Cold Plasma for Effective Fungal and Mycotoxin Control in Foods: Mechanisms, Inactivation Effects, and Applications

N.N. Misra, Barun Yadav, M.S. Roopesh, and Cheorun Jo

Abstract: Cold plasma treatment is a promising intervention in food processing to boost product safety and extend the shelf-life. The activated chemical species of cold plasma can act rapidly against micro-organisms at ambient temperatures without leaving any known chemical residues. This review presents an overview of the action of cold plasma against molds and mycotoxins, the underlying mechanisms, and applications for ensuring food safety and quality. The cold plasma species act on multiple 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 various pathways resulting in degradation products that are known to be less toxic. We argue that the preliminary reports from cold plasma research point at good potential of plasma for shelf-life extension and quality retention 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.

Keywords: antimicrobial, fungi, mycotoxins, nonthermal, plasma, yeast

Introduction

The past century has witnessed an increasing number of disease-causing fungi that infect plants, animals, and humans. Fungal diseases in crops have a major impact on food security and there are direct measurable economic consequences (Fisher et al., 2012; Fisher, Gow, & Gurr, 2016). Fungi have an ability to grow on all kinds of foods- cereals, meat, milk, fruit, vegetables, nuts, fats, and products of these commodities, where they cause food spoilage such as toxin production, discoloration, off-flavor development, rotting and formation of pathogenic and allergenic propagules (Filtenborg, Frisvad, & Thrane, 1996; Pitt & Hocking, 2009). As everyday examples, the blue and green mould frequently encountered during postharvest storage of fruits are *Penicillium italicum* and *Penicillium digitatum*, respectively. Most fungal infestations, once onset, are difficult to decontaminate as fungal cells and spores are characterised by quite resistant structures.

To prevent the fungal spoilage of many fruits, particularly those belonging to the citrus family, postharvest chemical fungicide application by spraying, or wax-coating is a common commercial practice. Examples of fungicides commonly employed include, imazalil (IMZ), thiabendazole (TBZ), sodium ortho-phenil phenate (SOPP), and benomyl. The use of wax treatments, however, has resulted in problems for the citrus industry such as health and environmental issues associated with chemical residues or the proliferation of pathogenic resistant strains (Palou, Valencia-Chamorro, & Pérez-Gago, 2015). In addition, fungicide/wax coating continues to grow unpopular among most consumers who prefer a "natural" product.

Fungal spoilage is not only problematic for fresh produce industry, but also for those relying on fruit products as ingredients (for example, minimally processed fruit yogurts containing berries) (Penney, Henderson, Blum, & Johnson-Green, 2004), cheese, as well as meat industry (for example, dry sausages) (Martín-Sánchez et al., 2011). Traditionally, the mold growth in processed foods is prevented using antifungal chemical agents. However, the growing "clean label" trend has resulted in a shift towards use of natural anti-mycotic agents, which are generally expensive and increase the product cost.

Given the diverse nature of the food industry, it is continually seeking and willing to adopt new technologies for sustainability, safety, profitability, consumer trust and continued success (Pal et al., 2016). In line with this attitude, to combat with the growing challenge of food safety and new consumer demands, several nonthermal technologies have been (and are being) explored by the food industry. The research efforts resulted in commercialization of purely physical and electro-technologies, viz. high-pressure processing (HPP) and pulsed electric field (PEF) processing. Others, such as ozone application and ultrasound processing have found limited industrial application in food

CRF3-2018-0150 Submitted 6/27/2018, Accepted 9/10/2018. *Author Misra is with the Center for Crops Utilization Research, Iowa State Univ., Ames, IA, USA. Authors Yadav and Roopesh are with the Dept. of Agricultural, Food & Nutritional Science, Univ. of Alberta, Canada. Author Jo is with the Dept. of Agricultural Biotechnology, Center for Food & Bioconvergence, Research Inst. of Agriculture & Life Science, Seoul National Univ., Seoul 08826, South Korea. Author Jo is with the Inst. of Green Bio Science and Technology, Seoul National Univ., Pyeongchang 25354, South Korea. Direct inquiries to author Syamaladevi (E-mail: roopeshms@ualberta.ca).*

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sectors, with several applications being under development. Within the context of nonthermal technologies, cold plasma is of contemporary interest to researchers in food science and physics, as well as the food industry. The definition and basics of plasma science will be introduced later in Section "Plasma Science and Technology". The key reason underlying the attractiveness of cold plasma technology is that it enables rapid decontamination at ambient temperature and pressure conditions, without causing significant perceivable changes in food quality or incurring huge costs when using ambient air as the working gas. The range of micro-organisms that have been shown to be inactivated using cold plasmas include Gram positive as well as Gram negative bacteria (Laroussi, 2003; Sysolyatina et al., 2014), biofilm forming bacteria (Puligundla & Mok, 2017), bacterial spores (Patil et al., 2014), yeast and fungi (Ishikawa et al., 2012), prions (Julák, Janoušková, Scholtz, & Holada, 2011), and viruses (Puligundla & Mok, 2016). The decontamination of foods and biomaterials using cold plasma technology has been reviewed by several authors (Misra, Schlüter, & Cullen, 2016; Misra, Tiwari, Raghavarao, & Cullen, 2011; Surowsky, Schlüter, & Knorr, 2014). Among these, the inactivation of fungi (molds and yeasts) by cold plasma remains a topic of high interest to the food industry, considering that they constitute a large class of food spoilage micro-organisms. Therefore, in this work, we will review the state of the science and practices pertinent to study of physical and chemical effects of cold plasma treatment on fungal organisms. Some recent reviews have briefly discussed the plasma assisted mycotoxin degradation (Hojnik, Cvelbar, Tavcar-Kalcher, Walsh, & Krizaj, 2017; Pankaj, Shi, & Keener, 2018; Schmidt, Zannini, Lynch, & Arendt, 2018). In this review, we will discuss in detail, the ability of cold plasma to effectively degrade the toxins produced by some fungi, the "mycotoxins."

The structure of this review is the following. We begin with a brief discussion of plasma physics and chemistry. We then discuss the mechanisms underlying the plasma led fungal inactivation evidenced from studies in model and real food systems. Subsequently, we present a critical analysis of the demonstrated applications in food systems and analyze the kinetics of fungal inactivation in plasma. Finally, we speculate future research needs, and close with conclusions.

Plasma Science and Technology

Plasma is an ionized gas containing atoms or molecules in a metastable state with a roughly zero net electrical charge (Turner, 2016). Examples of natural plasma include the sun and aurora in sky, whereas fluorescent lamps, plasma televisions and commercial ozonizers are man-made plasmas. The sun being at very high temperature, is an ideal example of thermal (hot) plasma. The aurora light (polar light) is an example of low temperature (cold) plasma. Plasmas can be induced in any neutral gas by providing sufficient energy capable of causing ionization of the gas. The developments in plasma physics and innovative designs of various plasma sources have enabled the realization of plasma at atmospheric pressure conditions and ambient temperatures; these are referred to as "cold plasma." A cold plasma, per se is partially (weakly) ionized, meaning that only a small fraction of all atoms and molecules in the gas is ionized (Turner, 2016).

Understanding gas breakdown

Cold plasmas (also called nonthermal or low temperature plasmas) are typically obtained by means of electrical discharges in gases at atmospheric pressure (or subatmospheric pressures). An electrical discharge results in breakdown of the gas, which may be described as follows. Under normal conditions, the constituent species in a gas (atoms/molecules/ electrons/ions) exist in a state of equilibrium, such that the rate at which charged particles are created is approximately countered by the rate of recombination (Misra et al., 2018). When the gas is exposed to an electric field, a small current develops owing to the small number of free electrons and ions within the gas. However, this current lacks the ability to disturb the equilibrium and the ion, and electron mobilities are nearly constant (Misra, Han, Tiwari, Bourke, & Cullen, 2014). Now, upon intensifying the applied electric field, the current density increases, thereby disturbing the equilibrium to result in propagation of ions and electrons. When a sufficiently large voltage (called "breakdown voltage") is reached, the current increases to an extent that a breakdown of the gas occurs, resulting in the formation of a cocktail of active anti-microbial species.

Plasma chemistry

The reaction mechanisms resulting in the formation of active plasma chemical species include electronic impact processes (vibration, excitation, dissociation, attachment and ionization), ion–ion neutralization, ion–molecule reactions, Penning ionization, quenching, three-body neutral recombination, and neutral chemistry, besides photoemission, photo-absorption and photo-ionization (Misra, Pankaj, Segat, & Ishikawa, 2016; Sahu, Han, & Kersten, 2017). In the plasma state, the free electric charges, viz. electrons and ions, make plasma electrically conductive, internally interactive, and strongly responsive to electromagnetic fields (Fridman, 2008). When the substrate gas has oxygen as one of its component, reactive oxygen species (ROS), such as ground-state atomic oxygen [$O(^3P)$], hydroxyl radicals ($^{\bullet}OH$), singlet oxygen molecules [$O_2(^1\Delta g)$], superoxide anions ($^{\bullet}O_2^-$), and ozone (O_3) are responsible for effective inactivation of micro-organisms; cf. Hashizume et al. (2015) and references cited therein. Among the ROS, ozone is a powerful oxidant, second only to the hydroxyl radical (Segat et al., 2014). The importance of reactive nitrogen species (RNS) and ultraviolet light, in addition to ROS, has also been reported (Lu et al., 2016; Moiseev et al., 2014).

Often quick insights into the plasma chemistry can be obtained using optical emission spectroscopy (OES). In OES, the pink, purplish or bluish glow of the plasma (emitted light) is evaluated using spectroscopy. OES is generally employed for obtaining a qualitative information about the type of reactive species in the plasma; for example, OES of air plasma often reveals the presence of excited nitrogen species, atomic oxygen, and hydroxyl radicals (Misra et al., 2014; Misra, Keener, Bourke, & Cullen, 2015). Sometimes, the temperature of the ionized gas can also be determined by studying the characteristics of the light emitted from plasma (Laux, Spence, Kruger, & Zare, 2003). Other inexpensive methods for measurement of plasma species include the use of optical absorption spectroscopy (OAS); cf. Moiseev et al. (2014), and Park, Choe, and Jo (2018).

Plasma sources

The essence of all cold plasma sources is to ensure the most efficient means of applying electric field to the gas for the intended application. Accordingly, many designs of plasma sources have evolved over time. The mode of operation of plasma sources, the power supply unit, and the configuration are unique to each type of plasma source. Examples of nonequilibrium atmospheric pressure plasmas include coronas, dielectric barrier discharges

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(DBDs), microwave discharges, and capacitive discharges. We will now briefly discuss the working principle of the plasma sources commonly employed for decontamination of food and biological materials.

Corona discharges are relatively low power electrical discharges that take place at or near atmospheric pressure (Chang, Lawless, & Yamamoto, 1991). The condition for corona discharge is the requirement of substantially different radii of curvature of emitter and collector, for example thin wire-plate or sharp pin-plate electrodes (Martynenko & Zheng, 2016). When strong electric fields are applied, the corona appears as a self-sustaining, non-luminous filamentary discharge starting at the discharge electrode and ending at the ground. Unlike coronas, a DBD consists of one or two dielectric plates separated by a distance (typically, 10^{-3} to 10^{-2} m) with the working gas occupying this inter-dielectric space. When a high voltage is applied (typically, 10^2 to 10^4 V; frequency: 10^0 to 10^4 Hz) to one of the electrodes, while the other is grounded (occasionally left at a floating potential), the gas in the gap experiences the increase in voltage and gas ionization is on-set (Pankaj, Bueno-Ferrer, Misra, Bourke, & Cullen, 2014). Depending on the power applied, the DBD could move into an arc regime, a microfilamentary discharge, or glow regime. The "in-package cold plasma technology" is based on the concept of DBD, where in the interdielectric space, a sealed package with the food material is introduced (Misra et al., 2015). The plasma is formed inside the sealed package, resulting in a myriad of chemically active antimicrobial species. These metastable species have lifetimes in the order of ns to few hours, recombining within few hours into the original gas, meanwhile decontaminating the sealed food (Misra, Ziuzina, Cullen, & Keener, 2013). Note that the sealed package treatment allows to prevent any post-processing contamination. An additional advantage of the high voltage DBD plasms technology is the abatement of pesticide residues on food surfaces (Misra, 2015; Misra et al., 2014; Sarangapani et al., 2016), without causing significant changes in the food quality (Misra, Pankaj, Frias, Keener, & Cullen, 2015). A DBD can also be configured with two concentric metallic cylinders with dielectrics in between. When a gas is forced through this inter-dielectric space and ionization is ensured, one obtains a "plasma jet." Likewise, a wire-cylinder configuration with flowing gas could also serve as a plasma jet. The key difference between DBDs and plasma jets from an application standpoint is that DBD plasmas are geometrically confined to interelectrode gaps or the containment enclosure, whereas plasma jets allow the ionized species to be launched outside (Misra et al., 2018). Besides corona and DBD, microwave powered plasma sources are also common for food and bio-decontamination applications. These plasma sources employ microwaves (915 MHz or 2.45 GHz) from magnetrons guided via waveguides to power the gas space, resulting in ionization and formation of plasma. A detailed review of microwave powered plasms sources at atmospheric pressure can be found in Leins et al. (2014).

Mechanism of Action of Plasma against Fungi

To understand the mechanisms of action of cold plasma against fungal cells, it is worthwhile to briefly revisit the structure of a fungus as relevant for this review. However, a detailed discussion of the fungal biology can be found in many books (Deacon, 2013; Kavanagh, 2017). Fungi are eukaryotic (nucleated), single celled or complex multicellular micro-organisms larger than bacteria. They enter the food chain mostly from agricultural fields (soil or infected seeds), water, or spores in air. The three major sub-divisions of fungi are: (i) multicellular filamentous moulds; (ii) macroscopic filamentous fungi, where the fruiting body is called "mushroom"; and (iii) single celled microscopic yeasts. Structurally, moulds comprise of fine threads, called "hyphae." The growing hyphae frequently bifurcate, resulting in long branched filaments which intertwine to form a network of threads called a mycelium. When foods are infected by fungi, they release digestive enzymes from the hyphal tip to digest the organic matter into smaller molecules for their growth. Sometime the hyphae grow into the air and specialized reproductive and propagative structures called "spores" form on these aerial branches. Spores carry a protective coat that shields them from harsh environmental conditions (for example, drying out, high temperatures). With this brief description of the structure of a fungus, we will now discuss the effects of plasma species on fungal cells.

A prominent role of atomic oxygen, $O(^1D)$ at 60 ppm for inactivation of *Aspergillus oryzae* and *Penicillium digitatum* has been reported (Hayashi, Yagyu, Yonesu, & Shiratani, 2014). The action of atomic oxygen has been found to result in non-culturable, but viable fungal cells, where the spores of the resulting hyphae appear wrinkled. Suhem, Matan, Nisoa, and Matan (2013) have reported results from light microscopic study of radio-frequency plasma jet treated *Aspergillus flavus* cells. They observed that post-treatment, the conidiophores and the vesicle were broken, which resulted in cell leakage and loss of viability. Avramidis et al. (2010) applied atmospheric pressure DBD plasma treatment to *Ascochyta pinodella* and *Fusarium culmorum* fungi. Via light microscopy, they observed that after plasma treatment the cell walls and cell membranes structures were damaged, resulting in leakage of cytoplasm. These changes became prominent only after 60 s treatment, and after 180 s the cells were flattened. Lee et al. (2015) observed through scanning electron microscopy (SEM) that plasma treatment (using an atmospheric jet) resulted in considerable morphological alterations in fungal spores of *Cordyceps bassiana*, causing rupture, flattening and shrinkage, with surface wrinkling. SEM observation of Dasan, Boyaci, and Mutlu (2017) have also revealed that the spores of *A. parasiticus* lose their integrity after plasma treatment (for 30 s using a fluidized plasma bed) and the cell contents disperse into clusters. Based on these observations, it may intuitively be hypothesized that plasma treatment results in cell wall destruction, making it permeable and thus allowing the leakage of intracellular components. This has been partially confirmed through circular dichroism (CD) and fluorescence studies of fungal spores where the spectral peak corresponding to cell wall protein was found to decay after plasma treatment. It has also been observed that plasma treatment results in destruction of the DNA in fungal spore, which has been confirmed from the decaying CD spectrum signature, and loss of band intensity after gel electrophoresis (Lee et al., 2015). It is worthwhile noting that the extent of changes in fungal spores is a function of the level of exposure to plasma species. For example, Panngom et al. (2014) reported that considerable changes in plasma treated spore morphology of *Fusarium oxysporum* were not observed under electron microscopy, although the germination levels decreased, and cells underwent apoptosis. This is likely due to use of argon plasma with relatively low antimicrobial activity of the resultant Ar ions. Even when the active species density is not sufficiently high to cause spore structure destruction, the cells could undergo physiological changes because of apoptosis, causing increase in accumulation of lipid bodies (Panngom et al., 2014). A pictorial summary of the mechanisms responsible for inactivation of fungal cells is provided in Figure 1.

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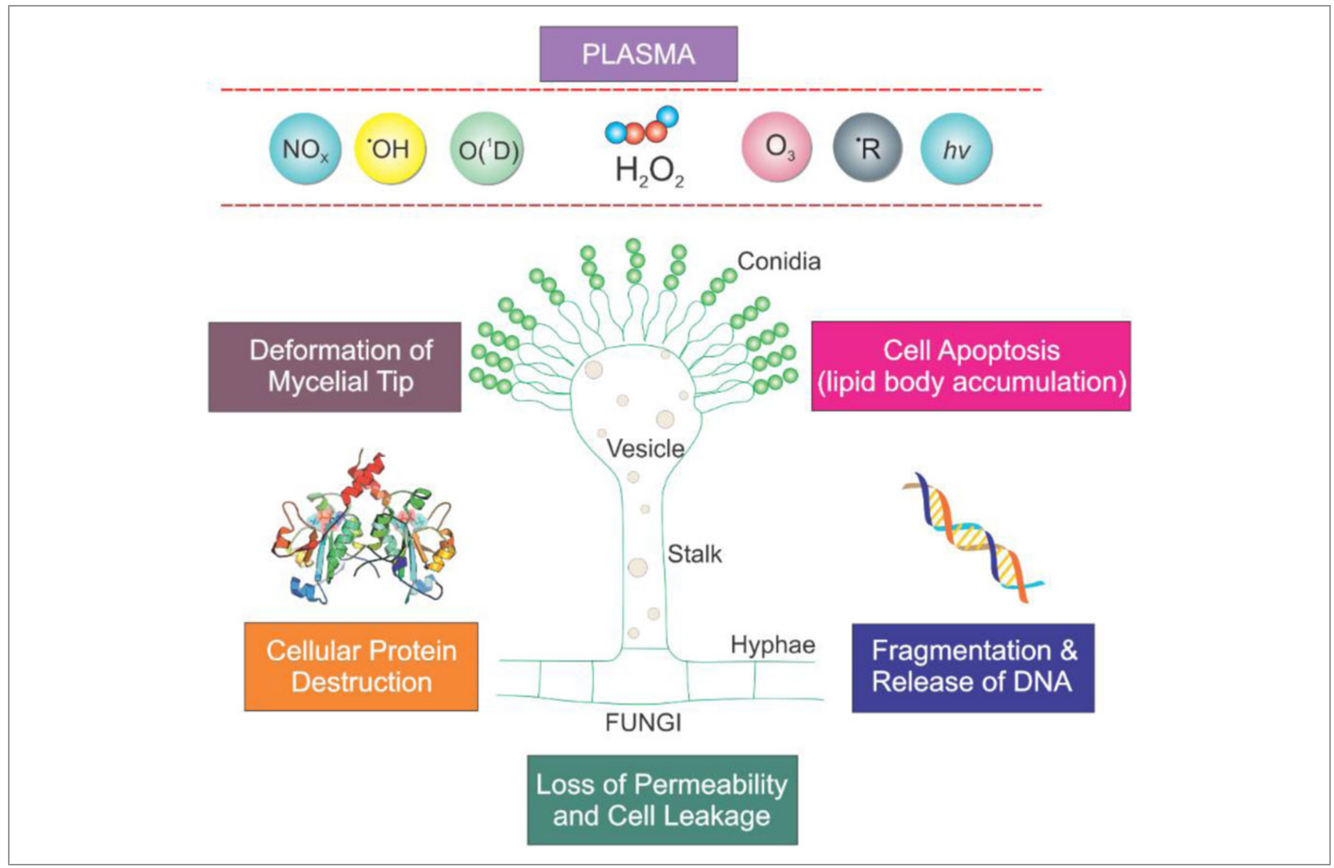

Figure 1–A summary of the effects of cold plasmaa species on fungal cells resulting in their inactivation.

Detailed studies to unravel the role of singlet oxygen species in inactivation of fungi have been carried out by Hori and co-workers (Hashizume et al., 2013, 2014, 2015; Iseki et al., 2011; Ishikawa et al., 2012). Through a comprehensive set of experiments, they reported that ROS, particularly $O(^3P_j)$, from an atmospheric pressure oxygen plasma potentially inactivates fungi. In summary, the fungal inactivation occurs through one or more of the following cellular effects:

(i) Inhibition of the cell membrane function for short plasma treatment times (ca. 30 s); subsequent treatment results in complete inactivation.
(ii) At low doses, fungal cells could undergo apoptosis (Hashizume et al., 2015).
(iii) Drastic morphological change of the cellular structures is not a necessity for fungal inactivation. However, intracellular nanostructural changes will be evident.
(iv) Morphological changes, if they occur, can be observed as distinct changes in the cell membrane and increase in its permeability (Cerioni, Volentini, Prado, Rapisarda, & Rodriguez-Montelongo, 2010; Dasan, Mutlu, & Boyaci, 2016; Kang et al., 2014).
(v) The ROS from plasma cause oxidation of intracellular organelles; particularly, lipid phosphates are oxidized by ROS to lipid peroxide through a chain reaction. In later stages, the oxidation of genomic DND and cellular proteins may also occur (Kang et al., 2014; Lu, Liu, Song, Zhou, & Niu, 2014; Panngom et al., 2014).

We wish to highlight that all the studies discussed in this section have emphasized on the dominant role of ROS, while air plasma is dominantly a mixture of ROS and RNS. The roles and contributions of RNS and ultraviolet radiation in cold plasma remain less researched considering the difficulty in separating the effects of ROS and RNS. It has been suggested that fungal spores contain the protective pigment melanin, in the cell wall layers, which confer resistance to external stresses, including UV (Eisenman & Casadevall, 2012). Therefore, further research to understand fungal interactions with UV and RNS from plasma sources is desirable.

Plasma Led Fungal Inactivation in Foods

Based on a review of recent literature, some of the notable food sectors which could benefit from antifungal efficacy of cold plasma include, the fresh produce, food grains, nuts, spices, herbs, dried meat and fish industries. A summary of the reports pertinent to inactivation of molds in food systems on exposure to cold plasma is provided in Table 1, basis which we delve into a discussion in the following subsections.

Fruits and vegetables

The fresh produce industry is frequently facing challenges of food-borne pathogen outbreaks. The pathogen inactivation effects of cold plasma potentially offer a treatment step for fresh produce to reduce the microbial load without adversely affecting the nutritional and other key characteristics (Min et al., 2018; Misra et al., 2015; Niemira, 2012). While much of research has focused on inactivation of specific pathogenic bacteria (which

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Table 1–Summary of research studies demonstrating inactivation of fungal species in food and model systems using cold plasma.

Product	Organism(s)	Plasma source	Process parameters	Salient results	Reference
Date palm	*Aspergillus niger*	DBD plasma jet	Pressure: 1 atm Gas: Argon Flow rate: 1.5–4.5 L/min Voltage: 20 kV (p-p) Flow rate: 25 kHz Time: 0.5–9.0 min	• Complete inhibition of *A. niger* spores were recorded after 9 min at 3.5 L/min argon flow rate • Flow rates > 3.5 L/min decreased inactivation efficacy of plasma	Ouf et al. (2015)
Mandarin (*Citrus unshiu Marc.*) fruits and peels	*Penicillium italicum*	Microwave plasma	Pressure: 0.7 kPa Gas: Helium; N_2+O_2 mixture (4:1) Flow rate: 1 L/min Power: 400–900 W Time: 2–10 min	• 84% reduction in disease occurrence with N_2 cold plasma (900W, 10 min) • Quality retained during storage study • Phenolics and antioxidant activity increased in peels	Won et al. (2017)
Hazelnuts, Peanuts, Pistachio nuts	*Aspergillus parasiticus*	Inductively Coupled Plasma (ICP)	Pressure: 500 mTorr Gas: Air, SF_6 Frequency: 1 kHz Voltage: 20 kV (p-p) Power: 300 W Time: 5–20 min	• SF_6 plasma was more effective than air plasma with a 5 $\log_{10}$ CFU/g decrease in fungal population • Shelled nuts require intense treatment than without • No significant organoleptic changes	Basaran et al. (2008)
Pistachios	*Aspergillus brasiliensis*	DBD	Pressure: 0.13-0.3 mbar Gas: Ar, O_2, Ar + O_2 (1:1, 2:1, 10:1 v/v) Frequency: 15.56 MHz Power: 40–400 W Time: 15 s – 30 min	• Ar/O_2 (10:1 v/v) mixture was most effective with complete inactivation after 15 s of treatment • 2 $\log_{10}$ reduction on pistachio can be achieved after 1 min of treatment	Pignata et al. (2014)
Hazelnuts	*Aspergillus flavus, Aspergillus parasiticus*	Atmospheric Pressure Fluidized Bed Plasma (APFBP) with stainless steel mesh based on jet from PlasmaTreat GmbH	Pressure: 1 atm Gas: Dry air; N_2 Flow rate: 50 L/min Voltage: 5–10 kV Frequency: 25 kHz Power: 460–665 W Time: 1–5 min Grain mass: 10 g	• Length/Diameter (L/D) ratio of the fluidized bed influenced the decontamination efficacy • After 5 min treatment with air plasma ○ For L/D = 49 mm/147 mm reductions were: *A. flavus*: 4.5 log CFU/g; *A. parasiticus*: 4.19 log CFU/g ○ For L/D = 65 mm/195 mm reductions were: *A. flavus*: 3.82 log CFU/g; *A. parasiticus*: 3.75 log CFU/g • Air was more effective compared to N_2 based plasma	Dasan et al. (2017); Dasan et al. (2016)
Onion powder	*Aspergillus brasiliensis*	Microwave plasma	Pressure: 0.7 kPa Gas: Helium Flow rate: 1 L/min Power: 400–900 W Time: 10–40 min	• At 400 W for 40 min, spores count was reduced by 1.5 ± 0.2 log spores/cm^2 • The color parameter, antioxidant activity, quercetin content was not significantly affected, while volatile loss was observed	Kim et al. (2017)
Saffron	*Aspergillus sp.*	Radio-Frequency plasma	Pressure: 13.5 mTorr Gas: oxygen Power: 10–90 W Time: 10 and 15 min	• Complete inactivation of *Aspergillus sp* after 15 min plasma exposure at 60 W. • Insignificant changes in color, antioxidant activity, odour and flavor of plasma treated saffron.	Hosseini, Farrokhi, Shokri, Khani, and Shokri (2018)
Tomato seeds, wheat, bean, chickpea, soybean, barley, oat, rye, lentil, corn	*Aspergillus spp., Penicillium spp.*	Inductively Coupled Plasma (ICP)	Pressure: 500 mTorr Gas: Air, SF_6 Frequency: 1 kHz Voltage: 20 kV (p-p) Power: 300 W Time: 5,10, 20 min	• SF_6 plasma for 15 min allowed decreasing both species by 3 $\log_{10}$ • Seed germination is retained after plasma treatment	Selcuk, Oksuz, and Basaran (2008)
Wheat and barley	*B. atrophaeus* *P. verrucosum*	DBD	Pressure: 1 atm Gas: Air Voltage: 80 kV, 50 Hz Exposure mode: direct and indirect Time: 5, 10 min	• Fungi population decreased on barley surface by 2.1 and 1.5 $\log_{10}$ CFU/ g and on wheat surface by 2.5 and 1.7 after 20 min direct and indirect exposure with 24 hours retention at 15 °C.	Los et al. (2018a)

(continued)

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Table 1–*continued*.

Product	Organism(s)	Plasma source	Process parameters	Salient results	Reference
Maize grains	*Aspergillus flavus*, *Aspergillus parasiticus*	Atmospheric Pressure Fluidized Bed Plasma (APFBP) with stainless steel mesh based on jet from PlasmaTreat GmbH	Pressure: 1 atm Gas: Dry air; N_2 Flow rate: 50 L/min Voltage: 5–10 kV Frequency: 18–25 kHz Power: 665 W (maximum) Time: 1–5 min Grain mass: 10 g	• Length/Diameter (L/D) ratio of the fluidized bed influenced the decontamination efficacy. • After 5 min treatment with air plasma ○ For L/D = 49 mm/147 mm reductions were: *A. flavus*: 5.48 log CFU/g; *A. parasiticus*: 5.20 log CFU/g ○ For L/D = 65 mm/195 mm reductions were: *A. flavus*: 5.08 log CFU/g; *A. parasiticus*: 4.99 log CFU/g • Air was more effective compared to N_2	Dasan et al. (2016)
Brown rice cereals	*Aspergillus flavus*	Radio-Frequency atmospheric cold plasma jet	Pressure: 1 atm Gas: Argon Power: 40 W Flow rate: 10 L/min Frequency: 50–600 kHz Voltage: 10 kV (max)	• Plasma power of 40 W for 20 min was effective in preventing *A. flavus* growth for 20 days under storage conditions of 25 °C and 100% RH	Suhem et al. (2013)
Beef jerky	*A. flavus* (KCTC6905)	Flexible thin layer DBD	Pressure: 1 atm Gas: Ambient Air Frequency: 15 kHz Time: 0–10 min	• *A. flavus* population reduced from 5.24 to 2.06 log CFU/g after 10 min exposure • Changes in color and flavor was noticed	Yong et al. (2017)
Dried filefish (*Stephanolopies cirrhifer*) fillets	*Cladosporium cladospori-odies*, *Penicillium citrinum*	UV induced photo-ionization plasma (from Biozone Scientific)	Pressure: 1 atm Gas: Air Time: 3, 5, 10, 20 min	• Surface fungal population reduced by >1 log_{10} CFU/g in 10 min • Lipid peroxidation and sensory quality were affected beyond 10 min of treatment	Park, et al. (2015)
Apple juice	*Z. rouxii*	DBD	Pressure: 1 atm Gas: Air Input power: 90 W Time: 20–140 s	• 5 log reduction of viable cells population in 140 s • Significant changes in color, pH and acidity • No influence on total soluble solids, total phenolic content, and reducing sugar after 140 s	Xiang et al. (2018)
Aqueous suspension	*Aspergillus brasiliensis*	Pulsed power discharge using a thyristor for pulsing	Pressure: 0.5 atm Gas: Nitrogen Pulsing frequency: 1.5 kp/s	• Fungal population decreased by a factor of 6 after 15 min of treatment	Sakudo, Toyokawa, Misawa, and Imanishi (2017)
Cellophane membrane	Hyphae of *A. flavus*	Diffuse surface coplanar barrier discharge	Pressure: 1 atm Gas: Ambient air Input power 400 W Dimension 8 × 20 cm Time: 0, 5, 15, 30, 60, 90, 180 s	• Complete loss of cell viability after 30 s of exposure • Loss of cell structure, damaged cell wall, leakage of cells content, intracellular oxidative stress, and damaged DNA • Increase in antioxidant enzyme activity after 15 and 30 s of plasma exposure	Simoncicova et al. (2018)
GSWP filters	*P. buchwaldii* *p. bialowiezense* *P. expansum*	Plasma jet	Pressure: 1 atm Gas: Nitrogen Flow rate: 15 l/min Time: 0, 5,10, 15, 20, 30 min	• 2.6, 2.2, and 1.9 log_{10} reduction of *P. buchwaldii*, *P. expansum* and *P. bialowiezense* respectively, after 20 min of plasma exposure. • Extended treatment beyond 20 min did not result in increased population reduction	Nierop Groot, Abee, and van Bokhorst-van de Veen (2018)

is undeniably very important), the inactivation of molds and yeasts is equally important for ensuring food quality and shelf-life. Therefore, we will review the potential of cold plasma for mold inactivation in fresh produce.

Lacombe et al. (2015) studied the effects of a cold plasma jet in air on blueberries inoculated with yeasts and molds over treatment durations ranging between 15 and 120 s where the samples were placed at a working gap of 7 cm from plasma jet. Results indicated that yeast and molds showed 0.8 to 1.6 log CFU/g order of reduction after 1 day and 1.5 to 2.0 log CFU/g reduction after 7 days. Plasma exposure longer than 60 s was reported to cause considerable reduction in firmness, anthocyanins and color. Sterilization efficacy of in-package indirect cold plasma generated in atmospheric pressure was also reported by Misra et al. (2014). They observed that a 5 min treatment at 60 kV resulted in 3.3 log cycle reduction of naturally occurring yeasts and molds on strawberry surface after 24 hr of in-pack storage. However, unlike Lacombe et al. (2015), they did not find significant differences in the respiration rate, color, and firmness in plasma treated strawberries. These differences in quality are a direct evidence of the

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differences that could arise from the nature of plasma source and the operating conditions. Notably, a plasma jet is more intense and localized than a DBD, and often operates in turbulence regime. A further evidence of the importance of operating conditions can be found in the study by Won, Lee, and Min (2017), where a microwave powered nitrogen cold plasma treatment of mandarins at 0.7 kPa pressure and 1 L/min flow rate for 10 min was reported to inactivate *Penicillium italicum* and reduce the disease occurrence by 84%. The inhibition of *P. italicum* was found to depend on the gas, power input, and treatment duration. The inhibition increased with increase in plasma power and treatment time, whereas maximum inhibition was attained using N_2 at 900 W for 10 min as compared to helium and 4:1 N_2/O_2 mixtures. A detailed study of the applicability of microwave plasma processed air for decontamination of the yeast *Candida albicans* in fresh produce has been reported by Schnabel, Niquet, Schlüter, Gniffke, and Ehlbeck (2015). They observed complete inactivation of yeast by 6.2 log_{10} steps on apple peel and strawberry, whereas only 3 log_{10} in apple pulp, indicating that the surface features have an impact on the efficacy of plasma. While detailed results are unavailable, the effectiveness of a large volume air plasma generated by applying 100 ns, 1 kHz high voltage pulse between two parallel electrodes separated by 48 mm gap against fungal contamination of grapes has also been reported (Mohamed et al., 2014).

A limited number of studies have also evaluated the evolution of spoilage micro-organisms in plasma treated plant produce during storage. Min et al. (2017), reported the inactivation of yeasts and molds ranging between from 0.9 to 1.7 log CFU/g in packaged lettuce exposed to high voltage (34.8 kV, at 1.1 kHz frequency) dielectric barrier pin type discharge system after 5 min. This study also demonstrated that the post packaged treatment storage of lettuce after 7 days did not have any effect on decontamination. Such time dependent effects point at the likelihood of fungal cell recovery postplasma treatments and deserve consideration for practical applications.

As a final note, we would like to point at the use of fungicides in-field or post-harvest for controlling fungi, which is associated with certain drawbacks. In order to be efficient, any fungicide must be totally lethal against the fungal sp. otherwise, it could stimulate mycotoxin production *in vitro* (Jouany, 2007). However, fungicide application raises serious concerns about residues of these compounds in food products, particularly because many fungicides are suspected or potential oncogens (Saharan, Kumar, Sharma, & Nagarajan, 2004). Interestingly, initial reports suggest that cold plasma can effectively dissipate fungicide residues in strawberries and blueberries (Misra, 2015; Misra et al., 2014; Sarangapani, O'Toole, Cullen, & Bourke, 2017). That said, further studies are encouraged for practical usage. Overall, cold plasma technology also offers opportunities for multiple points of application within the food chain, while ensuring microbiological as well as chemical safety.

Herbs and spices

Dry food systems with low water activity are often susceptible to fungal contamination and their safety remains a challenge for processors (Syamaladevi, Tang, & Zhong, 2016). Despite a higher resistance observed in dry food systems, plasma treatment has been found to be considerably effective in reducing molds vis-à-vis other known methods. Common examples of such food classes include herbs and spices which when contaminated, can cause rapid spoilage of the foods to which they are applied. A further challenge with decontamination of herbs and spices is their susceptibility to loss of volatiles on exposure to heat (Hertwig et al., 2015).

The ability of remote plasma treatment to reduce the yeast and mold counts on pepper seed and crushed oregano was first reported by Hertwig et al. (2015). It was found that 5 min plasma treatment completely inactivated yeast and mold on black pepper seeds, while 60 min treatment of crushed oregano reduced the population by 1.8 log_{10} CFU/g. Kim, Lee, and Min (2013) investigated the effect of treatment time, power and gas composition on decontamination of red pepper inoculated with *Aspergillus flavus* using a microwave powered cold plasma system. The highest inactivation of *A. flavus* of 2.5 log was observed using N_2 plasma at operating power of 900 W after 20 min, whereas for other gases such as He, N_2+O_2 and He-O_2 mixture, the inactivation levels were 2.0, 0.3, and 0.3 log, respectively.

Kim, Oh, Won, Lee, and Min (2017) explored the effects of microwave cold plasma treatment on *Aspergillus brasiliensis* inoculated into onion powder. They observed that plasma treatment following vacuum drying of the powder exhibited greater fungal reduction (1.5 log_{10} spores/cm^2) compared to that after hot air drying (0.7 log_{10} spores/cm^2). This difference was attributed to the surface cracks in hot air-dried samples versus a smooth surface in vacuum dried samples. While mold growth in tea is a rare incidence, tea leaves could serve as a model dry herb in research studies. Amini and Ghorannevi (2016) reported that the decontamination effect of argon cold plasma jet in black and green tea against mold and yeast was dependent on treatment time and initial microbial concentration. They observed a complete inactivation of the molds and yeasts after 7 min of treatment, starting from initial populations of 3.30 and 3.0 log_{10} CFU/g, respectively.

Overall, the success of cold plasma for herbs/spices is established, as far as inactivation of molds is concerned. However, no studies have attempted to look at the volatile profile of the plasma treated herbs/spices. This is crucial information for attracting industries, and further studies in this direction are desired.

Food grains and nuts

Owing to their extensive use as human foods and livestock feeds, the microbiology and safety of grains, seeds, nuts and their products deserve high importance. The fungal attack in cereal grains could be due to field fungi (which attack grains at high moistures) or storage fungi (which attack stored grains at relatively low moisture) (Los, Ziuzina, & Bourke, 2018b). Typical examples of field fungi include species of *Alternaria*, *Cladosporium*, and *Fusarium*, whereas storage fungi include *Eurotium*, *Aspergillus*, and *Penicillium*. Majority of studies reported in literature have focused on the cold plasma led inactivation of storage fungi in grains and nuts. Dasan, Boyaci, and Mutlu (2016) studied the sterilization effect of fluidized bed plasma treatment on *Aspergillus flavus* and *Aspergillus parasiticus* inoculated on maize grains. A maximum reduction of 5.48 and 5.20 log_{10} CFU/g in *A. flavus* and *A. parasiticus,* respectively after 5 min plasma treatment was observed. In a more recent study, an ambient air corona discharge plasma jet operating at 20 kV, 58 kHz at flow rate of 2.5 m/s was tested on rapeseed surface. About 1.8 and 2.0 log_{10} CFU/g reduction in yeast and mold cells respectively was observed after 3 min of plasma exposure (Puligundla, Kim, & Mok, 2017).

The aspergilli are ubiquitous invaders of nuts and can produce aflatoxins. Basaran, Basaran-Akgul, and Oksuz (2008) reported the sterilizing effect of low-pressure cold plasma against *Aspergillus parasitics*, where the cells were reduced by 1 log_{10} CFU/g after 5 min treatment and by an additional 1 log_{10} CFU/g after 10 min

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treatment. A 5 $\log_{10}$ CFU/g decrease for *A. parasiticus* population on hazelnut, peanut, and pistachio surfaces was recorded after 20 min while a 3-log reduction of *Aspergillus* spp. and *Penicillium* spp in the same time. Pignata et al. (2014) have reported a 2 $\log_{10}$ reduction in the fungal population of *Aspergillus brasiliensis* on pistachios after 1 min of low pressure plasma treatment in argon/oxygen (10:1 v/v) using a DC discharge (600 W, 15.56 MHz RF). Amini and Ghoranneviss (2016) reported complete destruction of *A. flavus* inoculated on fresh and dried walnut surface, after 11 min and 10 min of argon plasma jet exposure. Moreover, argon plasma jet exposed walnut showed insignificant residue, after 15 and 30 days storage period at 4 °C, and there were no changes in phenolic content and antioxidant activity of treated and control walnut after treatment and 30 days of storage. In another study, Dasan et al. (2016) reported a reduction in *A. flavus* and *A. parasiticus* cells on hazelnuts with 4.50 and 4.19 $\log_{10}$ CFU/g reduction in *A. flavus* and *A. parasiticus* population, respectively after 5 min plasma treatment in dry air. These studies also demonstrated that the sterilization efficacy increased with increased applied voltage and frequency of plasma generating system. *A. flavus* cells were more sensitive to plasma compared to *A. parasiticus*, suggesting that the difference in sterilization effect was not directly related to plasma system efficacy, but it would be essential to perform a molecular and structural dependent study against the response of plasma species to cells during the treatment process. It is worthwhile noting that although the external contamination can easily be decontaminated using gas plasma, whether the active species can penetrate the internal sites of colonization in large storages of grains and nuts remains unknown.

In a distinct study, Suhem et al. (2013) treated *A. flavus* inoculated brown rice snacks bar using low-pressure radio frequency plasma. They observed a 4 $\log_{10}$ CFU/g reduction in yeast and mold population in *A. flavus* inoculated brown rice snack bar after 30 min plasma exposure at an operating power level of 130 W.

Meat and meat products

A challenge in extending shelf-life of meat and meat products arises from the composition of meat that not only renders perishability, but also high sensitivity to loss of sensory traits when subjected to routine pasteurization processes. Several recent studies have demonstrated the potential of cold plasma technology as a novel intervention for ensuring meat safety, detailed accounts of which can be found from Misra and Jo (2017) and Lee et al. (2017). With regards to arresting yeasts and molds in meat or meat products there have been only few studies exploring use of cold plasma, mainly for dried or semi-dried products where fungal growth is a commonly encountered.

Ulbin-Figlewicz, Brychcy, and Jarmoluk (2015) reported that helium, argon and nitrogen generated plasma at lower pressure of 0.8 MPa reduced the growth of yeast and mold population on meat surface approximately 3, 2.6, and 1 log CFU/cm^2, respectively after 10 min treatments. They observed insignificant changes in color and pH values between plasma treated and control samples. Low-pressure helium and argon plasma exposure (10 min, 20 kPa) of pork and beef muscles against meat microbiota was studied by Ulbin-Figlewicz, Jarmoluk, and Marycz (2015). Results demonstrated that the highest inactivation efficacy for helium plasma jet and yeast and mold population were reduced by 1.90 and 0.98 log CFU on pork and beef muscles, respectively after 10 min treatment. Whereas application of argon plasma was less effective and the observed logarithmic reductions in population were 0.41 and 0.50 log cycle on pork and beef muscles, respectively after 10 min argon plasma treatment.

Yong et al. (2017) studied the mold inactivation efficacy of a thin, flexible surface barrier discharge plasma on beef jerky inoculated with *A. flavus,* where ambient air dielectric barrier discharge system was employed for plasma generation. They reported a significant reduction in initial microbial load up to 5.24 to 2.06 log CFU/g, after exposure of 10 min of plasma and the D-value for *A. flavus* population was 3.24 min. No significant difference was found in physicochemical properties such as shear force, myofibrillar fragmentation index and metmyoglobin of plasma exposed beef jerky sample. However, changes in sensory parameters (off odor, flavor, overall acceptability) of the samples were detected after 10 min of plasma exposure.

Dried fish is yet another product that is popular in many Asian countries and highly susceptible to mold attack. The decontamination of dried filefish fillets inoculated with spores of *Cladosporium cladosporioides* and *Penicillium citrinum* using cold plasma from a UV photo-dissociation source (from BioZone Scientific) in oxygen has been reported (Park & Ha, 2015). It was found that cold plasma resulted in reduction of the mold counts by 1 to 1.5 $\log_{10}$ CFU/g after 20 min treatment. However, more than two-fold increase in the thiobarbituric acid reactive substance (TBARS) concentration compared to untreated samples was also recorded in plasma treated samples. It may be recalled that TBARS is a measure of the extent of lipid oxidation measured as manolaldehyde equivalence. A critical review of the cold plasma induced lipid oxidation in foods and strategies to overcome the challenges were recently discussed (Gavahian, Chu, Mousavi Khaneghah, Barba, & Misra, 2018). In summary, there exists a need to explore the use of alternate plasma sources for decontamination of dried fish, and optimization of the process parameters to minimize quality losses.

Effect of Plasma on Mycotoxins

Traditional methods to degrade mycotoxins

Fungal species such as *Fusarium*, *Aspergillus*, *Penicilium*, *Alternatia* can produce mycotoxins. These mycotoxins are secondary metabolites of fungi, and generally exhibit high chemical stability. The inactivation of fungi or direct degradation of mycotoxins in foods and feed material can arrest their production and decrease exposure risk to animals or humans, respectively. The shelf life and quality of foods can be affected by fungi and mycotoxins as they can reduce the nutritional quality and seed viability. Some of the carcinogenic mycotoxins, such as aflatoxins, zearalenone, deoxynivalenol (DON, vomitoxin) and fumonisins are toxic to humans and can also cause mutations (Park et al., 2007). Aflatoxins are the notorious mycotoxins produced by the fungi *Aspergillus flavus* and *A. parasiticus*. Agricultural products such as corn, peanut, cotton seed can be susceptible to aflatoxins production especially at high temperature (25 to 32 °C) and humidity conditions (>85%). Vomitoxins or deoxynivalenol (DON) is produced by *Fusarium* species in grains such as wheat, corn and sorghum is a potential health hazard to humans.

Several of the post-harvest operations of grains and other food commodity can significantly reduce the quantity of mycotoxins in them; however, complete removal of mycotoxins or decontamination of these foods is impossible during these operations. Hence additional processing of these commodities may be required for safe consumption. The common methods employed to remove mycotoxins from food and feeds includes physical, and chemical, enzymatic and microbial decontamination methods (Karlovsky et al., 2016). The physical processing operations used are sorting

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(optical), sieving, floatation, washing, dehulling, steeping, milling, thermal treatments, Ultraviolet, gamma treatments. The physical separation process segregates inferior infected quality from bulk based on color and density difference, visual identification, shape and size. Unfortunately, these methods can be inaccurate, unreliable, laborious, time consuming, and not suitable for inline measurement.

Ultraviolet (UV) treatment has shown effectiveness in reducing fungi such as *P. expansum* and mycotoxins however, UV radiation cannot penetrate to food materials (Murata, Mitsumatsu, & Shimada, 2008; Syamaladevi et al., 2015; Syamaladevi et al., 2014). Gamma irradiation has been a promising intervention against mycotoxins and accepted by the food industry for treatment of dry food. Gamma irradiation produces high energy photons, which interact with microbial cells and induce DNA rupture (Udomkun et al., 2017). The chemical treatments using acetic acid, citric acid, lactic acid under simulated cooking (Aiko & Mehta, 2016) and bases such as NH_3, $Ca(OH)_2$, Na_2CO_3 (Park, Lee, Price, & Pohland, 1988), oxidizing agents (for example, ozone), reducing agents (for example, $NaHSO_3$) are also used to reduce mycotoxins (Temba et al., 2016). Enzymatic detoxification has also been commonly studied (for example, amylases, glucanases, proteases) to reduce mycotoxins in foods. The biological control of mycotoxins detoxification includes, fermentation, prevention of ingestion of mycotoxins content intake of contaminated food by microorganism of gastrointestinal track, introduction of active enzyme by genetic engineering in plant genes capable of detoxification (Halász, Lásztity, Abonyi, & Bata, 2009). It is worthwhile noting that the traditional mycotoxin degradation methods can take long processing time for effective mycotoxin reduction hence not energy efficient and expensive. Even though several methods have been proposed to degrade mycotoxins in foods, the food industry continues to seek a rapid and effective technology.

Degradation of mycotoxins using cold plasma

Reports pertinent to mycotoxin breakdown using plasma have focused on several aspects—the type of mycotoxin, the substrate, the plasma source and the process parameters. A summary of the reports pertinent to cold plasma led degradation of mycotoxin residues in various matrices is provided in Table 2. In an early study, Park et al. (2007) employed a microwave powered atmospheric pressure cold argon plasma treatment to evaluate the effects on mycotoxins. They found that mycotxins such as afltoxin B1 (AFB1), deoxynivalenol (DON, vomitoxin), and nivalenol (NIV) were degraded completely within 5 s of treatment. In the subsequent year, Basaran et al. (2008) investigated the possibility of dissipating mycotoxin from nut surfaces using a low pressure inductively coupled plasma source. They found that 20 min of air plasma allowed to decrease the concentration of a mixture of aflatoxins (B1, B2, G1, and G2) by up to 50%. Some years later, Ouf, Basher, and Mohamed (2015) reported a complete degradation of fumonisin B2 and ochratoxin A produced by *Aspergillus niger* inoculated on to date palm slices by double jet argon plasma treatment at 3.5 L/min. This treatment also eliminated the *A. niger* spores inoculated on to date palm fruit slices. The study not only suggested the potential of cold plasma treatment in degrading mycotoxins in food but also reducing the capability of harmful fungi in mycotoxin production in food and feed. This capability of cold plasma may be related to its significant influence on genes regulating the biosynthesis of mycotoxins in fungi. Recently, Aflatoxin B1 was shown to degrade up to 88% when treated using a 300 W RF plasma for 10 min and the degradation products of aflatoxin B1 were noted to be less toxic (Wang et al., 2015). Cold plasma treatment of aflatoxin inoculated hazelnut resulted in more than 70% reduction in concertation of aflatoxins after 12 min (Siciliano et al., 2016). Aflatoxins B1 and G1 were more sensitive to plasma treatments compared to aflatoxins B2 and G2, respectively. It was suggested by the authors that cold plasma can be scaled up and included into hazelnut processing line after dehulling and before roasting operations.

In a recent study, Devi, Thirumdas, Sarangapani, Deshmukh, and Annapure (2017) investigated the effect of cold plasma treatment on the growth of *Aspergillus flavus* and *Aapergillus parasiticus* and production of aflatoxins in groundnut inoculated with fungal species. Application of air plasma treatment at 60 W reduced the *A. flavus* and *A. parasiticus* growth in groundnut by 97.9% and 99.3%, respectively. Additionally, a reduction in aflatoxins B1 production by 70% and 90% was reported for plasma exposure at 40 W for 15 min and 60 W for 12 min, respectively. Ten Bosch et al. (2017) used atmospheric DBD cold plasma to degrade deoxynivalenol, zearalenone, enniatins, fumonisin B1, and T2 toxin produced by *Fusarium* spp., sterigmatocystin produced by *Aspergillus* spp. and AAL toxin produced by *Alternaria alternata*. The temperatures of the gas and substrate (which was a cover glass) during cold plasma treatments were lower than 60 °C. They observed almost complete degradation of mycotoxins in 60 s but the inactivation efficacy of cold plasma was dependent on the type of mycotoxins and the matrix. For instance, when extracts of rice cultures of fungal strains producing these mycotoxins containing approximately 100 μg/mL of each toxin were exposed to plasma, the mycotoxin degradation was smaller compared to mycotoxins in solution. This is most likely because the reactive species in plasma could be scavenged by the different components in the matrix. No stable residues of mycotoxins were observed with HPLC-MS after cold plasma treatment, which was suggested to be due to the conversion of mycotoxins to volatile compounds (Ten Bosch et al. 2017). In a recent study, Shi, Ileleji, Stroshine, Keener, and Jensen (2017) studied the degradation of aflatoxin in corn kernels using a high-voltage DBD plasma operating in air and modified atmospheres. They observed a rapid degradation of the toxins in a high oxygen atmosphere and at elevated humidity levels; cf. see Figure 2. This observation can be attributed to the favorable plasma chemistry involving production of greater amounts of hydoxyl radicals and ozone with high humidity and oxygen, as was experimentally demonstrated in previous studies (Moiseev et al., 2014; Patil et al., 2014).

Mechanism of mycotoxin degradation

Being an emerging area, the degradation products of mycotoxins and the pathways during cold plasma treatment are sparse the literature. The mycotoxin degradation pathways during cold plasma treatment are inevitably related to their molecular structure, the nature of the plasma chemistry and thus, the species interaction with toxin molecules (Pankaj et al., 2018). Drawing analogy from polymer science, one could argue that the presence of aromatic structures in polymers often slows down the degradation process during plasma treatments (Klarhöfer, Viöl, & Maus-Friedrichs, 2010; Ten Bosch et al., 2017). However, the mycotoxin degradation pathways during plasma treatment turns out to be remarkably different, being relatively small molecules. Wang et al. (2015) studied the chemistry of low pressure plasma treated AFB1 and based on mass-spectrometry proposed the degradation pathways. They anticipated the formation of an intermediate with $C_{17}H_{15}O_7$, which is also a major degradation product of AFB_1 after UV

Table 2–Summary of research studies demonstrating degradation of mycotoxin using cold plasma.

Product/Matrix	Aflatoxin	Plasma source	Process parameters	Salient results	Reference
N/A	Aflatoxin B1, deoxynivalenol and nivalenol	Microwave-induced argon plasma	Gas: Argon Gas flow rate: 100 L/min at 8 kgf/cm^2; Treatment time: 1–10 s	Mycotoxins were completely degraded and the mycotoxin induced cytotoxicity was completely decreased in 5 seconds of plasma treatment	Park et al. (2007)
Hazelnuts, peanuts, and pistachio nuts	Aflatoxins (B1, B2, G1 and G2)	low pressure cold plasma	Gas: Air Power: 300 W power Voltage: 20 kV Pressure: 100 mTorr and running pressure of 500 mTorr Treatment time: 5–20 min	20 min air plasma treatment reduced 50% of total aflatoxins (AFB1, AFB2, AFG1, and AFG2); SF_6 plasma reduced 20% of total aflatoxin after 20 min treatment	Basaran et al. (2008)
Date palm fruits	Fumonisin B2 and ochratoxin A	Atmospheric pressure argon cold plasma jet	Gas: argon Gas flow rate: 1.5–4.5 L/min Treatment time: 0.5–9 min; Sample-jet nozzle distance: 12 mm	Fumonisin B2 and ochratoxin A on date palm discs were completely degraded after 6 min and 7.5 min plasma treatments, respectively	Ouf et al. (2015)
Hazelnuts	Aflatoxin (B1, B2, G1, G2)	Dielectric barrier discharge	Gases: Pure N_2, and three mixtures of N_2/O_2 (21%, 1%, and 0.1% O2); Frequency: 100–150 kHz; Power: 0.4 and 2 kW; Distance to sample: 50 mm; Treatment time: 1, 2, 4, 12 min	The maximum detoxification efficacy (70%) of aflatoxins was achieved using N_2 or mixture of N_2 + 0.1% O_2; Higher effectiveness against AF B_1, AF G_1 vis-à-vis B_2 and G_2.	Siciliano et al. (2016)
Rice extracts containing fungal strain	Deoxynivalenol, zearalenone, enniatins, fumonisin B1 and T2, Sterigmatocystin, and AAL toxin	Dielectric barrier discharge	Gas: Air; Power density: 4 W/cm^2; Discharge gap: 2 mm; Flow rate: 130 L/min; Voltage: 38 kV	Pure mycotoxins were completely degraded by 60s treatment; Degradation rates of mycotoxin depends on their structure, and the presence of matrix	Ten Bosch et al. (2017)
Corn	Aflatoxins	Dielectric barrier discharge	Gas: Air, modified atmosphere (65% O_2/30% CO_2/ 5% N_2); Power: 200 W Frequency: 50 HZ Voltage: 90 kV Discharge gap: 4.5 cm; Treatment time: 1–30 min;	62 and 82% decrease in aflatoxin in corn with 1 and 10 min treatment at 40% humidity; Humid air (40, 80% RH) led to greater reduction than dry air (5% RH); Modified gas was effective than air	Shi et al. (2017)
Glass coverslip	AF B1	Direct gas breakdown using pulsed power from a static induction thyristor	Pressure: 0.5 atm Gas: Nitrogen Pulsing: 0–1.5 kpps Time: 0–30 min	After 15 min of treatment, the concentration reduced to <1/10th of initial (200 ppb)	Sakudo et al. (2017)

treatment. The cold plasma degradation of mycotoxins could be directly related to the free radicals (for example, O$^•$ and OH$^•$) produced during the treatments. In a very recent study, Shi, Cooper, Stroshine, Ileleji, and Keener (2017) also confirmed the same pathways when treating AFB1 using a high voltage DBD plasma source. The reactive gas species that have been identified as primary contributors to the aflatoxin degradation by humid air cold plasma include ozone, hydroxyl, and aldehyde radicals that form from ionization of oxygen, water molecules, and carbon dioxide precursors. A summary of the proposed pathways is captured in Figure 3, where majority of the reactions are due to ozonolysis, involving sequential addition and cleavage reactions. The degradation pathways primarily involve sequential addition of a water molecule, hydrogen atom, or aldehyde group to AFB1 or the epoxidation and oxidation reactions via action of the hydroperoxyl radical ($HO_2^•$). Several studies investigating the reaction of oxidative stressors with AFB1 have also suggested the breakdown of AFB1 at the C8 to C9 double bond of the dihydrofuran rings (Chen et al., 2014; Diao, Hou, & Dong, 2013). It may be noted that the emission intensity of ultraviolet light during cold plasma treatment is much less than the UV intensity required for the effective degradation of aflatoxin (Laroussi & Leipold, 2004; Liu et al., 2009).

Wang et al. (2015) proposed reduced toxicity of the degradation products of AFB_1 after cold plasma treatments, probably due to the loss of double bond in the terminal furan ring since the furfuran moiety of AFB_1 is important for its toxicity and carcinogenicity. The degradation of mycotoxins by changing their structure during plasma treatment could be related to the presence of UV photons, ozone or reactive ions and electrons. Significant contributions of reactive species other than ozone and UV photons to degrade mycotoxins or synergistic action of these species along with ozone and UV photons during cold plasma treatments could be possible, as the mycotoxin degradation efficacy of cold plasma technology is greater than that of ozone or UV treatments alone (Diao, Hou, Chen, Shan, & Dong, 2013; Luo et al., 2014; Mao et al., 2016). Further work in this direction is likely to generate better insights regarding the influence of each of the major reactive components

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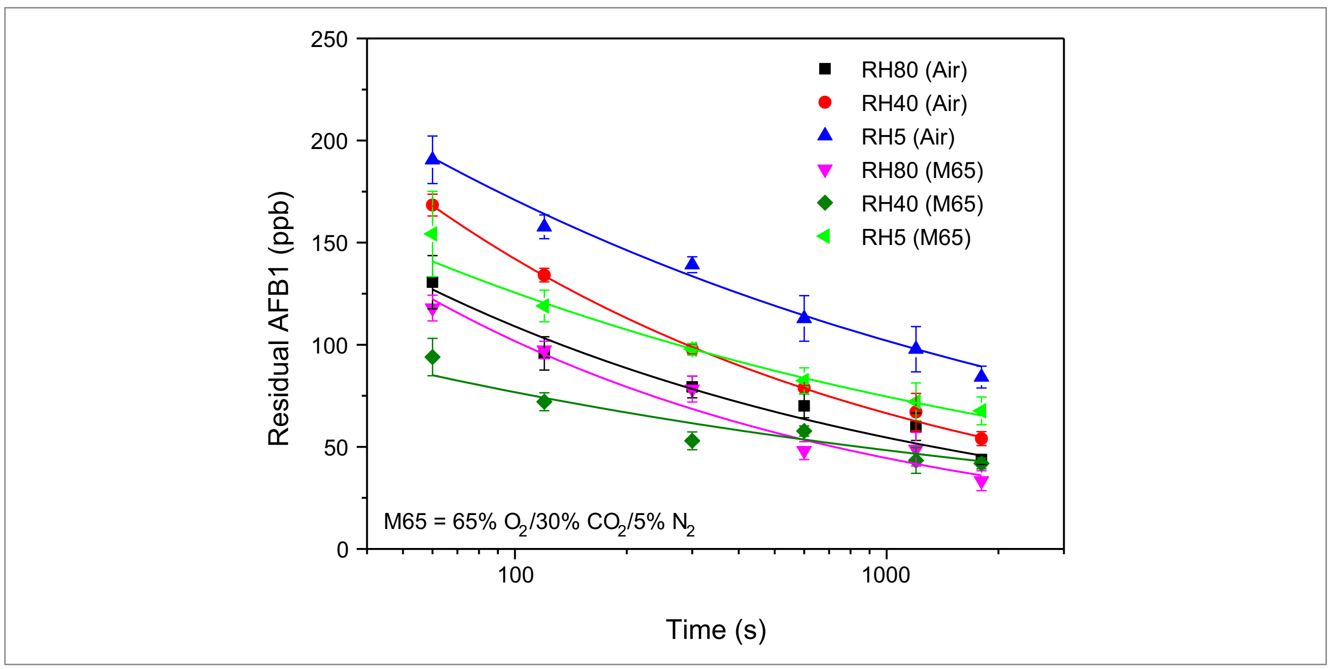

Figure 2–Degradation of aflatoxin in 25 g of contaminated corn kernels subjected to atmospheric pressure DBD cold plasma operating at 90 kV, 44 mm discharge gap. The data correspond to operation in air and a modified high oxygen gas mixture (65% O_2, 30% CO_2, 5% N_2) at three different relative humidities of 5, 40 and 80%. Data adapted from Shi et al. (2017), and the trendlines are meant to guide the eye. (color online).

Figure 3–Degradation pathways of aflatoxin (AFB1) on exposure to high voltage atmospheric cold plasma. Adapted from Shi et al. (2017).

in cold plasma and their synergistic activity to degrade various types of mycotoxins.

Future Perspectives

Cold plasma technology has the potential to reduce fungi and degrade mycotoxins in/on food and feed materials effectively as it could be a more sustainable method, requiring smaller energy input and investment. However, scaling up of cold plasma technology to reduce pathogenic fungi and mycotoxins in/on food and feed materials will need several aspects addressed. Cold plasma treatments should be able to handle the bulk quantities of food and feed materials. Therefore, studies exploring the feasibility of batch or continuous plasma systems to handle large quantities of food and feed materials are required.

The effectiveness of cold plasma treatment depends on multiple intrinsic and extrinsic parameters including, the surface characteristics, type of food and feed materials, nature and structure of mycotoxins, type of fungi and their attachment to the surfaces, ability of antimicrobial species to diffuse and spread on surfaces, their life time during and after treatments, time of treatment, cost effectiveness, and so on. A better understanding of the gas phase chemistry of air plasma is required as it depends on the characteristics of the surrounding air and the atmospheric conditions such as relative humidity and temperature.

Mycotoxins produced by fungi in cereals and feed materials can be confined to the surfaces, which can be effectively degraded using plasma treatments without inducing significant changes in the nutritional components. However, the effect of cold plasma on mycotoxins in flours and the resulting quality changes has not

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received the deserved attention. In addition, it is recommended that researchers should explore the suitability of cold plasma treatment as an additional decontamination step along with conventional approaches to understand the synergistic or additive potential to reduce fungi and mycotoxins.

Conclusions

Compared to several conventional and non-thermal approaches (for example, UV light, gamma irradiation, pulsed light), cold plasma treatments act rapidly against molds, require low energy input and have relatively milder impact on quality. Cold plasma could be a potential alternative to reduce fungi on food materials including grains, spices, fruits, vegetables, meat, etc. The differences in susceptibilities of fungi compared against bacteria to the action of cold plasma are likely associated with differences in cytology, morphology, reproductive cycle, and growth. Existing literature indicate that cold plasma could also serve as a rapid, effective and economically viable technology in degrading mycotoxins as compared to UV, heat, or chemical treatments. The total energy and the cost associated with the plasma treatments should be less than or comparable to the conventional mycotoxin reduction technologies used in food and feed industry. Future research activities are likely to yield more information for development of validation protocols for antimycotic or mycotoxin reduction action in food and feed paving the path for potential up-scaling of the technology to industrial adoption.

Author Contributions

Misra, Yadav and Syamaladevi compiled data and prepared the initial draft. Jo helped in building the overall concept. He also critically analyzed and revised the manuscript with attention to future perspectives and conclusions. All authors read and approved the final version of the manuscript.

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List of Abbreviations

AFB1	aflatoxin B1
APFBP	atmospheric pressure fluidized bed plasma
CD	circular dichroism
CFU	colony forming units
DBD	dielectric barrier discharge
DNA	deoxyribonucleic acid
DON	deoxynivalenol
HPLC-MS	high performance liquid chromatography-mass spectrometry
ICP	inductively coupled plasma
IMZ	imazalil
OAS	optical absorption spectroscopy
OES	optical emission spectroscopy
RNS	reactive nitrogen species
ROS	reactive oxygen species
SEM	scanning electron microscopy
SOPP	sodium ortho-phenil phenate
TBARS	thiobarbituric acid reactive substance
TBZ	thiabendazole
UV	ultraviolet

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