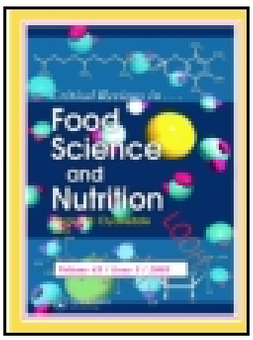

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# Factors influencing the antimicrobial efficacy of Dielectric Barrier Discharge (DBD) Atmospheric Cold Plasma (ACP) in food processing applications

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REVIEW



# Factors influencing the antimicrobial efficacy of Dielectric Barrier Discharge (DBD) Atmospheric Cold Plasma (ACP) in food processing applications

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**ABSTRACT**

Atmospheric cold plasma (ACP) is an emerging technology in the food industry with a huge antimicrobial potential to improve safety and extend the shelf life of food products. Dielectric barrier discharge (DBD) is a popular approach for generating ACP. Thanks to the numerous advantages of DBD ACP, it is proving to be successful in a number of applications, including microbial decontamination of foods. The antimicrobial efficacy of DBD ACP is influenced by multiple factors. This review presents an overview of ACP sources, with an emphasis on DBD, and an analysis of their antimicrobial efficacy in foods in open atmosphere and in-package modes. Specifically, the influence of process, product, and microbiological factors influencing the antimicrobial efficacy of DBD ACP are critically reviewed. DBD ACP is a promising technology that can improve food safety with minimal impact on food quality under optimal conditions. Once the issues pertinent to scale-up of plasma sources are appropriately addressed, the DBD ACP technology will find wider adaptation in food industry.

**KEYWORDS**

DBD; atmospheric cold plasma; food decontamination; in-package treatment; antimicrobial mechanisms

## Introduction

Occurrence of foodborne pathogens in food products is one of the biggest concerns for the food industry. Harmful bacteria, fungi, and viruses are the major pathogenic microorganisms related to foodborne outbreaks (Raybaudi-Massilia et al. 2009). Conventional methods, such as heat treatments, use of ethylene or propylene oxides, fumigation, irradiation, application of disinfectants (e.g., chlorination, hydrogen peroxide), and advanced decontamination methods, such as high-pressure processing, high-intensity pulsed electric field, pulsed light, and ozone technology, have the potential to reduce pathogenic microorganisms in food products (Baier et al. 2014; Olaimat and Holley 2012; Warning and Datta 2013; Ziuzina and Misra 2016); apart from the benefits that the new technologies present, they have certain limitations. High capital cost, hazardous chemical residues, thermal damages to flavor and nutrition, and low antimicrobial efficacy are the most prominent issues associated with current decontamination methods. With the increased demand for products with high quality, fresh appearance, and extended shelf life, potential of alternative technologies such as atmospheric cold plasma (ACP) are currently being explored.

To describe the plasma state, it is to be recalled that upon increasing the (internal) energy of a solid, it turns into a liquid, then subsequently into gas, and ultimately into an ionized state. This ionized state of a gas is described as "plasma". Any source of energy capable of ionizing a gas can be used for producing plasma (Mandal, Singh, and Singh 2018; Misra, Schlüter, and Cullen 2016). Ionization of a process gas leads to the concomitant formation of various reactive chemical species, such as ions and radicals, and UV light in plasma. Plasma can be classified into thermal or nonthermal. Nonthermal plasma, also referred to as cold plasma with a temperature of $<60\,^{\circ}C$, are characterized by a nonequilibrium temperature between the electrons and ions (Mandal, Singh, and Singh 2018). Plasma can be created at atmospheric pressure with the temperature of charged and neutral heavy particles being relatively low, close to room temperature, in comparison to very high electron temperatures in plasma.

ACP is a novel, non-thermal technology to treat food products to reduce foodborne microorganisms without affecting the quality. The reactive chemical species in ACP can inactivate microorganisms on food surfaces owing to their antimicrobial properties (Baier et al. 2014). The two main effective species in the air plasma process are reactive oxygen species (ROS) and reactive nitrogen species (RNS). Excited molecules of $N_2$ and nitric oxide radical (NO) are the main RNS species (Tappi et al. 2014). Ozone ($O_3$), singlet oxygen (O or $O^-$), superoxide ($O_2^-$), peroxide ($O_2^{-2}$ or $H_2O_2$), and hydroxyl radicals (OH) are the reactive oxygen species that can effectively inactivate microorganisms. The antimicrobial property of these oxidative species can be attributed to the lipid peroxidation in cell membranes and oxidation of proteins and DNA in microbial cells (Muranyi, Wunderlich, and Heise 2008).

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DBD is one of the most commonly employed non-thermal atmospheric gas discharge sources. DBD ACP exhibits surface decontamination effectiveness and has a huge potential to treat a variety of food products. A DBD ACP source contains a dielectric (insulator) material in the discharge path between the electrodes. While DBDs have been extensively employed for the study of food decontamination, the factors influencing their decontamination efficacy remain to be carefully analyzed. We will begin our review with a brief discussion of ACP sources, followed by a critical summary of the research studies dealing with application of DBDs in food processing. While doing so, our focus will be on the relevant process factors, food product characteristics, and microbiological factors influencing the antimicrobial efficacy of DBD ACP.

## Atmospheric cold plasma sources

### Corona discharges

Corona discharge occurs when a strong electric field is applied across two surfaces with drastically different radii of curvature, such as a sharp point or a thin wire electrode. The electrode with the smaller radius of curvature is referred to as the *emitter* electrode, while the other is known as the *collector* electrode. Coronas can be operated under positive or negative polarity, depending on the potential of the emitter relative to the collector electrode. In the positive corona, the high voltage electrode is the anode, whereas in the negative corona, the high voltage electrode is the cathode (Bogaerts et al. 2002; Lu, Cullen, and Ostrikov 2016). This type of plasma could be operated at atmospheric pressure with low gas temperatures and with relatively low power electrical discharges (Chang, Lawless, and Yamamoto 1991). Applications of corona plasma span across the areas of electrostatic precipitation, electrophotography, use as ionization sources for mass spectrometry at atmospheric pressure, ozone synthesis, destruction of toxic compounds, gas and liquid cleaning, etc. (Bogaerts et al. 2002; Chang, Lawless, and Yamamoto 1991). Coronas have remained the method of choice for ozone generation to treat vegetables and fruits (Guzel-Seydim, Greene, and Seydim 2004).

### Glow discharge

Glow discharge plasma is a non-thermal equilibrium discharge which generally operates at low pressure. In this type of plasma, the light emission pattern of the glow is divided into several layers between cathode and anode electrodes (Bogaerts et al. 2002; Conrads and Schmidt 2000). Different gap distances and gas pressure affect the pattern of glow. At elevated pressures, the glow is unstable and could be changed to a spark; hence, design considerations are important at atmospheric or high pressures (Bogaerts et al. 2002). Moreover, at elevated pressures, the temperature of the used gas could rise to a few thousand Kelvins, while at low-pressure glows, the temperature is close to room temperature. This type of plasma is used in glow discharge laser, as a molecular optical emission detector for gas chromatography, sterilization of microorganisms on surfaces in the healthcare industry, production of ozone, plasma polymerization, and material treatment (Bogaerts et al. 2002). The different types of glow discharge are low-pressure glow discharge, atmospheric pressure glow discharge, micro-discharges, hollow cathode discharge, and glow discharge with liquid electrodes (Lu, Cullen, and Ostrikov 2016).

### Plasma jet

In the case of atmospheric pressure plasma jets, the projection of the discharge plasma species is in an open environment and is operated in a non-sealed electrode arrangement (Winter, Brandenburg, and Weltmann 2015). The plasma jets can be used for direct treatment of various objects without the limitation of the treatment size. They have been used for the inactivation of bacteria, wound healing, and cancer treatment (Kong et al. 2009; Morfill, Kong, and Zimmermann 2009). Plasma jets can operate with a kHz AC power supply, microwave, RF, and pulsed DC supply. By using noble gases such as helium, the plasma jet can be generated more easily. Nevertheless, due to lower reactive species in noble gases, they are mixed with a small percentage of reactive gases, such as $O_2$ or air. They can be used for the treatment of bulk material or treatment in small sizes (Lu, Cullen, and Ostrikov 2016).

In high voltage pulsed discharge plasma, pulse rising time and pulse duration (width) are the most important factors. Implementing advanced pulsed power generators has facilitated the production of a high repetition rate of nanosecond pulsed discharges. The parameters of a typical pulsed voltage discharge are 100–500 ns pulse duration, 10–100 pulse rising time, and repetition rates up to tens of kHz. Besides, high voltage amplitudes could be applied to achieve a highly non-equilibrium state in a nanosecond pulsed discharge. DBD ACP, corona discharge, and glow discharge plasma could be driven by nanosecond pulsed voltages and exhibit distinctive discharge characteristics (Lu, Cullen, and Ostrikov 2016). They could be operated at much higher peak voltages and peak currents for the same average power and better efficiencies (Bogaerts et al. 2002). In pulsed DBDs, the discharge uniformity is higher and the operating temperature is lower than the AC power supply DBDs (Miao et al. 2018; Wang, Liu, et al. 2018).

### Dielectric barrier discharge (DBD) ACP

One of the widespread methods for the generation of ACP is the use of DBD. Here, the plasma discharge is produced between two parallel electrodes, where both or at least one electrode is wrapped by a dielectric (Becker et al. 2004; Klockow and Keener 2009). DBD presents numerous advantages in terms of flexibility of geometrical configurations and miniaturization, operating parameters, simple design, cost, safety, and characteristics of the power supply (Li et al. 2016; Tang et al. 2015; Tappi et al. 2014).

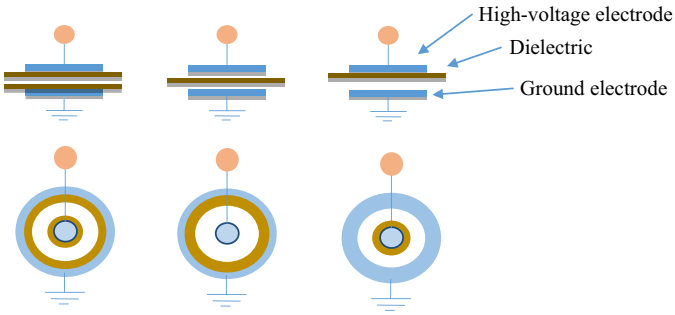


**Figure 1.** Basic configurations of DBD ACP systems adapted from (Tang et al. 2015).

Typical planar (plate-to-plate) DBD configurations are presented in Figure 1. This reactor is simple and could be easily scaled up for high production volume (Di, Zhang, and Zhang 2018). In DBD ACP, the discharge is blocked by a dielectric barrier layer, such as alumina, glass, silica glass, ceramic materials, and thin enamel or polymer layers (Kogelschatz 2003; Ragni et al. 2010), which covers one or both electrodes, or it can be suspended between two electrodes (Figure 1); hence, a spark or arc discharge can be avoided. The efficacy of this system strongly depends on the process parameters employed (gas pressure, gas type, gas flow, frequency and power of plasma excitation, time), device set-up (reactor geometry), type of the substrate, packaging material, and the exposure of the substrate (Misra et al. 2011; Misra, Yepez, et al. 2019; Suwal et al. 2019). At very high frequencies, the ability of the dielectric to limit current may be compromised and it may lose its dielectric properties. The operating conditions for DBD treatments typically fall in the range of the following: gas pressures of $\sim 10^4$–$10^6$ Pa; frequency band 50 Hz–10 MHz; AC or pulsed DC current; voltage amplitude $\sim$1–100 $kV_{rms}$; electrode gap $\sim$0.1 millimeter to several centimeters; and different dielectric material layers like glass, quartz, ceramics, and polymer layers. The DBD device is simple, stable, reliable, and economical (Kogelschatz 2003; Rybkin and Shutov 2017). In most high-power applications, liquid cooling of at least one of the electrodes is applied (Kogelschatz 2003). It is important to distinguish between volumetric DBD and surface DBD. In surface DBD, the electrodes are asymetricaly placed and separated by the dielectic, with no additional volumetric air gap. Consequently, the discharge occurs at the surface of the barrier. More details about surface DBD are provided by Misra, Yepez, et al. (2019).

Besides the planar DBDs, there are also annular (co-axial) ones in which discharge gaps between cylindrical electrodes and dielectrics are annular. Similar to planar DBDs, coaxial DBDs are simple, suitable for the treatment of large quantities, and could easily be scaled-up (Di, Zhang, and Zhang 2018). The shape of the coaxial DBDs allows for larger gas volume to be treated, resulting in the higher production of reactive species (Wang, Liu, et al. 2018). For chemical engineering applications, co-axial DBD reactors are preferred for decomposition of gaseous pollutants (Gadkari and Gu 2017; Miao et al. 2018), while planar DBDs are preferred for material processing (Kostov et al. 2009). The gas in DBDs' gap can either flow through the gap (ozone generation, surface treatment, pollution control), be fully encapsulated (excimer lamps, excimer-based fluorescent lamps and light panels, plasma display panels), or be recirculated ($CO_2$ lasers) (Kogelschatz 2003). The discharge produced in annular DBDs has been reported to be less homogenous and stable than planar DBDs (Subedi 2010). Planar and annular DBD are the most common DBD configurations. Recent advances with respect to other DBD configurations and understanding of the discharge characteristics were recently reviewed (Brandenburg 2017).

Dielectric barrier discharge (DBD) offers desirable efficiencies at short-term treatments of less than 1 min (Baier et al. 2014). Using ambient air in DBDs instead of noble gases (e.g., argon or helium) enables the application of ACP in large scales and reduces the operating costs (Baier et al. 2014). DBD ACP sources have been used for surface treatments including textile and polymer treatment, ozone synthesis, destruction of pollutants (e.g., volatile organic compounds, nerve gases, odors, $NH_3$, $H_2S$, $NO_x$, $SO_2$, etc.), plasma display panels, high-power $CO_2$ lasers, excimer UV/VUV lamps, hydrogen production from natural gas, and yeast and bacterial inactivation (Kogelschatz 2003; Morgan 2009). The electrical energy coupled into this system is transferred to electrons, and the surrounding gas remains close to ambient temperature (Mandal, Singh, and Singh 2018). Besides, the non-equilibrium plasma DBDs can be operated at elevated pressures. These features make them unique for many industrial applications. More details about the principle and applications of DBD ACP can be found elsewhere (Becker et al. 2004; Brandenburg 2017; Fridman 2008; Samoilovich, Gibalov, and Kozlov 1997).

## Application of DBD ACP in food preservation

### *In-package ACP treatment*

In-package treatment is a unique application of DBD ACP. Ambient air or modified gas mixture can be used inside the package that can be ionized, and significant amounts of reactive molecules can be generated and contained within the package. The ionized state of the gas, known as plasma, has antimicrobial effects without affecting the package material integrity (Misra, Yepez, et al. 2019). Multiple in-package ACP designs have evolved over the last few years. Among them, three main configurations with DBD are (i) volumetric DBD, (ii) surface dielectric barrier discharge (SDBD) inside the package, and (iii) SDBD placed firmly on the package containing the products to be treated (Misra, Yepez, et al. 2019). In this case, loss of reactive species due to diffusion to the open environment can be avoided although they have short lives. In-package ACP treatment has a huge benefit; this can be used as the final decontamination step after packaging as chances of post-packaging decontamination are significantly lower. Treatment of food products in packages up to 5.3-cm tall can be realized using DBD ACP with a power of 250 W (Yong et al. 2015). The common packaging materials used in DBD ACP applications are cardboard, glass, low-density polyethylene, high-density polyethylene, polyethylene terephthalate, polystyrene, rubber,

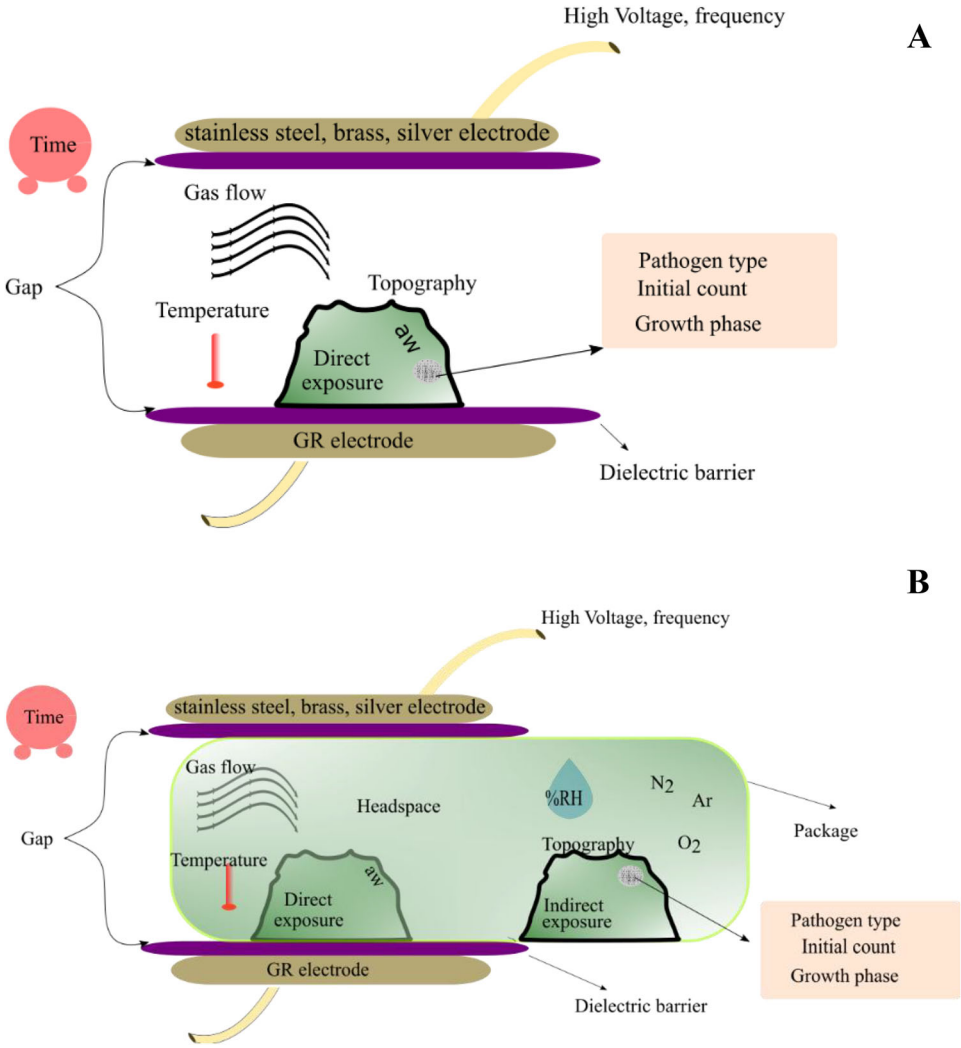


**Figure 2.** A pictorial summary of the factors influencing the efficacy of DBD ACP treatment (A: Open atmospheric treatment, B: In-package treatment).

tygon, etc. (Misra et al. 2011; Pankaj, Bueno-Ferrer, Misra, Milosavljević, et al. 2014). The decontamination efficacy of ACP treatment and its impact on food quality is dependent on the type of product. In-package decontamination of strawberries by indirect DBD has been demonstrated to reduce the population of total mesophiles and surface yeast/molds by 2.4 and 3.3 log, respectively, without any drastic change in respiration rate, color, firmness, and chemical quality of strawberries (Misra, Patil, et al. 2014). However, a 5-log reduction of inoculated *E. coli* O157:H7 on prepackaged spinach after DBD ACP treatment was reported to result in notable color degradation (Klockow and Keener 2009). It was reported that the DBD ACP treatment of romaine lettuce packaged in a conventional commercial plastic container reduced the populations of *E. coli* O157:H7, total mesophilic aerobes, and yeasts and molds during storage from 0.8 to 1.5, 0.7 to 1.9, and 0.9 to 1.7 log CFU/g, respectively. The color, $CO_2$ generation, weight, and surface morphology of lettuce during storage at 4 °C for 7 days did not change significantly (Min, Roh, Boyd, et al. 2017). Recently, in-package DBD ACP treatment at 100 $kV_{rms}$ (60 Hz) for 5 min was found to successfully reduce the population of native mesophiles and yeast and molds by 2 log on fresh-cut carrots, with no significant compromise in their physical and chemical quality (Kumar Mahnot et al. 2020). A detailed review of the in-package ACP technology, including the engineering aspects of the designs and their advantages, can be found in Misra, Yepez, et al. (2019).

### *Plasma activated solution*

A plasma-treated or -activated solution (PAS) produced by the treatment of aqueous solutions such as water, phosphate buffered saline, NaCl, and N-Acetylcysteine (NAC) by ACP can generate hydrogen peroxide ($H_2O_2$), nitrite ($NO_2^-$), and nitrate ($NO_3^-$) in the liquid phase (Ercan et al. 2016; Ma et al. 2015; Oehmigen et al. 2011). PAS contains reactive species such as ROS and RNS; hence, it can be used to inactivate microorganisms. Furthermore, the short inactivation times, lack of toxic chemicals, and low thermal loads make this an effective biological disinfectant (Shintani et al. 2010). The possibility of producing PAS by DBD ACP has been demonstrated in recent research (Brisset and Pawlat 2016; Zhang et al. 2017). PAS can be used in the treatment of cancer (Chauvin et al. 2018), the inactivation of bacteria (Shen et al. 2016), or the increasing of plant growth (Judée

et al. 2018). Characterization of tap and deionized water activated by the low-temperature plasma demonstrated that the treated tap water exhibited a larger conductivity, acidity, and concentration of reactive nitrogen and oxygen species in comparison to the deionized one (Bafoil et al. 2018).

The bactericidal activity of plasma-activated water (PAW) results from the synergistic effects of a high positive oxidation-reduction potential (ORP) and low pH (pH ≃2–3) (Kaushik et al. 2018; Oehmigen et al. 2010). $H_2O_2$, OH, O, $O_3$ and RNS contribute to the high ORP (Lukes, Brisset, and Locke 2012; Traylor et al. 2011; Van Gils et al. 2013). RNS also plays a dominant role in the acidification of PAW (Kaushik et al. 2018). Traylor et al. (2011) used DBD to activate water for the decontamination of *E. coli* for 15 min and 3 h of exposure time, and the antibacterial activity was correspondent to a ∼5-log reduction in cell viability both times. Other studies reported the antibacterial efficacy of PASs by DBD against *Candida albicans*, *E. coli*, *S. aureus*, *S. epidermitis*, and *E. faecalis* (Ercan et al. 2013; Laurita et al. 2015). The ROS and RNS produced in PAW were dependent on many factors, such as the discharge type, the plasma working gas, the treatment time, the storage time, and the chemical composition of the surrounding environment (Kaushik et al. 2018).

## Factors influencing the efficacy of DBD ACP treatment

Figure 2 presents a summary of the important process, product, and microbiological factors influencing the efficacy of DBD ACP treatment in open and in-package environments. The following sections provide a detailed description of these important factors.

### *Process factors*

#### *Voltage, frequency, and current*

Plasma process factors such as voltage, frequency, current, and electric field strength can influence microbial inactivation during ACP treatment. Higher microbial inactivation rates with an increase in power, voltage, and frequency during ACP have been reported (Albertos et al. 2017; Liao et al. 2018; Sarangapani et al. 2016; Wang, Zhuang, et al. 2018). The efficacy of ACP treatment for the reduction of *E. coli* on almonds was reported to increase at a higher applied voltage and frequency. The log reductions in *E. coli* were 1, 2.43, and 4.12, respectively, after 30 s of treatment at 16, 20, and 25 kV (Deng et al. 2007).

One of the main chemical species produced in ACP systems is ozone ($O_3$), a strong oxidizing agent which is used as a disinfectant in drinking water plants. At high voltages, dissociation of oxygen molecules occurs and ozone is produced; hence, the concentration of ozone is directly linked with applied voltage and frequency (Morgan 2009). Large amounts of ozone production at high voltages may be related to the improved antimicrobial efficiency. In order to generate ozone, a diatomic oxygen molecule must first be split by ultraviolet radiation, corona discharge, or DBD methods. Then the resulting oxygen free radical reacts with another diatomic oxygen to form the triatomic ozone molecule. Morgan (2009) reported that no ozone was detected at applied voltages below 2 kV due to the voltage not reaching the threshold value for the breakdown of the O–O bond, which requires high energy. The generated ozone concentrations were 1600, 2200, and 2800 ppm for the applied voltages of 60, 70, and 80 kV, respectively, after 8 min of ACP treatment (Sarangapani et al. 2016). However, Wang, Zhuang, et al. (2018) reported that an increase in voltage from 55 to 80 kV increased ozone concentration from 200 to 950 ppm, but the effectiveness of in-package ACP treatment against the growth of natural microflora on raw chicken fillets that were packed in the air and stored at refrigerated temperature did not alter. They assumed that the ACP treatment had injured the microbes instead of killing them and, during the enumeration on ideal media, the injured microbes were recovered and masked the treatment differences. Technological challenges, for instance high power consumption, need for better insulation, higher cost, and negative impacts on quality, must be prioritized in practice when increasing the voltage and frequency. Current also may have a significant impact on the antimicrobial efficacy of DBD ACP treatment. In a study on the inactivation of yeast by DBD ACP with different secondary current amplitudes (0.4, 0.8 and 1 mA), the lowest D-value was achieved with 1 mA current. With 0.4 mA current, complete inactivation of *S. cerevisiae* and *M. frigida* could not be achieved within an exposure time of 30 min (Morgan 2009; Morgan et al. 2009). In DBD ACP, using oxygen as the working gas, the concentration of the ozone was increased with an increase in the current (Garamoon et al. 2002).

#### *Type of gas*

The type of gas used is critical, especially in the case of in-package ACP treatment and DBD plasma jet systems, as the plasma chemistry is dependent on the properties of the gas medium. Any gas can technically be used to create ACP, and the inactivation rate of microorganisms will be different based on the type of gas used. For instance, applying direct ACP to an orange juice sample for 120 s followed by 24 h storage at 4 °C resulted in a 2.9-log reduction of *S. enterica* in the air and a 4.7-log reduction in a modified atmosphere of 65% $O_2$ + 30% $N_2$ + 5% $CO_2$ (Xu et al. 2017). After the indirect ACP treatment of the orange juice sample, a 2.2-log reduction in air and a 3.8 log reduction were achieved with the 65% $O_2$ + 30% $N_2$ + 5% $CO_2$ modified atmosphere. Greater reductions in the *E. coli* population were observed in spinach after ACP treatment when it was stored in an environment with oxygen in comparison to air (Klockow and Keener 2009). Air was more efficient than nitrogen for the inactivation of *Campylobacter jejuni* after ACP treatment. The presence of oxygen is suggested to be critically important during ACP treatments for achieving required inactivation, in comparison to nitrogen alone, to significantly reduce microorganisms (Kim, Lee, & Kim, Yong, et al. 2014). Rowan et al. (2007) reported that the order of effectiveness for inactivation of *Bacillus cereus* was $O_2$ >

$CO_2$ and air > $N_2$. In gas mixtures containing oxygen, ozone, and other effective antimicrobial reactive species such as singlet oxygen, superoxide anions and hydroxyl radicals can be produced during the ACP treatment. Ozone has a long life, is very oxidative in nature, and can react with water to produce peroxide. Hence, the presence of oxygen results in the generation of reactive oxygen species (e.g., ozone, peroxides) (Shi et al. 2017), which is one of the main reasons for the microbial inactivation. ACP reactive species can cause cellular protein degradation, fragmentation and release of DNA, and loss of permeability and cell leakage due to oxidative damage of the cell membrane. Intracellular oxidative stress leads to cell apoptosis and deformation of mycelial tips (in fungi) (Liao, Liu, et al. 2017; Misra, Yadav, et al. 2019). In another study, ACP treatment in the presence of modified atmosphere using 65% $O_2$ + 16% $N_2$ + 19% $CO_2$ resulted in reductions of 3.1 and 3.4 log for total mesophiles and yeasts/molds counts, respectively, in packaged strawberries, while using 90% $N_2$ + 10% $O_2$ resulted in 3.7 and 3.3 log reductions, respectively. The reductions in mesophiles observed with both gas mixtures were higher than those observed using air as the ACP gas medium (Misra, Moiseev, et al. 2014). McClurkin-Moore, Ileleji, and Keener (2017) used in-packaged high voltage ACP to extend the shelf life of distillers' wet grains. Using 65% $O_2$ + 30% $CO_2$ + 5% $N_2$ showed more antibacterial efficiency than pure $CO_2$ immediately after treatment. However, pure $CO_2$ suppressed the microbial growth more than 65% $O_2$ + 30% $CO_2$ + 5% $N_2$ after storage for 28 days. Argon has also been used for DBD ACP treatments. *L. innocua* on ready-to-eat meat was reported to be reduced by 1.6 log with 70% argon and 30% oxygen in 20 s of ACP treatment (Rød et al. 2012). Morgan et al. (2009) used ACP generated by argon and oxygen for yeast inactivation and the results suggested that the inactivation rate after ACP treatment using oxygen was more effective than when using argon. They reported that the D-values of *S. cerevisiae* and *M. frigida* were 3.2 and 3.4 min when oxygen was used as the gas during ACP treatment, while the D-values were 7.3 and 4.7 min, respectively, when argon was used. The proposed two inactivation mechanisms in oxygen DBD discharge were (1) oxidation of DNA and proteins by ozone, (2) etching and erosion by oxygen radicals ($O_2^+$, $O^+$) and atomic oxygen (O) (Morgan et al. 2009). Using pure argon for in-package ACP treatment has shown less antimicrobial effect in comparison to 93% Ar + 7% $CO_2$ (Chiper et al. 2011). This shows the importance of $O_2$ as an antimicrobial agent in the system. Helium is yet another gas that has been evaluated for DBD ACP treatment by Song et al. (2009). Song et al. (2009) used helium to improve the safety of sliced cheese and ham with ACP treatment. They reported a more than 8-log reduction of *L. monocytogenes* in cheese and a 1.73-log reduction in ham. Similarly, a reduction of 1.5 log population for *E. coli* K12 was reported (Connolly et al. 2013) when using helium with traces of air.

The concentration of the gas used in plasma is also important in achieving required inactivation of target microorganisms. Morgan et al. (2009) reported that the D-values of *S. cerevisiae* and *M. frigida* were decreased with increasing oxygen concentration. This may be due to a higher generation of ions and reactive species such as ozone, O, $O^+$ when elevated oxygen concentrations were used.

### Relative humidity (RH)

Environmental relative humidity is an important factor influencing plasma physics and chemistry. Increase in RH has been reported to increase the microbial inactivation during ACP treatment. This may be attributed to the increase in the concentration of OH radicals at high RH due to the presence of water molecules, which is one of the most effective reactive oxygen species, leading to high oxidation (Berardinelli et al. 2012). However, water vapor reduces the number of micro-discharges due to the reduction in the surface resistance of the dielectric material by the adsorption of water molecules (Falkenstein 1997; Falkenstein and Coogan 1997). In the presence of high humidity in the discharge, electron energy can be lost in electron-molecule collisions, and so, this quenching effect can weaken the plasma (Bruggeman et al. 2010; Butscher, Van Loon, et al. 2016). Maeda et al. (2003) treated *E. coli* K12 by ACP with a humidified working gas in the 0–70% RH range. The maximum inactivation was at 43%, and no inactivation at 0% and 70% relative humidity was detected (Maeda et al. 2003). This shows that the inactivation efficacy not only depends on the amount of water vapor but also on the type of microbiological strains.

Ragni et al. (2010) reported that modulating the RH level from 35% to 65% (at 25 °C) led to a rise in the concentration of OH radicals, which in turn increased the decontamination level from 2.5 to 4.5 log CFU/eggshell for *S. enteritidis*. Purevdorj et al. (2003) reported that *B. pumilus* spores showed increased spore mortality by more than two orders of magnitude by moistening the air in a low-pressure plasma setup. One study showed that, the inactivation levels of *Salmonella* by dry gas and air with RH of 80% were 3.90 log CFU/egg and more than 6 log CFU/egg, respectively (Georgescu, Apostol, and Gherendi 2017). Muranyi, Wunderlich, and Heise (2008) reported an optimum RH in the range of 70% for the inactivation of *A. niger* conidiospores. Interestingly, the inactivation efficacy decreased at a higher RH than 70%. In contrast to *A. niger*, the inactivation of *B. subtilis* endospores decreased with increasing humidity of the dry air with 0% RH. In their study, the negative effect of humidity was most apparent for a treatment time of 1 s at 80% relative humidity (Muranyi, Wunderlich, and Heise 2008). It was assumed that the reduced discharge homogeneity at high humidity, poorer transmissibility of UV radiation, and protective water film around the microbial cells could be the reasons for a lower inactivation rate of *B. subtilis* (Butscher, Van Loon, et al. 2016; Muranyi, Wunderlich, and Heise 2008; Ragni et al. 2010). The inactivation of oxidation-sensitive microorganisms such as *A. niger* is increased upon increasing the RH, while in the case of micro-organisms such as *B. subtilis*, where the UV radiation may be the main inactivation pathway, the inactivation rate

was decreased by increasing RH (Muranyi, Wunderlich, and Heise 2008).

### Temperature and flow rate

ACP treatments are generally carried out at relatively low temperatures (close to room temperature to 70 °C) and regarded as "cold" due to the low temperatures of heavy particles in plasma. Hence, inactivation by thermal effect is often negligible (Geyter and Morent 2012). Yadav et al. (2019) reported a slight increase in the surface temperature of ham from 21.0 °C to 26.1 °C and 25.1 °C during and after ACP treatments, respectively. An increase in the airflow rate causes the gas temperature to decrease and reach room temperature (~23 °C) (Laroussi and Leipold 2004). Furthermore, the relative concentration of atomic oxygen in the DBD decreases as the flow rate is increased. A correlation between flow-rate and antimicrobial inactivation is complicated to demonstrate as the concentration of $O_3$ and OH do not have a trend with flow rate (Laroussi and Leipold 2004).

### Material, thickness, and spacing of electrodes and barrier

The type of DBD electrode material can influence the decontamination efficacy of ACP treatment. For instance, Ragni et al. (2016) investigated the decontamination efficacy of ACP treatments using four DBD electrode materials (i.e., stainless steel, brass, silver, and glass/brass) to inactivate *L. monocytogenes* and *E. coli* after 20, 40, and 60 min using air as the working gas. Significant differences were observed in the reduction of *L. monocytogenes* after 40 and 60 min of treatment, where higher reductions were achieved by the silver and brass electrodes (up to about 8 log CFU/ml) in comparison to stainless steel and glass/brass electrodes after 60 min of treatment. The molecules from silver and brass could have played a role in the higher antibacterial properties when long treatment times were used, while the difference in the decontamination may not have been related to pH, electric features of the discharge, amount of ozone produced, or nitrate and nitrite concentrations. It was interesting to note that the authors did not observe any significant difference in decontamination efficacy when different electrodes were used for inactivating *E. coli* (Ragni et al. 2016).

The dielectric material used in the DBD electrode also could influence the decontamination efficacy. A higher inactivation of 3 log CFU in *E. coli* on alfalfa was observed after 10 min of ACP treatment using polymethylmethacrylate as the dielectric material, compared to polycarbonate with a 1.5-log CFU reduction (Butscher, Van Loon, et al. 2016). This may be attributed to differences in dielectric properties or plasma-surface interactions (Kogelschatz 2003). Ozkan et al. (2016) used four dielectric materials (i.e., quartz, pyrex, mullite, alumina) for ACP generation. Alumina (ceramic) was chosen as the best among them as the number and lifetime of micro-discharges and the plasma charge were the highest, while quartz was observed as the second best dielectric material. Aging of the dielectric material due to UV degradation (Andrady et al. 1998) or plasma etching (Egitto, Vukanovic, and Taylor 1990) also could be the other factors affecting the decontamination efficacy of DBD treatment. Moreover, for a dielectric barrier with a larger thickness, the capacitance of the dielectric decreases while the electrical charge is constant; hence, the voltage increases over the dielectric barrier and a greater number of micro-discharges is generated (Ozkan et al. 2016), which might enhance the antimicrobial efficiency. Ceramic plate has also been the material of choice for the dielectric barriers for seed germination applications using surface DBDs (Zahoranová et al. 2016; Zahoranová et al. 2018).

In general, a larger gap between the powered electrode and the substrate and a larger gap between electrodes can result in lower treatment efficacy (Hu and Guo 2012; Liao, Xiang, et al. 2017; Miao and Yun 2011) due to non-uniform production of plasma and by the recombination of active species before reaching the sample in larger gaps (Liao, Xiang, et al. 2017). However, in a simulation study by Iqbal and Turner (2015), the effect of gap spacing between 1 and 9 mm in DBD ACP discharge was assessed, and the electrons' and ions' densities were the highest when a 3-mm gap between the electrodes was used. In general, it remains unclear whether gap and voltage should be considered in isolation for DBDs or considered as a single parameter, "electric field," for comparison. While the latter is physically more accurate, much of the literature pertinent to ACP applications in food science employ gap and voltage as two independent factors.

### Treatment time and storage time

Increasing treatment time can improve the inactivation efficacy of DBD ACP treatment. For example, increasing the treatment time from 5 to 45 s increased the reduction rate of *S. auresus* from 0.09 to 4.95 log CFU/mL (Liao, Xiang, et al. 2017). A similar correlation has been reported in other studies (Ghomi et al. 2009; Hu and Guo 2012; Joshi et al. 2011). Microorganisms at high concentrations exist in multiple layers; hence, longer ACP treatment times are required to inactivate those residing in the inner layers. Extended ACP treatments generate higher concentrations of reactive species, a reduction in pH, and an increase in the mortality of pathogens, which explains the greater inactivation rates (Albertos et al. 2017; Berardinelli et al. 2016; Ghomi et al. 2009; Liao et al. 2018; Misra et al. 2012; Muranyi, Wunderlich, and Heise 2008; Ragni et al. 2016; Wang, Zhuang, and Zhang 2016; Xu et al. 2017; Zhang et al. 2017). Nevertheless, long treatment times could cause quality deterioration in the treated products (Berardinelli et al. 2012; Liao et al. 2018; Xu et al. 2017). However, Wang, Zhuang, et al. (2018) reported that increasing treatment time from 3 to 9 min at 80 kV did not alter the antibacterial effect of in-package ACP treatment against the growth of natural microflora on raw chicken fillets that were packed in the air and stored under refrigeration. Treating the sample for more than 3 min did not increase the ozone concentration significantly. Ozone is a very reactive agent, and once its concentration reaches a certain level, it reacts with other reactive agents generated in the package due to ACP

treatment to form other compounds. Treatment of more than 3 min resulted in changes in meat appearance by means of significant reductions in a* and b* values (Wang, Zhuang, et al. 2018).

Yadav et al. (2019) reported that ACP treatment time and post-plasma treatment storage significantly influenced the inactivation of *L. innocua* on RTE ham. Longer in-package storage time of food after ACP treatment can also cause higher reduction rates of microorganisms (Klockow and Keener 2009). A higher reduction in the *E. coli* population on spinach leaves was observed after treatment with increasing storage time from 0.5 to 24 h; however, change in the quality of the treated spinach leaves tended to increase with storage time (Klockow and Keener 2009). Greater inactivation of *E. coli* after 24 h compared to 0.5 h could be due to extended interactions of long-lived reactive species (e.g., ozone) inside the package with the microorganisms, thus preventing recovery from the injury and stress.

### Headspace and volume ratio of product in-package and product rolling

One of the advantages of DBD ACP is its capability to be used for in-package treatment. Min et al. (2018) investigated the effect of the volume ratio of the headspace (the space between the surface of food and the underside of the lid) of the grape tomatoes on the inactivation of *Salmonella*. A higher rate of inactivation was achieved when the headspace to tomato volume ratio was increased by either changing the container size or the number of tomatoes in the container. Moreover, incorporating rolling of the tomatoes to the treatment increased the inactivation rate impressively from $0.9 \pm 0.1$ log CFU/tomato to $3.3 \pm 0.5$ log CFU/tomato. This is possibly due to a better distribution of the reactive species throughout the package and an increase in the exposure of individual tomatoes to the reactive species, hence facilitating the diffusion of ozone and consequent microbial inactivation (Min et al. 2018). In another study investigating the inactivation of *E. coli* on romaine lettuce packed in 1, 3, 5, or 7 layers, the microbial reduction was 0.5–0.8 log CFU/g lettuce for the 1-, 3-, and 5-layer configurations. On the other hand, a greater reduction of 0.9–1.1 log CFU/g was achieved on the top layer in the 7-layer configuration. The reductions in the middle and bottom layers of the 7-layer lettuce did not significantly differ from each other or from the reductions in the other configurations. The number of reactive species in the 7-layer configuration was the highest due to the narrowest headspace; hence, enhanced microbial inactivation by increasing the contact efficiency was attained at the top layer. More uniform microbial reductions in these cases could be obtained by shaking. Overall, ACP treatment will be more uniform if the package has sufficient headspace. Alternatively, ample physical movement of the packaged food should be ensured when the headspace is limited to facilitate the diffusion of the reactive species throughout the package (Min, Roh, Boyd, et al. 2017).

### Direct and remote exposure

One of the determining factors in ACP efficiency is whether the substrate is in direct contact with the plasma or located remotely from it. Uniform three-dimensional ACP exposure to all the sides of large-sized food products is difficult, owing to the small gap between the electrodes unless high voltages are used. Remote plasma exposure could be potentially used in this case. In remote exposure, the amount of transmitted heat to the sample is reduced, and the charged particles recombine before reaching the sample; hence, they do not play a significant role in microbial inactivation. Moreover, many of the short-lived neutral reactive species also do not reach the sample; therefore, direct plasma exposure is generally more effective for microbial inactivation (Misra et al. 2011). Georgescu, Apostol, and Gherendi (2017) investigated direct and indirect ACP methods on the inactivation of *S. typhimurium* on egg surface. The population of *Salmonella* decreased below the detection limit ($10^2$ cells/egg) after 10 min and 25 min using direct and indirect modes, respectively. In treating plasma-activated water, the pH decreased and conductivity increased more rapidly with direct treatment than indirect treatment, demonstrating a clear difference in the concentration and antimicrobial activities of reactive species (Suwal et al. 2019). The higher inactivation efficiency in direct DBD ACP has been reported in other studies as well (Los et al. 2018; Xu et al. 2017; Zhang et al. 2017). In contrast to other studies, a recent work reported that *Bacillus atrophaeus* endospores inoculated on glass were reduced by 4.4 log through indirect mode and by only 3.5 log in direct mode. A greater reduction observed in indirect mode was correlated with more physical changes of spore structures and changes in the water contact angle of the spore in indirect mode. After ACP treatment, alteration from hydrophilic surface in untreated samples to hydrophobic surface in treated samples due to damage of spore coat proteins was reported (Los et al. 2017).

### Hurdle treatment

In hurdle treatment, different technologies can be combined to increase the inactivation of microorganisms and arrest their growth to enhance shelf-life of products. Research pertinent to use of ACP technology along with other treatments to enhance food safety is currently very limited. A combined ACP (10 min, 60 kV) and hydrothermal (10 min, 121 °C) treatment was used together to inactivate microorganisms on strawberry (Mehta and Yadav 2020). The use of ACP and hydrothermal treatment together resulted in a significant decrease in total bacteria count compared to applying ACP and hydrothermal treatment individually. In another study, ACP treatment along with peracetic acid as a hurdle antimicrobial intervention was used to reduce the total count of *S. typhimurium* on raw poultry meat (Chaplot et al. 2019). The combination of peracetic acid and ACP treatment exhibited a synergistic effect in reducing the *Salmonella* population. It was suggested that a higher concentration of reactive species could be produced both from the peracetic acid and atmospheric gases during plasma

treatment. Dissociation of peracetic acid to reactive species such as O, $H_2O_2$, $O_3$, $O_2^{\bullet-}$, $OH^{\bullet}$, and $HO_2^{\bullet}$ further improved the inactivation efficacy. Moreover, some injured bacteria could remain after ACP treatment, and the subsequent use of peracetic acid could be able to reduce the number of surviving cells to a greater extent. Peracetic acid can depolarize the bacterial cells and make the cell wall sensitive to reactive species. Applying peracetic acid and ACP treatments in sequence might have resulted in better antimicrobial effect due to enhanced penetration of reactive species produced by plasma (Chaplot et al. 2019). In ready-to-eat ham, hurdle approach consisting of ACP and post-treatment modified atmosphere storage was assessed by Yadav et al. (2020). They observed > 6 log reduction in cell counts of *L. monocytogenes* after 10 min ACP treatment using the gas composition of 20% $O_2$ + 40% $N_2$ + 40% $CO_2$ followed by 7 days of storage at 4 °C. During the post-treatment storage period, the long-lived reactive species produced by ACP treatment probably had longer time to react with bacterial cells, leading to higher cell damage and resulted in lower number of bacteria. In the context of DBD plasma assisted processing of fruit juices. Mahnot, Mahanta, Keener, et al. (2019) developed a strategy for achieving up to 5 $\log_{10}$ reduction of *Salmonella enterica* serovar Typhimurium LT2 in tender coconut water through hurdle approach involving addition of 400 ppm of citric acid. The acidification process resulted in additional stress to the bacterial cells. In a later study, it was reported that the hurdle approach involving addition of 400 ppm citric acid was also effective against *Escherichia coli* and *Listeria monocyotogenes* in tender coconut water when the plasma was induced in a modified gas environment (65% $O_2$ + 30% $CO_2$ + 5% $N_2$) (Mahnot, Mahanta, Farkas, et al. 2019).

## Product factors

### Product type and composition

Several outbreaks and recalls have been frequently related to meat and meat products due to the presence of foodborne pathogens. Fruits and vegetables are commonly consumed as fresh and need decontamination prior to packaging and distribution. Washing of fruits, vegetables, fresh produce, and meat using chlorine and other chemical disinfectants has long been used for disinfection. However, limited decontamination efficiency and the formation of potentially carcinogenic chlorinated compounds in water are their main drawbacks (Oliveira et al. 2012). Other available decontamination methods include hydrogen peroxide, washing with organic acids (e.g., lactic acid, peracetic acid, etc.), and application of ozone. Low direct antibacterial efficiency, pH dependence, and influence on quality factors have been reported as the disadvantages of these techniques (Ramos et al. 2013). Hence, alternative non-thermal methods such as ACP treatment may attract some interest from the food industry. In-package and open environment ACP has been used for decontaminating different food products including meat, fruits, vegetables, etc. (Table 1). The antimicrobial efficacy of ACP can be primarily dependent on the type, nature, and composition of the tested food products. The nature of food constituents (e.g., fat, protein, carbohydrate content) is the other important factor influencing decontamination by ACP.

*Animal-based products:* Animal-based products such as meat and meat products are widely consumed in the world, and they are highly susceptible to cross-contamination by pathogens, which can cause foodborne illnesses. Several studies reported the antimicrobial efficacy of DBD ACP when meat and meat products are treated. For instance, the total aerobic bacteria, *E. coli*, *L. monocytogenes*, and *S. typhimurium*, on chicken breasts were reduced after thin-layer DBD treatments by 3.36, 2.73, 2.14, and 2.71 log CFU/g, respectively (Lee et al. 2016). Lipid oxidation was found to be unaffected, but an increase in off-flavor and cohesiveness was confirmed, which should be taken into consideration regarding optimization of plasma treatment. Based on another study, the best treatment condition in the application of the in-package DBD-based ACP system was at 80 kV for 3 min to extend the shelf-life of raw boneless skinless chicken breast meat (Wang, Zhuang, et al. 2018). Following the use of flexible thin-layer DBD ACP on fresh pork and beef meat for 10 min, reductions of *L. monocytogenes*, *E. coli*, and *S. typhimurium* were 2.04, 2.54, and 2.68 log CFU/g, respectively, on pork-butt samples and 1.90, 2.57, and 2.58 log CFU/g, respectively, on beef-loin samples (Jayasena et al. 2015). A 2 log reduction in the native microflora of chicken breast subjected to in-package DBD ACP at 100 kV for 5 min in air was recently reported (Moutiq et al. 2020). Further, a 24 day storage study revealed that the DBD ACP treated chicken breast had 1.5, 1.4 and 0.5 $\log_{10}$ lower population of mesophiles, psychrotrophs and *Enterobacteriaceae* as compared to control. Inactivation of viable count on packaged fresh pork meat, natural microflora on broiler breast fillets, and total aerobic psychrotrophic bacteria, *Pseudomonas* and lactic acid bacteria on mackerel fillets, have been reported in other studies (Albertos et al. 2017; Huang et al. 2019; Jayasena et al. 2015; Kronn et al. 2015; Zhuang et al. 2019).

In cured pork meat processing, direct DBD treatment has successfully been demonstrated as a replacement for direct nitrite (antimicrobial agent) addition (Jung et al. 2017). Antimicrobial efficacy of ACP was reported to be lower on meat products in comparison to fruits and vegetables, which may be attributed to the different surface characteristics and composition of these products. A detailed review of the applications of ACP technology for decontamination of meat and meat products can be found elsewhere (Misra and Jo 2017).

Previous studies reported the potential of DBD ACP for decontamination of egg surface without a significant effect on quality attributes. Ragni et al. (2010) studied the decontamination of shell egg inoculated with *S. enteritidis* and *S. typhimurium* in 35% and 65% RH at 25 °C. A maximum log reduction of 4.5 log CFU/eggshell was observed for *S. enteritidis*, and no significant negative effect was detected on the egg quality traits after plasma treatment (Ragni et al. 2010). Likewise, 5.53 log CFU/egg reduction of *S. enteritidis* was

**Table 1.** Application of DBD ACP for the preservation of different food products.

| Gas | Power and frequency | Gap distance between electrodes | Treatment time | Max/average treatment temperature | Distance of produce from electrodes | Food product | Pathogen/enzyme | Maximum reduction | Drawback | Reference |
|---|---|---|---|---|---|---|---|---|---|---|
| **Air-Oxygen** | 40W and 60 Hz generating 12 kV of potential | 3 – 3.5 mm | 5 min | 22 °C | ND | Spinach | *E. coli* | 3–5 log | Notable color degradation | (Klockow and Keener 2009) |
| **20:60:20, 40:40:20 and 60:20:20 ($O_2$, $N_2$, $CO_2$),** | 85 kV | 40 mm | 1 min | ND | ND | Pork meat | Total viable aerobic flora | 0.38 log | Increases in lipid oxidation and carbonyl formation in protein oxidation– changes in meat surface color | (Huang et al. 2019) |
| **He- He + $O_2$** | 3 kV, 30 kHz | 0.5 mm | 5 and 10 min | ND | 3mm | Pork loin | *E. coli- L. monocytogenes* | 0.59 log | Decrease in pH, L*values and sensory quality parameters – Greater lipid oxidation | (Kim et al. 2013) |
| **65% $O_2$, 30% $CO_2$, 5% $N_2$** | 75 kV | 4.32 cm | 3 min | 22 °C | In contact with ground electrode | Broiler breast fillets | *Natural microflora* | 2.39 log | – | (Kronn et al. 2015) |
| **Air** | 70 kV | 4.32 cm | 0, 60, 180, or 300 s | 22 °C | In contact with ground electrode | Chicken breast meat | Campylobacter, Salmonella, Psychrophiles, | 1.1 log | Increased L*values- | (Zhuang et al. 2019) |
| **Air- 5% $O_2$, 5% $N_2$-% $O_2$, 5% N2** | 34.8 kV | 30 mm | 5 min | 24.5 °C | ND | Romaine lettuce | *E. coli- L. monocytogenes, Salmonella*, Tulane virus | 1.3 log | – | (Min et al. 2016) |
| **Air** | Potential differences of 15 kV, frequency of 12.7 kHz | ND | 10, 20, and 30 min | 22 °C | 9 cm | Apple | – | – | Alteration of the cellular respiratory pathway | (Tappi et al. 2014) |
| **ND** | Voltage from 16 to 30 kV and frequency from 1.2 to 2.4 kHz | 10 mm | 10, 20, and 30 s | ND | In contact with ground electrode | Almond | *E. coli* | 5 log | Considering technological challenges and limitation, cost, and negative impacts on quality in high voltage and frequency | (Deng et al. 2007) |
| **Air** | Potential difference of 15 kV | 1.5 mm | 10–90 min | 40 °C | 7 cm | Pear | Yeasts–molds | 2.5 log | Significant differences appeared for the peel color and the peel antioxidant capacity of pears treated for 90 mins after storage of 5 days at 20 °C | (Berardinelli et al. 2012) |
| **Oxygen- Argon- Ozone** | 1 – 30 kV, 1 kW power, operated at 50 Hz | 1.5 mm | 20 min | ND | ND | – | *Saccharomyces cerevisiae–Mrakia frigida* | 4.3 log | Complete deactivation of the pathogens could not be achieved within an exposure time of 30 min with 0.4 mA current | (Morgan et al. 2009) |
| **Air** | 30, 40, and 50 kV | 26 mm | 5 min | 25 °C | In contact with ground electrode | Tomato | Peroxidase | 100% | – | (Pankaj, Misra, and Cullen 2013) |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Air** | input voltage of 19 V | ND | 15, 30 min | 22 °C | 70 mm | Radicchio | *E. coli-L. monocytogenes* | 2 log | Significant changes to the external appearance of the radicchio leaves during storage | (Pasquali et al. 2016) |
| **Air** | Potential difference of about 15 kV, frequency of oscillation at 12.7 kHz | ND | 10, 20, 30, 45, 60, and 90 min | 25 °C | ND | Shell egg | *Salmonella enteritidis–Salmonella typhimurium* | 4.5 log | No significant negative effect was detected on the egg quality traits | (Ragni et al. 2010) |
| **Air** | 60 kV, 50 Hz | 40 mm | 5 min | 25 °C | Indirect exposure (at least 2.5 cm from the circumference of field) | Strawberry | Aerobic mesophiles, yeast/molds | 3.3 log | No drastic change in respiration rate, color, and firmness | (Misra, Sullivan, et al. 2014) |
| **Air** | 35 kV at 1.1 A, 1.0 kHz | ND | 3 min | 32 °C | In contact with ground electrode | Grape tomato | *Salmonella* | 3.3 log | No significant negative effect was reported on the quality traits | (Min et al. 2018) |
| **He/$O_2$ mixtures- Air** | 25-30 kV, 10-12 kHz | 60-80 mm | 25 min | 35 °C | Floated between electrodes-25 mm | Egg | *Salmonella typhimurium* | From $10^8$ CFU/egg to below $10^2$ cells per egg | No negative effects on egg quality | (Georgescu, Apostol, and Gherendi 2017) |
| **Air** | 60 and 70 kV | ND | 5 and 10 min | 20 ± 2 °C | ND | Wheat flour | – | – | – | (Misra et al. 2015) |
| **Air** | 35 V and 2 ± 0.2 A | 8 mm | 1, 2, 3, 4 min | 20 ± 2 °C | In contact with ground electrode | Peanut Protein | – | – | – | (Ji et al. 2018) |
| **Air** | 24 ± 1W | ND | 5 s,15 s, 30 s | 22 °C | In contact with ground electrode | Orange and carrot juice blend | – | – | Fluctuation in browning, nonenzymatic browning index, and browning index color | (Vukić et al. 2018) |
| **Air- 65%$O_2$ + 30%$N_2$ +5%$CO_2$,** | voltages up to 90 kV, 60 Hz. | 4.44 cm | 30, 60, 120 s | ND | In contact with ground electrode | Orange juice | *Salmonella typhimurium* | 5 log | Reduces vitamin C by 22%in air | (Xu et al. 2017) |
| **Air** | 70kV, 80kV, 50 Hz | 35 mm | 1, 3, 5 min | 15 °C | In contact with both electrodes | Mackerel fillets | Total aerobic psychrotrophic, *Pseudomonas*, lactic acid bacteria | ≈ 1.5 log | Increases oxidation | (Albertos et al. 2017) |
| **Air** | 80 kV, 50 Hz | 2.2 cm | 5 – 30 min | 27 °C | 10 mm distance from the top electrode- 120-160 mm between the samples and the center of the electrodes at indirect exposure | Cereal grain | *E. coli, Bacillus, lactobacillus, B. atrophaeus endospores* | 6 log | – | (Los et al. 2017) |
| **Air** | 1 ± 0.2 A and 50 V, 75 V, 100 V, 125 V | 8 mm | 2 min | 40 °C | In contact with ground electrode | Zein | – | – | – | (Dong et al. 2017) |
| **Air** | 15 kHz, input power of 250 W | ND | 5, 10, 20, 30 min | ND | Inside a container (240 × 200 × 100 mm) with electrodes | Quercetin | – | – | – | (Kim et al. 2017) |
| **Air** | 60, 70, and 80 kV , 50 Hz | 22 mm | 1, 2, 3, 4, 5 min | 19 °C | In contact with ground electrode | Chitosan film | – | – | – | (Pankaj et al. 2017) |
| **Air** | 60, 70, and 80 kV , 50 Hz | 10 mm | 5 min | 20 °C | In contact with ground electrode | High amylose corn starch film | – | – | – | (Pankaj et al. 2015) |
| **nitrogen and oxygen** | 550 W and 25 kHz | ND | 30 min | ND | ND | Meat | – | – | – | (Jung et al. 2017) |
| **Air** | 2500 Hz and 26 kV-17 KV, 500 Hz | ND | 10 min | ND | 1 mm | Bread | *E. coli* | 2 log | – | (Ranieri et al. 2018) |
| **Air** | 2 W average power, 15 kHz | ND | 10 min | ND | ND | Chicken breasts | | 3.36 log | Flavor decreased and off–flavor increased– | (Lee et al. 2016) |

(*continued*)

**Table 1.** Continued.

| Gas | Power and frequency | Gap distance between electrodes | Treatment time | Max/average treatment temperature | Distance of produce from electrodes | Food product | Pathogen/enzyme | Maximum reduction | Drawback | Reference |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | Total aerobic bacteria, *L. monocytogenes, E. coli, S. typhimurium* | | increased cohesiveness | |
| **Air** | 40, 50, and 60 kV, | ND | 5 min | 30 °C | ND | Alkaline phosphatase | – | – | – | (Segat et al. 2016) |
| **Air** | 15 kV peak to peak and a dominant frequency of 12.5 kHz | ND | 15 + 15 and 30 + 30 min | 22 °C | 70 mm | Melon | Mesophilic lactobacilli and lactococci, yeasts, total aerobic mesophilic and psychrotrophic bacteria | 3.4 log | Increased dry matter content, translucent appearance, and stress response of the tissue | (Tappi et al. 2016) |
| **Air** | 80, 90 kV- 50 Hz | 32 mm | 1, 3, and 5 min | 20 °C | In contact with ground electrode | Biscuit | – | – | – | (Misra, Sullivan, et al. 2014) |
| **Air** | Input power of 250 W at 15 kHz | ND | 0, 5, 10, and 20 min | ND | Inside a container (137×104×53 mm) among with electrodes | Naringin | – | – | – | (Kim, Yong, et al. 2014) |
| **Air** | 70 W for 80 kV treatment, 41 W for 65 kV, and 34 W for 55 kV | 5.2 cm | 9 min | ND | 3 cm with the top electrode | Chicken meat | Natural microflora | 1.5 log | Significant reductions in a* and b* value of color | (Wang, Zhuang, et al. 2018) |
| **Air** | 0.0, 9.0, 11.0, 13.0, 15.0, and 17.0 kV, 50 Hz | 8 mm | 4 min | 22 °C | ND | Wheat seed | – | – | – | (Guo et al. 2018) |
| **Air** | 34.8 kV at 1.1 kHz | 1.8 cm | 5 min | 28.3 °C | In contact with ground electrode | Romaine lettuce | *E. coli*, total mesophilic aerobes, yeasts, molds | 1.9 log | Without altering the color or leaf respiration during posttreatment cold storage | (Min, Roh, Boyd, et al., 2017) |
| **Argon** | Pulses of 2.5-10 KHz, 6-10 KV, 500 ns | 5 mm | 2-15 min | 85 °C | In contact with ground electrode | Sprout seeds of alfalfa, onion, radish, cress | *E. coli*, native microbiota | 3.4 log | Cracks and crevices can shield microorganisms from reactive species | (Butscher, Van Loon, et al. 2016) |
| **Air** | 60, 70, and 80 kV, 50 Hz | ND | 0, 2, 4, 6, 8 min | 27 °C | In contact with ground electrode | Pesticide (dichlorvos, malathion, endosulfan) | – | 78.98 % | – | (Sarangapani et al. 2016) |
| **Nitrogen and Oxygen** | 100-W peak power and 2-W average power at 15KHz | 0.28 mm | 0, 2.5, 5, 7.5, 10 min | ND | Inside a container among with electrodes | Pork and beef meat | *L. monocytogenes, E. coli, S. typhimurium* | 2.68 log | Significantly lowered a* values (redness) after 5 and 7.5 min exposures– taste was negatively influenced– increased lipid oxidation value | (Jayasena et al. 2015) |
| **Air** | input power of 250 W at 15 KHz | ND | 5, 10 min | ND | Inside a container (137 × 104 × 53 mm ) among with electrodes | Milk | *L. monocytogenes, E. coli, S. typhimurium* | 2.4 log | Decreased pH value– increased L* and b* values and decreased a* values | (Kim, Yong, et al. 2015) |
| **Air** | input power of 250 W, 15 kHz | ND | 60 s, 45 s, and 7 min | ND | ≈50 mm | cheese | *L .monocytogenes, E. coli, S. typhimurium* | 3.10 log | – | (Yong et al. 2015) |
| **Air- Nitrogen** | 5.5 kV and 30 kHz | 0.5 mm | 4 min | ND | 2.5 cm | – | *Campylobacter jejuni* | 4 log | – | (Kim, Lee, et al. 2014) |
| **Air** | Input power of 90 W | 10 mm | | ND | In contact with ground electrode | Apple juice | *Zygosaccharomyces rouxii* | 5 log | Significant changes in pH, titratable acidity, | (Xiang et al. 2018) |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | 0, 20, 40, 60, 80, 100, 120, 140 s | | | | | | and Certain color parameters of apple juice | |
| **$N_2$:$O_2$= 100:1** | 1000W, 4 – 10 kV peak amplitude voltages at 20-60 kHz in combined with radio frequency thermal treatment | ND | 5 cycles of 120 s+ 120 s | ND | 30 mm | Red pepper powder | *E. coli, S. aureus* | ≈ 8 log | Negligible changes in CIE L*, a*, b*, and ΔE* and extractable color values– significant differences in moisture content and water activity | (Choi et al. 2018) |
| **Air** | 30 kV | 9.5 cm | 15,30,45, 60 min | – | Floated in a packed-bed mode | – | Airborne viruses | 2.3 log | – | (Xia et al. 2019) |
| **Air** | 10 kV, 10 kHz | 4 mm | 0.5, 1, 2 min | ND | 20 mm | – | Feline calicivirus | 4.44 log ($D_{Value}$= 0.45 min) | – | (Yamashiro, Misawa, and Sakudo 2018) |
| **Air- Ar + 20% O2** | 20 kHz- | Integrated coaxial microhollow | 5 min | 43 °C | 2mm- 50 cm | – | Feline calicivirus | 4.5 log | – | (Nayak et al. 2018) |
| **Air- 5% $O_2$, 5% $N_2$- % $O_2$, 5% N2** | 34.8 kV | 30 mm | 5 min | 24.5 °C | ND | Romaine lettuce | *E. coli–L. monocytogenes, Salmonella*, Tulane virus | 1.3 log | – | (Min et al. 2016) |
| **Air** | 6 kHz and 10 kV for upper electrode, 50 Hz and variable voltage up to 24 kV at lower electrode | ND | 5–35 min | 30 °C | Between the two electrodes | Wheat germs | Lipase and lipoxygenase | 75% | – | (Tolouie et al. 2018) |
| **Air** | 80 kV at 50 Hz | 22 mm | 5, 20 min | ND | Direct: 10 mm- indirect: 23 cm | Wheat-barley | Bacteria and fungi | 2.5 log | Decreased surface hydrophobicity of wheat grains | (Los et al. 2018) |
| **Air** | Input power of 30, 40, and 50 W | ND | 40 s | 25 °C | 2 mm | Apple juice | *E. coli* | 4.34 log | Significant change of the pH, TA, color and total phenolic content at 50 W for more than 10 s | (Liao et al. 2018) |
| **Air** | 70 and 80 kV at 50 Hz | 52 mm | 1, 3, 5, 10 min | 27 °C | Between the two electrodes | sushi | total aerobic | 1.5 log | Increase in the oxidation rate | (Kulawik et al. 2018) |
| **Air** | 60 and 80 kV at 50 Hz | 47 mm | 0, 1, 2, 5 min | 27 °C | Inside a package ( 150 mm × 150 mm × 35 mm ) between electrodes | Blueberries | Pesticides | 80.18% | Decrease in ascorbic acid | (Sarangapani et al. 2017) |
| **Air** | 42.6 kV (1.5 A) | 5 cm | 10 min | 25.9 °C | In contact with ground electrode and 3 cm from the top electrode | Romaine lettuce | *E. coli* | 1.1 log | No significant differences in surface morphology, color, $CO_2$ generation, or weight loss | (Min, Roh, Boyd, et al. 2017) |
| **Air** | Input voltage of 50 V and the input power was 75 W | 6.5 mm | 1, 5, 10 min | ND | ND | Corn starch | – | – | – | (Bie et al. 2016) |
| **Air** | Supply voltage of 19.15 V | ND | 30, 60 min | 34 °C | 20 mm | Cut celery and radicchio leaves | *L. monocytogenes, E. coli* | 3.7 log | Significant decrease of the chroma color parameter in radicchio | (Berardinelli et al. 2016) |
| **65 % $O_2$+16 % $N_2$+19 %** | 60 kV rms (50 Hz) applied voltage | 40 mm | 300 s | 26 °C | Between the dielectrodes and outside the electrodes | Strawberries | Total aerobic mesophiles and yeast/molds | 3.7 log | Significant decreases in firmness and unpleasant dark | (Misra, Moiseev, et al. 2014) |

(*continued*)

**Table 1.** Continued.

| Gas | Power and frequency | Gap distance between electrodes | Treatment time | Max/average treatment temperature | Distance of produce from electrodes | Food product | Pathogen/enzyme | Maximum reduction | Drawback | Reference |
|---|---|---|---|---|---|---|---|---|---|---|
| **$CO_2$- 90 % $N_2$+10 % $O_2$** | | | | | | | | | color in high nitrogen environment | |
| **Air** | 3 W discharge power, 30 kV at the peak amplitude, 22 kHz | 6.6 mm | 0–180 s | 44.2 °C | In contact with ground electrode | Rice seed | *Gibberella fujikuroi* | > 99% | No adverse effects were detected on the seedling emergence and height | (Jo et al. 2014) |
| **Air** | 60 and 70 kV at 50 Hz | ND | 1–5 min | 22 °C | In contact with ground electrode | Sodium caseinate film | – | – | – | (Pankaj, Bueno-Ferrer, Misra, O'Neill, et al. 2014) |
| **Air** | 30 kV AC and a frequency of 30 kHz | 1.85 mm | 0–10 min | ND | In contact with ground electrode | Dried laver | Aerobic bacteria, marine bacteria, and molds | 99.68% | No significant changes in the color characteristics, total phenolic content, DPPH radical scavenging activity, and sensory characteristics | (Kim, Puligundla, et al. 2015) |
| **Air** | voltage output of 120 kV at 50 Hz | 40 mm | 10, 60, 120, 300 s | ND | Indirectly: 140–160 mm | Cherry tomatoes and strawberries | *E. coli, S .typhimurium and L. monocytogenes* | ≈6.7 log | Extended ACP treatment time was necessary on the more complex surface of strawberries | (Ziuzina et al. 2014) |
| **Air** | 30 kV in magnitude (peak to peak) and 0.5-kHz | 1.5 mm | 0–180 s | 22 °C | 1–2 mm | Chicken meat | *S. enterica and C. jejuni* | 3.11 log | Oxidation of lipids–limited size of the plasma probe–requirement of a flat surface of chicken meat | (Dirks et al. 2012) |

ND: none declared.

**Table 2.** Impact of ACP on bacterial cell structure.

| Bacterial cell structure | Impact by ACP | | |
|---|---|---|---|
| Proteins and enzymes | Protein denaturation | Amino acid oxidation | Loss of enzyme activity |
| Lipids and fatty acids | Membrane lipid peroxidation | | |
| Nucleic acids | Damage to DNA and RNA | Reduction in cell replication | |
| Cell membrane | Etching and perforation in membrane | Cell membrane damage by diffusion of reactive species | |
| Cell wall | Breaking of chemical bonds | Erosion due to reaction with radicals | Surface roughness increase |
| Cytoplasm | Deformation of the cytoplasm | Cell shrinkage | Cytoplasm leakage |

*Adapted from (Misra and Jo 2017).

attained by DBD ACP at 85 kV within 15 min using 65% $O_2$, 30% $CO_2$, and 5% $N_2$ (Wan et al. 2017).

*Plant-based products:* DBD ACP treatment has been used for decontamination of plant-based products, such as fruit and fruit juices, with a significant decrease in natural microflora, pathogens, and polyphenol oxidase residual activity and browning without a noticeable change in quality parameters such as total soluble solids, reducing sugar, and total phenolics (Berardinelli et al. 2012; Pasquali et al. 2016; Tappi et al. 2014; Xiang et al. 2018). By using atmospheric DBD ACP, mesophilic bacteria, yeasts, and molds were reported to decrease by 2.5 log CFU/fruit after 90 min of treatment without any significant adverse effect on quality traits immediately after the treatment. However, after 5 days of storage at 20 °C, the samples treated for 90 min had significant changes in the color and antioxidant capacity of the peel (Berardinelli et al. 2012). In radicchio leaves, decontamination without post-treatment antioxidant capacity change could be achieved using DBD ACP. A significant change in terms of visual quality was observed after 1-day of storage in comparison to the control (Pasquali et al. 2016). The reduction in microbial content is often attributed to highly reactive chemical species and release of intracellular nucleic acids and proteins in response to alterations in cell membrane permeability (Mahnot, Mahanta, Keener, et al. 2019; Xiang et al. 2018).

Jo et al. (2014) reported inactivation of *Gibberella fujikuroi*, a seed-borne pathogen, on rice seed surface by 50%, 90%, > 92%, and > 99%, after 9, 76, 120, and 180 s of ACP treatment, respectively. *Aspergillus flavus*, with an initial viable count of $1.2 \times 105$ CFU/sample on packaged pistachio, was completely removed after 18 min of ACP exposure (Sohbatzadeh et al. 2016). Also, DBD ACP treatment reduced the *E. coli* on almonds by almost 5 log after 30 s of treatment at 30 kV and 2000 Hz (Deng et al. 2007), and *Salmonella enteritidis* was reduced by > 5 log within 15 min using 20 kV and 15 kHz by diffuse coplanar surface barrier discharge (Hertwig et al. 2017). A suitable gas composition for ACP is critical, as Hertwig et al. (2017) observed color changes of unpeeled almond after ACP treatment with the working gas containing $N_2$.

### Surface characteristics

The substrate surface topology can influence the antimicrobial efficacy of the DBD ACP treatment. For instance, considerable differences in *E. coli* inactivation efficacies were observed on seeds with possibly different surface characteristics (Butscher, Van Loon, et al. 2016). The reduction of artificially inoculated *E. coli* on onion seeds was 1.4 log after 10 min of ACP treatment, while the identical treatment conditions resulted in a 3.4-log reduction on cress seeds. One of the main reasons for the observed difference is the different number of fissures, pits, and grooves on the surface of the seeds, which may protect the microorganisms from the plasma-generated species (Butscher, Van Loon, et al. 2016). On rough surfaces, bacteria may attach as multilayers, possibly making it difficult for plasma species to diffuse and inactivate. The antibacterial effect of ACP against bacterial endospores on polymeric model substrates was considerably more effective than on wheat grains (Butscher, Zimmermann, et al. 2016). This was attributed to the complex surface and the ventral furrow of wheat grains (Butscher, Zimmermann, et al. 2016; Los et al. 2018). In another study, better inactivation of bacteria inoculated on tomato surface was reported in comparison to strawberries (Ziuzina et al. 2014). This was due to the smooth surface of the tomato and lower concentration of ozone inside packages containing strawberries after ACP treatment. The presence of pores on strawberries exhibited more surface contact area; hence, the dissolution rate of ozone generated inside the packages containing strawberry increased, and subsequently, the antimicrobial efficacy reduced (Ziuzina et al. 2014).

### Water content

Bacteria become more susceptible to inactivation at high water contents with greater ROS (e.g., hydroxyl radicals) generation during ACP treatment in liquid water phase (Butscher, Van Loon, et al. 2016; Oehmigen et al. 2010; Van Gils et al. 2013). In addition, high water content of substrates increases the relative humidity of the gas phase (Butscher, Van Loon, et al. 2016). High water content facilitates the uptake of ozone by the product surface since ozone is water-soluble. The contact between the substrate and ozone can be increased at elevated water contents. In the presence of water, ozonation could be catalyzed by free radicals (i.e., hydroxyl ions) (Alexandre et al. 2017). However, Amini and Ghoranneviss (2016) reported that the inactivation rate of *A. flavous* on dried walnuts was higher than that on fresh walnuts after ACP treatment. Food-intrinsic factors such as osmotic stress and suboptimal pH induce stress hardening, creating cells resistant toward the subsequent ACP treatment that influence the inactivation efficacy (Smet et al. 2016). Hence, an optimum water content for each product should be considered in ACP treatment. For instance, the reported optimum water content for inactivation of native microbiota on alfalfa seeds was 17% (Butscher, Van Loon, et al. 2016). This is due to reactivation

of bacteria from the dormant state in the presence of water, a result which makes the bacteria susceptible to ACP. Furthermore, higher amount of ROS could be triggered, which contribute to the inactivation of microorganisms (Reuter et al. 2015; Van Gils et al. 2013). However, high humidity in the discharge may reduce the electron energy and density, quench the excited molecules, and weaken the ACP (Nikiforov, Sarani, and Leys 2011).

## Microbiological factors

### Pathogen type

Bacterial type, strain, and mode of existence are the main microbiological factors relating to inactivation efficacy by DBD ACP (Los et al. 2017). Yeasts and molds are more sensitive to treatments than mesophilic bacteria (Berardinelli et al. 2012). Applying DBD ACP on tomatoes reduced both the mesophilic aerobic bacteria and the yeast and molds by 1.3 and 1.5 log CFU/tomato, respectively (Min et al. 2018). Berardinelli et al. (2012) also reported 1 log CFU/fruit reduction of yeast and molds by DBD ACP in 10 min; however, no significant change was detected for the bacteria count within 30 min of treatment. In longer treatment times (60–90 min), the same decontamination level was observed for mesophile bacteria and for yeast and molds. In another report, psychrotrophs, total bacteria, and yeast and molds were reduced by 2.1, 2.3 and 2.6 log CFU/cm$^2$ within 10 min of ACP treatment (Ulbin-Figlewicz, Brychcy, and Jarmoluk 2015). Morgan (2009) reported D-values of 7 and 4.7 min for *S. cerevisiae* and *M. frigida*, respectively, by argon DBD discharge that demonstrated the faster inactivation of *M. frigida* than *S. cerevisiae*. In that study, *M. frigida* was completely inactivated in 15 min, while *S. cerevisiae* was completely inactivated in 20 min. In a study on *Pseudomonas fluorescens* and *Macrococcus caseolyticus*, after 1.5 min of treatment, DBD ACP treatment had more antimicrobial effects on gram-negative bacteria *P. fluorescens* than gram-positive bacteria *M. caseolyticus* (Wang, Zhuang, and Zhang 2016). In another study, cascaded dielectric barrier discharge (CDBD) was used to inactivate strains like *Salmonella* serotype *Mons*, *Staphylococcus aureus*, and *E. coli* and spores of *Bacillus subtilis*, *Aspergillus niger*, and *Clostridium botulinum*. *Aspergillus niger* was the most resistant strain with an inactivation level of about 3, 4, and 5 log after 1, 3, and 5 s. The highest reduction was observed for the vegetative cells with at least 6.6 log CFU/foil within 1 s, except for *E. coli*. For *E. coli*, reduction of 5.6 log CFU/foil was seen in the first second, and it was reported that the inactivation of the pathogens takes place in the initial seconds of the treatment (Muranyi, Wunderlich, and Heise 2007).

Generally, microbial spores exhibit high resistance against antimicrobial treatments. *Clostridium botulinum* endospores were reduced by 6.1 log CFU/foil within 1 s in comparison to 6.9 log CFU/foil reduction in *Staphylococcus aureus* count, and so, theoretically, the 12-D concept could be carried out after 2 s of CDBD plasma treatment (Muranyi, Wunderlich, and Heise 2007). Important factors in spore resistance against numerous sterilizing agents, in particular in *B. subtilis*, are the relative impermeability of the inner membrane against chemicals, high quantity of dipicolinic acid, the protection of the DNA by small acid soluble proteins, and the low core water content (Setlow 2006). Besides, bacterial spores contain detoxifying enzymes in their coat that play an important role in detoxifying chemicals (Henriques and Moran 2007; Setlow 2006). The reactive species in ACP cause damage to membranes and enzymes and, as a result, affect the germination process of the spore (Patil et al. 2014). Patil et al. (2014) reported the importance of the water and $O_3$ presence for higher spore inactivation. High values of RH accelerate the spore swelling and generate more reactive species during ACP treatment that damage the spore structure.

It was reported that the outer membrane of gram-negative bacteria was more sensitive to plasma-generated species than gram-positive bacteria (Deng, Shi, and Kong 2006; Laroussi 2002; Montie, Kelly-Wintenberg, and Roth 2000; Yong et al. 2015; Ziuzina et al. 2014) and that the thicker membrane of the gram-positive bacteria acts as a barrier against diffusion of plasma-reactive species to the bacterial cell. Other studies reported greater resistance of gram-negative bacteria against plasma exposure (Fan et al. 2012; Lu et al. 2014). Min et al. (2016) studied the inactivation efficacy of DBD ACP on *E. coli*, *Salmonella*, *L. monocytogenes*, and Tulane virus on romaine lettuce. Tulane virus was the most sensitive and *Salmonella* was the most resistant microorganism against ACP treatment, which could be due to the lack of cell wall in viruses. It was believed that a chemically reactive species such as $^1O_2$, $ONOO^-$, and ONOOH modify genomic RNA and leads to inactivation of the virus (Yamashiro, Misawa, and Sakudo 2018). The antiviral effect of DBD ACP was assessed for different viruses and, according to the process and microbial factors, different inactivation rates from 0.5 to 4.5 log were achieved (Ahlfeld et al. 2015; Nayak et al. 2018; Xia et al. 2019; Yamashiro, Misawa, and Sakudo 2018). By carefully reviewing the studies on anitimicrobial efficacy of ACP treatment provided in Table 1, it was observed that the sensitivity sequence of different microorganisms against ACP is yeast-mold-virus > bacteria > spores.

### Initial count

Surface microbial loading of the products is one of the microbiological factors that could influence the efficacy of DBD ACP treatment. The negative correlation of the initial count of microorganisms with inactivation rate by ACP treatment was reported in several studies (Ahlfeld et al. 2015; Deng et al. 2005; Kamgang-Youbi et al. 2008; Perni et al. 2008; Yu et al. 2006). The study conducted by Fernandez et al. (2012) by a nitrogen plasma jet clearly proved that increasing the concentration of *S. typhimurium* from 5 to 8 log CFU/filter reduced the inactivation efficacy of ACP (Fernandez et al. 2012). It was assumed that microbial inactivation depends on cell density, in particular in high initial microbial counts, due to the protective effect of overlaying cells and agglomerates against ACP (Ghomi et al.

2009; Muranyi, Wunderlich, and Heise 2007). In this circumstance, the inactivated top layers of microorganisms could form a physical barrier to shield the underlying microorganisms from ACP penetration (Deng et al. 2005).

### *Growth phase*

The *E. coli* cells at the logarithmic phase are more sensitive to ACP than those at stationary and declining phases (Deng et al. 2007). Similar results were documented for yeast cells exposed to high voltage electric pulses (Gášková et al. 1996; Jacob, Förster, and Berg 1981). In applying electric fields, it was assumed that in the logarithmic stage of yeasts, the amount of the cells in the state of budding was higher than that in the other phases. The budding area of the yeast was more sensitive to electric discharges, and so, they could be inactivated relatively easily (Jacob, Förster, and Berg 1981).

## Antimicrobial mechanisms of DBD ACP

Two main hypotheses for cell death caused by gas discharge are as follows: First, the total electric force caused by the accumulation of surface charge could exceed the total tensile force on the membrane and cause electrostatic disruption. Second, energetic ions, radicals, and reactive species generated by gas discharge cause oxidation, which provokes damage to the cell membrane or cellular components. Scanning electron microscopy of *Saccharomyces* and *Candida* yeast before and after treatment showed rupture in the cell surface after treatment, which resulted in the inactivation of the yeast (Morgan 2009). ROS from ACP alter the functions of biological membranes and cellular biomolecules via interaction with lipids, proteins, and DNA mostly by the oxidation process. The intense electric field of ACP ruptures the cell membrane and causes bacterial cell leakage and loss of cell functionality (Misra and Jo 2017). In contrast to most opinions that there was no UV inactivation at atmospheric pressure and that chemically reactive species are entirely responsible for the observed inactivation, it was proved that spore inactivation could be achieved either under the sole action of the reactive species or under dominant UV radiation (Boudam et al. 2006). In Table 2, a summary of the inactivation mechanisms of microorganisms associated with ACP treatment is presented.

## Challenges

Production of hot streamers (regions of highly ionized matter) in the air is a feature of DBD ACP. DBDs may be useful for treating hard solid foods such as nuts, but considering that these localized streamers may leave visible marks on the skins of fruits such as for mangoes and melons, the application of DBD ACP would be limited due to its influence on appearance and color (Perni et al. 2008). Another factor that should be considered is the high oxidative action of ACP treatment that can affect the bioactive compounds and other food components such as lipids and proteins in food products (Tappi et al. 2014). Plasma exposure can induce the oxidation of sugars into organic acids, peroxidation of lipids, and the modification of amino acid residues in proteins (Cullen et al. 2018). For instance, Baier et al. (2014) used ACP to treat plant leaves and observed that the argon plasma jet was a gentle and suitable ACP source for the application on leafy greens. On the contrary, a surface DBD and a remote exposure reactor, fed with plasma-treated air, had significant impacts on the leaf tissue. In ACP treatment, the presence of oxidizing agents could cause oxidation of lipids, thus limiting its potential for high-fat foods also.

During and after ACP treatment of in-packaged products, the generated ozone concentration could be reduced due to its interaction with the surface of the polymer bag. Also, energetic $Ar^+$ ions in ACP can dissociate the carbon atoms from the polymer when argon gas is used. Besides, ozone can interact with the dissociated carbon and produce carbon dioxide. Hence, the process should be switched off after the maximum ozone concentration is attained and before a significant amount of dissociated fragments form from the polymer surface of the package generated (Leipold et al. 2011). ACP decontamination of food products shares similarities with plasma medicine but also exhibits differences. The treated food products are sometimes more susceptible to plasma than human tissue and the volume of the products is larger in the food industry (Brandenburg et al. 2019).

Different DBD systems have been designed in the past for variety of applications in medical, electronics, and engineering fields. With the inherent complexity of ACP and the additional complexity of food products with different constituents, size, shape and surface characteristics, custom designed DBD systems should be developed for food product decontamination applications. Microbial survival during ACP treatment is greatly influenced by surface properties of the substrates used. On rough surfaces, microbial inactivation by ACP may be expected to be lower due to the shielding effect, although reactive species could diffuse on the surfaces. The ACP chemistry is greatly dependent on the electrode material, dielectric barrier and the substrate surfaces used. Hence, a great deal of work is required to understand the plasma chemistry when different food products are used in order to optimize ACP treatments for specific products. Effect of individual ACP components mainly, ROS, RNS, UV, and electric field on specific microorganisms is not clearly understood. Hence, additional research on microbial inactivation mechanisms by individual ACP components will help to identify the key components responsible for microbial inactivation. Other than the aforementioned, several process related factors will influence the decontamination efficacy of ACP treatments, adding another layer of complexity. Application of ACP in the food industry is quite a new field, and it requires considerable research for a better understanding of reaction mechanisms, antibacterial pathways, the impact of ACP on food quality and safety, and its probable side effects on consumer health. For defining standard ACP exposure conditions for treating the food products, focused research should be conducted on specific applications and the various factors described in this review. Hence, at the moment it is hard to define standard exposure

conditions for treating the food products. The information on important factors described in this review manuscript will help optimize the DBD ACP treatments for specific food decontamination applications.

## Scale-up of atmospheric pressure DBD plasma technology

At present, the scaling-up of atmospheric pressure DBD plasma systems is limited by technological and economical challenges. Only a few attempts have been made to scale-up the DBD plasma technologies. A continuous atmospheric pressure, high voltage DBD plasma system was developed by researchers from European Union and found to be successful in extending the shelf-life of cherry tomatoes and strawberries (Ziuzina et al. 2016; Ziuzina et al. 2020). Other attempts to scale-up include the surface DBD plasma technology developed by Anacail in UK (Anacail 2018) and a pilot-scale DBD developed and validated in Italy (Ragni et al. 2010).

Process and product parameters including treatment time, plasma density, power and current supply, gap between the sample and high voltage electrode, food properties, and cost of the system and the process should be considered for scaling-up an ACP based process without compromising the plasma uniformity. To keep the uniformity of the plasma in larger scales, much larger voltages (~130 kV) and customized dielectric materials are required (Cullen et al. 2018). For large volume treatments, a sustainable high voltage power source with sufficient lifetime is required. Process validation is a critical step to assure the safety of food products. In conventional treatments such as heat treatment, process validation is performed through temperature profiling of the heated product. However, in ACP treatment, there are numerous factors i.e. process, product and microbiological parameters (as described in previous sections) should be controlled, which is challenging. Yet another concern is controlling the amount and type of reactive species and tuning the plasma chemistry to ensure highest efficiency. The reactive species produced by ACP have limited lifetime, and no active residue should be left when the food products are on the shelf for sale; however, more studies need to be conducted toward this aspect. The diversity of mechanisms and species involved in ACP treatment is an important challenge to scale-up this technology and define a plasma treatment dose, specific to each product. Some of the reactive species such as ozone, produced during ACP treatment are stable over a period of time, and their exposure to workers and release from factories to the environment should be controlled. Regulatory approval may be required and toxicity studies in ACP treated foods need to be completed before the scale-up of ACP technology for various applications in food sector.

## Concluding remarks

The antimicrobial properties of ACP, primarily due to the radicals and reactive species, along with its lowest impact on food quality attributes, are the incentive factors for this technology to be used in food processing. DBD ACP can potentially be employed in the food production and processing lines for different products, including meat, fruit and vegetables, nuts, eggs, cereals, and packaged products, and scaled-up for surface decontamination of food products. However, DBD ACP treatment may not be able to completely replace conventional sterilization processes used in the food industry due to the limitations of the technology (i.e., poor penetration of reactive species inside foods). Since food products vary in size, shape, and nature, DBD ACP systems should be designed depending on the specific application and product type for future scale-up of this technology for food decontamination purposes. The antimicrobial properties of DBD ACP depend on process factors including voltage, frequency, relative humidity, temperature, flow rate and gas, type of electrode, interelectrode spacing, treatment time, headspace, and exposure pattern time; product factors including water content, type of product, surface characteristics; and microbial factors including growth phase, initial count, and type of the pathogen. Optimization of process conditions (e.g., in-package ACP treatment) and custom-designed DBD plasma systems for best outcomes require improved characterization and thorough knowledge about the important process, product, and microbiological factors as described in this review.

## Disclosure statement

The authors declare that they do not have any conflict of interest.

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