Chapter 5
Inactivation of Fungi and Fungal Toxins by Cold Plasma

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Abstract Over the last decades, foods contaminated by spoilage fungi and their mycotoxins have become a serious global burden, which not only cause huge loss of food production and quality but also severely threaten human health. Currently, a novel nonthermal technology of cold plasma exhibits great potentials as a cost-effective, efficient, chemical-free and environmental-friendly strategy to inactivate fungi and degrade mycotoxins on foods. Herein, this chapter mainly presents an overview of the decontamination of fungi and their mycotoxins by cold plasma and the possible mechanisms. The published literatures show that cold plasma can effectively inactive various fungi (yeasts and molds) and their biofilms. The reactive species in cold plasma can directly damage the external structure of cell, but also induce oxidative stress in cells, consequently damaging the intercellular components and destroying cell normal physiochemical functions. Besides reducing the mycotoxin production via inactivating fungi, cold plasma can also degrade mycotoxins to less or nontoxic molecules through complex chemical reactions. Furthermore, the last sector summarizes the studies of plasma-activated water (PAW) on fungi inactivation and mycotoxin degradation. Above all, although the effectiveness of cold plasma/PAW for fungi and mycotoxins inactivation has been evidenced, the exact mechanism (especially the degradation pathway of mycotoxin) is still not clear. Meanwhile, studies about the edible safety of plasma/PAW-treated food and the large-scale industrial plasma device development are rare, which need much more attention.

Keywords Cold plasma · Plasma-activated water (PAW) · Fungi · Mycotoxins · Inactivation mechanism · Reactive oxygen and nitrogen species (RONS)

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5.1 Introduction

Fungal contamination on crops can cause dramatic crop loss and damage the sensory and nutrient quality of foods, such as changing the color, decreasing the firmness, and producing bad smell (Palm 2001; Rossman 2009). Several types of spoilage fungi are pathogens, which can infect crops at different periods, including seeds, seedlings, and plants, leading to the decrease of germination rate, crop yield, and crop quality (Selcuk et al. 2008; Gonzales 2002). More importantly, most of the fungi can produce mycotoxins on crops and agricultural products, which seriously threatens the health of humans and animals (Lampel et al. 2012; Kuiper-Goodman 1995). Therefore, there is an urgent need to eliminate or minimize the food contamination by fungi and mycotoxins.

Traditionally, various approaches have been developed to inhibit the growth of fungi and decontaminate the mycotoxin on crops and agricultural products, including physical methods (e.g., UV, gamma rays, electron beam, ultrasound, pulsed light, microwave heating, thermal processing, sorting, cleaning, dehulling, and adsorption), chemical methods (e.g., acids and bases compounds, salts, oxidizing agents, chlorinating agents, electrolyzed water, and plant extracts), and biological control methods (e.g., microbial transformation, metabolization, fermentation and degradation, and atoxigenic microbial strains) (Basaran et al. 2008; Khamsen et al. 2016; Pankaj et al. 2018; Wielogorska et al. 2019; Ge et al. 2020). However, most of these methods have their own limitations. For example, some methods require expensive and sophisticated equipment and some approaches are inefficient, time-consuming, and impractical. More seriously, some chemical agents cause environmental pollution and threaten human health (Basaran et al. 2008; Wielogorska et al. 2019). Therefore, there is an urgent need to search for novel alternative control methods, which can overcome the limitations of these classical methods to some extent.

Recently, cold atmospheric plasma (CAP) is a promising green technology with potential applications in food sterilization. Initially, the antimicrobial effects of CAP on the heat-sensitive materials have been first reported at the beginning of the 1990s (Baier et al. 1992; Griffiths 1993; Chau et al. 1996; Laroussi 1996). CAP is an ionized gas, including charged particles, electric fields, ultraviolet photons, and reactive oxygen and nitrogen species (RONS) (Kogelschatz 2004; Laroussi and Leipold, 2004). Among those, RONS are considered as the major antimicrobial agents in CAP, which can inactivate a wide range of microorganisms including spoilage organisms and foodborne pathogens and almost without leaving any residues on foods due to its short lifetime (Ma et al. 2013, 2015; Xu et al. 2019; Du et al. 2020; Korachi et al. 2009; Yasuda et al. 2010; Sun et al. 2012a, b; Pan et al. 2013). RONS composition and concentration in CAP can be regulated via adjusting the voltage, frequency, power, gas type, and flow rate, which in turn can affect the sterilization efficacy of CAP (Xu et al. 2015; Xu et al. 2019). Numerous studies have evidenced the effectiveness of CAP sterilization on various foods (such as fresh fruits and vegetables, grains, nuts, spices, herbs, ready-to-eat meats, dried meats, and seafoods) and its degradation on various mycotoxins in solutions or foods (Misra

et al. 2018; Surowsky et al. 2014; Dasan et al. 2016a, b; Khamsen et al. 2016; Devi et al. 2017; Siddiqueab et al. 2018; Go et al. 2019; Scussel et al. 2019; Zhu et al. 2020). Meanwhile, ACP causes minor negative effects on food quality during sterilization. Additionally, air is the most widely used working gas for ACP sterilization, and the energy consumption of ACP discharge is relatively low (Dasan et al. 2016a, b; Khamsen et al. 2016; Devi et al. 2017, Go et al. 2019). Therefore, CAP technology might be a promising efficient, cost-effective and environmental-friendly candidate for the decontamination of fungi and mycotoxins on foods.

More recently, a novel promising alternative disinfection approach based on CAP has been developed, named as plasma-activated water (PAW). PAW is also rich of RONS, which is mainly derived from the delivery of gaseous RONS into the water, the secondary RONS generation in water via complex chemical reactions between gaseous RONS and water, and the UV photolysis of water. These RONS endow the PAW with excellent antimicrobial performance against a broad spectrum of microorganisms, which is similar to CAP (Ma et al. 2015, 2016; Xu et al. 2016). Moreover, compared with CAP treatment, PAW overcomes some drawbacks of practical application, e.g., uniformly treating the objects with irregular shapes and avoiding the adverse effects of CAP-generated electric fields, charged particles, UV photos and electrons on food quality and operators (Ma et al. 2015; Hojnik et al. 2019). Thus, fungi inactivation and mycotoxins degradation by PAW have also attracted much attention and some studies have evidenced its effectiveness.

Therefore, this chapter is mainly to give a detailed description of the effects of CAP/PAW on fungi inactivation and mycotoxins degradation and their possible mechanisms. Firstly, we summarize the antimicrobial effects of CAP against yeast, molds, and their biofilms. Then, the antifungal mechanisms are discussed from two aspects (the major antifungal agents in CAP and cellular response to CAP). Subsequently, we summarize the degradation efficiency of CAP against pure mycotoxins and mycotoxins in real food systems as well as the underlying degradation mechanisms. Lastly, the PAW effects on fungi inactivation and mycotoxins degradation are overviewed.

5.2 Cold Plasma Inactivation of Fungi

Until now, with respect to the antimicrobial effects of CAP, the CAP sterilization against the bacteria has been extensively researched, while the killing effects of CAP against the fungi are relatively less studied. When CAP is applied to eukaryotic microbes, the inactivation becomes less effective as exhibited in many studies compared to that of prokaryotic microbes, which may result from the fact that the eukaryotic microbes have different cellular structures and more elaborate defense mechanisms. Eukaryotic microorganisms possess membranous organelles and cell walls different from those of bacteria. These structural complexity may be able to provide an ability to protect cells from stresses caused by CAP. In addition, the sophisticated defense mechanisms can give eukaryotic microorganisms an ability to survive and maintain homeostasis under CAP stress.

Fungi are common eukaryotic organisms, which can contaminate foods at various media, such as soils, water, and air. Moreover, fungi can be mainly divided into three categories according to the different size and morphology: microscopic single-celled yeasts, microscopic multicellular filamentous molds, and macroscopic filamentous mushroom (Deacon 2013; Kavanagh 2017). Due to the different characteristics of yeasts and molds, this section is divided into two parts: antifungal effects against yeast and antifungal effects against molds.

5.2.1 Antifungal Effects against Yeast

Saccharomyces cerevisiae (*S. cerevisiae*) is a species of budding yeast and originally isolated from the skins of grapes. *S. cerevisiae* is not only commonly considered as a safe fermentative yeast in the baking and brewing industry but also used as a probiotic in humans. Various studies have explored the antifungal effects and mechanisms of CAP by using *S. cerevisiae* as a eukaryotic model organism.

Jin et al. (2006) compared the yeast inactivation treated by dielectric barrier discharge (DBD) plasma with three different working gas (helium (He), nitrogen (N_2), and air). The results showed that the DBD with these three gases all exhibited antifungal effects against yeast cells, and He plasma had the maximum inactivation efficiency. Morgan et al. (2009) investigated the effects of plasma discharge current on the inactivation rate against *S. cerevisiae* by using two kinds of working gases (argon (Ar) and oxygen (O_2)). The results show that the *D*-values of *S. cerevisiae* by Ar DBD plasma at 0.4, 0.8, and 1.0 mA were 7, 7, and 7.3 min, respectively, while the O_2 DBD plasma at 0.4, 0.8, and 1.0 mA led to the *D*-values of 7, 4.8, and 3.2 min, respectively. Chen et al. (2010) observed that 5-min air DBD plasma led to a 2-log reduction of *S. cerevisiae* cells in water. Ryu et al. (2013) reported that the antifungal effects of cold plasma against *S. cerevisiae* cells were closely related with the surrounding media and the inactivation efficiency was in the following order: water > saline solution > yeast extract peptone dextrose (YPD). Xu et al. (2019) found that the antifungal efficiency of He surface micro-discharge (SMD) plasma against *S. cerevisiae* cells was positively related with the treatment time while negatively related with the treatment distance. 10-min SMD plasma at 1 mm resulted in the best inactivation efficiency of 3.5-log reduction. Xu et al. (2020) reported that He/O_2 plasma jet could efficiently inactive the *S. cerevisiae* cells (about 99%) in water for 5 min. Du et al. (2020) also investigated the influence of working gas on the antifungal effects of SMD plasma against *S. cerevisiae* cells by using He and air gas. The results demonstrated that air SMD plasma achieved a markedly higher inactivation efficiency compared with that of He SMD plasma.

Different from *S. cerevisiae*, *Candida species* (spp) are common pathogenic yeasts resulted in various human mucocutaneous and cutaneous infections (Nield and Kamat 2007; Zaoutis et al. 2005). Sun et al. (2011) investigated the antifungal effects of He/O_2 (2%) plasma microjet against *Candida species* by using three drug-resistant species (*Candida albicans* (*C. albicans*), *Candida krusei*, and *Candida*

glabrata) on agar plate and in water. The results demonstrated that He/O_2 plasma achieved a high inactivation efficiency (>90%) in a shorter treatment time in water (1 min) than that on agar plate (10 min). Besides the planktonic fungi, many researches have also concentrated on the antifungal effects of CAP against *C. albicans* biofilms due to the fact that pathogens in biofilm have high resistance against antimicrobial treatment compared with pathogens in planktonic form, which is considered as a serious issue for *C. albicans* inactivation in medicine and industry (Cui et al. 2018). Koban et al. (2010) compared the antimicrobial effects of cold plasma on *C. albicans* biofilms with CHX and NaOCl. The results show that 10-min DBD plasma caused a 5-log reduction of *C. albicans* biofilms, while 10-min CHX or NaOCl exposure only led to a 1.5-log reduction. Sun et al. (2012b) reported that both He/O_2 plasma alone and in combination with common antifungal drugs can efficiently inactivate *Candida* biofilms. A strong antifungal effect of SMD plasma against *Candida* biofilms was also reported by Maisch et al., who observed that 6-log reduction was achieved by 8-min plasma treatment. Handorf et al. (2018) reported that cold plasma treatment had obviously negative effects on the cell viability of *C. albicans* SC5314 biofilms concomitant with the physical destruction of cell membrane and the leakage of intracellular components. In another study, the inactivation effects of cold plasma treatment for *C. albicans* biofilm are assessed in vitro and in vivo (He et al. 2020). The results show that the microbial counts of *C. albicans* biofilms in suspensions were reduced from 35.6×10^2 to 4.6×10^2 CFU/ml after 8-min cold plasma treatment and the microbial counts of *C. albicans* biofilms on OPC mice were deceased from 34.7×10^3 to 34.7×10^3 CFU/g after 4-min plasma treatment. Table 5.1 summarized the recent cold plasma inactivation of yeasts.

Based on the above results, it is obvious that CAP could efficiently inactivate planktonic yeasts (*S. cerevisiae* and *Candida species*) in solutions, on agar plate, and other artificial matrix as well as yeast biofilms.

5.2.2 Antifungal Effects against Molds

Phytopathogenic molds can be divided into two groups: field molds (e.g., *Fusarium*, *Cladosporium,* and *Alternaria*) mainly infecting crops in the fields and storage molds (e.g., *Aspergillus*, *Penicillium*, and *Eurotium*) mainly contaminating the crops during postharvest storage (Los et al. 2018). This section mainly summarizes the antifungal effects of cold plasma against the storage molds on foods after harvest (as shown in Table 5.2). For example, Basaran et al. (2008) investigated the influence of working gas on the inactivation efficiency of low-pressure cold plasma (LPCP) against *Aspergillus parasiticus* (*A. parasiticus*) inoculated on various nut samples (e.g., hazelnuts, peanuts, and pistachio nuts) by using air and sulfur hexafluoride (SF6) gases. The results demonstrate that SF6 plasma had a higher inactivation efficiency than that of air plasma, which achieved a 5-log reduction of *A. parasiticus*, while air plasma only led to 1-log reduction for the same treatment

Table 5.1 Summary of cold plasma inactivation of yeasts

Plasma source	Processing conditions	Yeast strain	Existence form	Inactivation results	Major inactivation agents	References
DBD plasma	Gas: He, N_2, or air, frequency: 6.5 kHz, voltage: 10 kV, time: 0–5 min	*S. cerevisiae*	Planktonic cells in sterile deionized water	• The survival amounts of yeast decrease at least by five orders of magnitude in N_2, six orders of magnitude in air, and seven orders of magnitude in He plasma exposure	• Low pH	Jin et al. (2006)
DBD plasma	Gas: He/O_2 at 2.5 L/min, frequency: 10 kHz, voltage: 6 kV, time: 2 and 5 min	*S. cerevisiae*	Planktonic cells on nitrocellulose filter membrane	• The D-value of yeast cells ranged from 1 min 24 sec to 3 min dependent on the initial inoculation concentration	• Oxygen radicals	Lee et al. (2006)
DBD plasma	Gas: Ar or O_2 at 5 L/min, frequency: 50 Hz, voltage: 0–8 kV, current: 0.4, 0.8, and 1.0 mA, time: 0–30 min	*S. cerevisiae*	Planktonic cells on sterilized slides	• Ar DBD plasma completely inactivated *S. cerevisiae* at 0.8 mA for 7 min and 1.0 mA for 7.3 min, while a complete inactivation of *S. cerevisiae* was not achieved for 30 min at 0.4 mA • Oxygen DBD plasma completely inactivated *S. cerevisiae* at 0.8 mA for 4.8 min and 1.0 mA for 3.2 min, while a complete inactivation of *S. cerevisiae* was not achieved for 30 min at 0.4 mA	• Ozone • Atomic oxygen	Morgan et al. (2009)
DBD plasma	Gas: air, frequency: 20 kHz, voltage: 12 kV, current: 3.8 mA, time: 1–5 min	*S. cerevisiae*	Planktonic cells in sterile deionized water	• There was a small decrease from the initial rate after first 2-min exposure, then	• RONS	Chen et al. (2010)

				followed by a 2-log reduction after 5-min exposure		
Plasma jet	Gas: He/O_2 (2% vol) at 2.5 L/min, voltage: 560 V, current: 30 mA, time: 2 and 5 min	*S. cerevisiae*	Planktonic cells in sterile deionized water	• The survival rate of wild-type yeast cells decreased to around 70% and 50% after 2- and 5-min plasma treatment, respectively	• RONS	Feng et al. (2010)
DBD plasma	Gas: air, frequency: 25 kHz, voltage: 2 kV, time: 0–5 min	*S. cerevisiae*	Planktonic cells on YPD agar plate or polystyrene plate	• A reduction in yeast cell number of more than 50% was observed after 5-min CAP exposure twice	• pH change in the plasma-treated background media	Park et al. (2012)
Plasma jet	Gas: He/O_2 (2% vol) at 3 L/min, voltage: 570 V, current: 30 mA, time: 0–3 min	*S. cerevisiae*	Planktonic cells in sterile deionized water	• After a 3-min treatment, the wide-type and plasmid control yeast cells both showed strongly impaired growth ability	• RONS	Ma et al. (2012)
Plasma jet	Gas: Ar at 0.4 L/min, frequency: 22 kHz, voltage: 4 kV, current: 13 mA, time: 0.5, 1, 2, and 3 min	*S. cerevisiae*	Planktonic cells in water, saline, or YPD medium	• Yeast cells in water were completely inactivated by plasma for 2 min • Yeast cells in saline solution were completely inactivated by plasma for 3 min • Yeast cells in YPD medium were not efficiently inactivated by plasma for 3 min	• Hydroxyl radical	Ryu et al. (2013)
Plasma jet	Gas: He/O_2 (2% vol) at 3 L/min, voltage: 560 V, current: 30 mA, time: 0–5 min	*S. cerevisiae*	Planktonic cells in sterile deionized water	• The survival rate of yeast cells was decreased to around 50% at first 1 min, and completely inactivated after 5-min plasma treatment	• RONS	Ma et al. (2013, 2014)

(continued)

Table 5.1 (continued)

Plasma source	Processing conditions	Yeast strain	Existence form	Inactivation results	Major inactivation agents	References
DBD Plasma	Gas: Ar at 0.8 L/min, frequency: 11 kHz, voltage: 9.3 kV, time: 0–10 min	*S. cerevisiae*	Planktonic cells on YPD agar plates	• 10-min plasma treatment led to a complete inactivation of yeast cells		Itooka et al. (2016)
DBD plasma	Gas: Ar at 0.8 L/min, frequency: 11 kHz, voltage: 20 kV, time: 0–25 min	*S. cerevisiae*	Planktonic cells in SD medium	• The survival rate of yeast cells was decreased to around 10% after 25-min CAP treatment	• UV • ROS	Itooka et al. (2018)
SMD plasma	Gas: He at 1.5 L/min, frequency: 8 kHz, voltage: 8 kV, time: 0–10 min	*S. cerevisiae*	Planktonic cells on YPD agar plate or agarose gel film	• 2.5-, 5-, and 10-min plasma caused 1.7, 2.7, and 3.4 log reduction of yeast cells at 1 mm discharge distance, respectively • 2.5-, 5-, and 10-min plasma caused 1, 1.5, and 2.5 log reduction of yeast cells at 2 mm discharge distance, respectively • The inactivation efficiency of plasma at 3 mm discharge distance was close to that of 2 mm	• Hydroxyl radical	Xu et al. (2019)
Plasma jet	Gas: He/O_2 (2% vol) at 5 L/min, voltage: 560 V, current: 30 mA, time: 0–5 min	*S. cerevisiae*	Planktonic cells in sterile deionized water	• The plasma treatment led to a 99% decrease in the survival rate of yeast cells in water.	• Singlet oxygen	Xu et al. (2020)
SMD plasma	Gas: He or air at 1.2 L/min, frequency: 5 kHz, voltage: 8 kV, time: 10 min	*S. cerevisiae*	Planktonic cells on YPD agar plate	• Air plasma led to a 4.5-log reduction of yeast cells on agar plate • He plasma led to a 2-log reduction of yeast cells on agar plate	• Hydroxyl radical for He SMD plasma • Ozone for air SMD plasma	Du et al. (2020)

BCD plasma	Gas: surrounding air, frequency: 12.5 kHz, voltage: 18 kV, time: 0–30 s	*C. albicans*	Planktonic cells on agar plate	• 5-s BCD plasma caused 4 log reduction of *C. albicans* on agar plates	• RONS	Morfill et al. (2009)
Plasma jet	Gas: He/O_2/N_2 at 2.0/1.2/1.5 L/min, frequency: 2.45 GHz, time: 0, 33, 63, and 91 s	*C. albicans*	Planktonic cells on agar plates or dentin slices	• Plasma caused 3–4 log reduction of *C. albicans* both on agar plate and dentin slices • Plasma had a lower inactivation efficiency against the microbes on the dentin slices compared with that on agar plates		Rupf et al. (2010)
Plasma jet	Gas: He/O_2 (3% vol) at 2 L/min, frequency: 8 kHz, voltage: 8 kV, time: 0–8 min	*C. albicans*	Planktonic cells on agar plates with or without cover	• Only a small fraction of fungal cells was effectively inactivated even after a long treatment time of 8 min without a cover on the Petri dish • 3.5-min plasma treatment resulted in more than 99.9% reduction of *C. albicans* when the petri dish was covered	• Oxygen atoms	Xiong et al. (2010)
DBD plasma and plasma jet	APPJ "kINPen09" Gas: Ar or Ar/O_2 (1% vol) at 5 L/min, frequency: 1.82 MHz, voltage: 2–6 kV HDBD Gas: Ar or Ar/O_2 (1% vol) at 1 L/min, frequency: 37.6 kHz, voltage: 9 kV	*C. albicans*	*C. albicans* biofilms on titanium discs	• kINPen09 almost had no antifungal effects against *C. albicans* biofilms • 10-min HDBD caused 2.9, 3.2, and 3.3 log reduction of *C. albicans* biofilms by using Ar plasma, Ar/O_2 gas, and Ar/O_2 plasma, respectively	• RONS for kINPen09 • RONS and electric fields for HDBD and VDBD	Koban et al. (2010)

(continued)

Table 5.1 (continued)

Plasma source	Processing conditions	Yeast strain	Existence form	Inactivation results	Major inactivation agents	References
	VDBD Gas: Ar at 0.05 L/min, frequency: 40 kHz, voltage: 10 kV, time: 1, 2, 5, and 10 min			• For VDBD, the population of *C. albicans* biofilms was reduced by 2.3 logs for 1 min, 2.2 logs for 2 min, 3.5 logs for 5 min, and 5.2 logs for 10-min plasma treatment • 10-min CHX or NaOCl treatment led to 1.5 logs reduction of *Candida* biofilm		
Plasma jet	Gas: He/O_2 (2% vol) at 2.5 L/min, voltage: 400 V, current: 35 mA, time: 0–4 min	*C. albicans, C. krusei, and C. glabrata*	Planktonic cells on SDA agar plate or in water	• Cold plasma caused 100% inactivation of *C. glabrata* for 2 min, while 91% inactivation of *C. krusei* for 10 min on SDA agar plate • In the untreated area of SDA agar plate, significant inactivation rates around 90% were observed after 10-min plasma treatment • Cold plasma caused a fast inactivation of all strains in water (about 100% inactivation after 1- and 2-min treatment)	• Charged particles and RONS for plasma inactivation on SDA agar plate • Hydroxyl radical and singlet oxygen for plasma inactivation in water	Sun et al. (2011)
SMD plasma	Gas: surrounding air, frequency: 1 kHz, voltage: 10 kV, time: 0–75 s	*C. albicans*	Planktonic cells on agar plate	• 30-s plasma treatment inactivated the yeast cells ranging from 4- to 6-log reduction on agar plates	• RONS	Klämpfl et al. (2012)

SMD plasma	Gas: surrounding air, frequency: 1 kHz, voltage: 9 kV, time: 0–15 min	*C. albicans*	Planktonic *Candida* cells on SDA agar plate and *Candida* biofilm in 6-well plate	• 40-s and 5-min SMD plasma caused a 3- and 5-log reduction of planktonic *Candida* cells, respectively • SMD plasma led to an effective inactivation of *Candida* biofilm ranging from 3- to 6-log reduction dependent on treatment time • 70% ethanol only caused 1.5-, 2.8-, and 3-log reduction of *Candida* biofilm after 5-, 7-, and 10-min treatment, respectively	• RONS	Maisch et al. (2012)
Plasma jet	Gas: He/O_2 (2% vol) at 2.5 L/min, Voltage: 400 V, Current: 35 mA, Time: 0, 30, 60 and 90 s	*C. albicans, C. krusei, and C. glabrata*	*Candida* biofilm in 96-well plate	• A complete inactivation of *Candida* biofilms was achieved by 1-min PMJ treatment	• Hydroxyl radical • Singlet oxygen • Superoxide anion	Sun et al. (2012b)
Plasma jet	Gas: Ar at 5 L/min, frequency: 1.1 MHz, voltage: 2–6 kV, time: 0–300 s	*C. albicans*	*Candida* biofilm in 96-well plate	• The inactivation efficiency reached a maximum of 2-log reduction after 300-s plasma treatment • For live/dead staining assay, 30-s plasma treatment caused a decrease of 0.97 in G/R ratio, and 60-s plasma treatment led to a maximum decrease of 1.78 in G/R ratio • The metabolic activity was decreased by 41 and 89% after 30 and 60-s plasma treatment	• RONS	Handorf et al. (2018)

(continued)

Table 5.1 (continued)

Plasma source	Processing conditions	Yeast strain	Existence form	Inactivation results	Major inactivation agents	References
Plasma jet	Gas: He at 2 L/min or He/O_2 (0.5% vol) at 10 mL/min, frequency: 1 kHz, voltage: 15 kV, time: 0–8 min	*C. albicans*	*Candida* biofilm in 24-well plate or OPC mice model	• After 2-min plasma treatment, the CFUs counting reduced to 17.6×10^2 CFUs/ml compared to 35.6×10^2 CFUs/ml in control and the CFUs number remarkably decreased to 4.6×10^2 CFUs/ml after 8-min treatment for *Candida* biofilm in suspensions • The CFU counting of *Candida* biofilm on OPC mice was decreased from 34.7×10^3 to 28.6×10^3, 4.9×10^3, and 6.4×10^3 CFU/g after 2, 4, and 6-min treatment, respectively	• RONS • Electric field • Charged particles	He et al. (2020)

Table 5.2 Summary of cold plasma inactivation of molds

Plasma source	Processing conditions	Yeast strain	Existence form	Inactivation results	Major inactivation agents	References
DBD plasma	Gas: air or SF_6, frequency: 1 kHz, voltage: 20 kV, time: 0–25 min	*Aspergillus* spp. and *Penicillium* spp.	Spores inoculated on grains and legumes	• 3-log reduction for both species was achieved after 15-min air or SF6 plasma treatment	• RONS	Selcuk et al. (2008)
Plasma jet	Gas: O_2/Ar at 0 to 1.4 L/h, time: 1, 3, and 5 min	*Penicillium digitatum*	*P. digitatum* spores	• The inactivation rate constant of *P. digitatum* spores by plasma-generated O (^3P_j) was on the order of 10^{-17} cm^3 s^{-1}	• O (^3P_j)	Iseki et al. (2011)
Corona discharge plasma	Gas: air at 300 to 900 L/min, time: 0–160 min	*Penicillium expansum*	*P. expansum* spores in bioaerosols	• The model developed described the experimentally determined inactivation kinetics of plasma disinfection of *P. expansum* spores well ($R^2 > 0.919$) • The relationship between the reciprocal of the square root functions of the corona energy density and the time taken to inactivate 90% of the spores in a population was linear ($R^2 = 0.987$)	• Heavy species • Light species	Ye et al. (2012)
Corona discharge plasma	Gas: air at 300, 450, 600, 750, and 900 L/h, discharge power: 0.65, 0.9, 1.15, 1.4, and 1.65 VA, time: 0–150 min	*Penicillium expansum*	*P. expansum* spore aerosols	• The D-value varied from 70 to 204 min at different discharge power (0.65–1.65 VA) and air flow (300–900 L/h)	• RONS	Liang et al. (2012)

(continued)

Table 5.2 (continued)

Plasma source	Processing conditions	Yeast strain	Existence form	Inactivation results	Major inactivation agents	References
Plasma jet	Gas: Ar at 10 L/min, frequency: 50–600 kHz, voltage: 10 kV, power: 0–40 W, time: 5, 10, 15, 20, and 25 min	*Aspergillus flavus*	*A. flavus* spores on agar media and brown rice cereal bars	• Plasma treatment (40 W and 20 min) inhibited *A. flavus* growth of 4-log CFU/g reduction on brown rice cereal bars • Plasma treatment (40 W and 20 min) inhibited the growth of mycelium on the brown rice cereal bars during 20-d storage • Plasma treatment (40 W and 20 min) completely inhibited the germination of *A. flavus* on the medium	• RONS	Suhem et al. (2013)
Inductively coupled plasma	Gas: O_2 at 0.05 L/min, pressure: 23 Pa, frequency: 13.56 kHz, time: 0–35 min	*Aspergillus niger*	*A. niger* spores on stainless steel petri dishes	• The D-value of *A. niger* through atomic oxygen exposure was 2.8×10^{16} atoms cm^{-2}	• Atomic oxygen	Yoshino et al. (2013)
DBD plasma	Gas: surrounding air, frequency: 22 kHz, voltage: 30 kV, time: 0–180 s	*G. fujikuroi*	Spore on PDA plate or inoculated on rice	• The clear zone without colonies on PDA was increased to 8 cm^2 after 180 s plasma treatment. • 180-s plasma caused more than 2-log reduction of fungi on the rice seeds • 10-min plasma efficiently inhibited the disease development caused by *G. fujikuroi* on seeds	• RONS	Jo et al. (2014)

DBD plasma	Gas: air or Ar at 1 L/min, voltage: 750 kV, current: 80 mA, time: 1, 5, and 10 min	*F. oxysporum*	Spores resuspended in saline or PBS on a petri dish	• During 10-min Ar plasma treatment, the germination rate of fungal spores in saline was decreased with treatment time	• Peroxynitrite • Nitrite	Panngom et al. (2014)
Plasma jet	Gas: Ar/O_2 (1% vol) at 1 L/min, frequency: 5–13 kHz, voltage: 5–12 V, time: 0–300 s	*Cladosporium fulvum*	*C. fulvum* spores on quartz plate or tomato	• Plasma can completely kill the resistant *C. fulvum* for 60 s • The rotting rates of *C. fulvum*-infected tomato seeds after 60-s plasma treatment were close to noninfected seeds (about 5%)	• RONS • Charged particles	Lu et al. (2014)
Plasma jet	Gas: Ar at 0.4 L/min, frequency: 22 kHz, voltage: 4 kV, time: 0.5, 1, 3, and 5 min	*Neurospora crassa* (bread mold)	*N. crassa* spores in ionic solutions	• Spore germination in water was dramatically reduced after 3-min plasma treatment • No significant reduction in germination was observed in NaCl solutions after 3-min treatment	• RONS • pH • Osmotic pressure	Kang et al. (2014)
DBD plasma	Gas: air, time: 3, 5, 10, and 20 min	*Penicillium citrinum and Cladosporium cladosporioides*	*P. citrinum* and *C. cladosporioides* on dried filefish fillets	• 3–20 min plasma treatment caused an average decrease of 0.91- and 1.04-log CFU/g for *C. cladosporioides* and *P. citrinum*, respectively • Plasma led to a decimal reduction time (d_R) of 9.32 and 7.42 min for *C. cladosporioides* and *P. citrinum* by Weibull model, respectively	• Negative and positive ions • RONS • Electrons • UV photons • Ozone	Shin and Sang (2015)

(continued)

Table 5.2 (continued)

Plasma source	Processing conditions	Yeast strain	Existence form	Inactivation results	Major inactivation agents	References
Arc discharge plasma	Gas: air, frequency: 6, 9, and 12 Hz, voltage: 10 kV, time: 1, 5, and 10 min for spores in water and 5, 10, 20, and 30 min for infected rice seeds	*Fusarium fujikuroi*	*F. fujikuroi* spores submerged in water or on infected rice seeds	• 10-min plasma treatment caused a 4-log reduction of *F. fujikuroi* spores in water • Dramatic reduction (over 80%) in fungal growth on infected rice seeds was observed after 20- and 30-min plasma treatment at 12 Hz	• RONS • Shockwave	Kang et al. (2015)
Plasma jet	Gas: Ar at 150 sccm, frequency: 22 kHz, output power: 9 W, time: 0, 1, and 3 min	*Cordyceps bassiana*	*C. bassiana* spores in water	• 3-min plasma treatment caused 99% decrease in the viability of *C. pruinose* spores	• Hydroxyl radicals • Singlet oxygen	Kim et al. (2016)
Plasmas jet	Gas: dry and filtered air or N_2, frequency: 18–25 kHz, voltage: 5–10 kV, time: 1–5 min	*Aflatoxigenic fungi* (*Aspergillus* spp.)	*A. parasiticus* and *A. flavus* spores on maize	• 5-min air plasma treatment caused a reduction of 5.48- and 5.20-log CFU/g in *A. flavus* and *A. parasiticus* on maize grains, respectively • 5-min N_2 plasma treatment caused a reduction of 4.62- and 4.68-log CFU/g in *A. flavus and A. parasiticus* on maize grains • 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	• Charged particles • RONS	Dasan et al. (2016a)

Plasma jet	Gas: dry air at 900 L/min, frequency: 18–25 kHz, voltage: 100–70% reference voltage, time: 1–5 min	*Aspergillus flavus* and *Aspergillus parasiticus*	*A. flavus* and *A. parasiticus* spores on hazelnuts	• 5-min air plasma at 100% voltage and 25 kHz caused a significant reduction of 4.50- and 4.19-log CFU/g in *A. flavus* and *A. parasiticus*	• RONS • Charged particles	Dasan et al. (2016b)
Plasma jet	Gas: pure dry air at 200 L/h, voltage: 6.75 kV, power: 5 W, time: 3, 6, and 9 min	*Penicillium digitatum*	*P. digitatum* spore suspensions	• The maximum of the germination inhibition rate of *P. digitatum* spores reached up to 91% after 9-min plasma treatment	• Hydroxy radical • Hydrogen peroxide • Low pH	Liu et al. (2016)
DBD plasma	Gas: air, O_2, N_2, He, or Ar at 500 mL/min, voltage: 16 kV, time: 5, 10, 20, 30, 40, and 50 min	*Fusarium oxysporum*	*F. oxysporum* spores in water or nutrient solution	• In water, 5-min air and O_2 plasma treatment can completely inactivate the microconidia of *F. oxysporum*, followed by He (10 min), Ar (20 min), and N_2 (50 min) • In nutrient solution, the microconidia had completely disappeared after 5-min O_2 plasma treatment, followed by He or Ar plasma (20 min), but the sterilization capability of air plasma was very weak and that of N_2 plasma was nonexistent	• Hydroxy radical • Atomic oxygen	Yasui et al. (2016)
Radio-frequency plasma	Gas: air at 0.2 mbar, frequency: 13.56 MHz, power: 40 and 60 W, time: 0–30 min	*Aspergillus flavus and Aspergillus parasiticus*	*A. flavus* and *A. parasiticus* spores inoculated on groundnuts	• Cold plasma at 60 W caused a 97.9 and 99.3% reduction in the growth of *A. parasiticus* and *A. flavus*, respectively	• RONS • Charged particles	Devi et al. (2017)

(continued)

Table 5.2 (continued)

Plasma source	Processing conditions	Yeast strain	Existence form	Inactivation results	Major inactivation agents	References
Plasma jet	Gas: air or N_2 at 1000–5000 L/h, frequency: 18–25 kHz, voltage: 5–10 kV, time: 1–5 min	*Aspergillus flavus and Aspergillus parasiticus*	*A. flavus* and *A. parasiticus spores inoculated on* hazelnuts	• 5-min air plasma caused a reduction of 4.17-log for *A. flavus* and 4.09-log for *A. parasiticus* • Cold plasma inhibited the growth of spores on hazelnuts during storage at 25 °C for 30 days	• RONS • Charged particles	Dasan et al. (2017)
Diffuse coplanar surface barrier discharge	Gas: ambient air, frequency: 14 kHz, voltage: 20 kV, time: 0–300 s	*Aspergillus flavus, Alternaria alternata, and Fusarium culmorum*	*A. flavus*, *A. alternata,* and *F. culmorum* spores on maize seeds	• 60-s plasma treatment caused a reduction of 3.79-log CFU/g in *F. culmorum* on artificially contaminated maize seeds • 300-s plasma caused a reduction of 4.21- and 3.22-log CFU/g in *A. flavus* and *A. alternata* • A complete devitalization of native microbiota on the surface of seeds was observed after a short treatment time of 60 s (bacteria) and 180 s (filamentous fungi)	• RONS • Charged particles • UV radiation	Zahoranová et al. (2018)
Diffuse coplanar surface barrier discharge	Gas: ambient air, input power: 400 W, time: 0–180 s	*Aspergillus flavus*	Hyphae of *A. flavus* on cellophane	• 15-s plasma caused 90% decrease in the cell viability of mycelia • 30-s plasma caused a complete inactivation of mycelia	• RONS • UV irradiation • Charged particles	Šimončicová et al. (2018)

Radio-frequency plasma	Gas: O_2 at 20 mL/min, power: 10–90 W, time: 10 and 15 min	*Aspergillus* spp., *Rhizopus* spp., and *Penicillium* spp.	Spores on saffron stigma	• 15-min plasma exposure effectively suppressed the fungal growth on saffron stigma	• RONS. • Charged particles	Hosseini et al. (2018)
Plasma jet	Gas: air, frequency: 28 kHz, power: 1000 w, time: 0–90 s	*F. oxysporum*	Spore suspensions on petri dish or spore inoculated on paprika	• 90-s plasma caused an inhibition ratio of 100% on the spores 30-s plasma significantly decreased the germination rate of fungal spores and completely inhibited after 90-s plasma treatment • The inhibition ratio on paprika significantly increased from 33 to 51% with the treatment time increasing from 30 to 90 s	• RONS	Go et al. (2019)
Surface barrier discharge	Gas: air, frequency: 40 kHz, voltage: 10 kV, time: 0–480 s	*Aspergillus flavus*	*A. flavus* spores in water	• 480-s plasma treatment at low and high power could both result in 5-log reduction of spores • The inactivation efficacy of PAB was significantly lower than that of the corresponding gas phase treatment • 24-h PAB treatment caused 1.04-log reduction of spores	• RONS • UV • Gas temperature	Hojnik et al. (2019)

(continued)

Table 5.2 (continued)

Plasma source	Processing conditions	Yeast strain	Existence form	Inactivation results	Major inactivation agents	References
DBD plasma	Gas: Ar at 0.8 SLM, frequency: 11 kHz, voltage: 9.3 kV, time: 0–60 min	*Aureobasidium pullulans*	Melanized and non-melanized *A. pullulans* cells	• More than 60% of cells were still alive after 30-min Ar plasma treatment • The non-melanized cells were efficiently inactivated (about 90%) after 10-min Ar plasma treatment	• RONS. • UV photons	Fukuda et al. (2019)

time. Moreover, this study also investigated the effects of different nut samples on the plasma inactivation of *A. parasiticus* and obtained the results that 5-min SF_6 plasma achieved an inactivation efficiency ranging from 3 to 6 log CFU/g on various nut samples. In the study of Selcuk et al. (2008), who also applied SF_6 cold plasma to treat *A. parasiticus* inoculated on the nut surfaces, a 5-log reduction of *A. parasiticus* was achieved after 20-min plasma treatment. Meanwhile, 15-min SF_6 plasma treatment caused a 3-log reduction of *Aspergillus spp.* and *Penicillium spp.* inoculated on the grains and legumes. Similarly, Suhem et al. (2013) reported that Ar cold plasma at a power of 40 W had an obvious inhibitory effect on the growth of *Aspergillus flavus* (*A. flavus*) on the medium after 25-min treatment, and 20-min Ar cold plasma at 40 W could efficiently inhibit *A. flavus* growth on brown rice cereal bars during 20-day storage at 25 °C and 100% RH. Ouf et al. (2015) investigated the antifungal effects of a double atmospheric pressure argon cold plasma (DAPACP) on *Aspergillus niger* (*A. niger*) inoculated on the date palm fruits and found that the *A. niger* population was decreased from 1000 to 20 CFU 100 mm^{-2} on date palm disc after 7.5-min treatment at 3.5 L min^{-1} and 9-min plasma treatment completely inhibit *A. niger* growth. Pignata et al. (2014) found that the fungi on the cellulose acetate was more easily inactivated by a low-pressure cold plasma compared with that contaminated on the food surface. The results show that the low-pressure cold plasma achieved a 5.4-log reduction of *Aspergillus brasiliensis* (*A. brasiliensis*) on cellulose acetate membranes after 15-s treatment at 400 W, 1-min treatment at 300 W, and 5-min treatment at 150 W by using the mixed gas of Ar and O_2, while the pure Ar or O_2 plasma treatment only led to a 3.45-log reduction. Moreover, with respect to the native fungi on pistachios, 1-min cold plasma treatment at 300 W only resulted in a 2-log reduction. Devi et al. (2017) investigated the effects of the discharge power on the inactivation efficiency against *A. parasiticus* and *A. flavus* artificially inoculated on groundnuts at different discharge power by cold plasma and found that 60-W cold plasma caused an inactivation efficiency of 97.9 and 99.3% against *A. parasiticus* and *A. flavus* respectively, while the low power led to a weaker antifungal effect. Furthermore, a first study presenting the antifungal effects of cold plasma on *A. flavus* biofilms was reported by Los et al. (2020), who found that the initial fungal population of the biofilms had a major effect on the inactivation efficiency of cold plasma by using CFU and cell viability assay. When the initial inoculum was 6 log CFU/mL, 20-min cold plasma resulted in a 2.3-log reduction and 11.7% survival rate; while the inactivation efficiency was decreased to 1.5-log reduction and 36.8% survival rate when the initial inoculum was increased to 7 log CFU/mL.

Taken together, although fungi are more resistant than bacteria, CAP still holds great potentials to inactivate various molds on foods with a high efficiency. However, it is still a challenge for cold plasma applied in large-scale industrial food sterilization due to the complex properties of foods, for example, size, shape, topography of food surface, the presence of shell, and solidness of food surface.

5.3 Antifungal Mechanism of Cold Plasma

Furthermore, cold plasma is considered as a novel technology for fungi inactivation. However, the fungal mechanisms of CAP have not yet been elucidated in detail. So far, the existing studies to investigate the antifungal mechanism of cold plasma are mainly from the following two aspects (Fig. 5.1). One is to investigate the major antimicrobial agents in cold plasma. The other is to investigate the action of CAP on cell components and functions in the eukaryotic model organism *S. cerevisiae* and molds, respectively.

5.3.1 The Major Antimicrobial Agents against Fungi in Cold Plasma

Cold plasma is a mixture of ionized gases, which mainly consist of electric fields, heat, charged particles, UV radiation, and RONS (Pankaj et al. 2018). Herein, this sector mainly discusses the roles of different agents in CAP inactivation of fungi. Among all of the agents in cold plasma, although charged particles and UV radiation are involved in the cold plasma inactivation, the RONS are responsible for its antimicrobial effects in the most of existing literatures. The RONS in cold plasma

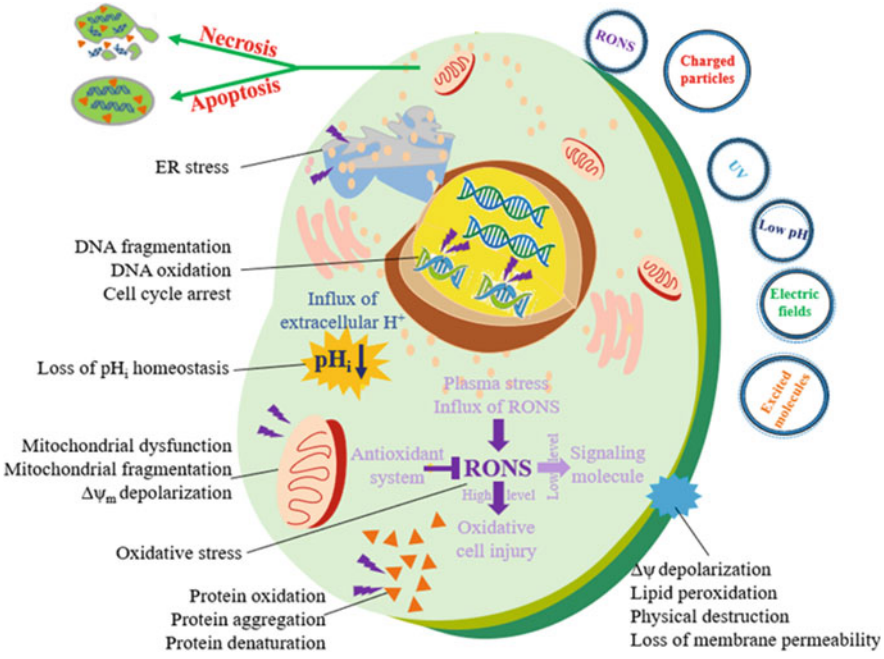

Fig. 5.1 The antifungal mechanisms of CAP

are complex and can be divided into long-lived RONS and short-lived RONS. The long-lived RONS mainly contains hydrogen peroxide (H_2O_2), nitrite (NO_2^-), ozone (O_3), and nitrate (NO_3^-), while the short-lived RONS mainly includes hydroxyl radical (·OH), superoxide anion ($\cdot O_2^-$), and singlet oxygen ($^1O_2$) (Graves 2012). Until now, the inactivation mechanism of individual RONS in cold plasma was not yet fully understood. Thus, it is urgent to investigate the contribution of each RONS to plasma inactivation and its corresponding killing mechanisms.

Lee et al. (2006) suggested that the sterilizing effects of cold plasma against yeast cells were not due to UV, but instead resulted from the action of ROS. Morgan et al. (2009) found that O_3 and atomic oxygen (O) play significant roles in the deactivation of *S. cerevisiae*. Similar results have also been reported by Iseki et al. (2010), who investigated the kinetic analysis of free radicals on fungal spores of *Penicillium digitatum* (*P. digitatum*) during plasma treatment including O by using real-time in situ electron spin resonance (ESR) spectroscopy and found that the ESR signal of *P. digitatum* was decreased only by applying oxygen plasma, which was also accompanied by the change in the shape and color of the mycelia, thereby conforming that O is the key antifungal agent. Iseki et al. (2011) found that O (^3P_j) was the major agent in the inactivation of *P. digitatum* spores by oxygen-radical source cold plasma. Sun et al. (2011) reported that the ·OH and $^1O_2$ detected by ESR in He/O_2 plasma-water system were considered as the major inactivation agents against fungi in water. Similar results were also reported by Sun et al. (2012b), who demonstrated that the *Candida* biofilms inactivation by He/O_2 cold plasma was mostly attributed to ·OH, $\cdot O_2^-$, and $^1O_2$. The results reported by Ryu et al. (2013) show that the change trend of ·OH concentration in water, saline, and YPD medium was consistent with that of yeast cell viability in these three solutions, indicating that ·OH was responsible for the yeast inactivation by Ar plasma jet. Atomic nitrogen and ·OH in cold plasma were responsible for microbial inactivation (Surowsky et al. 2014). Ouf et al. (2015) revealed that ·OH and O were the main antifungal agents for *A. niger* spores on date palm fruit discs by DAPACP due to the fact that the cell viability of *A. niger* spores was negatively correlated with the quantitative amount of ·OH and O with the extension of DAPACP treatment time. Itooka et al. (2018) demonstrated that the ROS and UV photons in cold plasma had synergistic effects on protein denaturation in yeast cells. Similar results were also obtained by Hojnik et al. (2019), who compared the inactivation efficiency of cold plasma against fungal spores with O_3 and UV treatments and found that the cold plasma sterilization was attributed to the synergistic effects of the multiple RONS, UV, and elevated gas temperatures. Xu et al. (2019) evaluated the roles of ·OH, H_2O_2, oxidation–reduction potential (ORP), and pH in the inactivation of yeast cells by He SMD plasma and revealed that ·OH contributed most to the cold plasma inactivation of yeast cells. Xu et al. (2020) employed specific ROS scavengers (superoxide dismutase (SOD), D-Manitol (D-Man), and L-Histidine (L-His)) to investigate the contribution of ·OH,$\cdot O_2^-$, and $^1O_2$ to the He/O_2 plasma inactivation of yeast by comparing their inactivation efficiency and found that $^1O_2$ contributed most to the yeast inactivation in this plasma-water system. Du et al. (2020) compared the antifungal effects of air and He SMD plasma against yeast cells and found that air SMD plasma had a

stronger inactivation efficiency than He SMD plasma and the main antifungal agent for air and He SMD plasma was ·OH and O_3, respectively.

Based on the above results, although the individual agents in cold plasma could inactivate microorganisms to some extent, the antimicrobial effects of cold plasma was mainly attributed to the synergistic effects of all these agents. Moreover, the major antifungal RONS are very different for these studies due to different cold plasma devices, treatment conditions, and fungi species. Therefore, establishment of a unique key antimicrobial agent for all plasma inactivation systems is impossible, and the detailed information of the major antimicrobial agents in every different plasma inactivation system against fungi has been listed in Tables 5.1 and 5.2.

5.3.2 *The Mechanism of Eukaryotic Cell Response to Cold Plasma*

S. cerevisiae is a well-studied eukaryotic model organism with high homology to humans, and it is genetically tractable and easy to manipulate in the lab; thus numerous studies have investigated the antifungal mechanism of cold plasma and the cellular responses of eukaryotic microorganisms to cold plasma by using *S. cerevisiae*.

A lot of literatures have evidenced that the RONS generated by cold plasma can cause oxidative stress in yeast cells, consequently resulting in cell dysfunction and death. For instance, it has been reported that the genes in oxidative stress pathway and cell cycle pathway were involved in the yeast response to cold plasma treatment by using a series of single gene mutants of *S. cerevisiae* (Feng et al. 2010). Chen et al. (2010) reported that cold plasma not only caused physical damage on cell surface of *S. cerevisiae*, but also induced oxidative stress and cell cycle arrest at G1 phase. Subsequently, similar observations have also been found by Ma et al. (2013), who reported that plasma-generated ROS could lead to cell apoptosis and cell cycle arrest at G1 phase in yeast cells through accumulation of intracellular ROS and calcium ion (Ca^{2+}), depolarization of mitochondrial cell membrane potential, and fragmentation of nuclear DNA. Ryu et al. (2013) reported that Ar cold plasma-induced intracellular RONS and phosphorylation of *HOG1* mitogen-activated protein kinase (MAPK) in yeast cells caused damages on cell morphology, membrane lipids, and genetic DNA. Chen et al. (2010) and Ma et al. (2014) revealed that cold plasma can enhance the antioxidant system (such as superoxide dismutase (SOD), catalase (CAT), and glutathione (GSH)) in yeast cells, which also confirmed that the yeast cells suffered from oxidative stress after plasma treatment. Itooka et al. (2016) found that cold plasma not only caused oxidative stress evidenced by the nuclear accumulation of the oxidative stress responsive transcription factor Yap1, mitochondrial fragmentation, and enhanced intracellular oxidation, but also led to protein denaturation, translational repression, and ER stress in yeast cells. Xu et al. (2019) reported that He SMD plasma could effectively inactive the yeast cells by damaging

cell membrane integrity and intracellular redox and pH homeostasis. Xu et al. (2020) also found that cold plasma can cause two kinds of cell death (apoptosis or necrosis) dependent on the treatment time. The short-time (3-min) cold plasma treatment caused cell apoptosis due to the accumulation of intracellular ROS, mitochondrial dysfunction, intracellular acidification, and DNA fragmentation, while the long-time (5-min) cold plasma caused cell necrosis due to the severe physical destruction on cell surface.

Besides yeasts, molds also belong to fungi, which comprise two forms (mycelium and spores). The spores have a protective coat to resist the external harsh environmental (Deacon 2013; Kavanagh 2017). Thus, fungi spores were more resistant to antimicrobial treatments. The existing literatures are mainly investigating the antifungal effects of cold plasma on fungi spores and its mechanisms.

For example, Gaunt et al. (2006) reported that the main inactivation mechanism of cold plasma against fungal spores was attributed to the cell wall rupture caused by the charged particles and RONS in cold plasma. Yong et al. (2014) reported that the RONS, UV photos, and other chemical reactive species in cold plasma worked together to destroy the natural defense system of spores, consequently resulting in the damage of intracellular components. Dasan et al. (2016b, 2017) also reported that cold plasma severely disrupted the cell membrane integrity of *A. flavus* and *A. parasiticus* on hazelnuts by scanning electron microscopy (SEM). Similarly, Devi et al. (2017) also reported that cold plasma-generated reactive species caused etching effects on the cell membrane of *A. flavus* spores, resulted in the electroporation and complete disintegration via SEM analysis. Furthermore, Šimončicová et al. (2018) firstly investigated the impact of cold plasma on young undifferentiated hyphae of *A. flavus* instead of asexual spores. Similar to the results of fungal spores, cold plasma also firstly interacted with the cell wall and membrane of *A. flavus* hyphae, causing cell membrane lipid peroxidation and damaging cell membrane integrity, followed by the leakage of intracellular contents and intracellular structures disintegration. Moreover, the oxidative stress induced by cold plasma enhanced the activity of intracellular antioxidant enzymes and compounds in *A. flavus* hyphae, which is consistent with the results in yeast cells.

Taken together, as shown in Fig. 5.1, the proposed antifungal mechanisms of cold plasma are that the active species can initially cause damage to the microbial cell wall and/or membrane, leading to membrane lipid peroxidation, cell membrane depolarization, and loss of membrane permeability, subsequently enabling further RONS or proton to enter the cell and damage the intercellular components such as organelles (mitochondria, nuclear and endoplasmic reticulum) and important biomolecules (DNA, RNA, and proteins) and meanwhile destroy the cellular ROS and pH homeostasis, consequently leading to cell death. Moreover, cold plasma can induce two different death modes (apoptosis and necrosis) dependent on the treatment doses. The detailed damage incidents happening in fungi in every plasma inactivation system have been summarized in Table 5.3. However, until now, most researches have only revealed the cellular physio-biochemical alterations during cold plasma inactivation, which is far from enough to uncover the antifungal mechanism of cold plasma. Thus, future work should employ the genomics,

Table 5.3 Summary of cellular responses of fungi to CAP

Damage incident and site	Plasma source	Treatment condition	Observations	References
Cell cycle	Plasma jet	Gas: He/O_2 (2 vol%), voltage: 560 V, current: 30 mA, fungi: *S. cerevisiae*	• 4 single gene deletion *S. cerevisiae* mutants in the cell cycle pathway were found to be hypersensitive to 5-min plasma treatment	Feng et al. (2010)
	Dielectric barrier discharges	Voltage: 12 kV, frequency: 20 kHz, peak current: 3.8 mA, fungi: *S. cerevisiae*	• Cell cycle arrest at G1 phase increased	Chen et al. (2010)
	Plasma jet	Gas: He/O_2 (2 vol%), voltage: 560 V, current: 30 mA, fungi: *S. cerevisiae*	• Cell cycle arrest at G1 phase through depolarization of mitochondria membrane potential and fragmenting nuclear DNA	Ma et al. (2013)
Cell apoptosis	Plasma jet	Gas: He/O_2 (2 vol%), voltage: 560 V, current: 30 mA, fungi: *S. cerevisiae*	66.1% of cells underwent apoptosis after 5-min plasma treatment	Ma et al. (2013)
	DBD plasma	Gas: air or Ar, voltage: 750 V, current: 80 mA, fungi: *F. oxysporum*	• 10-min plasma significantly enhanced the expression of several apoptosis-related genes (dynamic GTPase, metacaspase, and apoptosis-inducing factor) in *F. oxysporum* • A few spores after 5- and 10-min plasma treatment stained Annexin V positive • TUNEL assay results showed that plasma caused DNA breaks in spores and increased in a treatment-time-dependent manner	Panngom et al. (2014)
	Plasma jet	Gas: He/O_2 (2 vol%), voltage: 560 V, current: 30 mA, fungi: *S. cerevisiae*	• Yeast cells underwent apoptosis in the first 3-min plasma treatment	Xu et al. (2020)
Mitochondria	Plasma jet	Gas: He/O_2 (2% vol), voltage: 560 V,	• Plasma treatment can induce depolarization of mitochondrial	Ma et al. (2013)

(continued)

Table 5.3 (continued)

Damage incident and site	Plasma source	Treatment condition	Observations	References
		current: 30 mA, fungi: *S. cerevisiae*	membrane, causing the opening of the mitochondrial permeability transition pore (mPTP) and mitochondrial swelling, which leads to the dysfunction of mitochondria	
	DBD plasma	Gas: Ar, frequency: 11 kHz, voltage: 9.3 kV, fungi: *S. cerevisiae*	• CAP caused significant increase in the fragmentation of mitochondria in yeast cells after approximately 5-min exposure	Itooka et al. (2016)
	Plasma Jet	Gas: He/O_2 (2 vol%), voltage: 560 V, current: 30 mA, fungi: *S. cerevisiae*	• Mitochondria membrane potential was completely depolarized after the 5-min plasma exposure	Xu et al. (2020)
Cell morphology	DBD plasma	Gas: He/O_2, frequency: 10 kHz, voltage: 6 kV, fungi: *S. cerevisiae*	• Yeast cells exhibited peeling after plasma treatment	Lee et al. (2006)
	DBD plasma	Gas: He, N_2, or air, frequency: 6.5 kHz, voltage: 10 kV, fungi: *S. cerevisiae*	• He plasma caused severe cell destruction with a release of their cytoplasm into the suspension in yeast cells • Air plasma only caused some cracks on the cells' bodies • N_2 plasma showed a wizened configuration	Jin et al. (2006)
	Plasma jet	Gas: He/O_2 (3% vol), frequency: 8 kHz, voltage: 8 kV, fungi: *S. cerevisiae*	• The SEM results showed that plasma caused obvious changes in the external cellular structure • The TEM analysis also confirmed obvious changes to the interior of cells	Xiong et al. (2010)
	Plasma jet and DBD plasma	APPJ "kINPen09" Gas: Ar or Ar/O_2 (1% vol), frequency: 1.82 MHz, voltage: 2–	• 10-min HDBD caused massive perforations to cell walls in *C. albicans* and most	Koban et al. (2010)

(continued)

Table 5.3 (continued)

Damage incident and site	Plasma source	Treatment condition	Observations	References
		6 kV HDBD Gas: Ar or Ar/O_2 (1% vol), frequency: 37.6 kHz, voltage: 9 kV VDBD Gas: Ar, frequency: 40 kHz, voltage: 10 kV Fungi: *C. albicans*	cells seemed to be evenly split • 10 min VDBD caused similar destruction to those of HDBD in *C. albicans* • Only small areas of intact *C. albicans* cells could be examined after kINPen09 treatment	
	Plasma jet	Gas: He/O_2/N_2, frequency: 2.45 GHz, fungi: *C. albicans*	• Plasma jet damaged *C. albicans* cells with pores in their cell walls on the dentin surfaces independent on the treatment time	Rupf et al. (2010)
	Plasma Jet	Gas: He/O_2 (2% vol), voltage: 400 V, current: 35 mA, fungi: *C. albicans*	• *Candida* biofilm after 10-s PMJ treatment showed rough surface of sessile cells and some fragmented cells • 20-s PMJ treatment caused deformed and ruptured cells • Yeast cells were cracked and pseudohyphae were ruptured by 30-s PMJ treatment • *Candida* biofilm lost their original morphological characteristics and degraded to clusters of cell fragments after 60-s PMJ treatment	Sun et al. (2012b)
	DBD plasma	Gas: air, frequency: 25 kHz, voltage: 2 kV, fungi: *S. cerevisiae*	• Many crushed and donut-shaped yeast cells were observed by SEM	Park et al. (2012)
	Corona discharge plasma	Gas: air at 300 to 900 L/min, fungi: *P. expansum*	• SEM and TEM revealed noticeable defects in the external and internal structure	Ye et al. (2012)

(continued)

Table 5.3 (continued)

Damage incident and site	Plasma source	Treatment condition	Observations	References
			of the spores after plasma treatment	
	Corona discharge plasma	Gas: air at 300, 450, 600, 750, and 900 L/h, discharge power at 0.65, 0.9, 1.15, 1.4, and 1.65 VA, fungi: *P. expansum*	• TEM results showed that the cell walls of the plasma-treated spores were broken and exfoliated • SEM results demonstrated that the plasma-treated spores shrunk and leaked cytoplasm	Liang et al. (2012)
	Inductively coupled plasma	Gas: O_2, pressure: 23 Pa, frequency: 13.56 kHz, fungi: *A. niger*	• After plasma exposure, the spores were clearly ruptured and adhered to one another	Yoshino et al. (2013)
	Plasma jet	Gas: Ar, voltage: 4 kV, current: 13 mA, frequency: 22 kHz, fungi: *S. cerevisiae*	• The SEM analysis showed that the majority of yeast cells in water was severely crushed and shrunk after 3-min plasma treatment • Empty spaces were more often observed in TEM	Ryu et al. (2013)
	Plasma jet	Gas: Ar, frequency: 22 kHz, voltage: 4 kV, fungi: *N. crassa*	• The majority of 3-min plasma-treated spores were crushed and internally less dense by TEM analysis	Kang et al. (2014)
	Plasma jet	Gas: Ar/O_2 (1% vol), frequency: 5–13 kHz, voltage: 5–12 V, fungi: *C. fulvum*	• The spores were completely destroyed and the cell fluid completely spills out of the spores after 2-min plasma treatment	Lu et al. (2014)
	Arc discharge plasma	Gas: air, frequency: 6, 9, and 12 Hz, voltage: 10 kV, fungi: *F. Fujikuroi*	• Many plasma-treated spores exhibited much less electro-dense cytoplasm with empty spaces and more destroyed organelles by TEM analysis • Plasma-treated spores were crushed and shrunk compared to	Kang et al. (2015)

(continued)

Table 5.3 (continued)

Damage incident and site	Plasma source	Treatment condition	Observations	References
			control by SEM analysis	
	Plasma jet	Gas: Ar, frequency: 22 kHz, output power: 9 W, fungi: *C. pruinosa*	• Plasma-treated spores were shrunk, ruptured, and flattened	Kim et al. (2016)
	Plasma jet	Gas: dry and filtered air or N_2, frequency: 18–25 kHz, voltage: 5–10 kV, fungi: *Aspergillus* spp.	• Plasma treatment caused severe physical damage with disintegration, loss of spore integrity, and cell aggregation of *Aspergillus* spp. spores	Dasan et al. (2016a)
	Plasma jet	Gas: air, frequency: 18–25 kHz, voltage: 100–70% reference voltage, fungi: *A. flavus* and *A. parasiticus*	• The integrity of the cellular structure was completely broken, and cell contents were dispersed • The destruction of the phialide structure of *A. flavus* • The morphology of *A. parasiticus* spores altered and the integrity of cell membrane was damaged	Dasan et al. (2017)
	Plasma jet	Gas: Ar, frequency: 1.1 MHz, voltage: 2–6 kV, fungi: *C. albicans*	• Cold plasma caused membrane disruptions and intracellular fluid leakage in *C. albicans* biofilms by atomic force microscopy	Handorf et al. (2018)
	Diffuse coplanar surface barrier discharge	Gas: air, input power: 400 W, fungi: *A. flavus*	• SEM images showed that the hyphae after plasma treatment were more shriveled and crinkled than untreated sample	Šimončicová et al. (2018)
	Surface barrier discharge	Gas: air, frequency: 40 kHz, voltage: 10 kV, fungi: *A. flavus*	• Plasma-treated spores exhibited structural damage on cell surface and were surrounded by considerable cell debris • No significant differences were observed in the morphology	Hojnik et al. (2019)

(continued)

Table 5.3 (continued)

Damage incident and site	Plasma source	Treatment condition	Observations	References
			between PAB-treated and control spores	
	Plasma jet	Gas: He/O_2 (2 vol%), voltage: 560 V, current: 30 mA, fungi: *S. cerevisiae*	• Plasma caused physical destructions on the cell membrane of yeasts via SEM analysis	Xu et al. (2020)
Cell wall and membrane	DBD plasma	Gas: air, frequency: 20 kHz, voltage: 12 kV, current: 3.8 mA, fungi: *S. cerevisiae*	• For plasma-treated cells, intracellular protein concentration decreased, whereas extracellular nucleic acid concentration increased	Chen et al. (2010)
	Plasma jet	Gas: Ar, frequency: 22 kHz, voltage: 4 kV, current: 13 mA, fungi: *S. cerevisiae*	• Plasma-treated yeast cells in water showed damage to membrane lipids, which led to high level of lipid peroxidation	Ryu et al. (2013)
	Inductively coupled plasma	Gas: O_2, pressure: 23 Pa, frequency: 13.56 kHz, fungi: *A. niger*	• After plasma exposure, new peaks were observed around 200 nm and 250–280 nm in the eluted suspension of spores	Yoshino et al. (2013)
	Plasma jet	Gas: Ar/O_2 (1% vol), frequency: 5–13 kHz, voltage: 5–12 V, fungi: *C. fulvum*	• The MDA concentration rapidly increased from 0.44 to 2.1 mmol/g when the plasma treatment time was varied from 0 to 60 s • The concentration of intracellular proteins leaking out of the spores increased after plasma treatment	Lu et al. (2014)
	Plasma jet	Gas: Ar, Frequency: 22 kHz, Output power: 9 W, Fungi: *C. pruinosa*	• Fluorescence spectra of PI-stained spores indicated that plasma damaged the cell wall (and/or membrane) permeability • Plasma decreased the intensity of CD spectra of spores, which may	Kim et al. (2016)

(continued)

Table 5.3 (continued)

Damage incident and site	Plasma source	Treatment condition	Observations	References
			be attributed to structural alteration of the spore cell wall proteins and leakage of intracellular components • The decreased fluorescence intensity of tryptophan in spores indicated that plasma treatment caused structural alteration of the cell wall	
	Radio-frequency plasma	Gas: air 0.2, frequency: 13.56 MHz, power: 40 and 60 W, fungi: *A. parasiticus* and *A. flavus*	• Plasma caused complete disintegration of fungal spore membrane	Devi et al. (2017)
	Diffuse coplanar surface barrier discharge	Gas: air, input power: 400 W, fungi: *A. flavus*	• Plasma increased the membrane permeability by PI detection • Plasmas treatment led to higher level of MDA, indicating lipid peroxidation caused by plasma	Šimončicová et al. (2018)
	Plasma jet	Gas: air, frequency: 28 kHz, power: 1000 w, fungi: *F. oxysporum*	• Plasma resulted in the loss of plasma membrane integrity and upregulation of a membrane-related gene (*SHO1*)	Go et al. (2019)
	SMD plasma	Gas: He, frequency: 8 kHz, voltage: 8 kV, fungi: *S. cerevisiae*	• The MDA concentration significantly increased after 10-min plasma treatment, indicating that plasma caused membrane lipid peroxidation, consequently destroying cell membrane integrity	Xu et al. (2019)
	Plasma jet	Gas: He/O_2 (2 vol%), voltage: 560 V, current: 30 mA, fungi: *S. cerevisiae*	• 3- and 5-min plasma increased the relative MDA content by 1.15- and 2.65-fold higher than that of control samples in the water	Xu et al. (2020)

(continued)

Table 5.3 (continued)

Damage incident and site	Plasma source	Treatment condition	Observations	References
			• Plasma increased the leakage ratio of DNA/RNA and protein dependent on the treatment time	
Endoplasmic reticulum	DBD plasma	Gas: Ar, frequency: 11 kHz, voltage: 9.3 kV, fungi: *S. cerevisiae*	• CAP induced ER stress in yeast cells • Hsp30-GFP was not correctly transported to the plasma membrane • Ire1-GFP changed its intracellular localization and showed a dot-like distribution (activated Ire1) after plasma treatment	Itooka et al. (2016)
	DBD plasma	Gas: Ar, frequency: 11 kHz, voltage: 20 kV, fungi: *S. cerevisiae*	• Cold plasma caused ER stress in yeast cells • Cold plasma enhanced the levels of unfolded proteins in the ER as well as Ire1 oligomerization	Itooka et al. (2018)
DNA	Plasma jet	Gas: Ar, frequency: 22 kHz, voltage: 4 kV, current: 13 mA, fungi: *S. cerevisiae*	• The intensity of the genomic DNA bands decreased significantly after plasma exposure	Ryu et al. (2013)
	Plasma jet	Gas: He/O_2 (2% vol), voltage: 560 V, current: 30 mA, fungi: *S. cerevisiae*	• The DNA in plasma-treated wild yeast strain was fragmented into pieces, uniformly distributed in the whole cell	Ma et al. (2013)
	Plasma jet	Gas: Ar/O_2 (1% vol), frequency: 5–13 kHz, voltage: 5–12 V, fungi: *C. fulvum*	• The DNA can be severely destroyed by 60-s plasma treatment	Lu et al. (2014)
	Plasma jet	Gas: Ar, frequency: 22 kHz, voltage: 4 kV, fungi: *N. crassa*	• The amount of 8-OHdG (product of DNA oxidation) was increased in spores after 3-min plasma treatment	Kang et al. (2014)
	Plasma jet	Gas: Ar, frequency: 22 kHz, output power:	• Absorption spectroscopy, CD spectroscopy, and agarose gel	Kim et al. (2016)

(continued)

Table 5.3 (continued)

Damage incident and site	Plasma source	Treatment condition	Observations	References
		9 W, fungi: *C. pruinosa*	electrophoresis of the DNA extracted from the plasma-treated spores showed a decrease in the DNA content and DNA degradation	
	Diffuse coplanar surface barrier discharge	Gas: air, input power: 400 W, fungi: *A. flavus*	• 30-s or longer plasma treatment caused DNA fragmentation • Nonspecific cleavage of double-strand DNA was observed in plasma-treated fungal cells	Šimončicová et al. (2018)
	Plasma jet	Gas: He/O_2 (2 vol%), voltage: 560 V, current: 30 mA, fungi: *S. cerevisiae*	• The intensity of the DNA bands was significantly decreased with plasma treatment time	Xu et al. (2020)
Protein	DBD plasma	Gas: air, frequency: 25 kHz, voltage: 2 kV, fungi: *S. cerevisiae*	• Cold plasma increased the level of proteins with high molecular weights	Park et al. (2012)
	DBD plasma	Gas: Ar, frequency: 11 kHz, voltage: 9.3 kV, fungi: *S. cerevisiae*	• CAP induced the expression of heat shock protein (HSP) genes and formation of Hsp104 aggregates in yeast cells	Itooka et al. (2016)
	DBD plasma	Gas: Ar, frequency: 11 kHz, voltage: 20 kV, fungi: *S. cerevisiae*	• Cold plasma caused protein denaturation in yeast cells • Cold plasma increased the levels of insoluble protein aggregates and ubiquitinated proteins • GFP-tagged Tsa1 and Ssa1 and constitutively expressed Hsp70 formed cytoplasmic foci in CAP-treated cells	Itooka et al. (2018)
Intracellular pH homeostasis	SMD plasma	Gas: He, frequency: 8 kHz, voltage: 8 kV, fungi: *S. cerevisiae*	• 10-min plasma treatment caused a	Xu et al. (2019)

(continued)

Table 5.3 (continued)

Damage incident and site	Plasma source	Treatment condition	Observations	References
			significant decrease of 3.8 in pH_i	
	Plasma jet	Gas: He/O_2 (2 vol%), voltage: 560 V, current: 30 mA, fungi: *S. cerevisiae*	• The pH_i was slightly decreased from 8.24 to 6.74 during the first 3-min plasma treatment and then dramatically decreased to 2.03 after 5-min plasma treatment	Xu et al. (2020)
Intracellular redox homeostasis	Plasma jet	Gas: He/O_2 (2% vol) at 2.5 L/min, voltage: 560 V, current: 30 mA, fungi: *S. cerevisiae*	• 4 single gene deletion *S. cerevisiae* mutants in the oxidative stress pathway were found to be hypersensitive to 5-min plasma treatment	Feng et al. (2010)
	Plasma jet	Gas: He/O_2 (2 vol%), voltage: 570 V, current: 30 mA, fungi: *S. cerevisiae*	• Almost all wild-type cells were stained with ROS fluorescence probe and the intensity was very high • By contrast, in *Sod* overexpression strains, only a few cells were stained with ROS fluorescence probe and the intensities were much lower • However, the ROS in the extracellular scavenger groups were only slightly reduced	Ma et al. (2012, 2013)
	Plasma jet	Gas: He/O_2 (2 vol%), voltage: 520 V, current: 30 mA, fungi: *S. cerevisiae*	• Lethal-dose plasma, 100% of the cells were stained with ROS fluorescence probe and the intensities were very high • The SOD and CAT activities and GSH level in 3-min plasma-treated yeast cells increased to the maximum value at 3-h cultivation and then decreased with the	Ma et al. (2014)

(continued)

Table 5.3 (continued)

Damage incident and site	Plasma source	Treatment condition	Observations	References
			increasing cultivation time	
	Diffuse coplanar surface barrier discharge	Gas: Ar, frequency: 22 kHz, output power: 9 W, fungi: *C. pruinosa*	• Plasma-generated ROS decreased the amount of ergosterol in the spores, indicating that excessive oxidative stress destroys cellular antioxidant capacity	Kim et al. (2016)
	Plasma jet	Gas: Ar at 0.8 L/min, frequency: 11 kHz, voltage: 9.3 or 11 kV, fungi: *S. cerevisiae*	• The number of cells containing GFP-Yap1 in the nucleus increased with the plasma treatment time, and more than 50% of cells contained nuclear-accumulated Yap1 after 5-min cold plasma treatment, suggesting that cold plasma caused oxidative stress • CAP treatment increased intracellular ROS levels in yeast cells	Itooka et al. (2016, 2018)
	Plasma jet	Gas: air, input power: 400 W, fungi: *A. flavus*	• Cold plasma caused an increase of intracellular ROS levels • The antioxidant enzyme activities (CAT, SOD, and GPX) increased, while GSH level decreased in plasma-treated cells	Šimončicová et al. (2018)
	SMD plasma	Gas: He at 1.5 L/min, frequency: 8 kHz, voltage: 8 kV Gas: He/O_2 (2 vol%), voltage: 560 V, current: 30 mA, fungi: *S. cerevisiae*	• Intracellular ROS increased over the plasma treatment time	Xu et al. (2019, 2020)

transcriptomics, proteomics, and metabolomics approach to comprehensively study the inactivation mechanisms of cold plasma.

5.4 Mycotoxins Degradation by Cold Plasma

Mycotoxins are secondary metabolites produced by filamentous fungi (such as *Aspergillus*, *Penicillium*, *Fusarium*, *Alternaria*, *Claviceps*, and *Stachybotrys*), which can cause adverse effects (e.g., carcinogenic, mutagenic, estrogenic, nephrotoxic, neurotoxic, hepatotoxic, immunosuppressive, and gastrointestinal toxicity) in human and animal health. Among all mycotoxins, aflatoxin, fumonisins, zearelenone, ochratoxin, and deoxynivalenol are five most toxic mycotoxins severely threatening mammals (Karlovsky et al. 2016).

Traditionally, several strategies have been applied for the reduction of mycotoxins including physical methods (such as cleaning, heating, irradiation, adsorption, ultrasound, pulsed light, sorting, and dehulling), chemical methods (such as acidic and alkaline compounds, salts, oxidizing and chlorinating agents, ammoniation, ozonization, sulphitation, and electrolyzed water), and biological methods (such as applying atoxigenic microbial strains, microbial transformation, microbial metabolization, microbial fermentation, and enzymatic degradation) (Pankaj et al. 2018; Jalili 2015; Hojnik et al. 2017; Diao et al. 2013). However, almost all of the methods have considerable limitations. For example, physical methods are usually expensive, time-consuming, and easy to produce undesirable changes in foods (Khadem et al. 2012). Chemical methods usually produce undesirable toxic residues, which pose a threat to the environment and human health (Mendez-Albores et al. 2007). With respect to the biological method, the degradation efficiency is always low and the culture pigmentation is also the main factor limiting its application (Luo et al. 2014). Thus, it is necessary to seek new mycotoxins decontamination methods to overcome these limitations.

Besides microbial inactivation, cold plasma has also shown promising potential for the degradation of various mycotoxins, which also attracts much attention from researchers in food and agricultural science in the past few years. Thus, this section mainly discusses the degradation effects of cold plasma on the pure mycotoxins and mycotoxins inoculated or produced by fungi on the foods.

5.4.1 Degradation of Pure Mycotoxins

Park et al. (2007) reported that a complete degradation of aflatoxin B1 (AFB1), aflatoxin B2 (AFB2), aflatoxin G1 (AFG1), and aflatoxin G2 (AFG2) on glass substrate was achieved by microwave Ar plasma at atmospheric pressure in 5 s and meanwhile these plasma-treated mycotoxins exhibited no cytotoxicity on mouse macrophage cells. Wang et al. (2015) investigated the effects of cold plasma on

AFB1 degradation by using different AFB1 quantity (2, 10, and 50 μg) and plasma discharge power (100, 200, or 300 W). The results show that the initial AFB1 quantity and plasma treatment time almost had no effects on the degradation efficiency, while the plasma discharge power contributed most to the degradation rates, which increased from 58.2 to 88.3% with the discharge power ranging from 100 to 300 W. Similar results were also reported by Siciliano et al. (2016); the degradation efficiency of cold plasma was closely related with plasma discharge power. An increased treatment effectiveness was observed with the plasma discharge power increasing from 400 to 1150 W and the four standard AFs solutions were all completely eliminated after plasma treatment at 400 W for 12 min. Furthermore, AFB1 and AFG1 were more sensitive to plasma treatments compared to AFB2 and AFG2 due to the different molecule structures.

Besides plasma discharge power, Sakudo et al. (2017) also investigated the effects of discharge frequency of cold plasma on AFB1 mycotoxins degradation on a cover glass by using nitrogen cold plasma. The initial AFB1 concentration on cover glass was about 200.01 ppb, while 0.5, 1.0, and 1.5 kilo pulse per second (kpps) reduced the AFB1 content to 135.66, 56.03, and 12.4 ppm, respectively, which indicated that the degradation efficiency of AFB1 by nitrogen cold plasma was dependent on the discharge frequency. Bosch et al. (2007) studied the degradation effects of air cold plasma on six common mycotoxins (deoxynivalenol (DON), zearalenone (ZEN), enniatins A (Enn A), fumonisin B1 (FB1), T2 toxin, and AAL toxin) and found that all of these mycotoxins were almost completely degraded by 60-s cold plasma treatment. Moreover, the author also found a similar phenomenon with that reported by Siciliano et al. (2016), the degradation rates varied with mycotoxin structure. FB1 and structurally related AAL toxin with long aliphatic chains were degraded rapidly, while ST with a compact structure of condensed aromatic rings exhibited the highest resistance to degradation. The other three mycotoxins with intermediate decay rates possessed mixed structures of condensed rings and aliphatic chains. Similar conclusions were also obtained by Wielogorska et al. (2019); six mycotoxins (AFB1, FB1, ochratoxin (OTA), ZEN, DON, and enniatin B (ENB)) in solutions treated by cold plasma presented different degradation efficiencies due to the different structures in the following order: ENB $<$ FB1 $<$ OTA $<$ ZEN $<$ AFB1 $<$ DON. And the author also revealed that He plasma was more effective in the toxin's degradation than the mixture gas of He/O_2 (0.5% and 0.75%). Furthermore, Hojnik et al. (2017) compared the degradation efficiency of cold plasma against AFB1 on glass coverslips with two conventional methods (UV and thermal treatment). The results show that 15-s plasma treatment resulted in more than 80% degradation rate of AFB1, while the UV and thermal treatment could not significantly degrade AFB1, indicating that cold plasma holds great potential as an effective alternative to UV and thermal treatment for AFB1 degradation.

Based on the above results, it is concluded that the degradation efficiency of cold plasma was dependent on various parameters including working gas, treatment time, and discharge power and frequency. More interestingly, the mycotoxins structure can also affect the degradation efficiency by cold plasma. The mycotoxins with long aliphatic chains were more easily degraded than mycotoxins with the condensed

aromatic rings (Siciliano et al. 2016; Wielogorska et al. 2019). The in vitro cytotoxicity test evidenced that the cytotoxicity of plasma-treated mycotoxins was significantly reduced. The HPLC-MS results revealed that cold plasma could degrade the mycotoxins to small fragments or only change the structure of chemical bonds in mycotoxins. The detailed degradation mechanisms are discussed in the following sector.

5.4.2 Degradation of Mycotoxin in Real Food Systems

The first evidence of cold plasma degradation of mycotoxins on foods was reported by Basaran et al. (2008), who found that air cold plasma exhibited a better degradation efficiency (about 50%) of total AFs (AFB1, AFB2, AFG1, and AFG2) compared with that of SF_6 cold plasma (about 20% decrease). Ouf et al. (2015) investigated the effects of a double atmospheric pressure argon cold plasma jet on fungi spore germination and mycotoxin production in date palm discs. The results show that the amount of fumonisin B2 (FB2) and OTA produced by *A. niger* was gradually decreased with the extension of plasma treatment time and the mycotoxins were not detected after 6-min treatment for FB2 and 7.5-min treatment for OTA, which may be attributed to the inhibitory effects of cold plasma on the genes involved in the biosynthesis of mycotoxins. This study indicates that besides directly inactivating the fungi to reduce the mycotoxins production, cold plasma may also regulate the genes associated with the biosynthesis of mycotoxins, which should be verified via qRT-PCR or RNA-Seq analysis in the future. Siciliano et al. (2016) investigated the degradation effects of cold plasma on the artificially AFs contaminated hazelnuts without shell at four different discharge powers (400 W, 700 W, 1000 W, and 1150 W) and four different exposure times (1, 2, 4, and 12 min). The results show that the degradation rate of AFs by cold plasma was increased with the plasma discharge power and treatment time. Cold plasma at the highest power (1150 W) achieved the maximum degradation efficiency of AFB1 (29.1%) and total AFs (30.4%) for the longest exposure time (12 min). Devi et al. (2017) investigated the influence of air cold plasma on *A. parasiticus* and *A. flavus* growth and AFB1, B2, G1, and G2 production on groundnuts and found that the AFB1 contents were decreased by 70 and 90% in *A. parasiticus* after 40-W 40-min and 60-W 12-min plasma treatment, while 40-W 15-min and 60-W 12-min plasma treatment resulted in a 65 and 95% reduction in AFB1 in *A. flavus* samples. Bosch et al. (2007) investigated the effect of matrix on the degradation efficiency of four mycotoxins (FB1, EnnB, sterigmatocystin (ST), and ZEN) by cold plasma. The results show that the mycotoxins in matrix had a lower degradation efficiency than their pure compounds, which was similar to the plasma inactivation on foods. The reason may be that cold plasma-generated RONS can be consumed by the substances of matrix and the matrix could also provide physical shielding for the mycotoxins. Shi et al. (2017) investigated the degradation effects of high-voltage atmospheric cold plasma (HVACP) on AFs in corns by changing various treatment parameters (e.g., gas type (air and MA65 (65% O_2, 30% CO_2, and 5% N_2)), relative

humidity (5, 40, and 80% RH), treatment time (1, 2, 5, 10, 20, and 30 min), reaction mode, post-treatment storage, and stirring of corn material). The results show that the MA65 HVACP with longer treatment time and higher relative humidity led to a higher degradation rate of AFs in corns. Moreover, the degradation efficiency was significantly increased when the corns were stirred during HVACP treatment and under post-treatment storage. However, the reaction mode (direct or indirect HVACP treatment) had minor effects on the degradation efficiency. Subsequently, Iqdiam et al. (2019) used two different atmospheric pressure plasma jets (APPJ) to treat the AFTs (AFTB1 and AFTB2) produced by *A. flavus* inoculated on the peanut kernels and investigated the degradation rate and its effects on the physicochemical quality of peanuts. The results show that 2-min constant APPJ treatment decreased AFTs contents from 62.3 to 48.2 ppb and 5-min agitated APPJ treatment led to a 23% reduction. Both the constant and agitated APPJ treatment could elevate the surface temperature of peanuts, accordingly resulted in the burned peanut kernels. Wielogorska et al. (2019) performed a holistic assessment of cold plasma applicability for the most prevalent toxins on maize by performing degradation optimization, chemical and biological assessment of by-products, and the influence of cold plasma on the matrix molecular integrity. Results show that 10-min cold plasma treatment resulted in more than 66% reduction in AFB1 and FB1 concentration on maize. Degradation products of AFB1 and ZEN were nontoxic to the human hepatocarcinoma cells and small molecular mass components were observed in the cold plasma-treated maize matrix.

In summary, cold plasma could also effectively degrade different mycotoxins on foods, but the degradation rate was reduced compared with their pure mycotoxins due to the RONS consumed by the food matrix. Moreover, the mycotoxins degraded by cold plasma may be due to the following three reasons. Firstly, cold plasma could sterilize the mycotoxin-producing fungi, consequently reducing mycotoxin productions. Secondly, cold plasma may regulate the gene expression associated with the biosynthesis of mycotoxin with a low inactivation efficiency against the fungi. Thirdly, cold plasma could directly degrade mycotoxins on contaminated foods and feedstuffs.

Additionally, to promote the applications of cold plasma in decontamination of AFs on foods, the following issues should be paid more attention in future studies. Firstly, the toxicity of degradation products and the effects of cold plasma on food quality should be further investigated. Secondly, further research should be conducted by using naturally mycotoxins contaminated food. Thirdly, the cold plasma device should be optimized to improve the applicability for mycotoxins inactivation and enhance its economic feasibility.

5.5 Degradation Mechanism of Cold Plasma

Although there are some studies investigating the degradation mechanisms of cold plasma, it is still not well understood due to the highly complex plasma chemistry. For instance, air plasma involves around 500 reactions involving more than

75 reactive species (Pankaj et al. 2014). Thus, it is very difficult to determine the major agents responsible for cold plasma degradation and its degradation products. To our knowledge, most of the existing literatures focus on the degradation mechanisms of AFB1, with only one report investigating the degradation mechanisms of ZEN. In this sector, the degradation mechanisms of cold plasma are discussed from two aspects (the major agents contributed to plasma degradation and the degradation products).

5.5.1 The Major Agents in Cold Plasma Responsible for Mycotoxin Degradation

5.5.1.1 Short-Lived RONS

In the study of Wang et al. (2015), two speculated degradation pathways were obtained by the five degradation products (Fig. 5.2). For the second pathway, AFB1 was degraded through the addition of ·OH and ·H to its double bonds and the damage of carbon monoxide by O· and ·OH. Thus, these short-lived ROS were considered to play important roles in the degradation process by cold plasma. Furthermore, Wielogorska et al. (2019) revealed that the degradation efficiency of

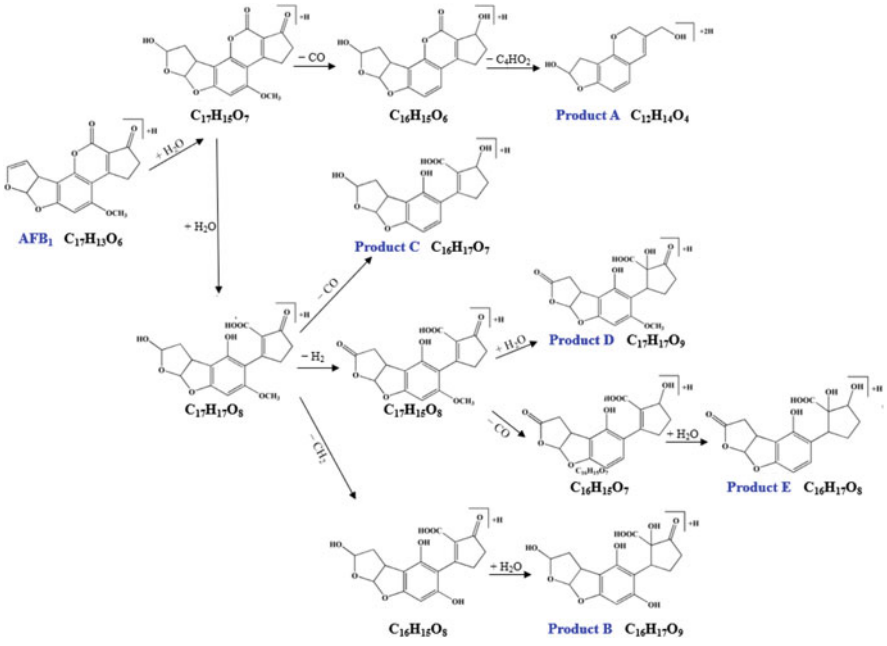

Fig. 5.2 The proposed degradation pathway of AFB_1 by low-temperature radio-frequency plasma (Wang et al. 2015)

cold plasma was decreased with the increase of oxygen ratio in He gas (0%, 0.5%, and 0.75%). And the presented spectra of He and different mixtures of He/O_2 (0.5% and 0.75%) clearly showed a decrease in intensity of the hydroxyl radical band, which had a similar change trend to that of degradation efficiency, thereby indicating that ·OH plays an important role in mycotoxins degradation by cold plasma.

5.5.1.2 Long-Lived RONS

Shi et al. (2017) investigated the degradation efficacy of HVACP treatment on AFs in corn by using different gas type (air and MA65 (65% O_2, 30% CO_2, 5% N_2)) and treatment modes (direct plasma treatment and indirect treatment). The results of different working gas show that MA65 HVACP had a higher degradation efficiency of AFs in corns compared with air HVACP, which was probably due to the higher concentrations of O_3 and NOx species in MA65 HVACP. Moreover, with respect to the different plasma treatment modes (direct plasma treatment vs. indirect plasma treatment), the direct plasma treatment with electric fields, charged particles, UV photons, short-lived and long-lived RONS caused a similar degradation efficiency to that of indirect plasma treatment only including long-lived RONS (such as H_2O_2, O_3, and NOx). Based on the above results, it was concluded that the degradation effects of cold plasma on AFs in corns is mainly attributed to the long-lived RONS like O_3 or NOx, instead of short-lived RONS, charged particles, electric fields, and UV photons.

5.5.1.3 High Energy Particles (Electrons and Excited Ions and Molecular Species)

Cold plasma is also widely used to degrade chemically stable pollutants in gaseous phase. For the degradation mechanisms of chemical pollutants, it was assumed that the energy dissipated during the cold plasma discharge could induce a series of complex chemical reactions between the plasma-generated RONS (such as O, O_3, ·OH, and NOx) and toxins Hopfe and Sheel 2007; Eliasson and Kogelschatz 1991) and/or decomposition after collision with electrons and ions (Efremov et al. 2004; Coburn and Kay 1979), which led to the cleavage of molecular bonds. Further reactions with plasma species can result in the fragmentation and generation of volatile compounds as no stable residues of toxin degradation could be detected with HPLC-MS. According to the aforementioned mechanisms, Bosch et al. (2007) also speculated that the energy of free electrons and excited ions and molecular species in cold plasma exceeds the dissociation energy of a C-C bond, which can lead to the fragmentation of the mycotoxins. And the mycotoxins molecules in the discharge were subjected to unspecific degradation. This hypothesis was in accordance with the study reported by Sakudo et al. (2017); the HPLC results confirmed the loss of AFB1 after 15-min plasma treatment and the generation of small fragments, possibly originating from the degradation process. Furthermore, the

author also assumed that the mycotoxin fragments could be converted into volatile compounds and then eliminated by plasma gas. Therefore, future work will be dedicated to analyze the plasma effluent by mass spectrometry.

5.5.1.4 The Synergistic Effects of Various Reactive Species in Cold Plasma

Hojnik et al. (2017) compared the degradation efficiency of cold plasma against AFB1 on glass coverslips with two conventional methods (UV and thermal treatment) and found that 15-s plasma treatment resulted in more than 80% destruction level of AFB1, while no significant transformation of AFB1 was observed under thermal or UV light treatments, even at the longest exposure times. Thus, it is reasonable to conclude that UV alone is not solely responsible for cold plasma. Furthermore, Wang et al. (2015) revealed that the degradation products ($C_{17}H_{15}O_7$; m/z 331) of AFB1 after cold plasma treatment have also been identified as a major degradation product by exposure to UV and Cobalt-60 gamma irradiation, and among the six cold plasma degradation products, two degradants were ozonized products of AFB1 (Wielogorska et al. 2019), indicating that UV and O_3 both contribute to cold plasma degradation. Taken together, the simultaneous exposure to multiple RONS and UV is likely to result in the synergistic effects of mycotoxin degradation by cold plasma.

5.5.2 The Degradation Products of Mycotoxins by Cold Plasma

The first study to investigate the degradation product structure of ABF1 after cold plasma treatment was reported by Wang et al. (2015). According to the deduced structure formulas of the five degradation products, two degradation pathways were proposed. One was that the degradation of AFB1 was initiated by an addition reaction of ·OH and ·H to the double bond in the terminal furan ring (C8-C9) and produced an intermediate with m/z 331 ($C_{17}H_{15}O_7$), which was considered as the major degradation pathway of AFB1 by cold plasma. The other was that carbon monoxide could be damaged by the reactive species in cold plasma (Fig. 5.2). Shi et al. (2017) investigated the AFB1 degradation efficiency and mechanism by air HVACP treatment, who proposed a different degradation mechanism of AFB1. As shown in Fig. 5.3, one degradation pathway involved the addition of ·H, ·OH, and CHO· radicals. The other degradation pathway involved the epoxidation by $\cdot HO_2$ radicals and oxidation of AFB1 by the combined effects of the oxidative species OH·, H_2O_2, and O_3. In a recent study conducted by Wielogorska et al. (2019), the degradation mechanism of AFB1 and ZEN by cold plasma was proposed based on the formulas, structures, and fragmentation spectra of by-products. The results show

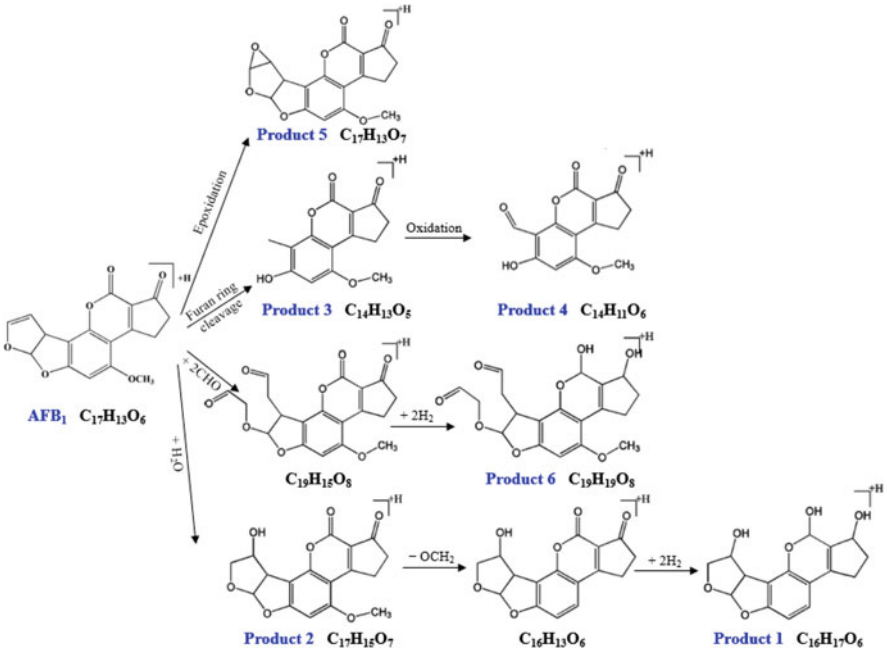

Fig. 5.3 The proposed degradation pathway of AFB_1 by air high-voltage atmospheric cold plasma (Shi et al. 2017)

that the main AFB1 degradation products were generated by the modification of the terminal furan rings and the methoxy group taking place during cold plasma treatment. The most prominent degraded product of AFB1 was AFB1-dihydrodiol ($C_{17}H_{14}O_8$). Singly hydroxylated terminal furan ring produced $C_{16}H_{12}O_7$, followed by the ring cleavage and remaining furan ring dihydroxylation ($C_{15}H_{12}O_7$) and dehydrogenation ($C_{15}H_{10}O_5$). The final degradation product was generated by the remaining furan ring cleavage ($C_{14}H_{12}O_5$) (Fig. 5.4). With respect to ZEN, the cold plasma degraded the ZEN to three main degradation products. One degradation product ($C_{21}H_{24}O_8$) at m/z 405 was produced via the opening of the lactone ring. The other two degradation products had a same molecular formula of $C_{18}H_{23}O_6$ at m/z 335 with different structure, which could potentially be isomers of a mono-hydroxylated product at C7 and C5.

In summary, the different degradation mechanisms of AFB1 by cold plasma may be due to the different plasma devices and treatment conditions (such as working gas, treatment time, power, frequency, gas flow rate, and so on). To date, there are still few studies investigating the degradation mechanisms of cold plasma; much work should be conducted in the future to realize the degradation pathways, especially considering widespread toxins lacking aromatic rings. Moreover, it is desirable to verify the postulated structural effects on degradation and identify reactive species in the plasma accountable for the degradation.

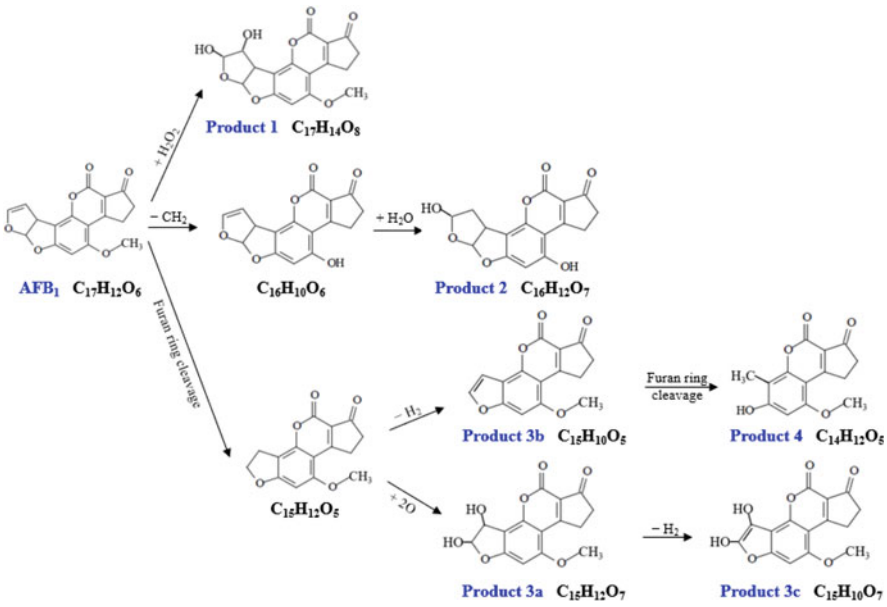

Fig. 5.4 The proposed degradation pathway of AFB_1 by He + O_2 pulsed DBD jet (Wielogorska et al. 2019)

5.6 PAW for Fungal and Mycotoxin Control

5.6.1 PAW

Recently, a new concept of plasma-activated water (PAW) has been put forward, which is generated by cold plasma reacting with water to produce various RONS. The RONS can be originated from the direct delivery of gas RONS into water, the primary RONS reacting with water or UV photolysis of water. A majority of studies have demonstrated that PAW also exhibited excellent broad-spectrum antimicrobial activity (Burlica et al. 2010; Kamgang-Youbi et al. 2009; Ma et al. 2015; Tian et al. 2015; Zhang et al. 2013). It is generally accepted that PAW inactivation was mainly due to the synergistic effects of RONS and pH, which is different from cold plasma sterilization (Ma et al. 2015; Naitali et al. 2010; Tian et al. 2015; Zhang et al. 2013). More importantly, compared with cold plasma treatment, PAW overcomes some drawbacks of practical application, e.g., uniformly treating the objects with irregular shapes and avoiding the adverse effects of cold plasma-generated electric fields, charged particles, UV photos and electrons on food quality and operators (Misra et al. 2014; Ma et al. 2015; Hojnik et al. 2019). Thus, the fungi inactivation and mycotoxins degradation by PAW have also attracted much attention, and some studies have evidenced its effectiveness. Thus, this section mainly summarized PAW application for the decontamination of fungi and mycotoxins.

5.6.2 PAW Inactivation of Fungi

Recently, although numerous studies have verified the effectiveness of PAW against various bacteria, there still few reports related to the fungi inactivation by PAW due to the high resistance of fungi to antimicrobial agents compared with bacteria. The first evidence demonstrating PAW with antifungal effects was reported by Kamgang-Youbi et al. (2009), who found that 5-min PAW could effectively inactivate *Hafnia alvei* as well as *Staphylococcus epidermidis*, *Leuconostoc mesenteroides*, and *S. cerevisiae* and 30-min PAW treatment led to a 3-log reduction of *S. cerevisiae* cells. Souskova et al. (2011) generated PAW by using the direct current corona electric discharge over the water surface and evidenced its effectiveness for the disinfection of molds (*Penicillium crustosum*, *Aspergillus oryzae*, *Cladosporium sphaerospermum*) and yeast cells. The results show that a complete inactivation of yeast cells with an initial concentration of 10^5 CFU/mL was achieved by 6-min PAW treatment, while 25-min PAW completely inactivated *C. sphaerospermum* spores and *A. oryzae* spores were still not completely inactivated after 30-min PAW exposure.

Until 2016, Ma et al. (2015) firstly investigated the antimicrobial effects of PAW against foodborne pathogens on fresh produces. Although there was no inactivation data of fungi in this study, the storage picture showed that almost no visual fungal spoilage was detected on the strawberries treated by PAW after 6-day storage, which directly indicated that PAW may also inhibit fungal growth on fresh produce. Subsequently, Xu et al. (2016) investigated the antimicrobial effects of PAW against the native microorganisms on button mushrooms and its effects on the postharvest quality. The results show that 10-PAW can efficiently inactivate the fungi on button mushrooms and led to a 0.5-log reduction CFU/mL after 7-d storage. Similarly, Ma et al. (2016) applied PAW to preserve the postharvest Chinese bayberry and found that 0.5-min PAW resulted in a 1.1-log CFU/g reduction of fungi on Chinese bayberry after 8-d storage and had minor effects on the postharvest quality of Chinese bayberry. Guo et al. (2017) investigated the antifungal effects of PAW against *S. cerevisiae* CICC 1374 inoculated on grape berries. The results show that PAW inactivated the yeast cells depending on the PAW treatment time. PAW-30 and PAW-60 achieved a 0.38- to 0.53-log CFU/ml reduction of yeast cells, respectively. Choi et al. (2019) investigated the synergistic effects of PAW and mild heating at 60 °C on the microbial inactivation of the native microorganisms and inoculated foodborne pathogens on the shredded salted Chinese cabbages. The results show that the combined PAW and mild heating treatment efficiently inactivated the mesophilic aerobic bacteria, lactic acid bacteria, yeast and molds on the shredded salted Chinese cabbages, which had a higher inactivation efficiency than that of individual PAW treatment.

In some more recent studies, researchers have compared the antifungal effects of cold plasma and PAW. For instance, in the study of Hojnik et al. (2019), a comparison of plasma-based decontamination techniques (a direct gas cold plasma vs. an indirect treatment using a plasma-activated aqueous broth solution

(PAB)) for inactivation of *A. flavus* spores was reported to highlight their respective efficiencies and corresponding inactivation mechanisms. The results show that the direct gas cold plasma had a stronger antimicrobial effect against *A. flavus* spores compared with PAB, which was probably due to the fact that the *A. flavus* spore had a hydrophobic surface, making RONS in PAB hard to contact with the spore surface. Consistent with the above results, Los et al. (2020) also reported that the direct gas plasma caused a higher inactivation efficiency against *A. flavus* spores than PAW. 20-min direct gas plasma achieved a 2.2-log reduction of *A. flavus* spores, while PAW only resulted in a 0.6-log reduction after 24-h treatment. The reason may be that PAW inactivation was only derived from the low pH and high contents of RONS, while direct gas plasma also contained various physical effects of high electric field, overpressure shock waves, and intense ultraviolet radiation, which also had antimicrobial effects.

5.6.3 PAW Degradation of Mycotoxin

Until now, according to our knowledge, there was only one study that investigated the degradation effects of PAW on mycotoxin (Chen et al. 2019). The author compared the degradation effects of intense pulsed light (IPL) and PAW treatments on DON in the raw and germinating barley. The results show that IPL treatment can degrade the DON in germinating barley faster than PAW treatment. 180-pulse IPL treatment achieved a 35.5% decrease in the DON contents for 60 s, while PAW treatment caused a 34.6% reduction for 5 min. With respect to the raw barley samples, 180-pulse IPL treatment could also significantly reduce the DON contents to 69.1%, while PAW had a lower degradation efficiency than that in the germinating barley.

This work indicates that PAW holds a great potential as an economical, eco-friendly, nontoxic method to degrade the mycotoxins on foods. However, more studies are needed to investigate its degradation effects on various kinds of mycotoxins and the toxicity of corresponding degradation by-products. Moreover, the effects of PAW on food quality should also be explored.

5.7 Conclusion

Decontamination of fungi and mycotoxins on agricultural products remains a significant challenge for the food industry. The existing literatures show that CAP holds great potential as an effective, economical, chemical-free and eco-friendly alternative to the traditional methods for fungi inactivation on foods and causes less effects on food quality. The proven antifungal activity of CAP may originate from the RONS, which can cause damages to the microbial cell wall and/or membrane, subsequently enabling further reactive species to enter the cell and damage the

intercellular components such as organelles and important biomolecules (DNA, RNA, and proteins). Cold plasma can reduce the mycotoxins production via inactivating the mycotoxin-producing fungi, regulating the gene expression associated with mycotoxin biosynthesis, or directly degrading the mycotoxins. For mycotoxin degradation, CAP has exhibited a comparable degradation efficiency to conventional UV, heat, and ozone treatments and also has some similar degradation products. But there are also many new degradation products generated by CAP via other specific chemical pathways due to the complex constituents in CAP, which has not been well understood and needs much more attention. Furthermore, PAW has a weaker antifungal effect compared with CAP probably due to lacking the physical effects of CAP (such as high electric field, overpressure shock waves, and intense ultraviolet radiation). The PAW effects on this subject are only a startup; thus the effectiveness of PAW on fungi and mycotoxins inactivation needs to be verified by much more different fungi and mycotoxins and the exact mechanisms should also be studied further. Additionally, in the future, no matter applying CAP or PAW technologies for decontamination of fungi and mycotoxins on foods, we should pay more attention on their effects on food nutrition and quality as well as the edible safety of CAP/PAW-treated foods. A large-scale industrial CAP device should also be developed to improve their applicability.

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