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INVITED REVIEW

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Inactivation of viruses related to foodborne infections using cold plasma technology

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Abstract

Globally, there is a rise in day-to-day demand for minimally processed foods to supply nutritious, wholesomeness and safe foods to the consumers. Contamination of food by pathogens is a serious problem resulting in several outbreaks. Food pathogens like molds, bacteria were detectable and can be inactivated. The virus detection in foods is always a difficult task as their presence could not alter any noticeable change in the quality. Norovirus, Hepatitis A viruses are well-known for their foodborne outbreaks and illnesses. Enveloped viruses are resistant and have the stability to the current traditional preservation methods due to the presence of a protective capsid layer and an envelope. The current thermal processing has shown significant effect on the product quality. The use of chemical disinfestation compounds is not suitable for food commodities. There is a need for alternative nonthermal food processing technologies for decontamination of food and food packages and preserving the food quality as well. Cold plasma is one of the emerging nonthermal, chemical-free residues, and eco-friendly technology widely being applied to the different food sectors. The main antiviral mechanism is the disruption of the capsid protein layer, the oxidation and denaturation of viral proteins. The method has also caused damage to the envelope layer and genetic material. This review focuses on cold plasma inactivation efficiency on different viruses.

1 | INTRODUCTION

With the emerging of new diseases and infections, there is a need for new and efficient technologies for decontamination and disinfestation. The recent outbreak of COVID-19 resulted in a pandemic and caused devastation across the world. To date, many countries are still suffering from the novel coronavirus and its mutant strains (Center for Disease Control and Prevention). The discovery of medicine for viral diseases is a time-taking process that involves several trials. Some of the viruses like Hepatitis E virus (HEV) are transmitted through the contaminated food substances, water and few through the infected food handlers. Although, the viruses cannot replicate on food substrates but they can survive for a time period which leads to food-person transmission. Many of the viruses survive during the food processing due to the presence of protein capsid layer and envelop on few viruses (Roos, 2020). Roos (2020) reported that some of the non-foodborne viruses like SARS-CoV-2 are also prevalent and can be transmitted through the food chains. Some concerns have been raised on the SARS-CoV-2 survival in food and on food packaging as a foodborne virus (Anelich, Lues, Farber, & Parreira, 2020). Capelli et al. (2021) also reported that the accidental contamination of food and food packages with SARS-CoV-2 is raising concerns about the food safety. However, SARS-CoV-2 transmission through the food chain is very low or nonsignificant attributing to it as a non-foodborne virus (Anelich et al., 2020).

Filipić et al. (2021) reported that the viruses are highly stable and can survive in water for a longer period of time compared to food substances. Some viruses like Norovirus, Rotavirus, Hepatitis A virus, Enterovirus, Coronaviruses, and Adenovirus caused foodborne illness through contaminated processed and raw meat, fresh produce, and seafoods (Govaris & Pexara, 2021). WHO (2008) reported that heating and drying methods of food preservation can inactivate virus but greatly depends on the complexity of the food matrix. Baert,

Debevere, and Uyttendaele (2009) reported that virus has survived in the heated milk cream for longer time compared to heated raw milk. This could be due to high fat globule content of the cream protected the virus from the heat treatment. Freezing is other efficient method for the preservation of food against viral infections (Papafragkou, D'Souza, & Jaykus, 2006). However, Baert et al. (2009) reported that viruses survived in different environment conditions like chilling, freezing, acidification, and modified atmospheric conditions. Govaris and Pexara (2021) reported that some of the traditional food preservation methods of smoking, drying, heating are efficient for the viral inactivation. However, these methods have a significant effect on product quality and consumer acceptance. There is a need for alternative food processing technologies for virus decontamination. Nonthermal food processing technologies could be possible solutions that are widely applied to minimally processed foods. Few nonthermal food processing technologies employed for viral inactivation are ionizing radiations, high pressure processing, and pulsed electric fields. The anti-microbial activity of light-based technologies is known for several decades. In recent times, the application of UV light, pulsed UV light, and LED UV light have gained importance for possessing anti-viral activity. However, the treatment time for the inactivation varies from pathogen to pathogen. Hadi et al. (2020) stated the virucidal activity of UV-C radiations that the light absorbed by the virus genetic material results in the photochemical reaction between the two adjacent pyrimidine bases and form dimers like thymine and cytosine of DNA. Similarly, Kumar et al. (2020) and Pendyala et al. (2020) reported that UV-C light affected the SARS-CoV-2 by disrupting the protein covering layer of the coronavirus and by developing a genomic model of pyrimidine dinucleotide frequency respectively. Govaris and Pexara (2021) reported that the high pressures have ruptured the capsid protein layer resisting the entry of the virus into the host cell. HPP mainly targets the protein-lipid layer present in the membranes attributing less resistance to enveloped viruses than the non-enveloped viruses. DiCaprio, Ye, Chen, and Li (2019) inactivated the human norovirus in strawberry puree using HPP, the authors have observed complete inactivation of virus at 600 MPa. The complexity of the food matrix, physicochemical properties of food, and presence or absence of protective covering of viruses greatly affect the anti-viral efficiency of HPP. However, there are some limitations in the application of the above technologies for viral inactivation. Roos (2020) reported that enteric viruses like the Hepatitis A virus and Adenovirus are resistant to processing conditions and to ionizing radiation. The capsid layer of rotavirus protected the virus against exposure to strong pulsed electric fields (Pexara & Govaris, 2020). The application of cold plasma could be a possible technology due to its high microbial inactivation efficiencies. Plasma is considered as the fourth state of matter which consists of ions, electrons, radicles, neutral and charged atoms, active species, UV light, and ozone (Thirumdas, Sarangapani, & Annapure, 2015). The excitation of gas molecules using electric discharges can produce the plasma at low or atmospheric pressures (Zhu, Li, Cui, & Lin, 2020). The antimicrobial efficiency of cold plasma depends on several factors like power applied, treatment time, feed gas, and mode of generation (Thirumdas et al., 2018). The reactive species formed in the plasma are responsible for the pathogen destruction. However, the formed species will revert back to the original state in short time without leaving any chemical residues on the treated substrates. The use of hot water, steam, and chemical compounds in the traditional methods may result in environmental pollution. From this point of view, cold plasma technology can be considered as an environmental friendly method. The antiviral activity of cold plasma on several foodborne viruses is still unexplored. In the present review, the efficiency of cold plasma for viral inactivation is discussed along with the foodborne virus pathogenesis and method of detection.

2 | SOME VIRUSES CAUSING FOODBORNE INFECTIONS

WHO (2008) has classified the foodborne viruses based on symptoms of infection into gastroenteritis, and entrically transmitted hepatitis groups. Similarly, based on the primary tissue tropism, the foodborne viruses are classified into Enterotropic, Heptotropic, Neutropic, Pneumotropic (Bosch, Pintó, & Guix, 2016). The different types of foodborne viruses that resulted in several foodborne outbreaks are given in Table 1. The presence of outer covering or membrane of the viruses they are recognized as enveloped or non-enveloped/naked viruses. A complete virus consisting of a genetic material (DNA/RNA), a capsid and a protective membrane capable to infect a host is called as virion. Unlike other pathogenic microbes, the viruses can exist as inactive virions outside the host cells but capable of replicating after transmitted to the host. However, the presence of virion or viral genome in food always could not confirm the virucidal activity after entering into the host through contaminated food (Roos, 2020). The exact mechanism for virus transmission to the host cells and pathogenesis through the contaminated food sources is still explored. One of the largest foodborne outbreaks reported in Germany is human norovirus transmitted through fresh produce, processed foods, and seafood (Li, Zhao, & Tan, 2021). The undercooking of infected pork and game meat like wild boars and deer are the main transmission route for several strains of HEV viruses (Di Cola, Fantilli, Pisano, & Ré, 2020). Hepatitis E virus is probably considered as the zoonotic foodborne virus transmitted from the consumption of infected animals and their meat products (Koopmans & Duizer, 2004). Seafood is the main sources for outbreak of Hepatitis A virus followed by berries, tomatoes and ready-to-eat foods. Similarly, Sánchez and Bosch (2016) stated that fresh fruits, leafy vegetables, oysters are the main food sources for norovirus outbreaks. Thippareddi, Balamurugan, Patel, Singh, and Brassard (2020) have raised a probability about the viral contamination of food and fresh produces through the contaminated water sources. Hall et al. (2011) reported that norovirus can also be transmitted through the use of virus contaminated water for the washing of fresh produce and for cultivation of seafoods. Moreover, a 50% of outbreaks of human norovirus in the United States are transmitted through infected food handlers (Greening & Cannon, 2016).

Bintsis (2017) categorized different foods acting as vehicles for pathogens are meat (bovine, pig) and poultry (broiler and egg) constituents around 23%, fish products—7%, dairy products including cheese—13%, and crustaceans—3%. Bosch et al. (2016) reported some

TABLE 1 Different foodborne viruses and their sources of contamination

Virus	Family	Particle	Genetic material	Source of food contamination
Hepatitis A virus	*Picornaviridae*	Non-enveloped	RNA	Sell fishes, unpeeled fruits, uncooked vegetables, salads, cheese, berries
Norovirus	*Caliciviridae*	Non-enveloped	RNA	Raw fruits and vegetables, lettuce, read-to-eat foods, sandwiches, cookies
Adenovirus	*Adenoviridae*	Non-enveloped	DNA	Milk, ice cream, cream, green onion, oysters, clams
Hepatitis E virus	*Hepeviridae*	Non-enveloped	RNA	Raw, uncooked pig and game meat, unpasteurized milk, sausages
Rotavirus	*Rotaviridae*	Non-enveloped	RNA	Juices, dairy products, shellfishes, sugary foods
Enterovirus	*Picornaviridae*	Non-enveloped	RNA	Oysters, raw shellfish, vegetables, fruits, and salads
Coronavirus	*Coronaviridae*	Enveloped	RNA	Fruits, vegetables, bread, meat products, dairy products
Tulane virus	*Caliciviridae*	Non-enveloped	RNA	Oyster, bivalve mollusks, shellfishes
Bacteriophage (T_4)	*Myoviridae*	Enveloped	DNA	Beef, chicken, pies, biscuits, cheese

Source: Papafragkou et al., 2006; WHO, 2008; Bosch et al., 2016; Roos, 2020

data of foodborne viruses contamination alters in different foods like bivalve molluscans (85%), frozen strawberries, blueberries, and mixed berries (8%). WHO (2008) has postulated the mode of foodborne virus transmission, that is, through the feces to oral route when the person is exposed to the human sewage and feces, consumption of contaminated meat products, especially undercooked meat, and contamination by the infected food handlers during the food preparation and distribution. The food handlers may be infected during the processing of contaminated food and slaughtering of the infected animals. The survival and transmission of the virus through food also depends on the intrinsic (infection dose, pH, moisture content, processing temperature of food) and extrinsic factors (relative humidity, temperature, nature of attached surface). As per the WHO (2008) report, the foodborne viruses are stable to a wide range of pH from 3 to 10 and survived up to several weeks to months in shellfishes and on fresh produces. Le Guyader et al. (2004) have stated that the oysters act as biofilters and trap the virus particles and acts as reservoirs for the transmission of the virus to the host. Many of the viruses are inactivated during the heating of food but it greatly affects the nutritional and organoleptic properties of the food materials. Some of the viruses survived the common food processing operation and preservation (heating, freezing) methods and transfer to the host (Papafragkou et al., 2006). However, it is very difficult to predict the presence of any virion as it does not affect the organoleptic characteristics of the food materials. According to Greening and Cannon (2016), enteric viruses can survive at pasteurization temperatures (60°C for 30 min) and even in acidic foods, pickles, jams, and jellies. However, the inactivation efficiency can also depends on constitute of the food material, and structure. Pexara and Govaris (2020) reported that the food ingredients may provide a shield around the viruses protecting them from the processing conditions. For example, Shi et al. (2012) observed that the proteins present in the serum have protected the Hepatitis B viral DNA from the DBD-generated plasma resulting in longer exposure times for complete destruction. The virus particles survived during the food processing and the acidic pH of the stomach after consumption reach the tissue tropism and cause the infection. Apart from the above reasons, the poor food handling by the virus infected food handlers is one of the key risks for many foodborne viruses transmission.

Pathogenesis defines the progression of viruses in the host causing infection. Doms (2016) reported that the virus pathogenesis depends on the site of replication and primary tropism for a tissue or organ. Primary tissue tropism of different foodborne viruses is given in Figure 1. The virus first adsorbs on the host cell and injects into the cell, replicates inside the cell and transfers to other cells in the tissues and organs (Thirumdas, Kothakota, Pandiselvam, Bahrami, & Barba, 2021). After adsorption, the virus enters into the host cells through the receptors like glycoproteins present in the cell membranes. For example, the pathogenesis of novel coronavirus enters into the host cell through the Spike- S protein (S protein is a glycoprotein) (Whittaker, Daniel, & Millet, 2021). The receptors on the host cell membrane for the entry of SARS-coronavirus are angiotensin-converting enzyme 2 (ACE2) expressed in the upper respiratory tract epithelial cells (O'Brien & Eger, 2021). The ACE2 receptors are expressed in alveolar epithelial cells of the lungs Wan et al. (2020), human enteroids (Lamers et al., 2020) and intestinal erythrocytes (O'Brien & Eger, 2021; Thirumdas et al. (2021) reported that SARS-coronavirus-2 infects the epithelial cells initially then it replicates and shows the onset of early symptoms. The pathogenesis of Norovirus involves the P2 domain present on the viral capsid binds to the receptor on the red blood cells (Histoblood group antigens [HBGA'S]) that initiates the point of viral attachment (MacCannell et al., 2011).

Respiratory foodborne viruses like avian influenza viruses involved localized replication in the upper respiratory tract causing pulmonary and respiratory diseases (Spackfman, 2018). Similarly, the rotavirus also replicates in the erythrocytes of the small intestine. Greening and Cannon (2016) reported that enteric viruses can be resistant to the high acidic pH of the stomach and proteolytic activity of the duodenum that passes to the small intestine and cause the primary infection. The enteroviruses are an example of an enteric virus that can survive the high acidic gastric pH and reach the

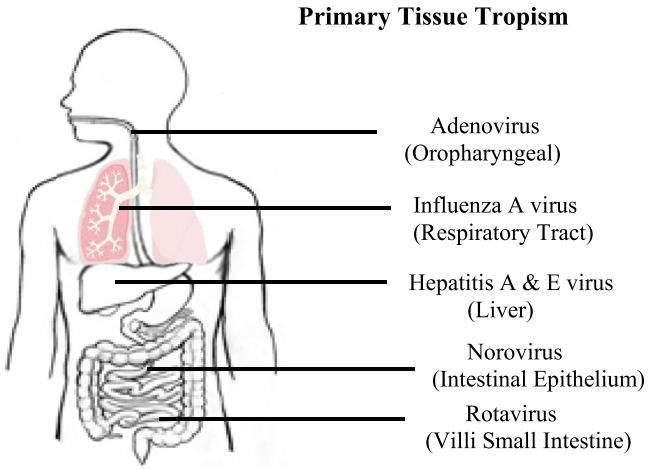

FIGURE 1 Primary tissue tropism of different foodborne viruses

oropharyngeal tissue for the genetic material (RNA) replication (Liu, 2018). Primadharsini, Nagashima, and Okamoto (2021) reported that the HEV might be replicated in the liver but the primary site of replication is still not clarified. HEV is a non-enveloped virus but these are associated with a lipid bilayer membrane free of viral glycoproteins in the membrane consider quasi-enveloped (Primadharsini et al., 2021). The pathogenesis of adenovirus was reported by Rosa and Suffrendi (2018), the virus after entering into the host complete replication cycles in the epithelial cells resulting in cell death and infection.

In recent days, the virus detection in the food substances is progressed very much and improved significantly. Several methodologies which are employed to detect foodborne viruses are electron microscope, cytopathic effects, immunological methods, and molecular detection methods (Rodríguez-Lázaro, Cook, & Hernández, 2013). Saravanan et al. (2020) also reviewed the general methods for foodborne pathogen detection in food products. Some of the detection methods used for foodborne pathogens: polymeric chain reactions, different types of biosensors, spectrophotometric methods, and biochemical methods. Among the several methods of detection particularly for viruses, reverse transcription PCR is more accurate, precise, and reliable used to quantify very minute concentrations. However, the extraction of viruses from the food matrixes is the most difficult step due to the complex food structure, and several food components restricting the virus extraction (Rodríguez-Lázaro et al., 2013). Many of the scientific evidence on the virus extraction was based on the three approaches are elution concentration method, direct viral RNA extraction and proteinase K treatment (Stals, Baert, Van Coillie, & Uyttendaele, 2012). After the extraction of viruses from food, it is followed by purification and molecular detection. Bosch et al. (2016) reported that International Standard Organization (ISO) has specified the use of quantitative reverse transcription PCR (RT-qPCR) for the detection of norovirus and HAV in different foods like leafy vegetables, berries, mollusks. However, the detected genetic material of viruses by RT-qPCR does not confirm the virulence in the host (Bosch et al., 2016). To enhance the performance of RT-PCR, Rajiuddin, Jensen, Hansen, and Schultz (2020) suggested adopting a direct lysis method for viral RNA extraction by increasing the volume of lysis buffer and the use of pectinases for better recovery. Ethidium monoazide coupled RT-qPCR method has been employed to evaluate the cold plasma inactivation efficiency of SARS-CoV-2 (Rasouli & Amini, 2021).

3 | COLD PLASMA AS A DECONTAMINATION TECHNOLOGY

Cold plasma has been applied in many food processing areas like microbial and enzyme inactivation, toxins degradation, seed germination enhancement, surface functionalization of biopolymers, starch modification, modification of proteins, pesticides dissipation *etc*. (Chaple et al., 2020; Devi et al., 2017; Sutar, Thirumdas, Chaudhari, Deshmukh, & Annapure, 2021; Sadhu et al., 2017; Thirumdas et al., 2015, 2016, 2020; Thirumdas, 2022). However, the extent of applications depends on several factors like fundamentals of plasma generation, the operating parameters, biological nature of food material and the surface topography of food materials. Based on the temperature of plasma it is divided into cold/nonthermal plasma and hot plasma/thermal plasma. In cold plasma, the temperature of electrons (Te) is higher compared to the temperature of ions (Ti), attributing to lower temperatures (Ti >> Te). Since the overall temperature of the generated plasma is near or below ambient room temperature designating it as cold plasma (Thirumdas et al., 2015). Misra, Yadav, Roopesh, and Jo (2019) also reported that the cold pertaining plasma when the temperatures of ions are lower to that of electrons of ionized gas. The formation of reactive species in plasma depends on the mode of generation like corona discharge, fluorescent tubes, radio-frequency plasma reactor, dielectric barrier discharges (DBD), and microwave discharges (Coutinho et al., 2021). Charoux et al. (2021) reported that in cold plasma, the temperature of electrons is higher than other species and bombardment of electrons results in the release of secondary electrons and ions which are capable to initiate several chemical reactions. The plasma can be generated from different energy sources like heating, application of electrical power and voltage, magnetic fields, radio-frequency waves and magnetic radiations. The reactive species of plasma particularly the reactive oxygen species (ROS), reactive nitrogen species (RNS), and other species that are capable of inactivation of a wide range of microorganisms includes gram-positive, gram-negative bacteria, yeasts, molds, bacterial biofilms, and viruses (Feizollahi, Misra, & Roopesh, 2021).

There are several ways that plasma active species show their antimicrobial activity by breakdown or rupture of the cell wall and plasma membrane, damaging the genetic materials, inducing the oxidative stresses by the generation of extra and intracellular ROS, overcoming the anti-oxidative mechanism of the cells, and formation of strong oxidizing agents like H_2O_2 both intra and extracellular. Plasma chemistry is very complex as the half-life of many reactive species formed is very short. Ionization, dissociation, excitation, ion-molecule interaction are the main processes responsible for the formation of different

reactive species (Misra et al., 2019). The reactive species formed in the plasma will revert back to the original state leaving no residue on the treated substrate. As there is no chemical residues left and zero waste management the cold plasma technology is generally considered as eco-friendly technology. Of the different reactive species formed in the plasma, Berardinelli et al. (2021) stated that atomic oxygen (O), hydroxyl radicals (·OH), ozone (O_3) are the important ROS whereas nitrogen oxide radicals and excited nitrogen molecules are important RNS formed in the atmospheric air plasma. However, the half-life of ROS like hydroxyl radicals and singlet oxygen ($^1O_2$) has a shorter live time but can create significant microbial destruction (Thirumdas, Kadam, & Annapure, 2017; Thirumdas, Trimukhe, Deshmukh, & Annapure, 2017). Wende, von Woedtke, Weltmann, and Bekeschus (2018) reported that singlet oxygen can live up to microseconds and H_2O_2, NO_2^-, and NO species are long-lived and stay up to few days. Guo et al. (2018) observed the concentration of H_2O_2, NO_2^-, and NO_3^- in plasma activated liquid to be 221, 8, 216 μM respectively after an exposure time of 2 min. Several reports have shown that RNS is long-lived reactive species than ROS. Similarly, the reactive species formed from the direct treatment (plasma plume direct contact with biological material) are long-lived than the indirect treatment (plasma-activated liquids). The plasma chemistry of plasma-activated liquids is complex that resulted in formation of different short-lived, long-lived and secondary reactive species (Chen, Garcia, Arumugaswami, & Wirz, 2020; Chen, Xu, Chen, & Wang, 2020). The formation of reaction species depends on the type of source, method of application, interaction of primary species with the surrounding environment and biological substances (Gorbanev, Privat-Maldonado, & Bogaerts, 2018). Optical emission spectroscopy is the widely used analytical method used for the quantification of different active species formed in plasma. From the optical emission spectroscopy, the emission of different active species both the ROS and RNS are observed between 200 and 900 nm (Yadav et al., 2020). The oxygen presence in feed gas results in the formation of higher concentrations of ROS like atomic oxygen and ozone possessing higher virus inactivation rates. The collision of high energetic electrons with the water molecules presents in the feed gas results in the formation of OH radicals that are observed at 306–309 nm in the optical emission spectrum (Yadav et al., 2020). The above authors have also stated that the excited nitrogen molecules have the ability to disassociate the water molecules and result in the formation of ·OH radicals. The higher relative humidity percentage of the feed gas regulates the formation of more hydroxyl ions and peroxyl acid groups that resulted in higher microbial inactivation (Coutinho et al., 2021). Thirumdas, Kadam, and Annapure (2017); Thirumdas, Trimukhe, et al. (2017) reported that due to the versatility nature of hydroxyl ions and atomic oxygen in forming covalent bonds with several compounds they are the most reactive species of ROS. Wende et al. (2018) reported that reactive species like singlet oxygen radicals and one-electron oxidants like peroxynitrite generated in plasma attack the nucleobase pairs of genetic material. The viruses are more susceptible to the active species compared to bacteria, molds and yeasts (Coutinho et al., 2021) due to the photocatalytic process that destroys the capsid layer. Mohamed et al. (2021) interpreted the data related to the research activities carried across the world on the application of nonthermal plasma for the viral inactivation. The above authors have reported that the 42% of research is carried in the United States followed by Japan 17%, China 11% and Germany 9%. The above data reported by Mohamed et al. (2021) include the nonthermal plasma inactivation of infection viruses and their surrogates.

3.1 | Virus inactivation using cold plasma

To inactivate a virion, the disruption or breakdown of the capsid protein layer or envelope is the main mechanism (Table 2). The interaction of plasma active species and the outer covering of viruses can attribute to the disruption of outer protective covering. The degradation or disruption of protein might be due to depolymerization, denaturation, and amino acid oxidation of capsid proteins. Sharma and Singh (2020) stated that the interaction of plasma reactive species with proteins results in changes in structural configuration, particularly the secondary and tertiary structural patterns. Coutinho et al. (2021) reported that the photocatalytic reactions caused by the advanced oxidation process of ROS of plasma disrupted the capsid protein structure consequently interacting with the genetic material (DNA/RNA) of the viruses. Feizollahi et al. (2021) reported that the ozone is one of the most active components of ROS formed in the cold atmospheric plasma possessing high microbial inactivation efficiency. The ozone has the ability of oxidizing the cysteine sulfhydryl bonds present in the virus capsid layer (Aghajanzadeh & Ziaiifar, 2021). Bermudez-Aguirre (2020) stated that a synergistic effect of ROS and ozone is beneficial in destroying the virus protein layer for easy facilitation of ROS to reach the genetic material for destruction. The author commented that ROS has limited access to penetrate the protein cover to reach the virus genetic material.

In the investigation carried out by Guo et al. (2018) on the cold plasma effect on T4 bacteriophage, there is a decrease in the protein probably due to the oxidation reaction caused by the reactive species particularly the short-lived leading the degradation of proteins of bacteriophages. The singlet oxygen ($^1O_2$) species formed has shown maximum antiviral activity by damaging the proteins and DNA. Wu et al. (2015) reported that the application of atmospheric cold plasma on MS2 bacteriophages resulted in a 95% decrease in the viral load at a treatment time of 1 min at 28 W power levels. Similarly, a 30 kV voltage applied to packaged bed-DBD discharge reactor to generate cold air plasma resulted in a 2 log reduction in MS2 bacteriophages present in aerosols. The plasma-activated water was used to inactivate MS2 bacteriophages by Guo et al. (2018) after activating water with cold atmospheric plasma for 30 s using feed gas (mixture of argon and air) that resulted in 4.6 fold decrease in the virus. These studies have revealed that the damage to surface protein and to RNA genes of bacteriophages is the important viral inactivation mechanism. Hirneisen et al. (2010) reported that the ozone has the ability to break down the protein capsid layer of bacteriophage into many subunits exposing the virus RNA and restricting the absorption of RNA into the host cells. However, the presence of O_2 in the feed gas has enhanced the inactivation efficiency. Alshraiedeh, Alkawareek, Gorman, Graham,

TABLE 2 Cold plasma inactivation of foodborne viruses

Virus	Plasma source	Plasma parameters	Virus reduction	Key findings	References
Adenovirus	High voltage pulse-static induction thyristor power	Feed gas: N_2 Time: 0.94 min Pressure: 0.5 atm Power: 1.5 kpps	1 log_{10}	H_2O_2, NO_2^-, NO_3^- are the key reactive species showed virucidal effect on genomic DNA	Sakudo, Toyokawa, and Imanishi (2016)
Feline calicivirus	Radio frequency-atmospheric pressure plasma jet	Feed gas: Ar + 1% O_2 Time: 15 s Power: 2.5 kW	6.6 log_{10}	Breakdown and oxidation of viral capsid and modification of dimeric interface of capsid layer restricting entry into host cell	Aboubakr et al. (2018)
	DBD plasma	Feed gas: Ar + 20% O_2 Time: 5 min Power: 14.5 W/cycle	3.4 log_{10}	Long-lived species (O_3 and NO_x) are important antiviral agents	Nayak, Aboubakr, Goyal, and Bruggeman (2018)
Hepatitis B virus	DBD-atmospheric pressure plasma	Feed gas: Air Time: 40 s Voltage: 20 kV	0.7×10^5 IU/ml decrease in DNA	Reactive species reacted with proteins present in outer capsid layer of virus resulted in death	Shi et al. (2012)
HIV	Cold atmospheric plasma jet	Feed gas: Helium Time: 45 s Voltage: 4.5 kV	2.5–3 fold decrease	Injury of genetic material due to destruction of viral capsid	Volotskova, Dubrovsky, Keidar, and Bukrinsky (2016)
Tulane virus	DBD-atmospheric cold plasma	Feed gas: N_2 + 10% O_2 Time: 5 min Voltage: 38.4 kV	1.3 log PFU/g	The high O_2 concentration resulted in O_2^-, O, O_3, O^- resulted in inactivation on lettuce. After the treatment, storage under MAP could be beneficial	Min et al. (2016)
	DBD-atmospheric cold plasma	Feed gas: Air Time: 3.5 min Voltage: 39 kV	2.2 log PFU/3.8 g cube	The reactive species and UV act upon the viral capsid and viral genome	Roh, Oh, Lee, Kang, and Min (2020)
Potato virus Y	Cold atmospheric plasma-single electrode	Feed gas: Ar + 1% O_2 Time: 10 min Voltage: 6 kV (peak-to-peak)	2.7×10^5 log	Damaging and destabilizing the viral protein coat	Filipić et al. (2019)
Norovirus	Cold atmospheric pressure plasma	Feed gas: Air Time: 15 min Voltage: 8.5 kV (peak-to-peak)	1.69 log	ROS (ozone, atomic oxygen, and singlet oxygen), RNS (NO_2, N_2O_3, NO_2^-) are important species for viral inactivation	Ahlfeld et al. (2015)
	Atmospheric pressure plasma jet	Feed gas: N_2 Time: 5 min Voltage: 3.5 kV (peak-to-peak)	>2 log	Active oxygen and nitrogen species affected the viral particles	Bae, Park, Choe, and Ha (2015)
	Cold plasma arc	Feed gas: Air Time: 90 s Power: 549 W	5 log	Photoinactivating light, gaseous antimicrobials, and reactive species have led to viral inactivation	Lacombe et al. (2017)
Pepper mild mottle virus	Cold atmospheric plasma	Feed gas: Ar + 1% O_2 Time: 5 min Voltage: 6 kV (peak-to-peak)	>1 log	Formation of H_2O_2 (5 mg/L) resulted in viral inactivation	Filipić et al. (2021)
Tobacco mosaic virus	DBD-atmospheric cold plasma	Feed gas: Air Time: 5 min Voltage: 20 kV (peak-to-peak)	100% reduction	Irradiation disrupted and fragmented TMV particles, thereby preventing infectivity	Hanbal et al. (2018)
Tulane virus	DBD-atmospheric cold plasma	Feed gas: N_2 + 10% O_2 Time: 5 min Voltage: 38.4 kV	1.3 log PFU/g	The high O_2 concentration resulted in O_2^-, O, O_3, O^- resulted in inactivation on lettuce. After the treatment, storage under MAP could be beneficial	Min et al. (2016)

TABLE 2 (Continued)

Virus	Plasma source	Plasma parameters	Virus reduction	Key findings	References
MS2 bacteriophage	Cold atmospheric pressure plasma	Feed gas: Air Time: 60 s Power: 28 W	1.3 log	Damage to surface proteins and RNA genes resulted in loss of viral infectivity	Wu et al. (2015)

and Gilmore (2013) observed that increase in O_2 concentration from 0 to 0.75% in the feed gas composition has increased the two fold $\log_{10}$ reduction of MS2 bacteriophage viability which is one of the surrogates for the human norovirus. The above authors have commented that the increase in O_2 concentration in feed gas attributed to high densities of ROS particularly $O^{\cdot}$, OH, O_3, $^1O_2$, O^-, O-O. Apple juice inoculated with Escherchia coli exposed to DBD atmospheric air plasma at 50 W power levels decreased a 4.34 $\log_{10}$ within 40 s of exposure. However, the cold plasma treated slightly affected the quality of apple juice in terms of pH, titratable acidity, color and total polyphenolic content.

Aboubakr et al. (2020) decontaminated the human norovirus on the surface of roman lettuce leaves using the DBD cold atmospheric plasma. After the treatment, the authors have used ethidium monoazide coupled RT-qPCR to quantify the viral genome copies and observed a reduction of more than 99.5% (up to 2.6 $\log_{10}$) after 5 min of exposure. The reactive species has oxidized some of the functional groups present in the capsid layer but left the layer intact not disrupting the layer completely (Aboubakr et al., 2020). However, the authors have used a two-dimensional array of integrated coaxial-micro hollow DBD plasma devices which is like an indirect way of plasma application to the viruses for their investigation. The direct application of plasma might have disrupted the capsid layer of viruses that was seen with other plasma devices. Similarly, the exposure of norovirus inoculated meat products to atmospheric plasma jet inactivated virus more than 2 folds within 5 min attributing to an overall 99% in reduction (Bae *et al*., 2015). Feline calicivirus is one of the widely used surrogates for the human norovirus responsible for foodborne outbreaks. Nayak et al. (2018) attempted to inactivate feline calicivirus using DBD air plasma, they have observed more than 5 $\log_{10}$ after 3 min of treatment. The authors commented that the synergistic effect of ozone and NOx species has played a main role in the inactivation. A study was conducted by Lacombe et al. (2017) to inactivate the norovirus on the surface of blueberries using cold plasma. A successful 5 $\log_{10}$ reduction in norovirus was found after treating with air plasma jet for 5 min. The reactive species along with the photo inactivating light targets the viral capsid layer and the genome could be the possible mechanism for inactivation (Lacombe et al., 2017). A peak-to-peak voltage of 8.5 kV applied to generate cold atmospheric pressure plasma resulted in a $\log_{10}$ reduction in human norovirus after 2 min of exposure (Ahlfeld et al., 2015). Similarly, 99.99% of norovirus inactivation was observed after the cold plasma treatment using argon gas plasma jet after 2 min of treatment (Aboubakr et al., 2015). However, the addition of 1% O_2 to argon gas has increased the reduction of norovirus by more than 6 $\log_{10}$ within 15 s of treatment. This shows the increase in O_2 concentration in feed gas composition can attribute higher inactivation efficiencies due to the formation of higher ROS densities. Aboubakr et al. (2018) analyzed the capsid integrity of calicivirus after the cold plasma treatment under a transmission electron microscope (TEM), the images showed debris and broken pieces of virus capsid within 15 s of exposure to argon and oxygen gas plasma.

The work conducted by Bae et al. (2015) to inactivate the Hepatitis A virus on fresh meat products using an atmospheric pressure plasma jet showed more than a $\log_{10}$ reduction in HAV without a significant change in meat quality (surface color, moisture content, and lipid oxidation were analyzed). The processing parameters (voltage: 3.5 kV, feed gas: N_2 [99.9%], and treatment time: 5 min) applied reduced the 1.45 $\log_{10}$, 1.49 $\log_{10}$, 1.47_{10} log on beef, pork, and chicken surfaces respectively. DBD generated cold plasma at 30 kV reduced the Hepatitis B viral DNA from 1.33×10^7 IU/ml to 0.74×10^2 IU/ml when applied to serum samples for 40 s of exposure attributing to a total 5 $\log_{10}$ reduction in viral DNA (Shi et al., 2012). Similarly, Alekseev, Donovan, Limonnik, and Azizkhan-Clifford (2014) found 90% of inhibition in Herpes simplex virus type-1 genome replication within 40 s of exposure to DBD atmospheric plasma. Human Adenovirus inactivation was carried out by exposing virus medium suspension to atmospheric cold argon gas plasma for various treating times from 30 to 150 s (Bunz, Mese, Zhang, Piwowarczyk, & Ehrhardt, 2018). The above authors have compared the cold plasma Adenovirus inhibition by using virus transduction efficiencies expressed as luciferase activity. After 120 s exposure to plasma, there is a 40% decrease in the luciferase enzyme activity but there is no significant difference exists among different exposure times and Adenoviruses types. Finally, the authors have drawn a conclusion that the cold plasma inhibition of Adenoviruses is species type-dependent. Sakudo et al. (2016) exposed the Adenoviruses to the short high voltage pulses of nitrogen gas plasma and observed a 1 $\log_{10}$ reduction in less than a minute. The virucidal effect of N_2 plasma is due to the formation of reactive species like nitrate and nitrite are effective in destroying the viral genomic DNA. Similarly, a 6 $\log_{10}$ decimal reduction in Adenovirus after treating for 240 s was reported by Zimmermann et al. (2011) in a solution that is activated by atmospheric cold plasma.

SARS-CoV-2 was inactivated using the atmospheric pressure plasma jet as decontamination technology on different surfaces by Chen, Garcia, et al. (2020); Chen, Xu, et al. (2020). Argon gas cold plasma treatment has inactivated coronavirus-2 on the different surfaces within 180 s. However, the authors found that the time of inactivation greatly depends on the surface topography of the exposed

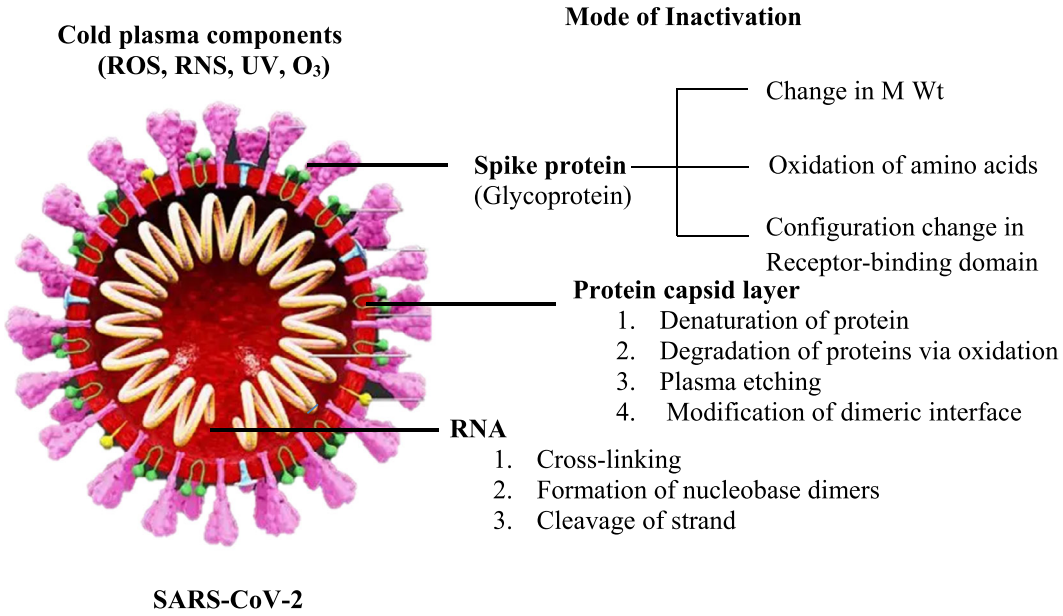

FIGURE 2 Cold plasma inactivation mechanism of SARS-CoV-2

surfaces. Capelli et al. (2021) observed complete inactivation of SARS-CoV-2 RNA genome after 10 min of exposure to DBD plasma treatment. Guo *et al*. (2021) activated deionized water for 5 and 10 min to generate plasma activated water and treated the SARS-CoV-2 proteins in a pseudovirus as a model to measure antiviral activity. From the results of the above investigation, the authors have postulated that PAW activated at lower treatment time resulted in slight morphological change whereas the longer activated water showed virus aggregates for large complexes. Chen and Wirz (2020) reported that the cold plasma antiviral mechanism on SARS-CoV-2 is due to surface wall disruption due to etching, damage to genetic material and denaturation of proteins. The other mechanism for SARS-CoV-2 inactivation by cold plasma was reported by Guo et al. (2020). The interaction of reactive species of plasma and SARS-CoV-2 resulted in the modification of receptor-binding domain (RBD) located on the S-spike protein making it unable to attach to ACE2 receptors restricting the viral entry into the host cells. From the results of Liu *et al*. (2021) on COVID-19 inactivation by e-beam irradiation, it was observed maximum viral destruction occurred at irradiation electron energy of 2 keV. The authors reported that the interaction of electrons and virion resulted in total damage of protein envelope layer rather the spike proteins and genetic material of SARS-CoV-2. The mechanism of cold plasma inactivation of SARS-CoV-2 is shown in Figure 2.

4 | CONCLUSION AND FUTURE PROSPECTS

In recent times, we have encountered many new foodborne pathogens outbreaks. For instance, the recent outbreak of novel coronavirus resulted in pandemics. The detection of viruses in food samples is a challenging task that requires robust and precise methodology. However, the advances in molecular-based quantification methods like RT-qPCR made it easy to interpret the test results. Some of the enteric viruses like norovirus, Hepatitis A virus that survived the common food processing operations generally followed for food preservation. Heating to high temperature could possibly kill all the viruses but it greatly affects the nutritional and sensory attributes. Cold plasma is an alternative technology that shows great antimicrobial activity. The reactive species (ROS and RNS) formed in plasma play an important role in viral destruction along with UV light and ozone. The destruction of the viral capsid protein layer is the main antiviral mechanism of cold plasma treatment. The damage to the genetic material, amino acids oxidation and oxidation of lipids present in envelope layer are the other important antiviral mechanism. However, the reactive species formed depends on the mode of application (direct or indirectly through plasma-activated liquids), nature of plasma reactor, feed gas type and composition, voltage and power applied, and exposure time. These parameters have to be optimized to achieve better inactivation efficiencies. Apart from the microbial inactivation, the food quality and sensory attributes of the treated samples need to be assessed.

As the cold plasma research in foods is increasing day-to-day, there is an immediate requirement to get approval from the regulatory agencies for commercialization. However, there is a need to work in the direction of standardizing the operating conditions as the efficiency of treatment varies between the plasma sources and method of applications. A point of concern has to be addressed in the occurrence of toxicity and mutagenicity of the cold plasma treatment. However, few scientific evidences published have shown the cold plasma treatment is safe to apply to different food products like fresh produce like fruits and vegetables, minimally processed foods, meat and milk products *etc*. There should be further research need to be focused on the scaling-up studies. Bourke, Ziuzina, Boehm, Cullen, and Keener (2018) commented that to get the approval for a new

technology like cold plasma, an application has to be directed to Food and Drug Administration (FDA, USA) through the Food Contact Notification (FCN) Program and also needs to get approval from Environment Protection Act (EPA), United State Agricultural Department (USDA) and the Food Safety Inspection Services (FSIS). Like other nonthermal food processing technologies like HPP, PEF, UV light and ultrasonication that have been approved by FDA and commercially available, we anticipate that cold plasma processing will get approval from the regulatory agencies within a short time as a chemical-free and environmentally friendly technology.

AUTHOR CONTRIBUTIONS

Rohit Thirumdas involved in Investigation, Conceptualization, and Writing.

CONFLICT OF INTEREST

None.

DATA AVAILABILITY STATEMENT

Data sharing not applicable to this article as no datasets were generated or analysed during the current study.

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