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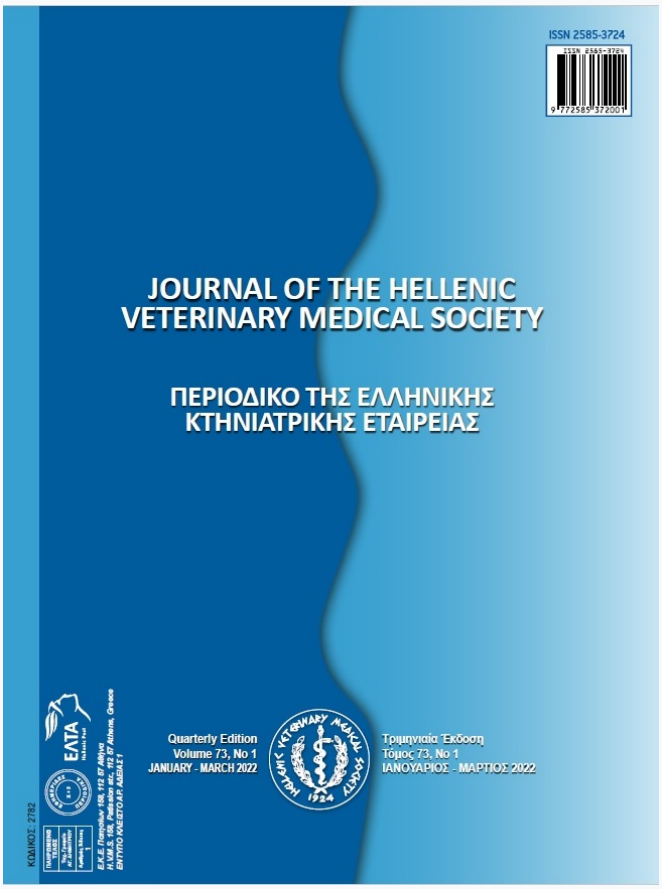

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Review article
Ανασκόπηση

Inactivation of foodborne viruses by the cold plasma technology

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ABSTRACT: Cold plasma (CP) is an innovative non-thermal food processing method. CP refers to a partially or completely ionized gas containing reactive chemical species, which are active against microorganisms, including viruses or enzymes of foods. CP has a minimal effect on the quality attributes of foods and can also elongate the shelf life of foods. Foodborne outbreaks caused by viruses have been increased in various countries in recent years. The research works on the inactivation effect of CP against viruses including foodborne viruses have been also increased in recent years. The most important foodborne viruses are human norovirus (HuNoV) and hepatitis A virus (HAV), involved in several outbreaks worldwide. Human astrovirus (HAstV), human adenovirus (HuAdV), Aichi virus (AiV), sapovirus (SaV) and enterovirus (EV) are also notable foodborne viruses and were associated in sporadic cases. The CP treatment proved efficient for the inactivation of foodborne viruses such as HuNoV and HAV. The present work reviews the CP as a non-thermal food processing technology and present the published data on the effect of CP process on foodborne viruses in foods.

***Keywords*:** Cold plasma, foodborne viruses, novel food processing technologies

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INTRODUCTION

In recent years, foodborne virus outbreaks have increased worldwide and have become an important concern for health authorities (Pexara and Govaris, 2020). Among recorded foodborne outbreaks, those caused by viruses reached 20.4% and 45% in EU (in 2014) and USA (in 2018), respectively (Yeargin and Gibson, 2019).

Human norovirus (HuNoV) and hepatitis A virus (HAV) are the most common etiological agents of recorded foodborne virus outbreaks worldwide (Yeargin and Gibson 2019). HuNoV has been also found as the leading cause of foodborne illness worldwide, with the highest range of deaths and highest incidence in children of less than 5 years old (Pires et al., 2015). HAV has been also found responsible for ca 5% of recorded foodborne outbreaks, and is the leading cause of viral hepatitis, with 1.4 million of new cases annually (Adefisoye et al., 2016).

Although hepatitis E virus (HEV) is primarily transmitted by contaminated water, it can also cause infections by the consumption of contaminated food, particularly undercooked meat or meat products. HEV has been associated with sporadic cases and small outbreaks, but it is characterized by severe symptoms in infected individuals (King et al., 2018).Other enteric viruses such as human rotavirus (HRV), human astrovirus (HAstV), human adenovirus (HuAdV), Aichi virus (AiV), sapovirus (SaV) and enterovirus (EV) are also notable foodborne viruses, causing sporadic outbreaks worldwide (Pexara and Govaris, 2020).

In foods, foodborne viruses are typically highly stable and can survive for a long time without any loss of infectivity (Sánchez and Bosch, 2016). In addition, traditional preservation methods such as drying, acidification or salting may not be efficient to inactivate foodborne viruses (Sánchez and Bosch, 2016). Thermal processing is an effective traditional method for virus inactivation since viruses are effectively killed at temperatures higher than 80 °C (Zhang et al., 2019). However, thermal processing can also change the physicochemical and organoleptic properties of foods and cause a reduction of the food quality characteristics (Aadil et al., 2019).

Since consumers demand high quality and safe foods, food industry tends to explore alternative preservation methods and replace the traditional processing methods of foods (Petrescu et al., 2020). Amongst novel, non-thermal food processing technologies is the cold plasma (CP) method.

CP is generated, when an electrical energy source is applied to a gas, resulting in the production of several reactive species such as ultraviolet photons, charged particles, radicals and other reactive species of nitrogen, oxygen, and hydrogen. CP has been found effective to inactivate enzyme and microorganisms and remove toxins or pesticide compounds in foods (Varilla et al., 2020). Although that most CP studies were initially focused on the inactivation of foodborne bacteria, recent studies on the effect of this technology on the inactivation of foodborne viruses has been showing promising results. This article reviews the CP as a non-thermal food processing technology and focuses on its effectiveness on foodborne viruses' inactivation.

COLD PLASMA (CP)

Plasma is the fourth state of matter. The term "plasma" was initially coined in 1927 by Irving Langmuir, a Nobel Prize awarded chemist from USA (Rajvanshi, 2008). CP refers to a partially or completely ionized gas containing reactive chemical species such as ions, electrons, neutral molecules, atoms, and charged species. The free electric charges (electrons and ions) make plasma electrically conductive, internally interactive, and strongly responsive to electromagnetic fields (Pignata et al., 2017).Generally, the two types of plasma are thermal or equilibrium and cold plasma. In thermal or equilibrium plasma, all particles have roughly the same temperature (average kinetic energy of random motion). In cold plasma (CP), the light electrons have much higher temperatures compared to heavy atoms and molecules, which often remain close to room temperature (Filipić et al., 2020). CP can be further classified into low pressure, which is also recognized as vacuum plasma, and atmospheric pressure plasma (Filipić et al., 2020). Typically, CP is generated at 1 atmospheric pressure with electron temperatures generally between 1 and 10 eV (Misra and Jo, 2017).

Various apparatus types have been used for the generation of CP such as corona glow discharges, dielectric barrier discharges, radio frequencies, gliding arc discharges, atmospheric glow discharges, inductively coupled plasmas and microwave induced plasmas (Guo et al., 2015).The most important active species generated by plasma discharge are neutral or excited molecules and atoms, UV photons, negative and positive ions, free radicals and electrons. The

presence of these active agents depends also on the plasma source, but the majority of reactive species are vibrationally and electronically excited oxygen and nitrogen, reactive oxygen species (ROS) such as singlet oxygen $^1O_2$, atomic oxygen O, superoxide oxygen O_{2-} and ozone O_3, reactive nitrogen species (RNS) such as atomic nitrogen N, nitrogen dioxide ($\bullet NO_2$), nitric oxide ($\bullet NO$), or peroxynitrite ($ONOO^-$). Also, if humidity is high, electrically charged components such as OH^- anion, H_2O^+, OH• radical, or H_2O_2 are present (Scholtz et al. 2015). The antimicrobial activity of CP is due to these active compounds (López et al., 2019).

CP has been examined by several scientists in recent years and has been used in different scientific fields such as medicine, agriculture, environmental protection and food industry due its ability to inactivate microorganisms as viruses, bacteria, spores, yeast or fungi (Niedźwiedź et al., 2019). According to recent research data, the plasma activated solutions (PASs) of H_2O, NaCl, and H_2O_2 are effective against microorganisms found in foods (Pignata et al., 2017).

Foodborne bacteria inactivation by CP

The antibacterial activity of CP has been examined by several researchers in recent years (Niedźwiedź et al., 2019).CP has been also found to possess antibacterial activities against major foodborne pathogens, such as *Listeria monocytogenes*, *Salmonella Typhimurium*, *Escherichia coli* O157:H7, *Campylobacter jejuni*, and *Salmonella* spp. (Leeet al., 2017).CP is active against foodborne bacteria at ambient temperature with no additional heat treatment (Varilla et al., 2020). CP is also able to inactivate different spores of spore forming bacteria, as reported by several workers (Varilla et al., 2020). The microbicidal properties of CP against foodborne bacteria have been found in various foods such as meat and meat products, eggs and eggshell, vegetables, fruits or spices (Apostol et al., 2015; Leeet at al., 2017; Pignata et al., 2017).

The inactivation of bacteria by CP is achieved through three modes of action: reactions between CP reactive species and positive or negative charge compounds of bacteria, the destruction of the membranes and the cell functions of bacteria, and destruction of or alteration DNA or RNA of bacteria (Moisanet al., 2002).

Effect of CP treatment on food quality

The effects of the CP treatment on the quality characteristics of foods have been extensively investigated (Surowsky et al., 2015; Misra et al., 2016; Pankaj et al., 2018). Among quality characteristics, the color of CP treated foods was examined in several studies. The color changes of meat, meat products, eggs, fruits and vegetables was dependent on CP treatment conditions (input voltage, time, power, working gas) (Bae et al., 2015; Surowsky et al., 2015; Pankaj et al., 2018; Roh et al., 2020). The product type (solid or liquid) and storage conditions are critical factors affecting the color. Overall, it is generally accepted that CP processing has a minimal effect on the color of food products at lower treatment times (Thirumdas et al., 2016; Pankaj et al., 2018). Yong et al. (2017) reported a similar red color in a pork jerky treated by CP to this achieved by supplementing sodium nitrite.

According to several studies, the CP treatment resulted in pH changes of food products (Fröhling et al., 2012; Kim et al., 2013; Almeida et al., 2015; Lee et al., 2016). CP buffering capacity can affect pH of CP treated foods (Pankaj et al., 2018).

CP can also adversely affect the quality properties of foods and should be properly applied to avoid a loss of food quality. CP treatment can enhance lipid oxidation in foods rich in lipid components. The CP ROS could interact with food lipids and initiate the oxidation process, especially when foods rich in unsaturated fatty acids are treated eg fish (Gavahian et al., 2018). However, conflicting results have been published on the lipid oxidation of fish, meat or dairy products caused by CP treatment (Bae et al., 2015; Pankaj et al. 2018). CP can also result in degradation of polymerized oligosaccharides in juices and the mechanism of this degradation should be examined in future studies (Almeida et al., 2015; Varilla et al., 2020).

The food enzymes, which can deteriorate the quality attributes of foods, are susceptible to degradation during CP treatment (Han et al., 2019). Food enzymes such as polyphenoloxidase, peroxidase, superoxide dismutase and lysozyme were also inactivated by CP treatment (Attri et al., 2015; Misra et al. 2016; Han et al., 2019). During CP treatment sensitive vitamins, such as riboflavin (B2), pyridoxine (B6), biotin or vitamin C, are usually stable during CP treatment (Pankaj et al. 2018). The structure of allergens, which are protein components, may be changed by reactive species produced by CP, while further studies are re-

quired to elucidate this beneficial effect of CP treatment (Gavahian and Khaneghah, 2020).

According to European Union regulations, CP treatment has been approved as an electronic preservative practice for organic foods (EC, 2014).

CP EFFECT ON FOODBORNE VIRUSES

Foodborne virus inactivation by CP

Published data on the effect of CP treatment on foodborne viruses are presented in Table 1. CP treatment resulted in a successful decrease of enteric viruses and their surrogates in aqueous solutions (Aboubakr et al., 2016; Aboubakr et al., 2018; Nayak et al., 2018), liquid media (Takamatsu et al., 2015), but also on food surfaces (Bae et al., 2015; Min et al., 2016; Lacombe, 2017; Aboubakr et al., 2020; Roh et al., 2020).

Table 1. Inactivation of foodborne viruses/surrogates in CP treatment

Virus/ surrogatestested*	Plasma Type	Plasma treatment parameters	Matrix	Reduction	References
HuNoV (GII.4)	Microdischarge	1 kHz/8.5 kV/ 30 mW/cm$_2$/Air/ 0.5, 1, 2, 3, 4, 5, 10, or 15 min	Medium	1.23 log genomic equivalents/ml after 10 min, 1.69 log genomic equivalents/ml after 15 min	Ahlfeld et al. (2015)
	A two-dimensional array of integrated coaxial microhollow dielectric barrier discharge	20 Hz/10.8 kV/ 14.5 W/ 16.4 slm air flow rate/ 10 cm exposure distance, wet-surface treatment	Romaine lettuce	~2.6 log$_{10}$ in genome copy number after 5 min	Aboubakr et al. (2020)
HAV	Atmospheric pressure plasma jet	28.5 kHz/3.5 kV/ N2 (99.9%) / 6 slm flow rate flow/ 10 s-20 min	Fresh meats (beef loin, pork shoulder and chicken breast)	90% reduction (1 log$_{10}$ PFU/ml) after 5 min	Bae et al. (2015)
FCV	Plasma jet	16 Hz/9 kV/ 10 W/ various gas species, including Ar, O_2, N_2, CO_2 mock air, and mixtures of these gases/ gas flow rate 1 L/min	Medium	>6 log TCID50/10 µl within 5 min (CO_2), >6 log TCID50/10 µl within 3 min (N_2)	Takamatsu et al. (2015)
	Radio frequency atmospheric pressure plasma	20 kHz/2.5W/, different gas mixtures (Ar, Ar + 1% O_2, Ar + 1% air, Ar + 0.27% H_2O) / 1.5 slm flow rate	Distilled water, Liquid medium	> 6.5 log$_{10}$ TCID50/0.1 ml after 15 s (Ar + 1% O_2 plasma), after 30 s (Ar and Ar + 1%)	Aboubakr et al. (2016)
	Plasma jet	20 kHz/2.5W/99% Ar + 1% O_2/1.5 slm flow rate/15, 30, 60, and 120 s/ distance 11.25 mm	Distilled water	>6.5 log$_{10}$ TCID50/100µl after 15 s	Aboubakr et al. (2018)
	A two-dimensional array of integrated coaxial microhollowdelectric barrier discharge	20 Hz/10.8 kV/ 14.5 W; Dry Air, Ar + 20% O_2/16.4 slm flow rate	Distilled water	>5 log$_{10}$TCID50/100µl after 3 min (dry air)	Nayak et al. (2018)

	A two-dimensional array of integrated coaxial microhollow dielectric barrier discharge	20 Hz/10.8 kV/ 14.5 W/ 16.4 slm air flow rate/ 10 cm exposure distance, wet-surface treatment	Romaine lettuce	>5 log_{10}TCID50 after 3 min	Aboubakr et al. (2020)
MNV-1	Atmospheric pressure plasma jet	28.5 kHz/3.5 kV/ N_2 (99.9%) / 6 slm flow rate flow/ 10 s-20 min	Fresh meats (beef loin, pork shoulder and chicken breast)	99% reduction (2 log_{10} PFU/mL) after 5 min	Bae et al. (2015)
	Atmospheric pressure plasma jet	47 kHz/549 W/Air	Blueberries	5 log PFU/g after 90 s	Lacombe et al. (2017)
TV	Dielectric barrier discharge	47.6 kV and 1 A for 5min/electrode distancewas 30 mm	Romaine lettuce	1.3 log PFU/g after 5 min (rigid package)	Min et al. (2016)
	Atmospheric pressure plasma jet	47 kHz/549 W/Air	Blueberries	3.5 log PFU/g after 120 s	Lacombe et al. (2017)
	Atmospheric dielectric barrier discharge	32 kV, 3.5 min/Air In-package	Chicken breast	2.2 PFU/cube after 3.5 min,	Roh et al. (2020)
CVA7	Plasma jet	16 Hz/9 kV/ 10 W/ various gas species, including Ar, O_2, N_2, CO_2 mock air, and mixtures of these gases/ gas flow rate 1 L/min	Medium	<2 log TCID50/10 µl within 10 min (CO_2) >6 log TCID50/10 µl within 10 min (N_2)	Takamatsu et al. (2015)
MS2	Plasma jet	20 kHz/6 kV/gas mixture of 0.0-1.0% and 100.0-99.0% helium/ 2 slm flow rate, 1- 9 min/distance 10 mm	Medium	3 log_{10} after 5 min, >7 log_{10} reduction after 9 min. (helium/oxygen: 99.25%/ 0.75%)	Alshraiedeh et al. (2013)
	Dielectric barrier discharge	30 V/20, 24, 28 W/ Air, 98% Ar + 2% O_2, 98% He + 2% O_2/ flow rate 2.5 liters/ min/ for up to 3 min	Distilled water	>0.69 log PFU/m^3 for all tested gas carriers and power levels for > 30 s	Wu et al. (2015)

* HuNoV, Human noroviruses; HAV, Hepatitis A virus; FCV, Feline calicivirus; MNV-1, Murine norovirus 1; TV, Tulane virus; CVA7, Coxsackie virus; MS2, bacteriophage MS2.

The majority of CP treatment studies was focused on the susceptibility of HuNoV since this virus has been associated with several food outbreaks and is a major problem in the food industry (Pexara and Govaris, 2020). A CP treatment for 5 min showed a reduction of 99% (2 log PFU/ml) of MNV-1, a surrogate of HuNoV, and 90% reduction (1 log PFU/ml) of HAV in fresh meat (beef loin, pork shoulder and chicken breast) (Bae et al., 2015). Ahlfeld et al. (2015) reported that HuNoVwas decreased by 1.23 log and 1.69 log in fecal samples treated by CP for 10 and 15 min, respectively. Alshraiedeh et al. (2013) studied the effect of CP on bacteriophage MS2, a surrogate for HuNoV, ingases (0-1% oxygen in helium) for the plasma generation. Overall, the highest reduction of MS2 was observed for treatments with 0.75% oxy-

gen, while MS2 was reduced by 4 log and 7 log, after 5min and 9 min treatments time, respectively. Aboubakr et al. (2015) reported a6 log reduction of Felinecalicivirus (FCV), a surrogate for HuNoV, with CP plasma generated in 1% oxygen for 90 s. In a CP treatment on blueberries at refrigerated temperature for 2 min, MNV-1 and Tulane virus (TV), two surrogates of HuNoV, were reduced by 3.5 log and 5 log PFU/g, respectively (Lacombe et al., 2017). Aboubakr et al. (2020) reported that CP treatment on stainless-steel surface and lettuce leaves caused almost the same reduction of HuNoV (ca 2.6 log) on both surfaces. The FCV on a stainless-steel surface was completely inactivated by CP treatment after 3 min (Nayak et al., 2018).Takamatsu et al. (2015 reported the inactivation of FCV in a nitrogen CP treatment in contrast to Coxsackie virus (CVA7) in laboratory media (Vero cells).

Mechanisms of foodborne virus inactivation by CP

The specific mechanisms of viruses' inactivation by CP have not yet been elucidated. The studies carried out so far, demonstrated that exposure to CP can lead to the modification or degradation of proteins and lipids of viral envelopes as well as nucleic acids of viruses (Pradeep and Chulkyoon, 2016).

The virucidal activity of CP is particularly affected by formed ROS and RNS. However, the formation of virucidal reactive agents vary and are highly dependent on the experimental conditions, such as the gas for the CP generation, the food matrix, or the virus type (Pignata et al., 2017).Among reactive species, UV radiation and charged particles (e.g. ions, electrons) may also have an important role in the inactivation of viruses by the CP treatment (Filipić et al., 2020).

According to recent studies, $^1O_2$ was the most significant ROS for the inactivation of FCV (Aboubakr et al., 2016; Aboubakr et al., 2018, Yamashiro et al., 2018) and phage T4 (Guo et al., 2018). Aboubakr et al. (2018) reported that $^1O_2$ causes oxidative modification of histidine residues and a shift in molecular mass of methionine residues. Guo et al. (2018) revealed that O_2 also reacts with cysteine, tyrosine, and tryptophan, and oxidizes guanine. The O_3 was one of the most important factors (Nayak et al., 2018) or an additional factor in FCV inactivation (Aboubakr et al., 2016; Aboubakr et al., 2018). The O_3 may also have a role in the inactivation of bacteriophages of MS2 (Xia et al., 2019) and HuAdV (Zimmermann et al., 2011).H_2O_2had a secondary role in inactivation of HuAdV (Sakudo et al., 2016) and FCV (Aboubakr et al., 2016). RNS such as ONOOH (in an acidic environment) (Aboubakr et al., 2016; Yamashiro et al. 2018), $ONOO^-$ (Yamashiro et al., 2018), or NOx (Nayak et al. 2018) have been found as the principal inactivation factors for the FCV surrogate. In future, the development of accurate methods for activity measurement of RONS and UV intensity will clarify viral inactivation by CP treatment in a better manner (Filipić et al., 2020).

The CP reactive species can also react with the capsid protein, leading to protein peroxidation and destruction of the virus. According to previous studies the damage of the capsid was the most important factor for the inactivation of bacteriophages λ (Yasuda et al., 2010), MS2 (Wu et al., 2015), T4 (Guo et al., 2018), and FCV (Aboubakr et al., 2018). The reactive species can damage or eliminate the viral nucleic acid and reduce gene expression (Pignata et al., 2017). The nucleic acid degradation was proved as an important mode of HuAdV inactivation (Sakudo et al., 2016). Yamashiro et al. (2018) reported that nucleic acid degradation was found to a significant factor for FCV inactivation. The damage of DNA in parallel to viral protein degradations are also responsible for foodborne viruses' inactivation by the CP treatment (Pignata et al., 2017).Additional studies are needed to clarify the mechanisms of viruses' inactivation by CP (Liao et al., 2017).

CONCLUSIONS

CP is an innovative non-thermal food processing method which has a minimal effect on the quality characteristics of foods and can also elongate the shelf life of foods. Several studies indicated the inhibition or inactivation activities against foodborne viruses of the CP process in foods. The CP treatment proved efficient for the inactivation of HuNoV and HAV, which are the major viraletiological agents of food-borne outbreaks worldwide.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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