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Inactivation of aflatoxigenic fungi (*Aspergillus* spp.) on granular food model, maize, in an atmospheric pressure fluidized bed plasma system

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ABSTRACT

Atmospheric plasma provides the advantages of high microbial inactivation that can be performed under ambient conditions; therefore, it is regarded as a potential alternative to traditional food preservation methods. The present work presents the results of a critical study conducted on the efficiency of a non-thermal atmospheric pressure fluidized bed plasma (APFBP) system used for decontamination of maize. Maize grains that were artificially contaminated with *Aspergillus flavus* and *Aspergillus parasiticus* spores were treated in APFBP system for 1–5 min at two differently designed fluidized bed reactors with air and nitrogen. Results indicate maximum significant reductions of 5.48 and 5.20 log (cfu/g) in *Aspergillus flavus* and *A. parasiticus* after 5 min air plasma treatment. The native microbial flora of the maize grains decreased to more than 3 log after 3 min APFBP treatment, and no viable cells were counted. During the storage of plasma treated maize samples at 25 °C for 30 days, the *Aspergillus* spp. spores log reduction was maintained with no occurrence of re-growth. Overall, this study shows that plasma treatment has a fungicidal effect on *A. flavus* and *A. parasiticus* spores associated with alterations in spore surface morphology and loss of spore integrity. APFBP can inactivate aflatoxigenic spores on maize grains and could be optimized to improve the safety and quality of produce.

1. Introduction

Filamentous fungi are ubiquitous in nature, but present a potential threat not only for humans, especially those who are immunocompromised, but also for economically important plants and animals. Maize (*Zea mays* L.) is the third most important cereal used as human food and animal feed worldwide. However, maize is a favoured host for the aflatoxin producing fungus *Aspergillus flavus* and *Aspergillus parasiticus* during their development in the field, as well as during their transport and storage (Amaike & Keller, 2011).

Several strategies have been tried to suppress or eliminate fungal contamination and reduce mycotoxin formation in food materials, including severe wet or dry heat, UV, gamma ray irradiation, ozone, modification of atmosphere conditions, microwave heating, antifungal chemicals and treatments with food preservatives (Al-Ahmadi, Ibrahim, & Ouf, 2009; Aziz, Souzan, & Azza, 2006; Perez-Flores, Moreno-Martinez, & Mendez-Albores, 2011). Although these methods can inhibit surface contamination and provide good results in a number of cases, their effects are various and often unsatisfactory, sometimes accompanied with undesirable side effects such as alteration of the sensory and nutritional quality. In addition, they are occasionally impractical as they are either expensive or too slow.

Recently, consumers demand food products, which contain fewer preservatives and other chemical contents. Thus, there is a need to produce minimally processed products of high quality without putting consumers' health at risk. Development of novel decontamination technologies assuring a high level of decontamination is essential for food industries. Physical non-thermal technologies such as irradiation, ultraviolet light, pulsed light, high pressure processing, and ultrasound are considered more promising alternatives. Among these, non-thermal atmospheric plasma

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technology has drawn a lot of attention as a minimal processing technology (Ziuzina, Petil, Cullen, Keener, & Bourke, 2014).

Non-thermal plasma (NTP) is generated by the input of energy to transform gas into plasma which contains neutral molecules, electrons, positive and negative ions, and exited species (Niemira, 2012). Different antimicrobial substances including UV photons, reactive oxygen species such as hydrogen peroxide, hydroxyl radical, superoxide, singlet oxygen, atomic oxygen and ozone and reactive nitrogen species such as peroxynitrite, nitric oxide and nitrite are generated (Rod, Hansen, Leipold, & Knochel, 2012). The concentrations in which the agents occur in the plasma depend greatly on device setup, operating conditions (gas type and power of plasma excitation) and gas composition (Brandenburg et al., 2009). The antimicrobial effect of cold plasma is the result of the action of charged particles and reactive species present in the plasma that can cause damage to the cell membrane, which can lead to further penetration of reactive species into the cell, DNA damage, and breaking of chemical bonds (Fernandez & Thompson, 2012). Even if the mechanism of interaction between the plasma species and the microorganism has yet to be clarified, plasma ions can catalyze processes such as oxidation and peroxidation that take place inside the cell as well as in the external environment (Dobrynin, Fridman, Friedman, & Fridman, 2009). Cold plasma efficiency also depends on biological parameters such as the type of substrate and microorganism characteristics (type, load, physiological state) (Misra, Tiwari, Raghavarao, & Cullen, 2011).

NTP is an emerging technology that could potentially decontaminate the surfaces of fresh produce. This antimicrobial intervention offers the advantage of being chemical and water-free, in addition to being able to operate openly and continuously at atmospheric pressure (Noriega, Shama, Laca, Diaz, & Kong, 2011). NTP can efficiently kill or inactivate bacteria, yeasts and molds and other hazardous microorganisms, as well as spores and biofilms that are generally very difficult to inactivate (Niemira, 2012). It has been employed without causing any damage during biodecontamination and sterilization of surfaces, medical instruments, water, air, food, and living tissues (Jiang, Schaudinn, Jaramillo, Webster, & Costerton, 2012).

The NTP method works at room temperature, and due to its high efficiency and safety, it has been demonstrated to be appropriate for various applications such as surface modification of polymers (Gunaydin, Sir, Kavlak, Guner, & Mutlu, 2010; Zigal, Akdogan, & Mutlu, 2011), sterilization for biological and medical purposes (Butscher, Zimmermann, Schuppler, & von Rohr, 2016; Hertwig, Reineke, Ehlbeck, Erdogdu, et al., 2015; Hertwig, Reineke, Ehlbeck, Knorr, & Schluter, 2015), degradation of food contaminants (Misra, Pankaj, et al., 2014), and enhancement of technological properties (Misra, Sullivan, et al., 2014). The term “cold plasma” is used for the treatment temperatures below 70 °C (Schluter et al., 2013).

However, compared to bacteria, the effect of non-thermal atmospheric plasma treatment on aflatoxigenic fungi in food systems has been generally neglected (Scholtz, Pazlarova, Souskova, Khun, & Julak, 2015). The focus of this study is to investigate the feasibility of plasma inactivation of aflatoxigenic fungal spores on maize samples in a newly designed atmospheric pressure fluidized bed reactor. The inactivation efficacy of non-thermal atmospheric plasma was investigated in two different fluidized bed reactors (various diameters at a constant L/D ratio of 3) with using varying plasma precursors. And also, surveillance of the fungal spores after a storage period of 30 days at 25 °C was studied.

2. Materials and methods

2.1. Food samples

Maize was selected as model granular food sample within the scope of this study. The maize samples that were used as animal feed were purchased from a poultry farm from the central Anatolia region of Turkey. They were 8–10 mm on average in diameter, 0.32 ± 0.07 g in weight, and 1.12 ± 0.03 g/cm^3 in density. Samples were stored at 4 °C.

2.2. Test fungus and inoculum preparation

Aflatoxin producing species of *Aspergillus* [*Aspergillus flavus* (ATCC 327) and *A. parasiticus* (ATCC 1041)] were obtained from TÜBİTAK MAM Culture Collection, Turkey, on slant agar media. The fungi strains were maintained on yeast extract glucose chloroamphenicol (YGC) agar (Merck KGaA, Darmstadt, Germany) slants at 4 °C. For inoculum preparation, *A. flavus* and *A. parasiticus* were subcultured on fresh YGC plates and incubated at 25–28 °C for 5 days. By the end of the incubation period, 10 g of maize sample, which had been exposed to a pre-decontamination process with 70% ethanol, was placed on *A. flavus/A. parasiticus* cultures grown on YGC agar and agitated for 30 s to allow the mold spores to adhere to the samples surface homogeneously (Dasan, Mutlu, & Boyaci, 2016). After the contamination process was pursued in a controlled manner, the artificially contaminated sample was incubated at 25–28 °C for 18–24 h to enable mold spores to adhere to the surface and remove the extra moisture gained during fungal inoculation.

2.3. Non-thermal plasma treatment conditions/inactivation of *Aspergillus spp.* on maize samples

The atmospheric pressure fluidized bed plasma system used in this study was clearly described in a previous study (Dasan et al., 2016). The atmospheric pressure plasma jet (Plasmatreat GmbH, Steinhagen, Germany) consists of a stainless steel nozzle with an inner diameter of 4 mm. The plasma was generated through an atmospheric pressure high-voltage discharge in the jet’s reaction chamber with a single electrode, forming a discharge that exits the jet nozzle at high velocity onto the surface of the part to be treated. Dry and filtered air and nitrogen were used as plasma precursors in the range of 5–10 kV electrode voltage and 18–25 kHz frequencies (at a maximum power of 655 W). A gas flow of 3000 L/h was used to generate plasma from the tip of the needle electrode to the inner wall of the nozzle. The generated plasma expanded to the outside of the nozzle with a length of 20 mm. Two fluidized bed reactors at different diameters, but with the same L/D ratio were designed to be compatible with APFBP system. The first and second fluidized bed reactors were manufactured from glass material with diameters of 49 and 65 mm and lengths of 147 and 195 mm, respectively. A sieve from stainless steel was designed and inserted between the plasma jet and the fluidized bed reactor to ensure a uniform gas distribution and to keep particles in touch with the discharge. This continuous movement and the avoidance from permanent contact points with the atmospheric plasma jet provide homogenous particle treatment. Besides, the effects of reactor diameters (D_1: 49 mm; D_2: 65 mm), treatment time (1–5 min) and the plasma forming gases (air, nitrogen) were investigated.

A certain amount of artificially contaminated maize grains (10 g) was placed in the APFBP reactor in direct contact with plasma afterglow. After the activation of gas flow and fluidization, the plasma was ignited for various treatment times. The pure gas flow had no effect on the inactivation of fungal spores. Following the