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Inactivation of Aflatoxins B_1 and B_2 in Peanuts by Pulsed Light

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Aflatoxins (AFTs) are secondary metabolites of *Aspergillus flavus* and *Aspergillus parasiticus* molds. They are toxigenic and carcinogenic compounds that contaminate globally important commodities. Therefore, destroying AFTs is a food-safety concern. Many methods have been studied to provide food free of AFTs. Pulsed light (PL) treatment is a relatively new technique, which has shown promising results in the degradation of AFB1, AFB2 in model solutions. In this study, the degradation of AFTs in contaminated peanuts was determined after PL treatment using three distances (5, 7, and 10 cm) from the PL strobe for different exposure times. AFB1 and AFB2 concentration was determined by an enzyme-linked immunosorbent assay kit. The temperatures of treated peanuts were monitored using an infrared thermometer. Peanuts were placed in small aluminum plates right below the xenon lamp on a conventional conveyor. Results showed that in-plate treatment of with-skin and without-skin peanuts for 240 s at a 7 cm distance degraded AFTs by 62.4% and 86% respectively. In-plate PL treatment for 300 s at 5 cm from the strobe reduced AFTs 82% for with-skin and 95.3% for without-skin samples. This study indicates that PL illumination could degrade AFTs in peanuts as a result of PL's photochemical and photothermal effects.

Fungi or molds have played a significant destructive role against agriculture commodities throughout history. Fungi can contaminate and colonize crops before harvest and during storage, usually under warm and high-humidity conditions or after exposure to a stressful environment such as drought. Molds have the ability to excrete extremely harmful secondary metabolites called mycotoxins on both inside and on crop surfaces. Of the hundreds of mycotoxins, a few have been detected in food and are considered to have a serious impact upon human health. Many fungi species have been reported as mycotoxin producers, however, the filamentous fungi *Aspergillus* is the dominant aflatoxins (AFTs) producer. Aflatoxins have the ability to pass through metabolic processes unaltered, accumulate in human or animal tissues, and cause potentially deadly cancer to various organs of the body, especially the liver (Piva et al., 1995; Serra et al., 2005). *Aspergillus* species produce AFTs as a live cell. However, the exact function of mycotoxins produced by this microorganism remains a mystery. It is hypothesized that mycotoxins act as a defense mechanism, protecting the fungus from plants, animals, and other competing fungi (Smith and Moss, 1985). The concern for human health mostly pertains to four types of AFTs – B1, B2, G1, and G2. The AFB series, especially B_1 is the most severely toxic AFT. It has multifactorial toxic and chronic effects. Peanuts (*Arachis hypogaea*) are considered one of the most susceptible crops for *Aspergillus flavus'* growth and AFT production. Peanuts become contaminated with AFTs when subjected to prolonged periods of preharvest heat and drought stress (Holbrook et al., 2000). Changes in the chemical composition of peanut kernels as a result of fungal infection are inevitable. Proteins, lipids, free and total amino acids, and free fatty acids are reported to change significantly when peanuts are infected by *A. flavus* (Chiou et al., 1997). Since AFTs have been discovered, numerous research projects have been conducted regarding detoxification and elimination methods. Aflatoxins are notoriously difficult to remove. Most AFTs are chemically stable and heat resistant. Thus, these toxins tend to survive thermal processing. Aflatoxins have been shown to tolerate temperatures encountered during baking, roasting, and breakfast cereal production. Most of the chemical, physical, biological, and irradiation approaches have never reached complete AFT elimination, especially in whole kernel grains (Manetta et al., 2011; Rustom et al.,1997). Pulsed light (PL) is one of the proposed new technologies for toxin inactivation. It is a non-thermal food treatment which can achieve microbial inactivation within a few seconds under mild temperatures. Pulsed light uses broad spectral wavelengths (200 to 1100 nm), including the ultraviolet (UV) spectrum. The lethal effect of pulsed light has been explained by several mechanisms, most of which are connected to the UV spectrum that has photochemical, photothermal, and photophysical effects (Elmnasser et al., 2007; Gómez-López et al., 2007). The infrared region has been shown to elevate the temperature and cause most of the thermal effect (Moreau et al., 2013; Wang et al., 2016; Zhao, 2013). Pulsed light has demonstrated ability to destroy or reduce harmful chemical compounds, bacteria, viruses, enzymes, allergens, and toxins (Gómez-López et al., 2007). This research demonstrates

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the ability of PL to reduce AFTs to significantly lower levels for peanut kernels with and without skin.

Materials and Methods

Sample Inoculation. Raw with-skin (with testa) and without-skin (w/o testa) peanuts were purchased from a local market. Peanuts were autoclaved for 15 min at 115 °C to eliminate any pre-contamination fungi. Peanuts were inoculated with *A. flavus* purchased from the American Type Culture Collection (ATCC, Manassas, VA). This specific strain anamorph (ATCC® 16875™) has a high efficiency for producing AFB1 and AFB2 (Wei and Jong 1986). The fungus was grown according to the methods specified by Doyle and Bracket (1982) and Nam et al. (2009). The inoculum was incubated at 30 °C. Fungi spores were harvested from petri dishes with a spatula, filtered using cheesecloth, and a suspension of fungal spores at approximately 100,000 conidia per milliliter were prepared. Conidia were counted using hemocytometer. Sterile peanuts (500 g) were placed in Erlenmeyer flasks (1000 mL), and the final moisture content was adjusted to 16%. Peanut containing flasks were inoculated with 25 mL of the spore suspension, incubated at 30 °C in the dark for 21 days. Flasks were shaken daily for good moisture and fungal distribution. After this long period of incubation, flasks were boiled in water for 1 h to stop AFT production.

Pulsed UV-light treatment. Pulsed light treatment was conducted using a Xenon PL applicator Model# LHS40 LMP HSG from Xenon Corp (Wilmington, MA). This system consists of an RC-747 power / control module, a treatment chamber that contains two xenon flash lamps (mercury free) and two blowers (air-cooling system) at the top of the lamp housing chamber, and one treatment chamber with a conveyor belt. The xenon lamp used has an electrical efficiency of 10% to 30% and UV intensity of 30.000 W/cm (Koutchma et al., 2009). The PL system generates a broadband spectrum between 100 and 1100 nm. Approximately 20%, 26%, and 54% of the energy is in the infrared, visible light, and UV regions, respectively. This system generates high-intensity PL at a pulse rate of three pulses per second and a pulse width of 360 μs. As per the manufacturer's specification, this system can produce an energy level of 1.27 J/cm^2 per lamp at 1.9 cm below the central axis of the quartz window of the PL xenon lamp (Wang et al., 2016).

The treatments were conducted in triplicate. Each time, 5 g samples of with-skin and without-skin raw runner type peanuts were arranged in small aluminum dishes (7.2 cm in diameter, and 1 cm high) obtained from Fisher Scientific, Inc. (Allentown, PA). A combination of several illumination durations and distances were tested to determine the most effective conditions for PL treatment of whole peanuts. The samples were treated with PL for several duration times 30, 60, 90, 120,180, 210, 240, and 300 s at three different distances 5, 7, and 10 cm, from the PL strobe. The treatment times were selected based upon the visual degree of roasting that occurred and AFT reduction for the peanut samples, ranging from lightly roasted to burnt, during preliminary tests.

Infrared thermometer temperature measurement. An Omega OS423-LS, (Omega Engineering, Inc., Stanford, CT) infrared thermometer was used for temperature measurement of treated peanuts before and right after PL treatment at the exact moment when the conveyor brought the sample out of the chamber.

Aflatoxin extraction. After the PL treatment, 5 g of peanuts were well milled in a grinder (Model LXC-150,50/60Hz,180W, Keunex, Korea). Then the peanuts were blended (31BL91, Waring, Dynamics Corporation of America, Hartford, CT) with 25 mL of 70% methanol (aqueous) for 2 min to extract AFTs. The extract was transferred to conical flasks and was shaken (MTS 2/4 D S1, IKA, Wilmington, NC) for 30 min at 300 rpm. The extract was allowed to settle, then was filtered through a Whatman Number 1 filter paper and moved to autoclaved containers, and stored in a freezer (–18 °C) until analysis for AFB1, AFB2 content. Extractions after storage were shaken before enzyme-linked immunosorbent assay (ELISA) analysis by using an orbital shaker (Heidolph, Schwabach, Germany) for 3 min similar to the method of Zheng et al. (2005) and Nyirahakizimana et al. (2013).

Competitive ELISA analysis. An AgraQuant® ELISA Aflatoxin Kit (Romer Labs, Getzersdorf, Austria) was used to determine the concentration of AFB1 and AFB2. All procedures for ELISA analysis were performed according to manufacturer specifications. The kit is able to quantitate AFTs in the range of 4-40 ppb.

Results and Discussion

This study's results indicated that temperatures increased parallel to increased exposure times (Table 1, Table 2) in both with-skin and without-skin treatments and decreased with increasing distance from the PL source. AFTs need high temperatures ranging from 237 °C to 306 °C to thermally decompose (Rustom 1997). Direct roasting 150 °C for 30–120 min never achieved high reduction for AFTs (Pluyer 1987; Yazdanpanah 2005). In this study, the highest temperature recorded by the infrared thermometer was 180 °C in without-skin peanuts treated by PL for 300s at 5 cm from the strobe, which led to 95.3 ± 3.47 % reduction of AFTs (Table 1). For with-skin samples, the highest temperature was 150 °C, and reduction of AFTs reached 82.0 ± 16.1% (Table 2).

Table 1. AFB1, AFB2 reduction in with-skin PL treated peanuts samples using different distances and times.

Time (s)	5 cm (ºC)	5 cm-R (%)[z]	7 cm (ºC)	7 cm-R (%)	10 cm (ºC)	10 cm-R (%)
0	23.0 ± 2.35	0 a[y]	23.0 ± 2.35	0 a	23.0 ± 2.35	0 a
30	58.5 ± 2.43	0 a	55.6 ± 0.89	0 a	53.1 ± 2.82	0 a
60	62.8 ± 4.26	26.9 ± 4.20 a, b	60.3 ± 0.25	20.9 ± 6.32 a, b	59.1 ± 1.50	1.20 ± 1.40 a
90	83.0 ± 1.64	33.8 ± 6.40 a, b	67.2 ± 1.83	23.1 ± 8.42 a, b	70.2 ± 4.10	3.63 ± 4.62 a
120	105 ± 8.65	39.3 ± 4.90 a, b	81.9 ± 7.21	34.5 ± 5.97 a, b	71.1 ± 13.8	4.16 ± 0.00 a
180	123 ± 4.85	40.1 ± 7.00 a, b	96.8 ± 2.62	39.3 ± 7.05 a, b	74.8 ± 15.4	4.39 ± 3.15 a
210	130 ± 12.2	43.4 ± 17.0 a, b	103 ± 3.25	40.3 ± 9.5 b a, b	92.0 ± 8.40	15.1 ± 5.05 a
240	136 ± 1.11	64.6 ± 19.4 b	105 ± 7.35	62.4 ± 15.0 b	98.3 ± 4.29	25.1 ± 15.0 a
300	150 ± 17.9	82.0 ± 16.1 b, c	114 ± 11.2	64.8 ± 14.5 b	103 ± 5.57	32.9 ± 16.2 a

[z]Mean ± standard deviation (n = 3), R (%) = reduction percentage, control was 129 ± 36.5 ppb.
[y]Means within a column followed by the same letter are not significantly different.

Table 2. AFB1, AFB2 reduction in without-skin PL treated peanuts samples using different distances and times.

Time (s)	5 cm (ºC) [z]	5 cm-R (%)[z]	7 cm (ºC)	7 cm-R (%)	10 cm (ºC)	10 cm-R (%)
0	22.1 ± 0.67	0 a[y]	22.2 ± 2.27	0 a	22.2 ± 1.45	0 a
30	58.7 ± 6.88	0 a	50.6 ± 0.29	0 a	45.1 ± 14.9	0 a
60	69.1 ± 4.41	32.6 ± 2.40 a	65.2 ± 0.98	23.5 ± 0.46 a, b	53.2 ± 3.69	13.0 ± 1.16 a, b
90	90.2 ± 5.03	36.2 ± 2.65 b	79.3 ± 2.26	28.8 ± 1.10 a, b	57.7 ± 1.20	3.00 ± 4.78 a, b
120	110 ± 6.47	48.6 ± 0.46 b, c	86.2 ± 0.73	44.6 ± 6.09 b	77.0 ± 0.81	30.4 ± 1.60 a, b
180	128 ± 3.97	68.6 ± 5.65 b, c	99.4 ± 0.82	60.2 ± 16.9 b, c	79.3 ± 1.10	41.3 ± 0.31 a, b
210	131 ± 3.66	76.3 ± 3.30 c	104 ± 2.56	70.3 ± 4.13 b, c	82.0 ± 2.34	45.2 ± 0.67 b
240	148 ± 3.02	86.9 ± 1.99 c, d	137 ± 0.01	77.9 ± 2.95 b, c	120 ± 1.59	50.1 ± 4.53 b
300	180 ± 12.9	95.3 ± 3.47 c, d	164 ± 2.54	80.1 ± 1.49 c	130 ± 1.61	55.1 ± 1.19 b

[z]Mean ± standard deviation (n = 3), R (%) = reduction percentage, control was 171 ± 22.2 ppb.
[y]Means within a column followed by the same letter are not significantly different.

Therefore, it is likely that the photochemical effect of pulsed light primarily led to the degradation of the AFTs in this instance since recorded temperatures were below levels known to cause thermal degradation of AFTs. Comparably, a UV light treatment using 43 mW/cm^2 for 24 hours reduced AFB1 in milled rice from 1100 ppb to 135 ppb, an 88% AFT reduction, while a higher intensity of 64 mW/cm^2 produced the same reduction of aflatoxins after 12 h of treatment (Nkama and Muller, 1988). Similar results for Wang (2016) revealed that PL treatment for 80s with an intensity ~ 0.52 J/cm^2/pulse at 9 cm distance from the lamp reduced AFB1 and AFB2 in rough rice by 75.0% and 39.2%, respectively.

Aflatoxins are known to be more stable against irradiation in a solid state (Aibara and Yamagishi, 1970). Therefore, one of the most crucial conclusions from this study is that the increase of peanut moisture content enhanced the irradiation efficiency. Water may have increased UV penetration since water is a highly UV-absorbent compound compared with other peanut constituents. For opaque foods such as peanuts, turbidity reduces the penetration capability of UV light (Guerrero-Beltran and Barbosa-Canovas, 2004). When the matrix is transparent, PL can penetrate easily, permitting decontamination of samples as was achieved when AFB1, AFB2 were treated in solvent by PL (Feuilloley et al., 2006). In general, PL can penetrate up to 2 mm in solid samples (Wallen et al., 2001). In this study, intrinsic factors of the sample attributed to the efficiency of AFTs detoxification in addition to the high intensity of the PL. For future work, since long treatments showed unequally distributed dark spots on the peanut surface, shaking the peanuts during PL treatment could result in a more uniform treatment without burning the surface of the samples.

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