a radiometer/photometer with a solar blind vacuum photodiode. The range was detected from 75 mW/cm^2 (minimum) to 102 mW/cm^2 (maximum) at a wavelength of 254 nm, which was close to that of our previous studies [9–11].

2.3. Mycotoxin degradation by microwave-induced argon plasma

The molecular structures of AFB1, DON, and NIV were shown in Fig. 1 [12,13]. For the degradation test, these mycotoxins were suspended in chloroform. The suspensions were inoculated onto slide glasses and allowed to dry at room temperature for 30 s. The inoculated slide glasses were placed in front of a nozzle and exposed to plasma for 1, 3, 5 and 10 s. After plasma treatment, the slide glasses were transferred into a screw cap glass vial containing 1 ml of chloroform, and thoroughly shaken for 60 s. The extracts were evaporated to dryness and dissolved in two different solutions, 1 ml of acetonitrile for determining the amount of mycotoxins and 1 ml of cell growth media for evaluating the cytotoxic effect of mycotoxins.

2.4. Mycotoxin detection by thin-layer chromatography and high performance liquid chromatography

TLC and high performance liquid chromatography (HPLC) were performed to determine the amounts of mycotoxins following plasma treatment. The extracts from the plasma-treated samples were spotted along with standards on TLC silicagel plates, developed in chloroform:acetone:2-propanol (93:5:2) as mobile phase for AFB1 or in chloroform:methanol (94:6) as mobile phase for DON and NIV. Afterwards, the plates were allowed to air-dry. For AFB1, the developed plate was heated for 10 min at 100–110 °C and then determined by blue fluorescence ultra-violet (UV) light (365 nm). For DON and NIV, the plate was heavily sprayed with 10% aluminum chloride in ethanol, heated for 10 min at 105 °C and then observed under UV light.

Confirmation and quantitative determination of the mytoxins were performed by HPLC according to the previously described

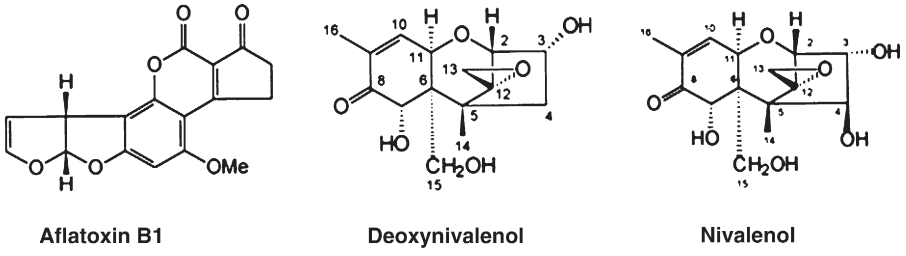

Fig. 1. Molecular structures of aflatoxin B1, deoxynivalenol and nivalenol.

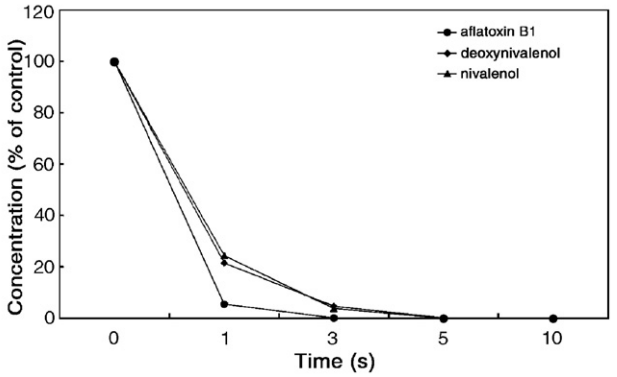

Fig. 2. Degradation effects of microwave-induced argon plasma at atmospheric pressure against aflatoxin B1, deoxynivalenol and nivalenol as assayed by TLC.

method [14]. The HPLC were performed by using a liquid chromatographer (Waters Co., Milford, MA) with a separation module (2695) and a photodiode array detector (2996) to provide confirmation of mycotoxin identity through the comparison of UV spectra with those of standards (Waters Co.). MassLynx, version V3.5 software (Waters Co.) was used to control the chromatograph and to process signal and data. The mobile phases were water:acetonitrile:methanol (55:30:15) for AFB1 and water:methanol:acetonitrile (90:5:5) for DON and NIV. The aliquot (10 μl) of the standard and sample solutions prepared was injected into the HPLC. The flow of mobile phases was 1.0 ml/min. AFB1 was detected and quantified by fluorescence detection at an excitation at 365 nm and emission at 430 nm.

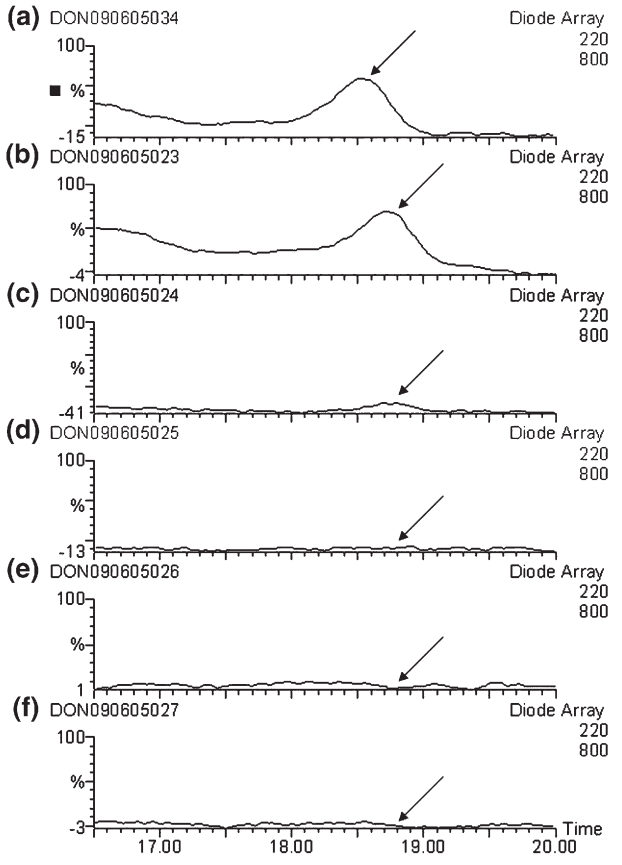

Fig. 3. HPLC chromatograms of deoxynivalenol (DON): standard (a) and DON treated with microwave-induced argon plasma at atmospheric pressure for 0 (b), 1 (c), 3 (d), 5 (e) and 10 (f) s. The arrow represents the UV spectrum of DON.

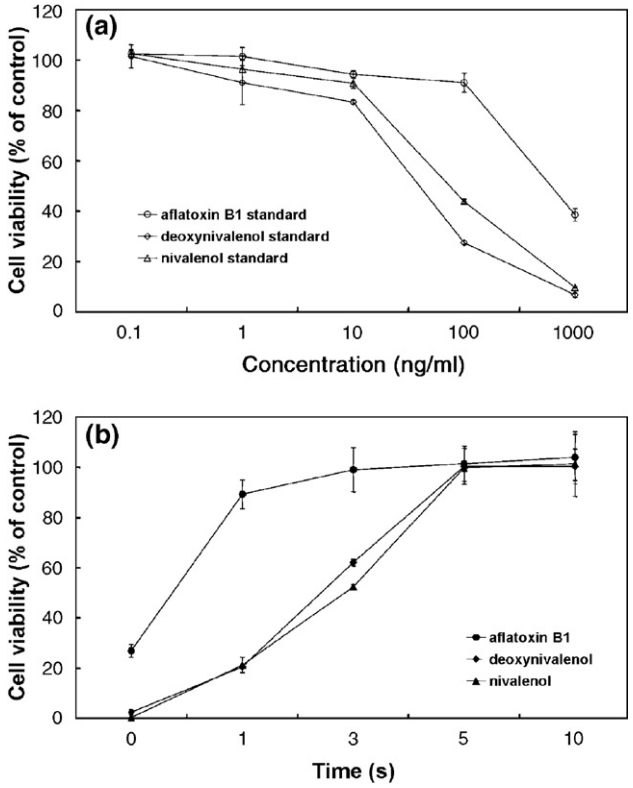

Fig. 4. Cytotoxicity of mycotoxins, such as aflatoxin B1, deoxynivalenol and nivalenol, to cultured mouse macrophage RAW264.7 cells. (A) mycotoxin standards and (B) plasma-treated mycotoxins.

2.5. Cytotoxicity of mycotoxins

In order to evaluate the cytotoxicity of mycotoxins following plasma treatment, mouse macrophage cell line, RAW264.7 (TIB-71, American Type Culture Collection, Rockville, MD) was routinely maintained in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum and a 1% antibiotic antimycotic solution (including 10,000 units penicillin, 10 mg streptomycin and 25 μg amphotericin B per ml) at 37 °C in a humidified atmosphere of 5% CO_2 in air. The cells were seeded in 96-well plate at 1.0×10^5 cells/well and then incubated for 24 h. After incubation, the extracts of mycotoxins in culture media were added and incubated for further 24 h. According to manufacturer's instruction, the cells were incubated with WST-8 in the last 4 h of the culture period tested at 37 °C in the dark. The absorbance was determined at 450 nm in an ELISA reader (Spectra Max 340, Molecular Device Co., Sunnyvale, CA). Parallel sets of plates received standard mycotoxins as the control.

3. Results and discussion

The most commonly encountered mycotoxins in foods and feedstuffs are aflatoxins, vomitoxin and zearalenone. They can be produced on a wide range of agricultural commodities and under a diverse range of situations. Due to their various toxic effects and their good thermal stability, the presence of mycotoxins in foods and feeds is potentially hazardous to the health of both humans and animals. They have been proven as cause of, or implicated in, mycotoxicoses of either animals or humans.

Various techniques have been devised to remove, destroy or suppress the toxicity of the mycotoxins. These techniques include physical removal of the contaminated portions of the foodstuffs [15], treatment with heat [16–19], chemicals [15,20–23] or radiation [24] in order to convert the toxins into relatively innocuous compounds or the addition of adjuvants [25–27] to suppress or otherwise mask the ill effects of toxins [28]. Although some workers have demonstrated the successful removal of toxins by these methods, these techniques are not always effective, since the mycotoxins can diffuse throughout the materials and are not associated exclusively with damaged, discolored or malformed seeds or materials. In addition, the technologies for removal can be labor- or equipment-intensive and, thus may not always be economically feasible [15–28].

In this study, the degradation effects of microwave-induced argon plasma at atmospheric pressure against mycotoxins, AFB1, DON and NIV, were investigated. TLC analysis revealed that plasma treatment resulted in a significant time-dependent decrease in the concentrations of AFB1 (R_F 0.79), DON (R_F 0.63) and NIV (R_F 0.48) (Fig. 2). The fluorescent spot of each mycotoxin showed that these mycotoxins were completely degraded within 5 s regardless of their types. These results were confirmed by HPLC analysis. Although DON and NIV were degraded relatively slowly compared to AFB1, plasma treatment resulted in a complete degradation of all the mycotoxins. The HPLC chromatogram of DON demonstrated that the UV spectrum of non-treated DON (Fig. 3b) had a peak of mountain shape like that of a standard (Fig. 3a), while the peaks of plasma-treated DON were rapidly decreased with the progress in the treatment time (Fig. 3c, d), and they were not detected after 5 s of treatment (Fig. 3e, f). The HPLC result of AFB1 or NIV showed the pattern similar to that of DON (data not shown).

The microwave-induced plasma system used in this study required much less exposure time for mycotoxin degradation than other methods, such as visible or UV light and gamma ray [15–24]. The UV irradiation and etching by plasma may be responsible for degrading and removing the mycotoxins [3,24]. This plasma system has many advantages, such as increased ionization by reactive species and relatively high intensity of UV light (75–102 mW/cm^2), low average temperature (75–130 °C) and easy operation.

As shown in Fig. 4(a), the standards of mycotoxins, AFB1, DON and NIV resulted in a significant dose-dependent decrease in the viability of RAW264.7 macrophages. These results imply that these mycotoxins are highly cytotoxic to mammalian cells even at micromolar concentrations. In contrast, plasma treatment appreciably reduced the viability loss of macrophages in a time-dependent manner, indicating that the mycotoxin-induced cytotoxicity was completely decreased after 5 s (Fig. 4b). These results suggest that the reduction of cytotoxicity may be involved in the degradation or removal of mycotoxins by plasma treatment. Although further study would not be performed on foods and feedstuffs contaminated with mycotoxins, the microwave-induced plasma system used in this study might be effectively used in the degradation or removal of mycotoxins.

4. Conclusion

This study confirmed the effects of our self-designed microwave-induced argon plasma system at atmospheric pressure on the degradation or removal of mycotoxins, such as AFB, DON and NIV. From the results, it would seem that the plasma system cannot only degrade mycotoxins from contaminated foods and feedstuffs, but also sterilize microorganisms as a source of mycotoxins. Although the physicochemical properties and structural changes of plasma-treated mycotoxins were not defined, this study represents a first step in showing that our plasma system can be applied to specific situations where the removal or inactivation of mycotoxins is required.

References

[1] J.W. Bennett, M. Klich, Clin. Microbiol. Rev. 16 (2003) 497.
[2] J. Varga, Z. Péteri, K. Tábori, J. Téren, C. Vágvölgyi, Int. J. Food Microbiol. 99 (2005) 321.
[3] M. Özdemir, M. Özilgen, Crit. Rev. Food Sci. Nutr. 41 (2001) 95.
[4] J.L. Richard, G.A. Bennett, P.F. Ross, P.E. Nelson, J. Anim. Sci. 71 (1993) 2563.
[5] J. Varga, B. Tóth, Acta Vet. Hung. 53 (2005) 189.
[6] A. Bata, R. Lásztity, Trends Food Sci. Technol. 10 (1999) 223.
[7] M.J. Sweeney, A.D. Dobson, Int. J. Food Microbiol. 43 (1998) 141.
[8] Z. Bronislaw, Int. Biodeterior. Biodegrad. 40 (1997) 43.
[9] B.J. Park, D.H. Lee, J.-C. Park, I.-S. Lee, K.-Y. Lee, S.O. Hyun, M.-S. Chun, K.-H. Chung, Phys. Plasmas 10 (2003) 4539.
[10] J.-C. Park, B.J. Park, D.-W. Han, D.H. Lee, I.S. Lee, S.O. Hyun, M.-S. Chun, K.-H. Chung, M. Aihara, K. Takatori, J. Microbiol. Biotechnol. 14 (2004) 188.
[11] K.-Y. Lee, B.J. Park, D.H. Lee, I.-S. Lee, S.O. Hyun, K.-H. Chung, J.-C. Park, Surf. Coat. Technol. 193 (2005) 35.
[12] J.C. Richard, A.S. Milbra, Handbook of Secondary Fungal Metabolites, vol. I, Academic Press, New York, 2003.
[13] J.C. Richard, B.J. Bruce, A.S. Milbra, Handbook of Secondary Fungal Metabolites, vol. III, Academic Press, New York, 2003.
[14] M. Trucksess, J. Giler, K. Young, K.D. White, S.W. Page, J. AOAC Int. 82 (1999) 85.
[15] L.S. Lee, J.B. Stanley, A.F. Cucullu, W.A. Pons Jr., L.A. Goldblatt, J. Assoc. Off. Anal. Chem. 57 (1974) 626.
[16] O.M. Hale, D.M. Wilson, J. Anim. Sci. 48 (1979) 1394.
[17] M. del C. De Arriola, E. De Porres, S. De Cabrera, M. De Zepeda, C. Rolz, J. Agric. Food Chem. 36 (1988) 530.
[18] H.K. Abbas, J.K. Mirocha, R. Rosiles, M. Carvajal, Cereal Chem. 65 (1988) 15.
[19] D. Cazzaniga, J.C. Basilico, R.J. Gonzalez, R.L. Torres, D.M. de Greef, Lett. Appl. Microbiol. 33 (2001) 144.
[20] A.F. Cucullu, L.S. Lee, W.A. Pons Jr., J.B. Stanley, J. Agric. Food Chem. 24 (1976) 408.
[21] R.F. Vesonder, A.C. Beckwith, A. Ciegler, R.J. Dimler, J. Agric. Food Chem. 23 (1975) 242.
[22] S. Haworth, T. Lawlor, E. Zeiger, L. Lee, D. Park, J. Am. Oil Chem. Soc. 66 (1989) 102.

[23] H. Maeba, Y. Takamoto, M. Kamimura, T. Miura, J. Food Sci. 53 (1988) 667.
[24] H.R. Pluyer, E.M. Ahmed, C.I. Wei, J. Food Prot. 50 (1987) 504.
[25] R.B. Harvey, L.F. Kubena, T.D. Phillips, W.E. Huff, D.E. Corrier, Am. J. Vet. Res. 50 (1989) 416.
[26] B.M. Colvin, L.T. Sangster, K.D. Haydon, R.W. Beaver, D.M. Wilson, Vet. Hum. Toxicol. 31 (1989) 46.
[27] R.W. Beaver, D.M. Wilson, M.A. James, K.D. Haydon, B.M. Colvin, L.T. Sangster, A.H. Pikul, J.D. Groopman, Vet. Hum. Toxicol. 32 (1990) 16.
[28] R.W. Beaver, Trends Food Sci. Technol. 2 (1991) 170.