Chapter 11
Application of Cold Plasma in Liquid Food Products

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Abstract Cold plasma has garnered much attention in the last decade as food processing technology with potential in food preservation and managing the safety of food with minimum influence on quality characteristics. The action of cold plasma on pathogens is multi-targets, mostly including the cellular envelopes, cell membrane, lipids, proteins, and DNA via interaction with the reactive species in cold plasma and liquid medium. The multi-target high germicidal action of cold plasma on important food pathogens will undoubtedly play a vital role in the smooth adoption of this technology in fruit and vegetable juice and milk processing industries. Also, pH decline increased lipid oxidation, and changes in color, antioxidant activity, phenolic, and flavonoid concentrations were observed after cold plasma treatments. Therefore, intensified efforts are needed to address some of the quality issues relating to pH decline and heightened lipid oxidation in oily liquid food products, as this has a considerable influence on safety and consumer perception.

Keywords Nonthermal plasma · Plasma-treated liquid food · Plasma-activated water chemistry · Microbial inactivation · Quality attributes

11.1 Introduction

Food spoilage and poisoning from pathogens have become a topic of concern to food processors and researchers. If these pathogens are not controlled, they can contaminate food products and pose a significant threat to human health as a result of food-borne illnesses. The estimation of foodborne illnesses can be utilized to create food safety policies and strategies that can drastically reduce the episodes of food-borne outbreaks. The World Health Organization data of 2010 showed food-borne diseases have led to about 600 million illnesses and 420,000 premature deaths in most of the

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low- and middle-income countries (Sub-Saharan Africa, South Asia, and Southeast Asia). These translated to an estimated cost of $95.2 billion per annum, with an annual food-borne illness treatment cost of around $15 billion (World-Bank 2018). Likewise, the European Union reported about 88 million tons of food wastage every year estimated at a loss of $177.12 (€143) billion in value (Scherhaufer et al. 2018; Stenmarck et al. 2016). The economic burden of food-borne illnesses in the United States is over $15.5 billion, with about 9.4 million illnesses annually. The United State Center for Disease Control (CDC) food-borne estimates in 2011 showed that about 40 million people have become sick due to food-borne outbreak, among which 128,000 cases were hospitalized, and 3000 death were recorded (CDC 2018; Hoffmann et al. 2015).

Numerous pathogens have been associated with food-borne diseases and food spoilage, and these include *Salmonella typhi* that causes typhoid, *Vibrio cholera* that results in cholera, and *Escherichia coli* O157:H7 which causes illnesses including hemolytic uremic syndrome, characterized by renal failure and hemolytic anemia which can lead to permanent kidney dysfunction. The spores of *Bacillus* species were also associated with many food spoilage and poisoning (Lin et al. 2005; Malik et al. 2014). These pathogens and many others must be contained to prevent cross-contamination along the food value chain as well as during handling operations in the food industry. Therefore, the need for an effective processing strategy capable of decontaminating or killing of food pathogens is one of the most critical steps for the hazard analysis and critical control point (HACCP) system in the food industry.

In this regard, the importance of cold plasma in ensuring food safety has been studies on many food products. The cold plasma produced a variety of reactive species that come in contact with food constituents and microorganisms, thus resulting in many reactions, which leads to microbial inactivation. Some chapters in this book have discussed some of the reactions involved in the antimicrobial efficacy of cold plasma. Cold plasma has been applied in various fruit and vegetable beverages (Fig. 11.1). This chapter will focus on the interactions of cold plasma reactive species with liquid foods and its antimicrobial effects from a food safety perspective.

11.2 Cold Plasma at Gas-Liquid Interphases

In order to fully understand the role of cold plasma in ensuring the safety of food products, one needs to understand its behavior in air and liquid phases. Cold plasma generated at gas-liquid interphase produces reactive species such as hydrogen peroxide (H_2O_2), hydroxyl radical (OH^-), nitric acid (HNO_3), and ozone (O_3) that have high antimicrobial efficacies (Liao et al. 2018b; Muhammad et al. 2018c). Liu et al. (2015) demonstrated how fast plasma species could be transferred from the point of generation to the air region. At the air gap region, reactive oxygen species (ROS) such as O_3 and H_2O_2 can diffuse to the downstream liquid surface over a distance of 0.01 m within 1 s of discharge. However, the concentration of H_2O_2

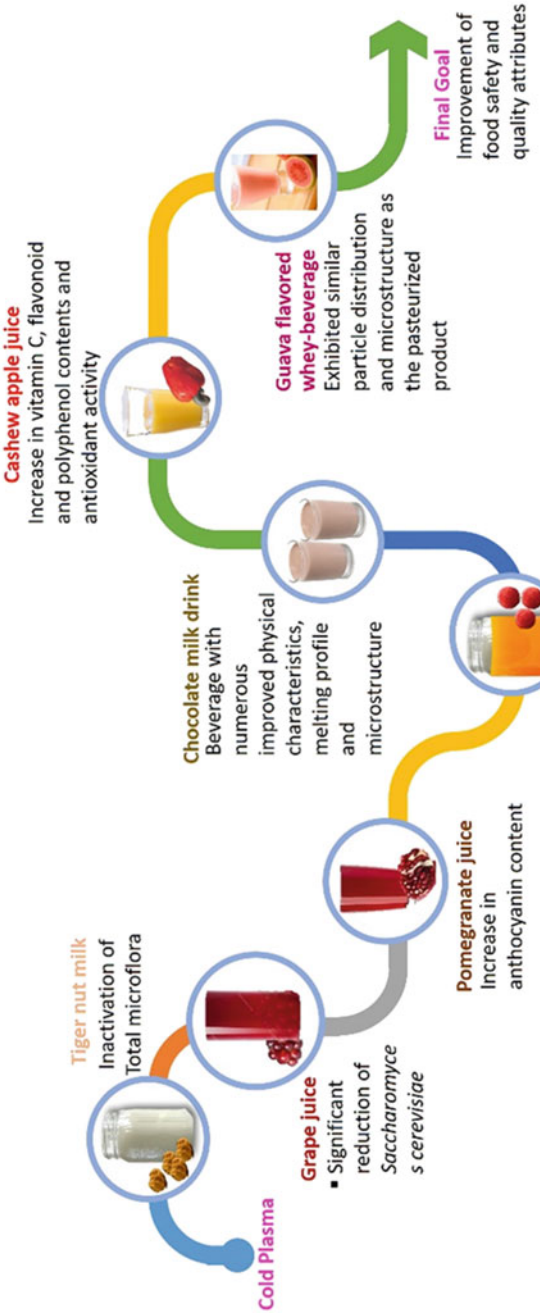

Fig. 11.1 Cold plasma application to some liquid food products

declined due to larger Henry's coefficient, whereas the concentration of the O_3 remained similar to that at the exit of the plasma generation region. H_2O_2 is highly soluble in water compared to O_3 and $OH^{\cdot}$, and has a longer lifespan, thus making it an important ROS in microorganisms inactivation (Liao et al. 2018b; Liu et al. 2015). To further explain the diffusion of reactive species in air and water, Shimizu et al. (2011) studied the thermal flow pattern and chemical components transport using the platinum electrode under a water surface dielectric barrier discharge plasma system. The researchers found that the diffusion of reactive species towards the water surface was influenced by the airflow rate visibly demonstrated by the circular pattern. The plasma flume interaction with water resulted in pH reduction, thus affirming the dissolution of reactive species, including O_3, H_2O_2, and nitrous acid (HNO_2). These reactive species are distributed within the water molecules via convective transport, as explained by the discoloration of methyl red solution as the flow pattern progressed. This phenomenon highlighted how microorganisms in liquid could be inactivated when in contact with plasma-activated liquids. It is worth noting that only long-live plasma reactive species such as atomic oxygen (O), O_3, H_2O_2, or nitric oxide (NO) can diffuse through the liquid medium and interact with microorganisms (Zuizina et al. 2012). Other reactive species of antimicrobial importance that are found in plasma-treated water include $OH^{\cdot}$, peroxynitrite ($ONOO^{-}$), nitrite (NO_2^{-}), and nitrate (NO_3^{-}) (Julák et al. 2018; Muhammad et al. 2019a). Their generation and importance with regard to food safety will be discussed in subsequent sections.

11.2.1 Understanding the Interaction of Cold Plasma Reactive Species with Water Molecules Using Atmospheric Air as Inducer Gas

Water subjected to cold plasma treatment at predefined condition is termed plasma-activated water (PAW) (Liao et al. 2020). Reactive oxygen and nitrogen species (RONS) are generated in PAW by several chemical reactions involving the plasma flume and the water molecules with atmospheric air as working gas. In cold plasma generation, atomic oxygen (O), nitrogen (N_2), and $OH^{\cdot}$ are excited at the gaseous plasma interphase and are transported into the water and converted to other RONS, including H_2O_2, NO_2^{-}, and NO_3^{-} at the liquid interphase (Lukes et al. 2014). The attack of high-energy electrons (e^{-}) creates the reactive species in plasma discharge and combine with water molecules to produce many aqueous reactive species and radicals. The acidic RONS among them such as NO_2^{-} and NO_3^{-} increase the water acidity, and consequently enhanced the bactericidal efficacy of the water (Cao et al. 2018; Shen et al. 2016). Some of the reactions involved in the formation of reactive species are summarized below. When the e^{-} energy is higher than that of the ionization energy of water molecules, the water molecules dissociate as a result of

electron collision, thereby forming hydrogen radicals ($H^{\cdot}$), $OH^{\cdot}$, and other cations, as shown in reactions (11.1), (11.11), and (11.12) (Cao et al. 2018; Zhou et al. 2018).

$$e^- + H_2O \rightarrow OH^{\cdot} + H^{\cdot} + e^- \tag{11.1}$$

According to Joshi et al. (1995), Laroussi and Leipold (2004), and Cao et al. (2018), the radicals can react with each other and produce other reactive species as illustrated in reactions (11.2)–(11.14).

$$N_2 + e^- \rightarrow 2N + e^- \tag{11.2}$$

$$O_2 + e^- \rightarrow 2O + e^- \tag{11.3}$$

$$N + O \rightarrow NO \tag{11.4}$$

$$NO + O \rightarrow NO_2 \tag{11.5}$$

$$O + O_2 \rightarrow O_3 \tag{11.6}$$

$$NO + O_3 \rightarrow NO_2 + O_2 \tag{11.7}$$

$$O + H_2O \rightarrow 2OH^{\cdot} \tag{11.8}$$

$$2OH^{\cdot} \rightarrow H_2O_2 \tag{11.9}$$

$$e^- + H_2O_2 \rightarrow OH^- + OH^{\cdot} \tag{11.10}$$

$$H_2O + e^- \rightarrow H_2O^+ + 2e^- \tag{11.11}$$

$$H_2O^+ + H_2O \rightarrow OH^{\cdot} + H_3O^+ \tag{11.12}$$

$$N_2O + e^- \rightarrow H_2O^+ + 2e^- \tag{11.13}$$

$$N_2{}^+ + H_2O \rightarrow NO + H^{\cdot} \tag{11.14}$$

$OH^{\cdot}$ is among the crucial species in cold plasma discharges and is formed mainly as a result of electron striking water molecules with a short lifetime of few nanoseconds. Nevertheless, $OH^{\cdot}$ can recombine to give a dimer H_2O_2 as in reaction (11.9) (Cao et al. 2018). Meanwhile, O_3 is another essential ROS with strong oxidizing power similar to that of O and is produced from the reaction of O and O_2 is demonstrated in reaction (11.6) (Muhammad et al. 2019b; Surowsky et al. 2014b). The solubility of H_2O_2 is higher in water compared to O_3 and $OH^{\cdot}$ and has a longer lifespan for microorganisms inactivation (Liao et al. 2018a; Liu et al. 2015). In addition to the above-mentioned RONS, $ONOO^-$ is another RONS of antimicrobial importance. Its instability has rendered this compound to have a less biological effect (Julák et al. 2018). In contrast to this, Machala et al. (2013) established that $ONOO^-$ is produced in plasma-treated liquids and correlated its antimicrobial efficacy with the amount that is produced. Although few researchers have reported its biological activity against some important food pathogens, its instability and production level is too low to be regarded as a key antibacterial agent alone and therefore is less

explored during PAW studies. Notwithstanding, the contribution of $ONOO^-$ in PAW is the decomposition of the solution into highly reactive $OH^{\cdot}$ and nitroxyl ($NO_2^{\cdot}$) (Slosky and Vanderah 2015). Shen et al. (2016) also reported $ONOO^-$ contribution during the cell death of *Staphylococcus aureus* by interacting with H^+, NO_2^-, and H_2O_2 as indicated in reaction (11.15). On the other hand, studies have demonstrated that *Escherichia coli* cytochrome bd is resistant to damages caused by $ONOO^-$. This rapidly triggers the degradation of $ONOO^-$ to NO_2 thus, suggesting that $ONOO^-$ was more of a catalyst for the formation of more reactive NO_2 (Borisov et al. 2015; Dezest et al. 2017).

$$H_2O_2 + NO_2^- + H^+ \rightarrow ONOOH + H_2O \tag{11.15}$$

11.3 Antimicrobial Mechanisms of Cold Plasma in Liquid Media

The mechanism of inactivation varies depending on the microorganisms (vegetative cells and spores). Bacterial spores are generally more resistant to cold plasma treatment than vegetative bacteria. Also, Gram-positive bacteria are more resistant to cold plasma treatment than Gram-negative bacteria. This is because they possess thicker peptidoglycan that allows them to resist external stress, thus block the influx of cold plasm reactive species through the cellular envelopes. Peptidoglycan is an essential biomolecule in the cell wall that increases bacterial rigidity and survival instinct (Liao et al. 2018b; López et al. 2019). The roles of reactive species in cold plasma for microbial inactivation are in different pathways, as showcased in Fig. 11.2. There are the physical means via the generation of electric fields, and

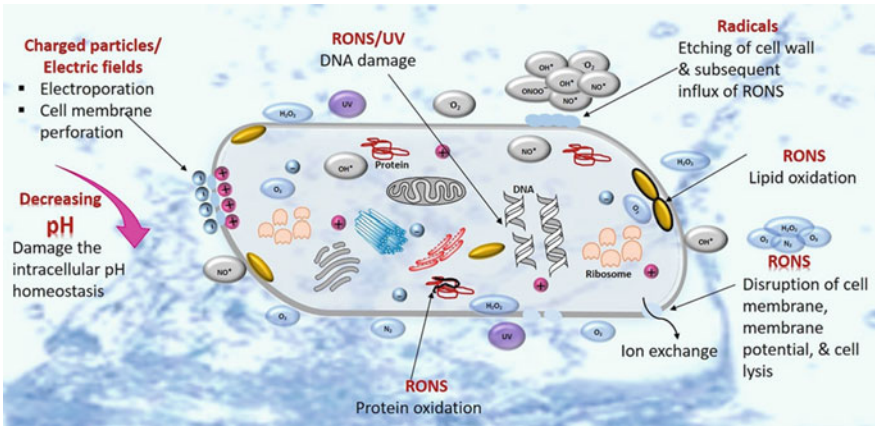

Fig. 11.2 Mode of action of cold plasma reactive species during microbial inactivation

ultraviolet (UV) radiation, and chemical means that are as a result of low pH and the action of RONS and their interaction with water.

Microorganisms exposed to cold plasma experienced continuous bombardment, shrinkage, and etching by charged particles due to electrostatic field at the cell membrane (Lunov et al. 2016; Schlüter and Fröhling 2014). Some microorganisms change shape from rod-shape to round shape to minimize the effect of electrostatic forces from the charged particles. When this effect becomes too high and unbearable, the integrity of the cell membrane is compromised, thereby allowing the inflow of RONS (Dezest et al. 2017; Fröhling and Schlüter 2015; Ha et al. 2016; Joshi et al. 2011). Electric fields generated in cold plasma have lethal effects on microorganisms such as electroporation (Lukes et al. 2012). This phenomenon rendered the cell membrane permeable and encouraged the influx of RONS into the cell. Cell shrinkage and damages of the cell membrane with loss of cytoplasmic membrane integrity are other pathways in which microorganisms are inactivated (López et al. 2019). The role of UV in microbial inactivation is to prevent bacterial replication via dimerization of thymine bases in DNA strands (Laroussi and Leipold 2004). The interaction of UV with genetic constituents, including DNA, increases with the continuous etching of the cell membrane (Moisan et al. 2002).

The low acidic pH has a synergistic antibacterial effect with RONS in PAW. Lower acidic environmental is more favorable for the reactive species to penetrate cell walls, and the presence of reactive species in such acidic conditions reduces the resistance of bacteria. Furthermore, the H^+ in PAW flow into the intracellular of the bacterial cell, thereby disrupting the intracellular pH homeostasis, eventually lead to cell death (Xu et al. 2019, 2020; Zhang et al. 2016).

On the other hand, RONS as the most active components of cold plasma have caused the oxidation and degradation of cell biomolecules (Laroussi and Leipold 2004). RONS such as $OH^{\cdot}$, $NO^{\cdot}$, excited N_2 and O_2, O_3, and H_2O_2 induced etching and erosion of cell membrane, lesion, and subsequent reaction with cell macromolecules (Park et al. 2015). Membrane lipid, especially polyunsaturated fatty acid, is one of the first to be attacked by RONS because it is located near the cell surface and has a high affinity to RONS. The peroxidation of lipid by O_2 oxidation leads to lipid hydroperoxide that resulted in the production of Malondialdehyde (MDA) as a secondary product. MDA is used as the biomarker for oxidative stress. The formation of MDA in *E. coli* submitted to plasma increased with exposure time and can react with protein and DNA, thereby causing irreversible damage and eventual cell death (Alkawareek et al. 2014; Joshi et al. 2011; Liao et al. 2017).

Protein oxidation is another path in which microbial cells are targeted and can occur in different processes such as direct amino acid oxidation by H_2O_2 and recombination of lipid peroxidation by-products during the oxidation of cell membranes (Weber et al. 2015). Protein carbonylation is an irreversible change in the microbial cell, and this type of oxidation was reported in *E. coli* due to the interaction with H_2O_2 (Dezest et al. 2017).

11.4 Cold Plasma Processing of Fruit and Vegetable Juices

11.4.1 Effects on Microorganisms Inactivation

The safety of fruit and vegetable juices is critical in controlling food-borne outbreaks. The selection of appropriate processing techniques that will minimize microbial spoilages is essential. Generally, the presence of spoilage microorganisms in the range greater than 4 log CFU/mL in food products is responsible for their spoilage. Therefore, the choice of biological decontamination strategy that will drastically reduce the population of the microorganisms and minimize quality changes is needed. Thermal pasteurization of fruit and vegetable juices has been utilized for decades in the fruit juices preservation and extension of shelf life. However, this is accompanied by some quality changes that impaired the nutritional and organoleptic properties of the juices (Petruzzi et al. 2017). Fruit and vegetable juices are healthy, functional food products with several health benefits and disease prevention properties (Muhammad et al. 2018b). Cold plasma, due to its low temperature and high bactericidal efficacy, has been utilized for the treatment of thermolabile fruit juices to ensure their microbial safety, functionality, and nutritional attributes are preserved. Table 11.1 summarized the recent cold plasma inactivation of food pathogens in fruit and vegetable juices. Hosseini et al. (2020) reported 6 log CFU/mL inactivation of *Escherichia coli* in sour cherry juice after 9 min of cold plasma treatment. Apple juice exposed to cold plasma treatment for 30 min resulted in a 5.6 log CFU/mL reduction in *Zygosaccharomyces rouxii*. The scanning electron microscopy (SEM) images of the plasma-treated yeast cells showed some pores formation and contraction, with visible damages to the cell wall (Wang et al. 2020). The inactivation of a similar microorganism in apple juice resulted in about 5 log CFU/mL reduction after 90 W and 140 s of plasma treatment (Xiang et al. 2018). Another cold plasma treatment of apple juice by Liao et al. (2018a) showed 3.98–4.3 log CFU/mL inactivation of *E. coli* in less than 40 s at input powers ranging from 30 to 50 W. The researcher attributed the high microbial inactivation to the accumulation of H_2O_2, O_3, and NO_3^- in the treated apple juice. Similarly, Surowsky et al. (2014a) obtained 5 log CFU/mL reduction of *Citrobacter freundii* in apple juice subjected to cold plasma treatment for 480 s after 24 h of storage. The treated cells showed severe deformation with rough surfaces, discrete ridges, and pores. Dasan and Boyaci (2018) compared the inactivation efficacy of cold plasma on *E. coli* in different fruit juices. The results revealed inactivation efficacies of 1.59, 1.43, 4.02, and 3.34 log CFU/mL reductions in orange, tomato, apple, and sour cherry juices, respectively. The study further linked the lower inactivation obtained in orange and tomato juices to different food matrices. Clear juices such as apple juice result in more log reduction than cloudy juices or juice with high suspended solids such as tomato juice. DBD plasma used in the inactivation of *Staphylococcus aureus*, *E. coli*, and *C. albicans* in orange juice showed more than 5 log CFU/mL after 25 s of treatment. The researchers explained that the reduction rate in the viable counts were higher in *E. coli*, then followed by *S. aureus*, and

Table 11.1 Summary of cold plasma inactivation of microorganisms in fruit and vegetable juices

Type of cold plasma device	Processing conditions	Liquid food products	Test strain	Inactivation results	Quality changes	References
Dielectric barrier discharge (DBD) plasma	Inducer gas: air Voltage: 60 kV Time: 10 and 15 min Discharge gap: 30 mm	Strawberry juice	Total aerobic bacteria	• About 1 log CFU/mL reduction after plasma treatment	• Increase in total phenolic content • Reduction in vitamin C content	Mehta and Yadav (2020)
Cold plasma	Inducer gas: air Voltage: 0–30 kV Time: 0–3 min Discharge gap: 1 cm	Tangerine juice	*Escherichia coli* ATCC 700891	• *E. coli* was reduced by 4.8 log CFU/mL after 2 min of plasma exposure	• Marked changes in phenol content, total acidity, and ascorbic acid were observed	Yannam et al. (2018)
DBD plasma	Inducer gas: air Input power: 90 W Time: 0–140 s	Apple juice	*Zygosaccharomyces rouxii*	• 5 log CFU/mL reduction after 140 s treatment	• Drastic changes in pH, color values, and acidity • Insignificant changes in phenolic contents and reducing sugar	Xiang et al. (2018)
Atmospheric pressure cold plasma (APCP)	Inducer gas: Ar and O_2 Time: 1, 5, and 9 min Discharge gap: 2 cm Flow rate: 5 L/min	Sour cherry juice	*E. coli*	• 6 log CFU/mL in after exposure to 9 min plasma treatment	• Reduction in total anthocyanin content by 4% • 21% reduction in vitamin C	Hosseini et al. (2020)
Cold plasma jet	Inducer gas: O_2 Voltage: 11 kV Time: 2, 4, and	Blueberry juice	*Bacillus* sp.	• 7.2 log CFU/mL reduction after 6 min treatment at 1% O_2	• Significant increase in phenolic contents • Decrease in antioxidant	Hou et al. (2019)

(continued)

Table 11.1 (continued)

Type of cold plasma device	Processing conditions	Liquid food products	Test strain	Inactivation results	Quality changes	References
	6 min Discharge gap: 2 cm Flow rate: 1 L/min				activity • Decrease in vitamin C content	
Cold plasma	Inducer gas: air Voltage: 15, 18, and 21 kV Time: 0–30 min Flow rate: 150 L/h	Apple juice	*Z. rouxii*	• A 5.6 log CFU/mL reduction after 30 min plasma treatment was recorded	• Slight increase in pH • Insignificant changes in soluble solids, titration acidity, and reducing sugar • No significant changes in volatile compounds	Wang et al. (2020)
DBD plasma	Inducer gas: air Input power: 30, 40, and 50 W Time: 0–40 s Discharge gap: 2 mm	Apple juice	*E. coli*	• 3.98–4.3 log CFU/mL reduction in less than 40 s	• Slight changes in pH, titratable acidity, color, total phenolic content, and antioxidant capacity	Liao et al. (2018a, b)
Cold plasma gliding arc discharge	Inducer gas: nitrogen Voltage: 3.8 kV Input power: 40 W Time: 30–300 s Flow rate: 440 L/h Discharge gap: 1 cm	Tomato juice	Total aerobic mesophilic bacteria, yeast and mold, *Candida albicans*, and *Saccharomyces cerevisiae*	• 3.45 log CFU/mL reduction for the mesophilic bacteria after 5 min • 3.55 log CFU/mL reduction in yeast count after 5 min plasma exposure • 3.32 log CFU/mL reduction for molds after 5 min treatment	• Decline in pH • 11% increase in lycopene content following 2 min of treatment • 13% increase in carotenoid content after 2 min treatment • 5% loss in vitamin C contents after 5 min of exposure to plasma	Starek et al. (2019)

				• 2.93 log CFU/mL reduction in *C. albicans* after 5 min treatment • 3.69 log CFU/mL reduction in *S. cerevisiae* after 5 min treatment		
High-voltage atmospheric cold plasma (HVACP)	Inducer gas: air and 65% O_2 + 30% N_2 + 5% CO_2 (MA65) gas Input power: 90 kV Time: 30, 60, and 120 s Discharge gap: 4.44 cm	In-package orange juice	*Salmonella enterica* serovar Typhimurium	• A reduction of >5 log CFU/mL after 30 s treatment with both air and MA65 gas and no storage • 2.9 and 4.7 log CFU/mL reduction in air and M65 gas, respectively, after 120 s treatment and 24 h storage	• Insignificant changes in pH and °Brix after 120 s treatment • A 22% reduction in vitamin C in air	Xu et al. (2017)
Atmospheric pressure plasma jet	Inducer gas: air Input power: 650 W Time: 30, 60, 90, and 120 s Discharge gap: 3.5 cm Flow rate: 3000 L/h	Orange, tomato, apple juices, and sour cherry nectar	*E. coli*	• 1.59 log CFU/mL reduction in orange juice • 1.43 log CFU/mL reduction in tomato juice • 4.02 log CFU/mL reduction in apple juice • 3.34 log CFU/mL reduction in sour cherry	• Visible color changes in all the juices • Slight change in pH • 10–15% increases in phenolic contents	Dasan and Boyaci (2018)
HVACP	Inducer gas: air Voltage: 80 kV Time: 1, 2, 3, and 4 min	White grape juice	*S. cerevisiae*	• 7.4 log CFU/mL reduction after 4 min of treatment	• Slight change in pH and acidity • Decrease in total phenolic and flavonoid contents • Decrease in scavenging and antioxidant capacity	Pankaj et al. (2017)

(continued)

Table 11.1 (continued)

Type of cold plasma device	Processing conditions	Liquid food products	Test strain	Inactivation results	Quality changes	References
Cold atmospheric plasma jet	Inducer gas: Ar, Ar + O_2 Voltage: 65 V Time: 0–480 s Discharge gap: 10 mm Flow rate: 5 L/min	Apple juice	*Citrobacter freundii*	• 5 log CFU/mL reduction of plasma treatment of 480 s and 24 h storage • Antimicrobial effect was via permeabilization and RNA damage of the cell	Not determined	Surowsky et al. (2014a)
DBD low-temperature plasma	Inducer gas: air Voltage: 30 kV Input power: Time: 3–25 s Discharge gap: 3 mm	Orange juice	*Staphylococcus aureus*, *E. coli*, and *C. albicans*	• >5 log CFU/mL reduction was achieved for *S. aureus*, *E. coli*, or *C. albicans*	• Slight reduction in pH and vitamin C content • Shelf life extended	Shi et al. (2011)

C. albicans being the least (Shi et al. 2011). In plasma-treated tangerine juice, *E. coli* at an initial concentration of 7 log CFU/mL was inactivated below the detection limit of 1 log CFU/mL after 3 min of plasma treatment (Yannam et al. 2018).

Starek et al. (2019) carried out cold plasma inactivation of numerous microorganisms in tomato juice. The inactivation results obtained were 3.45, 3.55, 3.32, 2.93, and 3.69 log CFU/mL reductions for mesophilic bacteria, yeast, molds, *Candida albicans*, and *S. cerevisiae*, respectively. Similarly, when a strawberry juice was exposed to cold plasma treatment for 10 min, a 1 log CFU/mL reduction in total aerobic bacteria was recorded (Mehta and Yadav 2020). A high-voltage atmospheric cold plasma (HVACP) employed in the inactivation of *Salmonella enterica* serovar *Typhimurium* in orange juice revealed more than 5 log CFU/mL reduction after 30 s of treatment using a mixture of 65% O_2 + 30% N_2 + 5% CO_2 (MA65) as working gas. Meanwhile, treatment of 120 s using air and M65 gas with subsequent storage for 24 h inactivated the pathogen by 2.9 and 4.7 log CFU/mL, respectively. The morphological changes observed in the treated *S. enterica* included shrinkage and lysed cells, thus demonstrated obvious damages and loss of integrity (Xu et al. 2017). A similar HVACP cold plasma equipment was utilized by Pankaj et al. (2017) for the inactivation of *S. cerevisiae* in white grape juice. The microorganism was inactivated by 7.4 log CFU/mL within 4 min of plasma exposure. In cold plasma treatment of blueberry juice, Hou et al. (2019) obtained a 7.2 log CFU/mL reduction during the inactivation of *Bacillus* sp. after 6 min treatment at 1% O_2 concentration. The antimicrobial potency and mode of action of plasma reactive species are based on cell wall etching and perforation, and cell permeabilization. ROS such as H_2O_2 and hydroperoxy radicals ($^{\cdot}HO_2$) have induced DNA and RNA damage and oxidation of protein and lipid at the cell level (Liao et al. 2018b; López et al. 2019; Surowsky et al. 2014a).

One of the main challenges facing cold plasm inactivation of pathogens in liquid food products is the availability of standardized equipment that can ensure uniformity in the generation and distribution of RONS within the liquid media that can achieve efficient inactivation. With the current trend in the activation efficacy, it is difficult to unify the pathogens inactivation due to many factors including microorganisms strain (bacteria, spores, fungi, or yeast), initial microbial load, Gram-positive or Gram-negative, treatment conditions (input voltage or power, gas type, duration, mode of exposure, and discharge type), and claim high pasteurization as stipulated by authorities for liquid foods. The United States Food and Drug Administration (FDA) has specified a minimum benchmark of 5 log CFU/mL for pathogens inactivation in fruit and vegetable juices or milk by novel emerging technologies be achieved before claiming pasteurization (FDA 2004).

11.4.2 Effects of Cold Plasma on Quality of Juices

The quality of processed food products determined consumers' acceptance. Processing strategies should be applied to promote food safety, as well as preserved

the quality of the treated food products. pH, acidity, and color are quality parameters that determine the freshness of beverages. Many studies have reported declines in the pH of fruit juices after cold plasma treatments (Dasan and Boyaci 2018; Liao et al. 2018a; Mehta and Yadav 2020; Pankaj et al. 2017; Shi et al. 2011; Starek et al. 2019; Wang et al. 2020; Xu et al. 2017). The acidification of plasma-treated foods usually depends on the treatment duration, input power, juices type, and inducer gas used. The increase in acidity has been associated with numerous plasma RONS reactions with food substrates and bioactive compounds. Among them is the degradation of amino acid by-products by ROS, with the formation of acidic compounds (Bußler et al. 2015). In addition, the interaction of water molecules and with charged ions also led to the formation of hydronium ions (Muhammad et al. 2018c).

Other notable changes in cold plasma-treated juices included changes in the color of apple juice after cold plasma treatment. The color of the apple juice became yellowish and lighter, with no dramatic changes in the volatile compounds as compared with the untreated juice (Liao et al. 2018a; Wang et al. 2020). Dasan and Boyaci (2018) also observed the quality of orange, tomato, apple, and sour cherry juices not visibly impaired in terms of color after cold plasma treatment. A similar assertion was made by Hou et al. (2019), where the original color of the treated blueberry juice remained unchanged.

11.4.3 Effects on the Functional Components, Vitamins, and Antioxidant Constituents

Phenolic compounds are bioactive components in fruit and vegetable juices. Their uptake into the body induces numerous health benefits such as anti-inflammatory, antimicrobial properties, and antioxidant. Cold plasma processing of fruit and vegetable juices caused some qualitative and quantitative changes in the composition and functionality of the bioactive components. Anthocyanins are water-soluble bioactive components found in the cell vacuole of plants. The exposure of these plant materials to cold plasma breaks up the cell membrane and leads to the release of intracellular substances, including bioactive compounds into the extracellular environment. This leads to enhanced mass transfer and reactions with RONS; consequently, the bioactive components are extracted faster (Muhammad et al. 2018b). Many studies have recorded accumulation or otherwise in total phenolic and anthocyanin contents in plasma-treated food products. The total phenolic content of the treated blueberry juice was significantly increased following plasma treatment from 2 to 4 min in the presence of 1% O_2. On extending the treatment time, a significant reduction in anthocyanin and antioxidant activity was obtained (Hou et al. 2019). In contrast, Liao et al. (2018a) did not observe significant changes in total phenolic content and antioxidant capacity of apple juice treated at the lower input powers of 30 and 40 W. Accumulation of 10–15% in phenolic contents of orange, tomato, apple, and sour cherry juices was described by Dasan and Boyaci (2018) after cold

plasma processing. In plasma-treated strawberry juice, an increment in total phenolic contents was discovered following 10 min of plasma treatment (Mehta and Yadav 2020). Likewise, the total phenolic and flavonoid contents and antioxidant capacity of white grape juice were found to reduce with an increase of plasma treatment time. The researchers opined that these decreases were comparable to those recorded with thermal pasteurization of the juice (Pankaj et al. 2017). The anthocyanin content of processed sour cherry juice was reduced by 4% after 9 min of cold plasma treatment (Hosseini et al. 2020).

Fruit and vegetable juices are natural sources of vitamins such as riboflavin (B2), pyridoxine (B6), biotin, vitamin A, C, and E, carotenoids, thiamin (B1), and lycopene. Some of these vitamins are stable during processing while others (carotenoids, lycopene, thiamin (B1), and vitamin A, C, and E) are degraded (Altemimi et al. 2017; Muhammad et al. 2018b; Pankaj et al. 2018). Cold plasma can exert its effect on vitamins present in juices during processing. Starek et al. (2019) also observed increases of 11% and 13% in the quantity of lycopene and carotenoid, respectively, during cold plasma processing of tomato juice. Sour cherry juice subjected to 9 min of cold plasma treatment experienced a 21% reduction in vitamin C content (Hosseini et al. 2020). The vitamin C content of the orange juice was also reduced by 22% after cold plasma treatment with air as inducer gas (Xu et al. 2017). A similar loss of 5% in vitamin C contents was observed in tomato juice after 5 min of treatment (Starek et al. 2019). Following the cold plasma treatment, a slight decrease in vitamin C content was noticed, and the shelf life of the orange juice was extended (Shi et al. 2011). Contrarily, a significant reduction in vitamin C in blueberry juice was obtained with an increase in cold plasma treatment time (Hou et al. 2019), meanwhile a decrease in vitamin C content of strawberry juice was equally recorded (Mehta and Yadav 2020). These changes in the concentrations of vitamins were mostly linked to the oxidation influence of ROS such as O_2, O_3, and $OH^{\cdot}$.

11.5 Cold Plasma Processing of Milk

11.5.1 Effects of Cold Plasma on Microbial Inactivation

The presence of background microflora in milk determines its microbial safety limits and freshness. The total background microflora in such products ranging from 3.0 to 5.2 log CFU/mL for unpasteurized milk (Corrales et al. 2012). Even though consumption of milk can boost the health and well-being of individuals, they can also harbor pathogens that could pose health hazards to consumers due to poor handling. Several pathogens have been associated with milk in the past, and some, including *L. monocytogenes* and *Bacillus cereus* spores, can withstand and survive post-pasteurization environments (Olivier et al. 2005). The pasteurization technique that is employed for controlling milk pathogens should be in such a way that it does not harm the milk quality while providing the necessary antimicrobial effects required.

In the last decades, studies have demonstrated that achieving a higher inactivation rate in milk is more complicated than in juices or model solutions due to the complex microstructure of milk. Corrales et al. (2012) associated the low microbial inactivation by ultraviolet radiation (UV-C) in tiger nut milk to turbidity due to suspended solids. Likewise, the inactivation of milk with pulsed electric field was also influenced by the presence of fat in the milk (Grahl and Märkl 1996). In cold plasma preservation of milk, encouraging results were recorded with regard to microbial safety. Table 11.2 has summarized cold plasma-related studies of milk inactivation. Gurol et al. (2012) recorded a 4.18 log CFU/mL reduction in the *E. coli* population in milk after 3 min of plasma treatment. Reductions of 2.43, 2.40, and 2.46 log CFU/mL for *E. coli*, *L. monocytogenes*, and *S. typhimurium*, respectively, were reported in cold plasma-treated milk by Kim et al. (2015) following 10 min exposure. Another study explored an important milk pathogen (*Prototheca zopfii*) that causes mastitis, which results in low milk production and quality deterioration in cows. The cold plasma treatment of this milk revealed more than 2 log CFU/mL reduction of *P. zopfii* (Tyczkowska-Sieron et al. 2018).

Unlike in the studies mentioned above, higher logarithmic inactivation from the milk of plant origin was reported. This could probably be due to their lower fat and protein contents as compared to dairy milk. When tiger nut milk was submitted to cold plasma treatment, the microflora of the milk was reduced to an undetectable limit after 12 min of treatment (Muhammad et al. 2018a). Similarly, a 5.28-log reduction of *Bacillus cereus* at input powers ranging from 39 to 46 W was obtained during the cold plasma processing of tiger nut milk (Muhammad et al. 2019b). It is worthwhile mentioning that irrespective of the milk origin, cold plasma can be relied upon to ensure the microbial safety of the treated milk.

11.5.2 Effects of Cold Plasma Treatment on Quality Attributes of Milk

The cold plasma-treated milk also experiences some quality changes, as indicated in Table 11.2, mainly due to reaction with some plasma immanent species. Similar to fruit juices, the pH of plasma-treated milk was found to decline with increases of treatment time and input power (Gurol et al. 2012; Kim et al. 2015; Muhammad et al. 2018a, 2019b). Besides, slight changes in color parameters were observed in both treated milk from animal and plant origin (Gurol et al. 2012; Kim et al. 2015; Muhammad et al. 2019b).

Table 11.2 Cold plasma inactivation of microorganisms in milk and milk products

Type of cold plasma device	Processing conditions	Liquid food products	Test strain	Inactivation results	Quality changes	References
Low-temperature corona plasma	Inducer gas: air Time: 3, 6, 9, 12, 15, and 20 min	Whole, semi-skimmed, and skimmed milk	*E. coli*	• 4.18 log CFU/mL (54%) reduction of *E. coli* after 3 min regardless of fat content	• No significant change in pH • Slight changes in color	Gurol et al. (2012)
Encapsulated DBD plasma	Inducer gas: air Input power: 250 W Time: 5 and 10 min	Milk	*E. coli*, *L. monocytogenes*, and *Salmonella* Typhimurium	• 2.43, 2.4, and 2.46 log CFU/mL reductions in *E. coli*, *L. monocytogenes*, and *S.* Typhimurium, respectively, after 10 min treatment	• Decline in pH • Slight color change • Increased lipid oxidation	Kim et al. (2015)
Cold plasma jet	Inducer gas: Helium gas Input power: 17 W Time: 1–12 min Flow rate: 1.9 L/min Discharge gap: 5 mm	Milk	*Prototheca zopfii*	• More than 2 log CFU/mL reduction after 12 min	Not determined	Tyczkowska-Sieron et al. (2018)

(continued)

Table 11.2 (continued)

Type of cold plasma device	Processing conditions	Liquid food products	Test strain	Inactivation results	Quality changes	References
Cold atmospheric plasma	Inducer gas: air Input power: 38, 43, and 46 W Time: 0–270 s	Tiger nut milk	*Bacillus cereus*	• 5.28 log CFU/mL reduction at input powers of 39–46 W	• Significant decrease in pH, total flavonoid content, and antioxidant activity at 43 and 46 W • Changes in color • Marked increase in total phenolic content	Muhammad et al. (2019b)
DBD plasma	Inducer gas: air Voltage: 30 V Time: 2, 4, 6, 8, and 12 min Discharge gap: 6 mm	Tiger nut milk	Total background bacteria and molds and yeast	• Microflora was reduced below the detection limit	• Significant drop in pH after 8 and 12 min • Increase lipid oxidation	Muhammad et al. (2018a)

11.5.3 Influence of Cold Plasma on Bioactive Components of Milk

Milk is among the healthy food with abundant functional ingredients. The bioactive components are altered due to cold plasma treatment. The concentrations of the fatty acids in the plasma-treated milk were not altered except butyric acid and caprylic acid (Kim et al. 2015). Meanwhile, the total phenolic content of tiger nut milk was significantly increased in a time- and input power-dependent manner after cold plasma treatment. In contrast, the antioxidant capacity and total flavonoid content were markedly reduced in similar trends (Muhammad et al. 2019b).

Another critical quality issues associated with cold plasma treatment of milk is lipid oxidation. Many researchers have reported an escalation of lipid oxidation in cold plasma-treated foods (Kim et al. 2015; Muhammad et al. 2018a). This is expected as plasma contains many oxidizing ROS. The lipid oxidation induced by ROS could negatively influence the acceptability and shelf life of food products. Although it is a general belief that cold plasma does not produce waste by-products, the level of lipid oxidation occurs in high oxygen plasma food called for safety scrutiny. To date, no document has an in-depth study of the safety issues of the secondary metabolite generated in plasma-treated milk. One study, however, emphasized the need to investigate the cold plasma influence on lipids (Gavahian et al. 2018). This could reduce the adverse effects on the sensory characteristics of the food products and clear the related health concerns.

11.6 Conclusions

Cold plasma is a nonthermal strategy for managing the safety of food products and has achieved tremendous bactericidal efficacy in the last two decades concerning liquid food products. This chapter presented a recap of cold plasma inactivation of pathogens with high microbial risks in liquid food products by the lethal actions of cold plasma. Even though cold plasma has enormous contributions to food safety, it still presents some challenges related to the quality of the treated products. Already, some of the products such as milk and juices are complex multi-component food products containing proteins, starch, vitamins, lipids, phenolics, and antioxidant constituents, and their reaction with cold plasma RONS become more complicated to understand the chemical reaction mechanisms. This is one of the drawbacks that require further attention to realize its full-scale application in food industries. A tailored cold plasma treatment that targets high inactivation efficacy for a specific food matrix (liquid or solid foods) with negligible quality changes can offer a solution to this challenge. Therefore, the design of cold plasma equipment, standardization of protocols for pathogens inactivation using the various plasma equipment, choice of inducer gas, and treatment duration are critical process conditions

that should be optimized to achieve effective pathogens inactivation with minimum or negligible quality changes.

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