ATMOSPHERIC PRESSURE COLD PLASMA ON AFLATOXIN MITIGATION OF GROUNDNUT

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ABSTRACT

Experiments were conducted on *Aspergillus flavus* inoculated groundnut to evaluate the effect of cold plasma on the mitigation of aflatoxin. A dielectric barrier discharge type of cold plasma equipment was used to generate plasma at voltage levels of 12, 15 and 18 kV. The exposure time of groundnut samples to plasma was kept at 5, 10 and 15 min in the trials. Argon gas at a flowrate of 15 L/min was used for cold plasma generation. There was a significant reduction of AFB_1 in groundnut when treated with cold plasma. The voltage level and exposure time were found to affect the level of aflatoxin degradation. A maximum of 60% reduction of AFB_1 was observed at a voltage of 18 kV and exposure time of 15 min. It is concluded that cold plasma treatment can be an effective tool for mitigation of aflatoxin in groundnut.

Keywords: *groundnut, atmospheric pressure cold plasma, Aspergillus flavus*, *aflatoxin, AFB_1.*

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1. Introduction

Groundnut is the third largest oilseed produced in the world. Aflatoxin contamination is one of the most challenging and serious food safety issues in food and agricultural commodities. Groundnut is reported to be a high risk commodity as for as aflatoxin contamination is concerned. Natural occurrence of aflatoxin poses economic risks worldwide which reflects in the export of groundnut (Guchi, 2015). The shortage of vegetable oil in the world can be addressed by increase in groundnut production to some extent. Aflatoxin degradation in groundnut is of great concern for the safe and good quality production of vegetable oil too.

Aflatoxin is considered as hidden poison due to its slow and adverse effect on various biological pathways in humans (Pandey *et al*., 2019). The most commonly occurring mycotoxins in postharvest handling of groundnut are aflatoxins produced by *Aspergillus* species including *A.flavus* and *A. parasiticus*. The naturally occurring aflatoxins are classified into four classes as Aflatoxin B1, B2, G1, and G2 (Nawas *et al*., 2017). Aflatoxins can contaminate different agricultural commodities such as nuts, cereals and oilseeds (Siciliano *et al*., 2016). Due to its stability and presence in foods, it is becoming a burden to food safety management in global food industry. It is essential to develop adequate methods for the degradation or removal of aflatoxins in groundnut to permitted safe levels (Suresh *et al*., 2021).

Conventional strategies for mycotoxin reduction includes both prevention and decontamination strategies. To degrade the aflatoxin level in groundnut, many physical, chemical and thermal treatments are followed which cause several changes in the sensory and quality attributes. Thermal processing and chemical treatments are not adequate methods to cause aflatoxin decontamination in food products. Novel processing methods are continuously explored to achieve complete aflatoxin degradation in food products such as microwave heating, gamma and electron beam irradiation, ultraviolet and pulsed light, electrolyzed water and cold plasma (Pankaj *et al*., 2018). The application on food products need further studies for confirmation of the effectiveness of decontamination process. By following non thermal treatments such as cold plasma, aflatoxin can be degraded to the safe level for satisfying the export regulations required by countries like United States of America (20 µg/kg), European Union (4 µg/kg) and Republic of South Africa (15 µg/kg).

Cold plasma has more recently become a subject of research as an intervention to improve the safety of foods. Some of the notable food sectors which could benefit from antimycotic and antimycotoxin efficacy of cold plasma include, the fresh produce, food grains, nuts, spices, herbs, dried meat and fish industries. In cold plasma treatment of aflatoxin contaminated foods, the different species generated act on the sites of a fungal cell resulting in loss of function and structure, and ultimately cell death. Likewise, the species cause chemical breakdown of mycotoxins through ozonolysis of the aflatoxin resulting in degradation products that are known to be less toxic (Misra *et al*., 2019).

Several authors have reported that atmospheric cold plasma is effective against aflatoxin and can be used for detoxification in foods such as hazel nuts, onion powder, wheat and barley (Siciliano *et al*., 2016; Dasan *et al*. 2017; Kim *et al*., 2017; Los *et al*., 2018). In this study, atmospheric cold plasma has been used for aflatoxin mitigation in groundnut. The research focused on effects of cold plasma parameters such as voltage and exposure period on aflatoxin degradation in groundnut.

2. Materials and methods

2.1 Microbial culture

Aflatoxin producing strain of *Aspergillus Flavus* (NRRL 3357) was obtained from ARS culture collection, National Centre for Agricultural Utilization Research. The fungal strain was maintained on potato dextrose agar (PDA) medium and stored in refrigerator at 4°C. To prepare inoculum for the fungi, fungal strains were grown on PDA plates for 7 d at 30±2°C. Fungal spore suspension was prepared from individual cultures by flooding the mycelia surface with 10 mL of sterile saline solution using 0.1% (v/v) Tween-80. Sterile saline washed suspension was centrifuged to collect spores. Spores were re-suspended in sterile saline water and serially diluted to get spore suspension of 10^5 CFU mL^{-1} spores' concentrations with the help of a hemocytometer. Spore suspension was stored at 4°C. for use of study.

2.2 Inoculation of fungal spores on groundnut

Raw groundnuts of TNAU CO6 variety were procured in June 2022 from the Department of Oil Seed, Tamil Nadu Agricultural University, Coimbatore. The groundnut samples were stored in gunny bags and kept in ambient conditions throughout the period of study. The moisture content was maintained at 7.50% (db). The decorticated groundnut kernels were surface disinfected to eliminate possible growth of other organisms. Spore suspension was spread on to the disinfected groundnut kernels. The inoculated groundnut kernels were dried inside laminar

chamber and stored at 25°C for 3 d before exposing to cold plasma treatment.

2.3 Plasma equipment and treatment

Atmospheric pressure dielectric barrier discharge system was used for the study in which plasma was generated by Argon gas. The cold plasma system consists of a plasma chamber (40 cm x 40 cm x 20 cm) with two parallel electrodes (30 cm x 30 cm); one ground electrode covered with a dielectric material of 3 mm thickness and other high voltage electrode covered with 10 mm thick acrylic sheet. During plasma treatment, the distance between electrodes was fixed at 10 mm. An AC high voltage power supply (Vmax: 40 kV; Frequency: 50Hz) was used to generate plasma between the two electrodes.

Artificially inoculated groundnut samples were exposed to atmospheric pressure dielectric barrier discharge cold plasma (DBD). For each experiment, 20 g of sample was placed inside the chamber. Plasma was generated at a voltage of 12, 15 and 18 kV. A gas flow rate of 15 L/min was used. Exposure period was maintained as 5, 10 and 15 min. According to experimental design, nine experiments with triplicate samples were performed for different combinations of voltage level and exposure time. The cold plasma treated groundnut samples were stored at room temperature for 24 h. Control samples were not subjected to plasma treatment.

2.4 Determination of aflatoxin content

The samples were pulverized to extract Aflatoxin B_1 (AFB_1). About 25 g of finely ground samples were added with 5 g of sodium chloride and 100 mL 80% (w/v) methanol and shaken vigorously for 30 min at 150 rpm to extract AFB_1 from samples. Then 4 mL filterate were diluted with 16 mL of phosphate buffered saline (PBS). The sample was allowed to pass through immunoaffinity column (Aflarhone) with a flow rate of 1 mL/min and washed with 20 mL of PBS at a flow rate of 5 mL/min. The purified extract was collected after elution with 1 mL of methanol and final washing with 1 mL of water and made upto 2 mL.

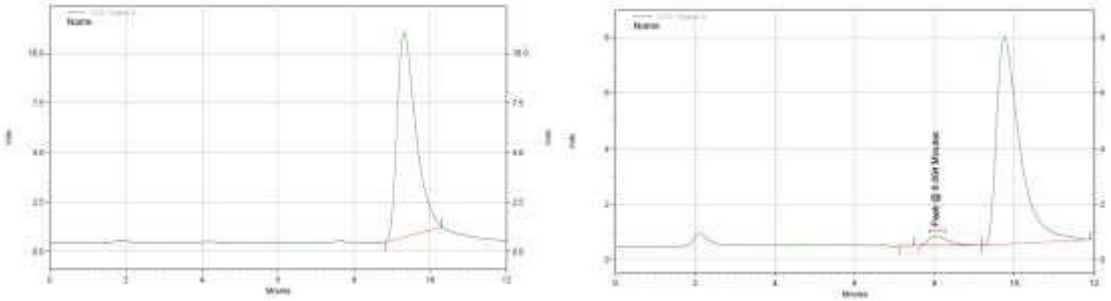
The AFB_1 content in groundnut was determined by a reverse phase high-performance liquid chromatography (Agilent 1200 HPLC system, Agilent Tech USA) equipped with a fluorescence detector and a reverse phase silica packed C-18 HPLC column (150 x 4.6 mm, 5 µm particle size; Agilent Technology, USA). The mobile phase was 40% methanol with a flow rate of 1 mL/min at 40°C. The level of AFB_1 in sample was detected by fluorometry with excitation and emission wavelength at 365 nm and 425 nm, respectively. The injection volume was 50 µL. KOBRA cell (R-Biopharm, Germany) was used for electrochemical derivatization. The level of AFB_1 was obtained by comparing the standard curve established from different concentrations of standard solution.

2.5 Statistical Analysis

Response Surface Methodology (RSM) was adopted in the experimental design (Design Expert 13). The results were statistically analyzed using one-way analysis of variance (ANOVA).

3. Results and Discussion

The effect of atmospheric pressure cold plasma on the degradation level of AFB_1 present in groundnut is shown in Table 1. HPLC method of analysis was used to determine the AFB_1 content in control and cold plasma treated groundnut. HPLC chromatograms of control and cold plasma treated sample are illustrated in Fig. 1. The initial level of AFB_1 in the control sample was 7.02 µg/kg. When treated at 12 kV, the level of AFB_1 of groundnut decreased from 6.71 µg/kg to 3.64 µg/kg when the exposure time was increased from 5 to 15 min. Similarly, for the same increase in exposure time, when treated at 15 kV, the AFB_1 content decreased from 5.84 µg/kg to 3.52 µg/kg and at 18 kV plasma treatment, the level of AFB_1 decreased from 4.05 µg/kg to 2.71 µg/kg. From these results, it is seen that the AFB_1 content decreased with increase in exposure period and voltage., in the range of conditions experimented.



(a) **(b)**

Fig. 1. Chromatograms: (a) for control (b) for cold plasma treated groundnut (18 kV; 15 min)

Table 1. Effect of atmospheric cold plasma on AFB_1 content in groundnut

Voltage (kV)	Exposure period (min)	AFB_1 level (µg/kg)	AFB_1 level of Control (µg/kg)
12	5	6.71 ± 0.79	7.02 ± 1.28
12	10	4.25 ± 0.54	
12	15	3.64 ± 0.74	
15	5	5.84 ± 0.95	
15	10	4.81 ± 0.58	
15	15	3.52 ± 0.86	
18	5	4.05 ± 1.65	
18	10	2.76 ± 0.43	
18	15	2.71 ± 0.66	

The relationship between the level of voltage and period of exposure on the AFB_1 content is shown in Fig. 2. A maximum reduction of 60% of AFB_1 content was observed in groundnut treated with cold plasma at 18 kV for 15 min when compared to control sample. The reduction in AFB_1 content in plasma treated groundnut is significantly affected by treatment voltage level and exposure period. Like previous reports on aflatoxin degradation in groundnut by Iqdiam *et al*. (2019) and Devi *et al*. (2017), the results revealed that the level of AFB_1 decreased significantly ($p < 0.01$) in cold plasma treated groundnut.

Iqdiam *et al*. (2019) investigated on the effect of atmospheric pressure plasma jet on groundnut inoculated with *Aspergillus flavus*. Constant and agitated plasma treatment was conducted at a power of 650 W. Total aflatoxin level showed a reduction of 23% for 2 min of constant treatment and 38% reduction for 5 min of agitated treatment. Devi *et al*. (2017) researched on the influence of cold plasma on fungal growth and aflatoxin production on groundnuts. Fungal inoculated groundnuts were treated with low pressure air plasma at a frequency of 13.56 MHz. The results of the study showed more than 65% reduction in AFB_1 content of groundnut at 15 kV of plasma generated at a power level of 60 W for an exposure time of 12 min. The reduction in AFB_1 was 95% when the plasma was generated at 19.5 kV for an exposure time of 15 min at a power level of 40 W.

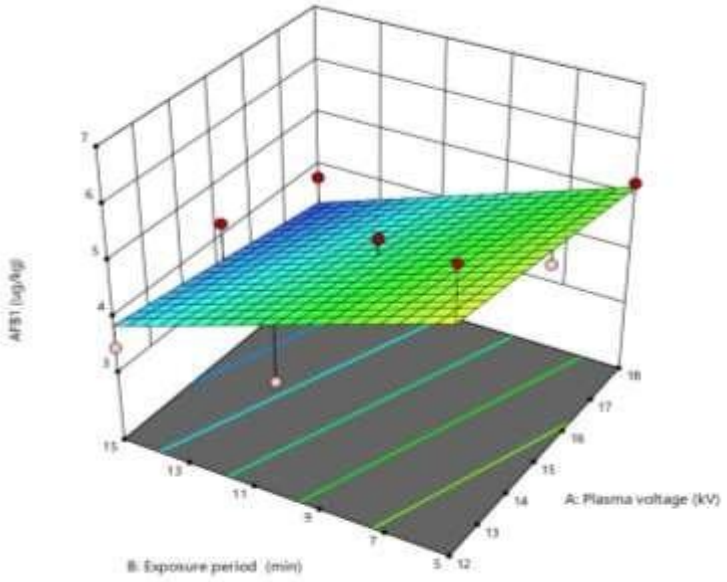


Fig. 2. Effect of process parameters of cold plasma on AFB_1 content of groundnut

The results confirm the results reported earlier by Makari *et al*. (2021) on elimination of *Aspergillus flavus* from pistachio nuts with dielectric barrier discharge cold plasma. The effects of different durations of cold plasma treatment were studied at 130 W power, 20 kHz frequency and 15 kV voltage level. The distance between electrodes and sample was 3 mm. The maximum reduction of 52.42 % of AFB_1 was observed after 180 s of the treatment in nut samples. The study reported that the generation of reactive species in cold plasma treatment can degrade aflatoxin content effectively. The molecular structure of toxin molecules and their interactions with the reactive species affect the

degradation of toxin. The process named ozonolysis during atmospheric cold plasma treatment might be the main cause for degradation in which the structure of AFB_1 changes.

Several other studies also reported on effect of cold plasma for the degradation of aflatoxin on different surfaces such as nuts, rice, wheat, maize, chilli and spiked glass slides. Sasireskha, R. (2021) studied on the effects of dielectric barrier discharge and low pressure plasma on aflatoxin decontamination in chilli pepper. A maximum of 41.57% reduction in aflatoxin was achieved in the DBD plasma treatment. A voltage of 14 kV, treatment time of 12 min, and Argon gas flow rate of 1 L/min attained a better reduction in aflatoxin. Low pressure cold plasma treatment of chilli pepper resulted in 43.37% reduction in aflatoxin content.

Puligundla *et al*. (2020) used corona discharge plasma jet to degrade AFB1 on glass slides and in spiked food commodities, namely rice and wheat. The sample to electrode distance was set at 15 mm. A degradation of 95% was observed in AFB_1 concentration for 30 min of plasma treatment at 1.50 A current. Sen *et al*. (2019) studied atmospheric pressure cold plasma treatment of contaminated hazel nuts. Plasma was generated using air with a flow rate of 50 L/min at a frequency of 25 kHz and power level of 655 W. The content of AFB_1 in hazel nuts decreased by 72% for 1.7 min of plasma treatment. From this study, it is inferred that atmospheric pressure cold plasma treatment is an effective method for degradation of aflatoxin in groundnut.

4. Conclusion

The research evaluated the degradation effect of atmospheric pressure cold plasma on AFB_1 content of groundnut artificially inoculated with *Aspergillus flavus*. A maximum reduction of 60% was observed in AFB_1 content in groundnut after 15 min of cold plasma treatment at 18 kV compared to control sample. The AFB_1 content of groundnut significantly decreased ($p < 0.01$) with voltage and exposure time. The results confirmed that atmospheric pressure cold plasma system could be utilized to degrade aflatoxin in groundnut effectively.

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