# Chapter 14 Cold Plasma Hurdled Strategies for Food Safety Applications



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Abstract The application of hurdle interventions can improve the microbicidal efficacy as well as assure the food quality. Cold plasma (CP) is a promising decontamination technology and has gained a lot of interests in food industry. In recent few years, the combination of CP with other techniques (e.g., mild heat, organic acids, essential oils, ultrasound, bacteriophage) has been proposed and attracted a lot of interest. In this chapter, a comprehensive summary about the current status of cold plasma-based hurdle technologies was provided. Furthermore, the factors (e.g., treatment sequence, equipment configuration, microbial properties) affecting the antimicrobial efficacy of cold plasma-based hurdle have also been presented in detail, which should be carefully considered during designing and optimizing the hurdle technique. The effects of cold plasma-based technologies on the quality attributes (e.g., color, texture, flavor) of food were also evaluated in this chapter. More cold plasma-based hurdle technologies with efficient antimicrobial efficacy and good quality retainment require further research to discover.

Keywords Cold plasma · Hurdles · Mild heat · Organic acids · Essential oils · Bacteriophage · Affecting factors · Quality attributes

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## 14.1 Introduction

Hurdle technology was first proposed by Leistner in 1978 as a novel strategy to control microbial contamination and assure food safety (Leistner [1978\)](#page-14-0). The aims of a hurdle technology are combining multiple fence factors to inhibit the growth and reproduction of pathogenic bacteria and delay the deterioration of food (Fig. 14.1) (Gómez et al. [2011\)](#page-13-0). Hurdle technologies have been widely applied in meat and vegetables (Singh and Shalini [2016\)](#page-15-0). The most commonly used fence factors include water activity  $(a_w)$ , pH (acid or alkaline), temperature (heat or cold), and preservatives (Leistner [1994](#page-14-0)). For an individual fence factor, a slight increase in the intensity might cause a significant compromise in food quality. With hurdle treatments, each fence factor requires only moderate levels to achieve efficient microbial inactivation as well as avoid nutrient loss and retain maximum food quality. In recent years, hurdle technologies integrating novel nonthermal decontamination techniques (e.g., high pressure, electrolyzed water, ultrasound, pulsed light) have been proposed to achieve a synergism on microbial inactivation (Ross et al. [2003](#page-14-0)). The suitable combination of fence factors to achieve maximum microbial reduction and minimal quality loss is the key point for the development of a novel hurdle technology.

Cold plasma (CP), also called nonthermal plasma (NTP), is an emerging disinfection technology for environmental and food applications (Liao et al. [2017\)](#page-14-0). Plasma, is a wholly or partially ionized gas, containing a vast mixture of various species, including charged particles, electrons, reactive species (e.g., reactive oxygen/nitrogen species [RONS], excited molecules), and ultraviolet (UV) photons (Liao et al. [2019\)](#page-14-0). In CP, only the electron temperatures are elevated, and the overall temperature of CP remains relatively low. In recent years, CP technology has been widely explored for the efficient inactivation of a wide range of microorganisms, including Gram-positive and Gram-negative bacteria, fungus, viruses, spores, and biofilms (Bourke et al. [2018\)](#page-13-0). The abundant reactive species in plasma are thought to contribute to the microbial inactivation through the damages in multiple cellular targets, including cell walls and membranes and intracellular components (e.g., proteins, nucleic acids, adenosine triphosphate-ATP) (Georgescu et al. [2017](#page-13-0)). CP laboratory scale systems with direct, indirect, and in-package application modes have been developed specifically for food decontamination (Toyokawa et al. [2017\)](#page-15-0).

Fig. 14.1 The basic concept of hurdle technology



Antimicrobial factor 1, 2 and 3

The advantages of CP technology include operation at low temperatures, energy efficiency, short processing times, free of chemical residues, flexible treatment modes, and high antimicrobial efficacy with minimal impact on food quality and the environmental matrix. The food can be exposed to CP in a direct and indirect (plasma-activated water [PAW] or PAW ice) mode (Liao et al. [2020a](#page-14-0)). In a direct mode, the reactive species produced from CP directly contact with the treated food (Fernández et al. [2013](#page-13-0); Liao et al. [2018a](#page-14-0)).

During the last several years, the combination of CP and other techniques as novel hurdle technologies has been developed for food decontamination. The purpose of this chapter is to make a summary about the development of cold plasmabased hurdle technologies, including the current status, the factors affecting the hurdle efficacy as well as the effect of cold plasma-based hurdles on food quality (Table [14.1\)](#page-3-0).

#### 14.2 Current Status of Cold Plasma-Based Hurdles

## 14.2.1 Cold Plasma and Mild Heat

In the last 2 years, CP, particular PAW has been used in combination with mild heat (40–60 °C) as a novel hurdle technology (Choi et al.  $2019$ ; Muhammad et al.  $2019$ ; Xiang et al. [2019](#page-15-0), [2020;](#page-15-0) Liao et al. [2020b](#page-14-0); Bai et al. [2020\)](#page-12-0). In the study of Xiang et al. [\(2019](#page-15-0)), the alliance of PAW and mild heat was employed to inactivate the foodborne pathogenic bacteria-Escherichia coli O157:H7. The results indicated that the combination of PAW and mild heat at 60  $\degree$ C achieved more than 8.28-log reduction level of E. coli O157:H7, whereas the inactivation levels of 0.77 and 1.78 logs were caused by the individual PAW and mild heat exposure, respectively. The synergistic effect might be attributed to the enhanced disruption of the outer structure and intracellular components of E. coli cells. Apart from the simultaneous treatment, our group developed a sequential exposure of PAW and blanching (60  $\degree$ C, 5 min) to remove the background bacteria on the tiger nuts. It is found that the sequential treatment led to 3.65–3.7 log reduction, while the single PAW or blanching treatment achieved 1.7–3.22 logs (Muhammad et al. [2019](#page-14-0)). In addition to the vegetative bacteria, our group also employed the combination of PAW and mild heat (40 and 55 $^{\circ}$ C) to tackle the bacterial spores, which are extremely resistant to heat and chemicals and are of concern to the food industry (Bai et al. [2020\)](#page-12-0). The decimal reduction time (D value) to kill  $90\%$  (1 log) *Bacillus cereus* spores was decreased from 35 to 20 min when the mild heat  $(55 \degree C)$  was added to PAW.

<span id="page-3-0"></span>

**Table 14.1** Cold plasma-based burdle rechnologies for microbial decontamination  $\pi$  is o et al. 2020c) Table 14.1 Cold plasma-based hurdle technologies for microbial decontamination (Liao et al. [2020c](#page-14-0))







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## 14.2.2 Cold Plasma and Organic Acids

Among the natural preservatives, organic acids and their salts are important components, including ascorbic acid, citric acid, propionic acid, lactic acid, and their salts, etc. (Hugo and Hugo [2015\)](#page-14-0). Generally, the acidity of an organic acid mainly depends on the conjugate base of the molecule. Organic acids can be divided into monocarboxylic acids (e.g., formic, acetic, propionic, sorbic acid), dicarboxylic acids (e.g., adipic, fumaric, succinic acid), alpha hydroxyl acids (e.g., citric, lactic, malic acid) and sugar acids (e.g., ascorbic, gluconic, lactobionic, tartaric acid) (Theron and Lues [2011\)](#page-15-0). The most commonly used organic acids in foods include acetic, benzoic, citric, formic, lactic, and propionic acids (Lianou et al. [2012\)](#page-14-0). Organic acids can be used alone or in combination during food preservation (Huang and Chen [2011;](#page-14-0) Ricke [2003\)](#page-14-0). The antimicrobial efficacy of weak organic acids is affected by the dissociated extent of the acids (Lianou et al. [2012\)](#page-14-0). Bacterial inactivation caused by organic acids is mainly attributed to the lipophilic undissociated acid molecules, which can penetrate through the cytoplasmic membrane and dissociate inside the bacterial cell. Generally, organic acids exhibit higher antimicrobial activity in the undissociated forms than that in their dissociated forms. After entering into the bacterial cell, the dissociation of acid molecules occurs to lead to the decrease in the intracellular pH, which subsequently disrupts the membrane proton-motive force as the accumulation of charged ions within the bacterial cells (Brul and Coote [1999\)](#page-13-0). The combined effects of damages in membrane integrity, reduction in intracellular pH and compromise in essential metabolism contribute to the microbial inactivation by organic acids. The combination of organic acids and CP has been proposed by several studies in recent years. The alliance of CP combined with sodium dodecyl sulfate (SDS) and lactic acid (LA) has been employed for the inactivation of Listeria monocytogenes and verotoxigenic E. coli (VTEC) by Trevisani et al.  $(2017)$  and the completed inactivation was achieved. In the study of Chaplot et al. [\(2019](#page-13-0)), CP combined with peracetic acid was found to result in 3.8–5.3 log reduction of Salmonella in raw poultry, while individual peracetic acid exposure led to 0.6–1.3 log reduction levels. In addition, Vilchèze et al. ([2013\)](#page-15-0) proposed the combined treatment of ascorbate (5 mM vitamin C), a common food additive, with CP for enhancing the reduction level of pathogenic bacteria, including E. coli, Pseudomonas aeruginosa, Staphylococcus epidermidis and Bacillus subtilis. The authors hypothesized that the addition of reactive radicals by CP could further help enhance the antimicrobial efficacy of ascorbate.

## 14.2.3 Cold Plasma and Essential Oils

Essential oils (EOs) are secondary metabolites of plant origin and are aromatic substances with volatile oily liquids extracted from different tissue parts such as

leaves, flowers, roots, stems, or fruits of plants (Burt [2004](#page-13-0)). In 1881, De la Croix first reported the antimicrobial efficacy of EO vapors (Boyle [1955](#page-13-0)). The compositions of essential oils is very complicated, usually mainly composed of over 300 different compounds, such as monoterpenes, sesquiterpenes, lipids, aldehydes, ketones, alco-hols, and other components (Calo et al. [2015\)](#page-13-0). Multiple mechanisms have been proposed to be involved in antimicrobial mode of EOs, including the damages in cell structure, disturbance of intracellular enzymatic system and metabolism pathways, and the disruption of intracellular nucleic acids. The hydrophobic nature renders EOs with the ability to move easily across the lipid bilayer of the cell membranes and disrupt the integrity of cell wall structures (Cosentino et al. [1999](#page-13-0)). The subsequent impermeability in cell membrane results in the leakage of intracellular components (e.g., proteins), which brings out the final cell death (Dorman and Deans [2000\)](#page-13-0). In general, EOs are more effective for the inactivation of Gram-positive bacteria than Gram-negative bacteria. It is demonstrated that the presence of lipopolysaccharides (LPS), linking the outer and inner membranes in Gram-negative bacteria is the major contributor to the resistance toward EOs. However, the flavor of EOs might change the food taste and compromise the consumers' acceptance (Echegoyen and Nerín [2015\)](#page-13-0). In addition, some constitutes in EOs (e.g., trans-cinnamaldehyde), also cause the damages in metabolism and result in the bactericidal effect (Shen et al. [2015\)](#page-15-0). The alliance of CP and EOs can reduce the use of EOs in high concentrations and achieve the efficient antimicrobial efficacy. The low concentration EOs (lime oil, sweet basil oil, and clove oil) combined with CP has been used for the inactivation of E. coli, Salmonella Typhimurium, and S. aureus on chicken eggs (Matan et al. [2014\)](#page-14-0). It is speculated that the CP treatment could form functional groups of EOs, which enhanced the antimicrobial efficacy. In the study of Cui et al. [\(2016b](#page-13-0)), it is found that the combination of CP and clove oil showed a synergistic effect on inactivation of E. coli O157:H7 biofilms on fresh lettuce with a reduction level of 5.48 logs. The individual CP and clove oil treatments brought out 0.81- and 2.2-log reduction, respectively. For another study from the same research group, the combination of CP and lemongrass oil was used for removing L. monocytogenes in poultry loin. The individual nitrogen CP (500 W, 120 s) or lemongrass oil (5 mg/mL, 30 min) resulted in less than 1 log reduction of  $L$ . *monocytogenes*, while the combined treatment achieved 2.80 logs inactivated level (Cui et al. [2017\)](#page-13-0).

#### 14.2.4 Cold Plasma and Bacteriophage

The discovery of bacteriophages (phages) and its application in food have enriched the types of bacteriostatic agents and provided a safe, efficient, and highly targeted biological bacteriostatic agent for food safety (Bigwood et al. [2008;](#page-13-0) Modi et al. [2001\)](#page-14-0). Bacteriophages are the most abundant organisms found in nature with an estimate of more than  $10^{31}$ , in which lytic phage can lyse and kill host bacteria during reproduction (Fig. [14.2](#page-9-0)). Bacteriophages have a high specificity to a certain

<span id="page-9-0"></span>

Fig. 14.2 The lytic cycle of bacteriophage

bacterial species through recognizing and attaching the specific receptors on the bacterial surface. In addition, bacteriophage enjoys the advantages of low inherent toxicity, harmlessness to humans as well as easy isolation. To date, various bacteriophages have been applied on a variety of foods to assure the microbiological safety (Abuladze et al. [2008](#page-12-0); Bandara et al. [2012](#page-12-0); Goode et al. [2003;](#page-13-0) Soffer et al. [2017\)](#page-15-0). BCP1-1 phage was used to decrease Bacillus cereus counts in fermented soya bean paste (Bandara et al. [2012\)](#page-12-0). Goode et al. ([2003\)](#page-13-0) reported the application of Campylobacter jejuni typing page 12673, P22, 29C, and Salmonella typing phage 12 for the decontamination of chicken. EcoShield™ (formerly ECP-100), a phage cocktail, was applied for controlling  $E$ . *coli* 0157:H7 on tomatoes, broccoli, and spinach (Abuladze et al. [2008](#page-12-0)). In 2006, the U.S. Food and Drug Administration approved the use of a bacteriophage mixture (ListShield™) as a food additive for the control of L. *monocytogenes* in meat and poultry products (Lang [2006\)](#page-14-0). Subsequently, Salmonella (SalmoFresh™), and E. coli O157:H7 (Ecoshield™) received the Generally Recognized As Safe (GRAS) recognition from the FDA for the direct application onto foods (Sukumaran et al. [2015\)](#page-15-0). However, the host specificity of bacteriophage limits its application. In the study of Cui et al. [\(2018](#page-13-0)), the sequential treatment of bacteriophages and cold nitrogen plasma was employed as a hurdle technology to remove the E. coli O157:H7 biofilms on vegetables. It is found that the hurdle treatment could achieve over 5-log biofilm reduction, while the individual treatment resulted in a reduction of around 2 logs. The CP treatment could assist the disruption of biofilm matrix, which made it easier for the penetration of bacteriophages to achieve efficient inactivation.

## 14.2.5 Cold Plasma and Ultrasound

Ultrasound is a sound wave that is inaudible to the human ears and has a frequency of higher than 20 kHz (Piyasena et al. [2003](#page-14-0)). The area of space where the ultrasound waves propagate is called the ultrasonic field (Piyasena et al. [2003\)](#page-14-0). At present, it is generally believed that the cavitation effect generated in the medium is the major contributor to the microbial inactivation by ultrasound. CP in combination with ultrasound has been proposed by Chen et al. ([2009\)](#page-13-0), who developed an ultrasoundassisted plasma simultaneous treatment system. It is found that the combination treatment could achieve over 5-log reduction of E. coli and Saccharomyces cerevisiae, while individual exposure of ultrasound or CP resulted in a reduction of approximate 2 logs. Discharge of the gas inside the bubbles produces abundant reactive species and once the bubbles explode, the reactive species are subsequently released into the medium and react with microbial cells. The sequential treatment of CP and ultrasound was conducted by our group to inactivate pathogenic S. aureus (Liao et al. [2018b](#page-14-0)). It is found that the combined treatment resulted in over 6 logs reduction of S. aureus, while it was 2.55- and 0.55-log reduction for individual CP and ultrasound treatments, respectively. The hurdle treatment of air CP and ultra-sound was also used to inactivate L. monocytogenes (Pan et al. [2020\)](#page-14-0). The study demonstrated that the ultrasound helped weaken the cell membrane, and the CP posed oxidative stress on bacterial cells, which resulted in synergism on the inactivation of L. monocytogenes.

#### 14.2.6 Cold Plasma and Other Techniques

In addition to the aforementioned techniques, other decontamination methods, such as antimicrobial nanomaterials, could be used to be combined with CP to develop novel hurdle technologies. Nanomaterials refer to the materials in which at least one dimension in three dimensions is in the order of nanometers (approximately 1–100 nm) (Duncan [2011\)](#page-13-0). The size of nanomaterials is extremely small, and the structure is also very special, so it will show many special properties and functions different from traditional materials in chemical, mechanical, electrical, optical, and biological aspects. The gold nanoparticles (AuNP) combined with CP has been confirmed to cause synergistic effect on cancer cell death (He et al. [2018](#page-13-0)). It is hypothesized that the pretreatment of CP could help the penetration of nanomaterials into cells to react with the intracellular components, enhancing cell inactivation efficacy. Therefore, the alliance of nanomaterials and CP can be considered for the hurdle development for microbial decontamination.

## 14.3 The Factors Affecting the Hurdle Efficiency

## 14.3.1 The Treatment Sequence

The sequence of the successive hurdle treatment has been associated with the antimicrobial performance. Chaplot et al. [\(2019](#page-13-0)) compared the efficacy of various order of CP and peracetic acid exposure on S. Typhimurium. It is found that the pretreatment of CP could result in higher reduction than the pretreatment of peracetic acid did. In addition, the exposure of CP-ultrasound treatment could bring out stronger antimicrobial effect on S. aureus than the ultrasound-CP treatment did (Liao et al. [2018b](#page-14-0)). It is hypothesized that the pretreatment of CP produced abundant reactive species in the medium, which were further injected into bacterial cells with the assistance of ultrasonic microjet effect.

## 14.3.2 Equipment Configuration

The equipment configuration for the cold plasma-based hurdle treatment is related to the antimicrobial efficacy and should be carefully considered when developing the hurdle technologies. In the study of Chen et al. [\(2009](#page-13-0)), the effect of various reactor types of the ultrasound-assisted plasma system on the inactivation efficiency of E. coli was evaluated. It is found that the hybrid mode resulted in higher reduction than the submerged mode did, which might be attributed to the higher productive capacity of reactive species (e.g., singlet oxygen, hydrogen peroxide, ozone) and UV photos when the plasma produced upon the water.

#### 14.3.3 The Microbial Properties

The antimicrobial efficacy of cold plasma-based hurdle also depends on the characteristics of the target microorganisms. Pan et al. [\(2020](#page-14-0)) found that the inactivation effect of combining ultrasound and CP was compromised when targeting on L. monocytogenes with lower growth temperature (10  $^{\circ}$ C). This study further revealed that the anteiso-C15:0 and branched chain fatty acids (BCFAs) showed more abundant under the low growth temperature, resulting in higher cell membrane fluidity and higher resistance toward the hurdle exposure.

## <span id="page-12-0"></span>14.4 The Effect of Cold Plasma-Based Hurdles on Food **Quality**

The effect on food quality by cold plasma-based hurdle technologies should be carefully evaluated during the development and optimization of the novel hurdle technologies. In the study of Cui et al. ([2016b\)](#page-13-0), it is found that the combination treatment of clove oil and CP resulted in a slight loss of greenness of the fresh lettuce compared with the individual clove oil or CP exposure. In addition, it is also reported that the alliance of CP and peracetic acid caused a compromise in the redness of poultry meat (Chaplot et al. [2019](#page-13-0)), while no reduction in the moisture content. It might be explained by the reaction of the oxidative agents and the myoglobin. Some studies demonstrated that there was no significant change in food quality when applied with the cold plasma-based hurdles. In the study of Cui et al. [\(2017](#page-13-0)), it is found that the values of  $L^*, a^*, b^*$  for color characteristics exhibited no significant different among pork loins treated by the combination exposure of CP and lemongrass oil or the individual treatments. Various process parameters might contribute to the different results from various studies. Therefore, the optimization of process parameters should be carefully conducted to maximumly retain food quality.

## 14.5 Conclusions

In recent years, the hurdle technologies combining cold plasma and other techniques have been proposed and developed for efficient decontamination of food and retain the food quality. So far, the data about the cold plasma-based hurdle is still limited and required more investigation for seeking other potential techniques allied with CP. In addition, the design of the hurdle treatments and their optimization should be further conducted for minimizing the compromise in the food quality attributes as much as possible.

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