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REVIEW PAPER

ENHANCEMENT OF FOOD SAFETY – ANTIMICROBIAL EFFECTIVENESS OF COLD PLASMA TREATMENTS

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Cold plasma treatment proved to be a flexible, efficient, chemical-free antimicrobial process and it can represent an easy to use sanitizing method for the food industry that does not require special temperature, humidity or pressure conditions. This paper reviews the classification of plasma and the main cold plasma generating devices used in the recent years to enhance food safety. A research of available literature was also conducted to identify the antimicrobial mode of action of cold plasma treatment as well as advantages and key limitations of this technique when applied to different food products such as fruits, vegetables, meat and milk. The study revealed that further development of this method will have to be carried out, allowing better understanding of the complex interactions during applications and its restrictions, as well as practice outlook.

Keywords: cold plasma; antimicrobial treatment; food safety

1. Introduction to cold plasma

In recent years, the need for enhancing food safety, quality and shelf life without affecting the nutritional and sensory attributes of foods has increased the interest in chemical-free and low temperatures technologies for food preservation. Different innovative preservation methods, suitable for this type of commodities have been studied and tested, such as the use of electrolysed water (Izummi, 1999; Koseki *et al.*, 2001; Restaino *et al.*, 1995), UV radiations (Bintsis *et al.*, 2000; Jun *et al.*, 2003; Yaun *et al.*, 2004) or ozone (Olmez and Akbas, 2009; Graham, 1997; Kim *et al.*, 1999), modified atmosphere packaging (Farbert, 1991; Oliveira *et al.*, 2010) or the use of natural essential oils (Burt, 2004; Martín-Diana *et al.*, 2008; Mexis, 2009; Gutierrez *et al.*, 2009). Remodelling the already applied methods and ascertaining innovative solutions to improve the food safety and to extend the shelf life of different commodities focus on the development and assessment of an

alternative and emerging antimicrobial technique, i.e. cold atmospheric plasma treatment. The present review aims at tackling principles of action, applications and some recent results obtained with this innovative technology.

Although plasma is practically considered an energetic form of gas, it is actually a distinct state of the matter (Niemira and Sites, 2008). Defined as the fourth state of the matter, plasma is formed by adding energy to a gas till when the intra-atomic structures of its components break, generating a static system consisting of a very large number of particles, presenting neutral electronic and photonic components and characterized by a collective behaviour of its free charge carriers (Laroussi *et al.*, 2006; Park *et al.*, 2008; Critzer *et al.*, 2007; Niemira, 2012a).

Plasma is created naturally or artificially and can exist in various forms. In nature, it is found as the *aurora borealis* or it might appear for a short while during lightings or thunders. Regarding the plasma state, several temperatures could be defined, associated to numerous forms under which energy is absorbed by the system. Generally, plasmas could be divided into cold (low temperature plasmas) and hot plasmas (high temperature plasmas), where the temperature of heavy particles, neutrons and ions, is considered. In turn, low temperature plasmas are divided in thermal and non-thermal plasmas. In non-thermal plasmas, also called non-equilibrium plasmas, the gas remains at low temperature, because the energy transfer from electrons is less effective than cooling of ions and uncharged molecules, while in thermal plasmas, the energy is sufficiently high that all the particles are in equilibrium and the temperature of heavy particles becomes almost equal to the electron temperature (Fridman *et al.*, 2008; Niemira, 2012a; Laroussi, 2005). According to the nature of the dominant field which supplies energy for creating plasma, there are electric, magnetic and electromagnetic plasmas. Non-thermal plasmas may be produced by a variety of electrical discharges at different pressure levels. Here we can identify low-pressure plasmas ($p<1Pa$), moderate pressure plasmas ($p\approx100Pa$) and atmospheric pressure plasmas.

2. Cold plasma generating devices

Cold plasma generating devices may vary a lot and there are numerous plasma jet devices that have been described in literature (Brandenburg *et al.*, 2007; Critzer *et al.*, 2007; Deng *et al.*, 2008; Laroussi, 1996; Niemira and Sites, 2008; Park *et al.*, 2008; Schütze *et al.*, 1998; Weltmann *et al.*, 2008). Moreover, the diversity of the devices and their complexity can make the evaluation process of their potential quite difficult (Moreau *et al.*, 2005). Besides the working gas, many differences in operation parameters have to be taken into account for a comparison of these plasma sources. Atmospheric pressure plasma is commonly generated in the kHz regime by corona discharge (CD) or dielectric barrier discharge (DBD), in the RF regime by inductive coupled plasma (ICP) or atmospheric pressure plasma jet (APPJ) and in the microwave regime by plasma torches (Fröhling *et al.*, 2012).

One atmosphere uniform glow discharge plasma (OAUGDP) was used to inactivate microorganisms such as *Escherichia coli* O157:H7, *Salmonella* spp. or *Listeria* sp. inoculated on different types of surfaces or commodities. Critzer *et al.*

(2007) used this kind of plasma generating system to expose fresh fruits and vegetables to antimicrobial active species produced in the OAUGDP exhaust. Kelly-Wintenberg *et al.* (1998) also assessed the sterilization capabilities of an OAUGDP system by treating solid surfaces, fabrics or filter paper inoculated with *Staphylococcus aureus*, *Escherichia coli* or endospores of *Bacillus stearothermophilus* and *Bacillus subtilis*. An OAUGDP blower exposure unit can operate at radio frequency using air or other gas. It produces uniform, steady state glow discharge plasma inside a tubular configuration that allows the airflow to pass through the electrodes. The airflow can be maintained inside the chamber in order to promote plasma uniformly. There are an interior and an exterior electrode, and the dielectric of the last one establishes the plasma volume. Both electrodes are cooled using recirculated oil and a cold-water radiator, mounted onto the bottom of the device, which allows the exhaust to be maintained at a uniform temperature, around 25°C. The treated samples are placed in a rectangular chamber, which is placed onto the radiator. This type of device creates uniform or diffuse glow discharge plasma at atmospheric pressure and room temperature, without using a vacuum system and the optimum uniform glow discharge plasma can be obtained by adjusting the RF frequency or the RMS voltage. Another type of atmospheric glow discharge system is represented by an atmospheric dielectric-barrier discharge tube jet (DBD-tube jet). It consists of a dielectric tube wrapped with a metallic strip as a powered electrode and a sample holder, which plays the role of the ground electrode. The plasma plume is produced from a gas flow through the two parallel-plate electrodes (Deng *et al.*, 2007). The same device, but this time consisting of a quartz condenser tube, with a copper rod as an internal electrode and using sodium chloride (NaCl) solution in the outer layer as the external electrode was used by Deng *et al.* (2008) in various plasma conditions to estimate the inactivation of microorganisms.

The atmospheric pressure plasma jet (APPJ) has been intensively investigated also, being a very promising plasma for various applications, such as biological decontamination (Lee *et al.*, 2011), treatment of surfaces (Weltmann *et al.*, 2008) or deposition and processing of thin films (Xiong *et al.*, 2008). Recently, this device has also been applied on fresh commodities, in order to prevent microbial growth and enhance their shelf life (Grzegorzewski *et al.*, 2009). According to Anghel *et al.* (2010), a wide range of frequencies of the electric field was covered by this type of device, from direct current (DC) to radio frequency (RF), or microwave, and a large variety of electrodes configuration was used. There are different designs of this device, but generally, a plasma jet consists of a nozzle made of ceramics. There are two electrodes: the powered electrode usually is mounted inside the nozzle (Brandenburg *et al.*, 2007; Fröhling *et al.*, 2012), or wrapped around the ceramic tube (Noriega *et al.*, 2011), being connected to the power supply, while the ground electrode can be placed near the outlet surrounding edge of the nozzle (Fröhling *et al.*, 2012), placed with a certain distance downstream of the nozzle (Noriega *et al.*, 2011), or the treated sample itself can take the place of the second electrode (Brandenburg *et al.*, 2007).The configuration of the device also includes a power supply and a gas supply. Typically, the used

plasma gases are Ar (Brandenburg *et al.*, 2007; Fröhling *et al.*, 2012; Weltmann *et al.*, 2008) and He (Lee *et al.*, 2011), or mixtures of them, but depending on the possible applications, small quantities of O_2, N_2 or simply atmospheric air can be added in order to produce active species (Lee *et al.*, 2011;). The gas flows between the electrodes, being ionized, and then it is ejected from the source (Fröhling *et al.*, 2012).

A gliding arc is one of the atmospheric pressure plasmas designed to operate in open air that have been used mostly for surface treatment. Because in the beginning, this type of treatment used to cause serious damages to the treated sample, Shiki *et al.* (2008) experimented a split gliding arc which was designed with a hole plate placed at the exit window in order to prevent any arc spot damages. Niemira and Sites (2008) used a gliding arc plasma generator system in order to outbreak strains of *Salmonella* Stanley and *Escherichia coli* O157:H7 inoculated on agar plates and apples. The system consisted of an AC power supply, represented by a centre taped customized ground transformer and a plasma emitter, which in this case was a custom-made modification of a gas-injected gliding arc system, respectively. The two components were connected by an external high-voltage-insulated cabling. The oxygen-free cooper electrodes were attached to the plasma emitter at its top and bottom. The plasma generated device used air as working gas.

3. Antimicrobial effectiveness of cold plasma treatment

Cold atmospheric plasma is a novel method that demonstrated its applicability as antimicrobial treatment. It is a sanitising method that uses electricity and carrier gases such as air, nitrogen, oxygen or argon. Because of the presence of free radicals, charged particles, photons, UV radiations and chemical reactive species, the antimicrobial effectiveness of cold plasma treatment can be beneficial to food producers and retailers, in order to extend the shelf life of fresh commodities and to maintain the food safety all along the food chain. Gaseous plasmas are mixtures of electrons, ions and free radical species, all capable of affecting the microorganisms (Chirokov *et al.*, 2005; Lerouge *et al.*, 2001; Perni *et al.*, 2008). When a gas passes through plasma, it becomes excited, ionized or dissociated by the collision between electrons or ions and the respective gas, which leads to forming new active species (Critzer *et al.*, 2007; Keener, 2008).

The generated plasma contains strong oxidizing agents such as atomic oxygen and ozone (Kelly-Wintenberg *et al.* 1998) as well as charged particles, photons, UV radiations and chemical reactive species (Fröhling *et al.*, 2012) that affect the integrity of the cellular membranes of microorganisms and generate the antimicrobial effect of the plasma treatment (Brandenburg *et al.*, 2007; Perni *et al.*, 2008). Depending on the plasma sources, process parameters and the used gases, the reactive species and their concentration may vary (Fröhling *et al.*, 2012) and therefore, there can result different inactivation kinetics between plasma devices or even when the same device is used. The atomic oxygen species are considered to be the most important in inactivating the microorganisms, due to the destruction

mechanism, consisting in the oxidation of the microbial constituents. Oxidation of amino acids and nucleic acids causes modifications leading to the death of the microorganism or to its damaging. Lipid membranes are the most affected by the oxygen reactive species due to their localization along the surface of the bacterial cell, which allows them to be easily attacked by such strong oxidizing agents (Critzer *et al.*, 2007). Using SEM images, electrophoresis experiments or inactivation kinetics, Deng *et al.* (2007) presented clear evidence of protein destruction after using a low-temperature atmospheric dielectric-barrier discharge jet on BSA protein-coated stainless steel surfaces, these results supporting the prospect of cold atmospheric plasma as a generic sterilization technique.

Transmission electron microscopy and spectrometric measurements confirmed the release of cellular constituents after plasma treatment was applied on *E. coli*, *S. aureus* or *P. aeruginosa*, while a bacterial virus was mentioned as the most difficult type of microorganism to kill (Kelly-Wintenberg *et al.*, 1998). Using a DBD plasma jet device, Deng *et al.* (2008) noted a total inactivation of over 7 log units in less than 5 s for an *E. coli* population that was exposed to the afterglow generated plasma. While using the same conditions, the author mentioned only one log unit reduction for *B. subtilis*.

The inactivation mechanism also depends on bacterium type and its differentiation state (Fröhling *et al.*, 2012). Theoretically, the Gram-positive microorganisms are more resistant to sterilization treatments than the Gram-negative ones, but when comparing the tolerance to the plasma treatment, Critzer *et al.* (2007) noticed that the results showed no significant differences. It was reported that *L. monocytogenes* was generally more sensitive to cold plasma treatment, but its resistance was not appreciated as weaker than the resistance of *E. coli* O157:H7 or *Salmonella* spp. Fröhling *et al.* (2012) used *L. innocua* and *E. coli* inoculated on a polysaccharide gel to compare the antimicrobial effectiveness of a non-thermal plasma jet driven by a RF generator with Ar as working gas. After 1 min treatment using a power of 10 W it was determined a less than 1 log unit reduction for both microorganisms, while the maximum antimicrobial effectiveness of more than 6 log units was achieved at a plasma operating power of 40 W after 2 min and 1.5 min, respectively. The most promising plasma operating power with real application to food surface decontamination was 20 W applied for 4 min, which led to a microbial reduction to the detection limit.

Antimicrobial plasma effects have also been tested by Brandenburg *et al.* (2007), by using polyethylene strips punctually contaminated with suspensions of *Escherichia coli* or *Bacillus atrophaeus* spores. In this case, a RF-plasma jet using argon as working gas stated a reduction factor of 3.8 log units after 7 min treatment for *E. coli*, while *B. atrophaeus* spore strips registered an increased time-dependent spore reduction up to 4.3 log units, after 11 min plasma treatment. Weltmann *et al.* (2008) used a plasma jet to treat *Staphylococcus aureus* inoculated catheters and he noticed better decontamination results when a small amount of oxygen gas (0.1 vol%) was added to argon. Furthermore, Ziuzina *et al.* (2013) noticed the inactivation effectiveness of dielectric barrier discharge atmospheric cold plasma

generated inside a sealed package for *Escherichia coli* ATCC 25922, the author underlining the advantage of this in-package disinfection approach in eliminating post-processing contamination. Kelly-Wintenberg *et al.* (1998) reported the effectiveness of an OAUGDP as a sterilization process. It was noted a distinct alteration in morphology of the remaining viable *E. coli* population after 5 s OAUGDP exposure, which suggests the mutant or injury effect of the applied treatment. Up to 10 log units reduction after 14 min treatment, using a N_2 and O_2 gas mixture, was obtained by Moreau at al. (2005) with a gliding arc cold plasma system on *Erwinia carotovora carotovora*, *Erwinia carotovora atroseptica* and *Erwinia chrysanthemi* inoculated on LB broth. Vlugels *et al.* (2005) observed that *Pantoea agglomerans* embedded inside biofilms is still amenable to inactivation after using atmospheric pressure discharges, this technique being superior to the use of low-pressure UV sources.

4. Cold plasma applications to foods

Plasma treatment, as any other treatment applied to fresh commodities, should not cause any level of discoloration, dehydration or affect in any way the sensorial and quality characteristics of the food products, so that it can become an acceptable technology for the food industry (Vlugels *et al.*, 2005; Schwabedissen *et al.*, 2007). Because cold plasma treatment is a waterless, contact-less, chemical-free method that can reduce and prevent microbial growing (Niemira, 2012b), it has successfully been applied to different food products in order to provide antimicrobial effectiveness (Barasan *et al.*, 2008; Fernández-Gutierrez *et al.*, 2010; Grzegorzewski *et al.*, 2010; Perni *et al.*, 2008).

Critzer *et al.* (2007), using an OAUGDP system determined its effect on inactivation of *Escherichia coli* O157:H7, *Salmonella* spp. and *Listeria* sp. inoculated on the surface of apples, cantaloupe and lettuce, respectively. It was observed a sharp decrease in the microbial population right after the plasma treatment was applied, followed by a decline in inactivation rate which was observed when longer treatments were applied. After 1 min plasma treatment, the *Escherichia coli* O157:H7 population inoculated on apple sections registered an approximate reduction of 2 log units, while *Salmonella* spp. inoculated on cantaloupe rinds was inactivated with more than 3 log units, respectively. *Listeria monocytogenes* inoculated on lettuce leaves registered only an approximate 1 log unit decrease, while the microbial population was eliminated after applying a 5 min plasma treatment.

Klockow and Keener (2008) studied the antimicrobial effectiveness of an atmospheric non-equilibrium plasma (ANEP) on inoculated (*E. coli* H157:O7) spinach leaves placed inside a polyethylene bag. Under refrigerated conditions, plasma treated samples showed a microbial reduction of 2.47 and 3.55 log units, using air and oxygen as working gases, respectively. The applied treatment method used maximum concentrations of ozone and nitrous oxides without causing the heating of the electrodes. Regarding the exterior aspect of the treated leaves, all samples showed discoloration and wilting effects compared to control samples.

These changes increased with longer storage times and with the use of oxygen as a working gas. Therefore, further investigations are needed in order to find out the optimal process parameters and to lessen quality degradation when ANEP treatment is applied to this type of commodity.

Applying a cold plasma treatment generated in a gliding arc, Niemira and Sites (2008) noted an effective inactivation of *Escherichia coli* O157:H7 and *Salmonella* Stanley both inoculated on apple surface. In this case, *Salmonella* Stanley was more effectively inactivated than *E. coli* and it showed a time-dependent reduction for all the flow rates that were used. Using 1, 2 and 3 min treatment times at a flow rate of 10, 20, 30 and 40 L/min, was observed that an increased duration of exposure to the plasma treatment and of the applied plasma flow rate led to an increased rate of the inactivated microorganisms. Treating the samples for 1 min at a flow rate of 10 L/min, it was obtained the lowest microbial inactivation, but the authors mentioned that each longer treatment increased significantly the inactivation. After 1 min plasma treatment at 40 L/min, *Salmonella* Stanley was reduced by 2.4 log units, while applying the same treatment for 3 min, it was registered a microbial reduction of 3.7 log units. *E. coli* O157:H7 registered a reduction of 3.4 log units after using a 40 L/min flow rate for 3 min.

Vlugels *et al.* (2005) studied the application of atmospheric pressure glow discharges to fresh food decontamination. Because the use of high concentration of reactive plasma species can affect the material integrity of food surface or may lead to a depletion of the nutrient content and textural qualities of the commodities, the effect of atmospheric pressure glow discharges on surface colour of bell peppers was assessed. The author noticed that the colour variation resulting from the plasma treatment was quite small and similar to what people can normally observe regarding the vegetables from the supermarket.

The *PlasmaLabel*^{TM} concept, based on atmospheric pressure dielectric barrier discharge was applied by Schwabedissen *et al.* (2007). It consists of three system components represented by package, the plasma exiting dielectric barrier discharge electrodes and the power supply and it was used as a conservation method for cherry tomatoes and strawberries. There was noted no sign of degenerated tomatoes on the ozone-treated samples after 14 days storage, the same result being obtained for the strawberries also, but at a shorter storage time.

Cold plasma reduction of *Salmonella* spp. and *E. coli* O157:H7 on almonds was also noticed by Niemira (2012b), using an AC plasma jet device based on a form of gliding arc plasma. In this case, longer treatment times did not always result in enhanced reductions, but using nitrogen as working gas increased the antimicrobial efficacy compared to dry air. The greatest *E. coli* O157:H7 reduction observed was of 1.34 log units after 20 s treatment at 6 cm spacing. Regarding the reduction of *Salmonella* PT30 inoculated on almonds, the 20 s showed also great result, at 6 cm but also 4 cm spacing, under air and nitrogen, respectively.

Listeria monocytogenes strains were selected by Lee *et al.* (2011) to inoculate slices of chicken breast and ham. An atmospheric pressure plasma jet was used to decontaminate the samples covered by a cone-shaped glass container. The device

used different working gases, such as He, He + O_2, N_2 and N_2 + O_2, respectively. He and N_2 (7 L/min) were used for discharging the plasma, and in order to increase its antimicrobial properties, O_2 (0.07 L/min) was added. The combination of N_2 + O_2 was by far the most effective, and there was noted a microbial reduction of 1.37-4.73 log units after 2 min plasma exposure on slices of chicken breast and 1.94-6.52 log units on ham, respectively. The study presented the potential of an APP jet to inactivate *L. monocytogenes* on different types of meat products and also to prolong their shelf life. Another atmospheric pressure plasma jet apparatus was used by Noriega *et al.* (2011) for decontaminating chicken skin and muscle inoculated with *Listeria innocua* and the results were compared to those of inoculated membrane filters. He (5 L/min) and O_2 (0.1 L/min) were the feeding gases of this device and higher values of AC voltage, excitation frequency and the presence of O_2 in the carrier gas resulted in the greatest inactivation efficiency. The effectiveness of the same plasma treatment decreased in the transition from membrane to chicken muscle and then to chicken skin, which suggests that the surface matrix plays a significant role in microbial inactivation using this type of treatment. A microwave plasma setup was used for indirect plasma treatment of fresh pork by Fröhling *et al.* (2012b), and a plasma exposure time of 2 x 2.5 min was noted to be sufficient to prolong the shelf life of porcine *musculus longissimus* (MLD) and to maintain its aerobic viable count at the detection limit of 10^2 CFU/g over the storage period of 20 days at 5 °C.

Shell eggs inoculated with *Salmonella enteritidis* and *Salmonella thyphimurium* were treated in a plasma after-glow chamber at atmospheric pressure using a resistive barrier discharge (RBD) (Ragni *et al.*, 2010). Different decontamination times and two relative humidity values (RH) of the gas mixture in the chamber were considered for this study. There was noted a microbial reduction of 1.0-1.6 log units/eggshell after 10-20 min plasma treatment, when BGA and TSA media were used, respectively. A maximum reduction of 2.2-2.5 log units/eggshell in *S. enteritidis* levels was obtain after 60-90 min plasma treatment at 35% RH, while a 65% RH and 90 min of plasma treatment enhanced the effectiveness of the treatment up to 3.8-4.5 log units/eggshell. Even if different inactivation dynamics were observed for the two microorganisms, *S. thyphimurium* was also more sensitive when higher RH values were performed and a reduction of 3.5 log units/eggshell was registered for 90 min treatment.

The time dependent efficiency of inactivating *E. coli* in milk with different fat content was investigated using atmospheric corona discharge plasma (Gurol *et al.*, 2012). It was found that a 3 min plasma treatment registered a 54% reduction in the population of *E. coli* colonies in different types of milk (whole, semi-skimmed and skimmed) without affecting the colour and pH properties of the product.

5. Further research needs and conclusions

Further development of cold plasma technology will have to be carried out, allowing a better understanding of the complex interactions during applications, such as food surface interactions, impact on food composition, optimisation of gas

composition and other processing parameters according to the treated sample. Also, additional information regarding food quality must be considered with respect to the cold plasma treatment, and changes concerning the nutrient content, toxic residues or textural qualities should be investigated.

Cold plasma treatment proved to be a flexible, efficient, chemical-free antimicrobial process and it can represent an easy to use sanitizing method for the food industry that does not require special temperature, humidity or pressure conditions. The application of a plasma treatment on different commodities represents a relatively new decontamination approach of this technology and more research studies are needed if it is to provide a commercial applicability for the food industry.

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