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Effect of Cold Plasma on Storage Toxigenic Fungi - *Aspergillus flavus*

Silva[1], Jr.; Medeiros[2], M; Pereira[1], Mn; Barcelos[2], Ks; Cubas[2], Alv; Moecke[2], Eh; Scussel[1]*, Vildes M.

[1]Mycotoxicology and Food Contaminants Laboratory, Food Science and Technology Department, Center of Agricultural Sciences, Federal University of Santa Catarina, Florianopolis, SC, Brazil
[2]Environmental Engineering, University of Southern Santa Catarina, Palhoça, SC, Brazil
*Corresponding author: vildescussel_2000@yahoo.co.uk
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Abstract

Cold plasma is a novel non-thermal food processing technology that uses energetic, reactive gases to inactivate contaminating microorganisms such as fungi and bacteria. This flexible sanitizing method uses electricity and a carrier gas (air, oxygen, nitrogen, or helium) antimicrobial chemical agents are not required. The primary modes of action are due to UV light and reactive chemical products of the cold plasma ionization process. *Aspergillus flavus* is the predominant species responsible for fungal contamination and subsequent production of aflatoxins mainly in grains during postharvest operations and storage. Due to their relatively high contamination risk, decontamination methods for fungi are of great interest for economic and environmental reasons, as well as in public health. Improved post-harvest processing followed by further prevention of fungal growth is an effective way to restrict aflatoxin contamination and would have major impact on reducing health related risks and on production economics. Thus, the objective is to evaluate the inactivation of *A. flavus* by cold plasma. The experiment was conducted with 3 mm sample *A. flavus*in PDA culture medium. Plasma was applied at different durations (2, 5, 10, 12, 15 and 20 min). After application, the Petri dishes with treated samples were stored at 25°C for 6 days. There was fungal growth after 2 days in the treatments with2 and 5 min durations, 4 days with the treatments with 10 and 12 min durations and there was no fungal growth with the treatments of 15 and 20 min after 6 days. The durationof 15 and 20 min with the plasma parameters tested, were efficient for the inactivation of *A. flavus*. Cold plasma may be a promising green method to be applied in this microorganisms present in grains and other products during storage.

Keywords: cold plasma, fungi, storage, inactivation

1. Introduction

The term "plasma" applies to an ionized gas, containing neutral and electrically charged species, electrons, positive and negative ions, atoms and molecules (Alves, 1995). It is formed from the excitation of a gas or gas mixture by the application of a pressure and energy, which may be the latter mechanical, thermal, nuclear or most common, electric current (Misra et al., 2014).

Cold plasma is a novel non-thermal food processing technology that uses energetic, reactive gases to inactivate contaminating microorganisms such as fungi and bacteria. This flexible sanitizing method uses electricity and a carrier gas (air, oxygen, nitrogen, or helium) antimicrobial chemical agent are not required (Niemira, 2012; Pankaj et al., 2017).

Plasma sterilization can offer an alternative for disinfection methods. This gas presents uniform treatment, can perform the activity at low temperature and without food alteration (taste, odor, structure), and finally, the plasma does not require chemicals, therefore, they do not leave toxic residues (Selcuk; et al. 2008).

Cold plasma has a variety of applications for the food industry, including decontamination of microorganisms in foods such as meats, dairy products, fruits and vegetables, granular and particulate foods (grains, herbs and spices) and germinated seeds. This technology has also been successfully applied for surface sterilization in packaging materials (Mir et al., 2016; Misra et al., 2015, Pankaj et al., 2014, Scholtz et al., 2015).

Cold plasma inactivates microorganisms by three primary mechanisms. The first is the chemical interaction of radicals, reactive species, or charged particles with cell membranes. The second is by damage to membranes and internal cellular components by UV radiation. Finally, DNA strands may be broken by UV generated during recombination of the plasma species. While on a given commodity, one mode of action may be more significant than another, the greatest sanitizing efficacy results from plasma with multiple antimicroorganism's mechanisms (Geyter and Morent, 2012; Fridman et al., 2007; Choi et al., 2006).

Aspergillus flavus is the predominant species responsible for fungal contamination and subsequent production of aflatoxins mainly in grains during postharvest operations and storage (Scussel, 2017). *Aspergillus flavus* is an aflatoxin producer (AFLs) in storage grains in tropical and subtropical climates, especially AFB_1 which is the predominant and most potentially mutagenic, teratogenic and hepatocarcinogenic mycotoxin according to the International Agency for Research on Cancer (IARC, 1993).

Due to their relatively high contamination risk, decontamination methods for fungi are of great interest for economic and environmental reasons, as well as in public health. Improved post-harvest processing followed by further prevention of fungal growth is an effective way to restrict aflatoxin contamination and would have major impact on reducing health related risks and on production economics.

2. Materials and Methods

2.1. Fungi strains

The fungi strains *A. flavus* were obtained from the Food Mycology Laboratory of Mycotoxicology and Food Contaminants (LABMICO) culture collection at the Federal University of Santa Catarina, Florianopolis, SC, Brazil.

2.2.Culture media and chemicals

Culture media - potato dextrose agar (PDA), Kasvi (São Jose dos Pinhais, PR, Brazil) andchloramphenicol, Vetec (Duque de Caxias, RJ, Brazil).

2.3.Equipment

Microbiologicalincubator, Quimis (Diadema, SP, Brazil), autoclave, Phoenix (Araraquara, SP, Brazil), microwaveoven, Philco (Sao Paulo, SP, Brazil); laminar flowcabinet, Veco (Campinas, SP, Brazil). Cold plasma reactor corona discharge type built in borosilicate glass (11.5 x 10.5 cm). The geometry of the reactor is tip-plane in relation to the metal electrodes. The electrical system used consisted of a variac for input voltage adjustments and a 16 kV power supply. For the plasma generation, alternating current was used with no oxygen gas inlet.

2.4. Cold plasma application

A disc (3 mm) with *A. flavus* mycelia material and conidia, taken from the edge of 7-days-old-fungal culture was placed individually inside the reactor. Plasma was applied at different time durations in duplicate (2, 5, 10, 12, 15 and 20 min), whereas the fungi in the control received room air at the same exposure times. Afterward, the Petri dishes containing fungi, including the control, were held in an incubator at 25◦C for 6 days. The efficiency of cold plasma treatment was evaluatedafter 2, 4 and 6 days by measuring the fungi colony diameter (Fraternale et al. 2003; Savi and Scussel, 2014).

2.5. Statistical analysis

The data of fungi colonies growthwere analyzed by analysis of variance (ANOVA).

3. Results and Discussion

The strain of *A. flavus* that received cold plasma exposure for 15 and 20 min did not grow during the 6 days incubation, showing that the plasma parameters used were effectiveat these durationsto inactivate *A. flavus*. For the treatment durationsof 2 to 12 minutes, growth was significantly lower than controls (Figure 1).

After 2 days application of the plasma, there was growth of fungi in the times of 2 and 5 min (10 mm) and control (18mm). In the treatments of 10 and 12 min, the growth was observed after 4 days (16 and 19 mm, respectively).

Allresultscomparedwiththecontroltreatmentdifferedstatistically. In Figure 2, images show the growth of fungi in petri dish containing PDA after 6 days of incubation after treatment.

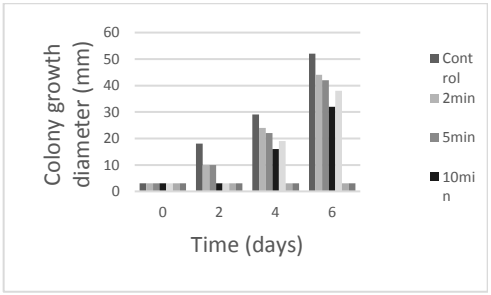

Figure 1. Effect of cold plasma (2, 5, 10, 12, 15, 20 min of exposure) on *A. flavus* growth.

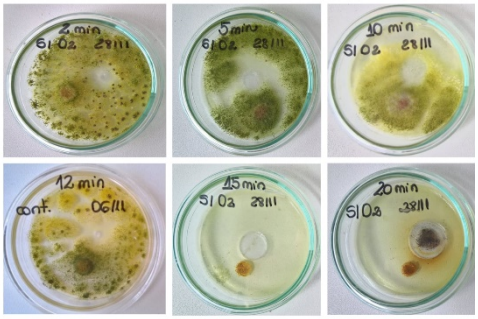

Figure 2. Colony growth of *A. flavus* in Petri dish containing PDA after 6 days of incubation after treatment.

There are fewer studies on the use of cold plasma as a decontaminant agent in fungi in foods than studies involving bacteria. The most studied foods were oilseeds (pistachios, almonds, peanuts and hazelnuts), dates and seeds (tomato, wheat, beans, oats, soya, barley, maize and rye). In relation to fungi, the genus *Aspergillus* and *Penicillium* were the most tested.

No studies were found in the literature to evaluate fungal growth after cold plasma treatment. However, many papers report their efficiency in decontamination and / or reduction of fungi on food surfaces. Dasan et al. (2016) investigated the decontamination of inoculated fungi (*A. flavus* and *Aspergillus parasiticus*) on cold plasma hazelnut surfaces. Significant reductions of 4.50 log (cfu / g) in *A. flavus* and 4.19 log (cfu / g) in *A. parasiticus*were achieved after 5 minutes of 655 W treatments. No growth was observed in *A. flavus* and *A. parasiticus* during storage of plasma-treated hazelnuts, while in the control samples the fungi continued to grow under storage conditions (30 days at 25°C).

In rice cereal bars, Suhem et al. (2013) studied the effect of cold plasma treatment to inhibit the growth of *A. flavus*. The treatment was applied to the surface of the cereal bars with 40W potency and exposure time of 20 min, reducing approximately 4 logcfu/g and also preventing growth of the fungus on the surface of the bars for at least 20 days.

Selcuk et al. (2008) successfully decontaminated the seeds of wheat, bean, chickpea, soybean, barley, oat, rye, lentil, and corn, contaminated with *Aspergillus* and *Penicillium sp.* to less than 1% of initial count depending on treatment times. The treatment times varied from 30 s to 30 min. The results suggested that after plasma treatment food quality of wheat and beans were not affected or only marginally affected. The seeds were found to be viable post plasma processing.

The mechanism by which plasma causes the cell death of microorganisms is due to reactive species of O_2, OH, NO2, etc. which have been extensively associated with direct oxidative effects on the outer surface of microbial cells. Reactive oxygen species are expected to significantly affect membrane lipids due to their location along the cell surface of the microorganisms, allowing them to be bombarded by these strong oxidizing agents (Scholtz et al., 2015; Laroussi, 2005; Mendis, Rosenberg, Azam, 2000). Oxidation of amino acids and nucleic acids can also cause changes that result in death or injury of the microorganism such as bacteria and fungi (Mir; Shah; Mir, 2016; Guo et al., 2015).

4. Conclusions

*Aspergillusflavus*treated was significantly reducedby cold plasma treatment application. The best results of antifungal effect were observed on 15 and 20 min of exposure, that were completely inhibited showing to be an effective method for this storage fungus. Cold plasma may be a promising green method to be appliedto control this microorganism present in grains and other

products during storage. It also could be a promising method of decontamination in industries and storage units, in order to avoid contamination and ensure food security to the consumer.

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Computer-Aid Molecular Docking Technology in Cereal Mycotoxin Analysis

Jinying Chen [a]*, Fusheng Gong [c], Zi Tai Sang [b]

[a] SinoGrain Chengdu Storage Research Institute Co.Ltd.
[b] State Key Laboratory of Biotherapy and Cancer Center, Collaborative Innovation Center for Biotherapy, West China Hospital, Sichuan University, Chengdu, China
[c]China Grain Reserves Group Ltd.Company
*Corresponding author: chen2331738@yeah.net
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