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Effectiveness of pulsed light treatment for degradation and detoxification of aflatoxin B_1 and B_2 in rough rice and rice bran

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ABSTRACT

Aflatoxins primarily accumulate in the hull and bran layers of rough rice making these by-products of rice milling unsuitable for animal feed or human consumption. Contaminated rough rice is also a potential source of aflatoxin exposure to workers handling the grain during post-harvest storage and processing. Currently, no technologies are available to remove or detoxify these toxic and mutagenic fungal metabolites from contaminated rough rice. Pulsed light (PL) is a novel technology with the potential to degrade and detoxify aflatoxins in foods and their processing by-products. Rough rice was inoculated with *Aspergillus flavus* to produce aflatoxin B_1 (AFB_1) and B_2 (AFB_2) contamination, followed by PL treatments of 0.52 J/cm^2/pulse for various durations. A PL treatment time of 80 s reduced AFB_1 and AFB_2 in rough rice by 75.0% and 39.2%, respectively; while a treatment time of 15 s reduced AFB_1 and AFB_2 in rice bran by 90.3% and 86.7%, respectively. Since PL treatments result in the degradation of aflatoxins *in situ*, the toxicity and mutagenic activity of the residual by-products of AFB_1 and AFB_2 after PL treatment were evaluated. Toxicity was estimated using the brine shrimp (*Artemia salina*) lethality assay and mutagenicity measured by the fluctuation test with *Salmonella typhimurum* tester strains TA98 and TA100. The mutagenic activity of AFB_1 and AFB_2 was completely eliminated by PL treatment, while the toxicity of these two aflatoxins was significantly decreased. The obtained results suggest that PL technology has a promising potential to degrade, detoxify, and inactivate the mutagenic activity of aflatoxins in rough rice and rice bran.

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1. Introduction

Cereal grains, tree nuts, peanuts, and cottonseed are susceptible to colonization by the filamentous fungi *Aspergillus flavus* (*A. flavus*) and *Aspergillus parasiticus* (*A. parasiticus*), which produce aflatoxins as secondary metabolites. At least 18 different aflatoxins have been isolated and characterized, with aflatoxin B_1 (AFB_1) of most concern and the most highly regulated in agricultural commodities due to its toxic and carcinogenic properties. The International Agency for Research on Cancer (IARC) has classified AFB_1 in Group I as a human carcinogen (IARC, 2002). All animals, especially poultry, livestock, and fish, can be vulnerable to the acute toxicity of AFB_1 from contaminated feed (Eaton & Groopman, 1994). A variety of chemical treatments (including ammonia, hydrogen peroxide, sodium bisulfite, chlorine, ozone, acids, and alkali) and physical treatments (including gamma irradiation, heat, microwave radiation, and ultraviolet and visible light) have been studied to degrade and detoxify aflatoxins and eliminate fungal viability, but all have significant limitations due to lack of suitability for use in solid food substrates, incomplete detoxification, retention of residual toxicity, chemical alteration of nutrients in the food substrate, or the creation of undesirable residual by-products (Samarajeewa, Sen, Cohen, & Wei, 1990).

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Pulsed light (PL) is an FDA-approved, "non-thermal" technology (FDA, 1996) with the potential to alternate conventional processes. PL technology creates short, high-intensity flashes of broad-spectrum white light. The full spectra include ultraviolet, visible and infrared. With the synergy of these spectra, the cell wall and nucleic acid structure of microorganisms are destroyed in a few seconds (Oms-Oliu, Martín-Belloso, & Soliva-Fortuny, 2010). Recently, researchers found the potential application of PL for degradation of mycotoxins. Moreau et al. (2011) reported that eight flashes of PL degraded solutions of zearalenone by 85%, deoxynivalenol by 72%, AFB_1 by 93%, and ochratoxin by 98%. Funes, Gómez, Resnik, and Alzamora (2013) treated McIlvaine buffer, apple juice and apple purée by PL and obtained significant decrease in patulin levels. However, the degradation of aflatoxins by PL treatment on grains such as rough rice and rice bran has not been tested.

Rough rice is commonly contaminated with aflatoxins, with the FAO reporting that on a yearly basis 25% of grain crops are contaminated with mycotoxins. There are no current treatments available to eliminate the adverse effects of aflatoxin contamination in rough rice. *A. flavus* is an obligate aerobe capable of colonizing the surface of grains (Moreau et al., 2011). Surface inoculation of rough rice with either *A. parasiticus* or *A. flavus* under conditions in which moisture was adjusted for optimal fungal colonization resulted in aflatoxin accumulation primarily in the hull and bran layers (Breckenridge & Arseculeratne, 1986; Castells, Ramos, Sanchis, & Marin, 2007). Inoculated rough rice stored under humid conditions developed aflatoxin contamination in all milling fractions, with the highest accumulation in the bran layer (Trucksess, Abbas, Weaver, & Shier, 2011). Aflatoxin contamination in rough rice could present a hazard to workers during post-harvest handling and milling operations. It also limits the use of the milled fractions for any further use as products for human consumption or as animal feeds. Accordingly, this study evaluated the suitability of PL as a treatment to degrade AFB_1 and AFB_2 contamination in rough rice and rice bran. In addition, the cytotoxicity and mutagenicity of the resulting degradation products of AFB_1 and AFB_2 after PL treatment were determined using a brine shrimp lethality assay and the Ames fluctuation test.

2. Materials and methods

2.1. Regents and chemicals

The tests used solvents and aflatoxin standards from Sigma Aldrich (St. Louis, MO, USA), AflaTest® columns from Vicam (MA, USA), Grade A brine shrimp from Fisher Scientific (NY, USA) and B5051 Muta-Chromo plate™ kit from EBPI Environmental Bio-Detection Products Inc. (Brampton, Ontario Canada). All chemicals and solvents were analytical grade.

2.2. Samples

Dried medium-grain rough rice, variety M206, was obtained from the Farmers' Rice Cooperative (West Sacramento, CA). The initial moisture content of the rough rice was 13.3 ± 0.2% wet basis (w.b.).

A. flavus NRRL-3357 strain was obtained from the National Center for Agricultural Utilization and Research, USDA-ARS, Peoria, IL. This strain only produces AFB_1 and AFB_2. *A. flavus* was grown in potato dextrose agar (PDA) (Sigma, St. Louis, MO, USA) at 28 °C for 7 days (Kim, Campbell, Mahoney, Chan, & Molyneux, 2004). A conidial suspension of 10^5 cfu/mL was prepared by flooding the culture plates with 0.03% Tween 80 (w/w) (Wang et al., 2014). A volume of 10 ml of the spore suspension was pipetted onto 500 g rough rice in a conical flask, sealed by a double layer aluminum foil, and kept in a 28 °C incubator for 14 days until the mold growth was observed. The flask was shaken every day to ensure that *A. flavus* grew uniformly. The rice samples were frozen at −20 °C until analyzed.

AFB_1 and AFB_2 stock solutions were separately prepared according to the protocol of the AOAC 971.22 (18th edition) (AOAC, 2005). To prepare the aflatoxin contaminated rice bran, a 5 mL of 1 μg/ml of AFB_1 and 0.5 mL of 1 μg/ml of AFB_2 were added to 100 g of rice bran in a 200 ml beaker, mixed uniformly and put in hood for 30 min to evaporate the solvent before the PL treatment.

2.3. Pulsed light device

The SteriPule-XL® 3000 Sterilization System (Xenon Corp., Wilmington, MA, USA) was used as the pulsed light generator. The system consists of a linear xenon flash lamp, a treatment chamber and a power/control module. The system generates 3 pulses per second (pulse width 360 μs) of polychromatic light in the wavelength range of 100–1100 nm, which includes the ultraviolet, visible and infrared regions. As per manufacturer's specifications, the energy produced approximate 1.27 J/cm^2/pulse at 1.9 cm from the quartz window surface for an input voltage of 3800 V. The distance between the quartz window and the central axis of the lamp was 5.8 cm and the distance between the sample and the quartz window can be changed using adjustable trays setting in the chamber with 11 shelves. The intensity at the distance 9 cm was 0.52 J/cm^2/pulse.

2.4. Pulsed light irradiation on rough rice, rice bran and filter paper

A sample of 50 g inoculated rough rice was spread uniformly on the tray in a single layer. Then the tray was placed on the chamber shelf at a vertical distance of 9 cm from quartz window. The treatment time was 20 s and then cooled to the room temperature (25 °C). This treatment was repeated 2, 3, and 4 times for each sample to achieve a total treatment times of 20, 40, 60 and 80 s. The irradiated rough rice was kept at a temperature of −20 ± 1 °C until further analysis. The high peak temperatures were measured and recorded during PL irradiation using an infrared thermometer (Fluke 568, Fluke Corp., WA, USA). Treatments and measurements were made in triplicate and an average of the results is reported.

A sample of 3 g of contaminated rice bran was spread uniformly with a thickness of 1 mm on the tray. The treatment durations were 5, 10 and 15 s. After that, the rice bran samples were kept refrigerated at 4.0 ± 0.5 °C until further analysis.

In order to investigate the toxicity of PL treated AFB_1 and AFB_2, aflatoxins in pure form were treated. Three methanol solutions (0.5 mL) containing 10 μg/ml of AFB_1, AFB_2 and a mixture of equal volume of AFB_1 and AFB_2 (AFB_1 + AFB_2) were prepared, respectively. Filter paper strips with 1 × 3 cm (Grade 1, Whatman International Inc., Maidstone, England) were dipped in the solutions and air dried in a fume hood for 5 min. The dried strips were exposed to the pulsed light at intensity of 0.52 J/cm^2/pulse for different times of 5, 10 and 30 s at room temperature. Untreated strips were used as a control. After treatments, the strips were immersed in 1.0 ml methanol to collect the residual aflatoxins for the preparation of HPLC analysis.

2.5. Aflatoxin extraction and clean-up

Rough rice (50 g) was blended in an MC3 minicontainer (Waring Products Div., Dynamics Corporation of America, USA) with 100 ml of methanol/water (80: 20, v/v) for 1 min. Filtered aliquots of 1 ml were added to 4 ml of 2.5% NaCl. The mixture was gravity filtered through fluted filter paper (Folded Grade 1, Whatman International