Review Paper

A STUDY ON COLD PLASMA FOR FOOD PRESERVATION

ABSTARCT:

Cold plasma is an electrically energized matter composed of highly reactive species includes molecules of charged and gas particles in the form of positive ions, negative ions, photons electrons, free radicals at room temperature. It is an emerging technology in non-thermal food preservation in the application of sterilization. An increase in the plasma based treatment for food to inactivate the food borne pathogens clearly seen in the recent years. The present study reviews the action of plasma agents on the microbial classes, surface decontamination of the raw produce in the food processing and novel technologies with future view in applications of food.

Key words: cold plasma, Food, preservation, sterilization.

Introduction

Matter on earth exists mostly in three distinct phases (gas, liquid and solid) but when universe is considered as fourth state of matter which abundantly exists. So, Plasma is hence referred to as the fourth state of matter, next to solids, liquids and gases. The term ‘Plasma’ was first employed by Irving Langmuir in 1928 to define this fourth state of matter which is partially or wholly ionized state of gas and discovered plasma oscillations in ionized gas. The phase change from solid to liquid and further to gas occurs as we increase the energy input likewise increasing the energy input beyond a certain level in gas state causes ionization of molecules which yields the plasma state. d Agostino et al. reported that plasma can be obtained either in low temperature, non-equilibrium glow discharge or high temperature, equilibrium thermal plasma.

From the properties of plasma, it is used in various fields such as textile, electronics, life sciences, packaging etc. *Roth et al.* Application of the plasma technology as a surface cleaning tool has been commercially adopted for the removal of disinfection chemicals applied to medical devices manufactured from heat sensitive plastics. Moisan et al. In the biomedical sector plasma technology issued for cold sterilization of instruments and prostheses as well as many thermo labile materials used in the biomedical technology sector for its particular advantages, including its moderate or negligible impact on substrate materials and use on nontoxic compounds. Conventionally, sterilization methods such as heat, chemical solutions are used for the surface disinfection of fruits, seeds, and spices etc., which are often time-consuming and damaging or have toxic residues. Van de Veen et al. reported that the effect of cold plasma on bacterial spores is more than the conventional techniques like heat, chemicals and UV treatment. One of the important challenges associated with cold plasma technology is ensuring high microbial inactivation while maintaining sensory qualities that ensure there fresh appearance

Conventional chemical treatments are familiar to food processors, as are conventional energy based processes, such as heating. The three conventional states of matter are solids, liquids, and gases; plasma has been described as the fourth state of matter, an unfamiliar designation that warrants explanation. As materials acquire energy (such as by heating), they change state, from solid (lowest energy) to liquid and then ultimately to gas. The melting points and boiling points of materials widely vary. For all materials, however, at each phase transition, the interactions and structures between molecules become loose rand ultimately breakdown entirely (Niemira 2012). Gases are collections of molecules (e.g., $N2$,O 2, CO 2) or single atoms (e.g., He, Ne,Ar)without large scale structure.

At still higher energies, the intra molecular and intra-atomic structures breakdown, liberating free electrons and ions. Plasma may be though to fasan ionized gas consisting of neutral molecules, electrons, and positive and negative ions. Plasmas generated in conventional devices do not ionize all of the atoms in a gas, even for hot (i.e., thermal) plasmas, such as welding arcs and spark plugs (Fridman et al. 2005). Within these hot plasmas, all species are extremely reactive. Within cooler (i.e., nonthermal) plasmas, such as those found in neon signs and plasma display screens, some of the chemical species are more reactive than others. For this reason, the chemical composition of the feed gas becomes a determining factor in the types of reactions that the plasma can initiate (Lieberman &Lichtenberg 2005, Niemira & Gutsol 2010).

The energy required to ionize gases into plasma can come from a variety of sources, such as heat, electricity, laser light, radiation, and extremely rapid compression. As a cloud of active particles, the plasma retains the imparted energy for a period of time. When the active particles recombine with each other, the energy is released as visible and UV light in the process of recombination (Lieberman & Lichtenberg 2005, Niemira 2012). Of more interest to food processors, the active particles in the plasma can react with the food substrate, releasing the stored energy into the bacteria or viruses to be targeted. How much energy a plasma has to impart will depend on its chemical composition, density, and temperature.

Plasma Science (Plasma- Definition, Physics and Chemistry)

In 1922, the American scientist Irving Langmuir proposed that the electrons, ions and neutrals in an ionized gas could be considered as corpuscular material entrained in some kind of fluid medium and termed this entraining medium "plasma", similar to the plasma, introduced by the Czech physiologist Jan Evangelista Purkinje to denote the clear fluid which remains after removal of all the corpuscular material in blood. However, it emerged that there was no "fluid medium" entraining the electrons, ions, and neutrals in an ionized gas (Bellan 2015), nevertheless the name prevailed.

The term "plasma" refers to a partially or wholly ionized gas composed essentially of photons, ions and free electrons as well as atoms in their fundamental or excited states possessing a net neutral charge. The plasma possesses a net neutral charge because the number of positive charge carriers is equal to the number of negative ones (Kudra and Mujumdar 2009). Electrons and photons are usually designated as "light" species in contrast to the rest of the constituents designated as "heavy" species. Due to its unique properties plasma is often referred to as the fourth state of matter according to a scheme expressing an increase in the energy level from solid to liquid to gas and ultimately to plasma.

Definition of Technology

Thermal plasma, operating at many hundreds or thousands of degrees above ambient, would be immediately detrimental to the quality of food products. Non thermal plasma is therefore the focus. For the sake of clarity, however, a distinction must be made between what non thermal means to a plasma physicist and what the same term means to a food processor. To the physicist, non-thermal means that the plasma has a distinctly non uniform distribution of energy (a non-equilibrium) among the constituent particles. Electrons are likely to transfer energy via collisions with heavier particles, exciting the larger particle in to a state of reactivity (Fridmanetal. 2005, Niemira & Gutsol 2010). To a food processor, non-thermal means that the mode of action of the antimicrobial process does not rely on thermal kill for inactivation of associated pathogens. As a practical matter, non-thermal processes are generally regarded as those that cause little or no thermal damage to the food product being treated. There are three primary mechanisms by which cold plasma inactivates microbes (Moisanetal. 2002).

- The first is the chemical interaction of radicals, reactive species, or charged particles with cell membranes.
- The second is by damage to membranes and internal cellular components by UV radiation.
- Finally, DNA strands may be broken by UV generated during recombination of the plasma species.

While on a given commodity, one mode of action may be more significant than another, the greatest sanitizing efficacy results from plasma with multiple antimicrobial mechanisms (Moisan et al. 2002, Laroussi 2003). As a food processing technology, cold plasma is new enough that the terminology is still evolving. The terms cold plasma (Noriega et al. 2011), cool plasma (Tran et al. 2008), atmospheric pressure plasma(Chirokovetal.2005),cold atmospheric gas plasma (Moisanetal.2001),and other comparable terms have been used in recent publications. In other cases, the plasma is described by the generative technology, e.g., dielectric barrier discharge (Fridman et al. 2006), plasma jet (Lu etal.2009), uniform glow discharge plasma(Gadrietal.2000),gliding arc discharge (Burlicaetal. 2010), etc.

Types of Plasma

Two classes of plasma, namely thermal and Non-thermal plasma (NTP) can be distinguished on the basis of conditions in which they are generated. This classification of plasma is based on the relative energetic levels of electrons and heavy species of the plasma. NTP (near ambient temperatures of 30-60^{o}C) is obtained at atmospheric or reduced pressures (vacuum) and requires less power. NTPs are characterized by an electron temperature much above that of the gas (macroscopic temperature) and consequently do not present a local thermodynamic equilibrium. NTP is also generated by an electric discharge in gas at lower pressure or using microwaves.

Typical illustrations for plasma generation at atmospheric pressure include the corona discharge, Dielectric barrier discharges (DBD), Radio-frequency plasmas (RFP) and the gliding arc discharge. To the contrary, thermal plasmas are generated from higher pressures and require high power. Thermal equilibrium may exist in between the electrons and the heavy species. Plasma generation at atmospheric pressure is of interest, both technically and industrially for the food industries because this does not require extreme conditions.

A thermal plasma is characterized by the existence of a thermodynamic equilibrium between the electrons, ions and neutral particles. The temperatures of a thermal plasma at atmospheric pressure generally are above 6000 K. This corresponds to a mean kinetic energy of less than 1 ev.

A non-thermal plasma has significantly different electron and gas temperatures. For example, the electron temperature may be several 10,000 K, which corresponds to a mean kinetic energy of more than 1 eV, whereas the gas temperature can be close to ambient. In spite of their low temperature, such plasmas can trigger chemical reactions and excitation states via electron impact. In Contrast thermal plasma, non-thermal plasma can also be applied directly to thermally sensitive surfaces.

Plasma Sources

Usually, plasma treatments was carried out under vacuum conditions, but researchers have developed atmospheric pressure plasma system, resulting reduced cost, increased treatment speed, and industrial applicability (Yoon and Ryu 2007; Yun et al. 2010). The ability to generate non-thermal plasma discharges at atmospheric pressure makes the decontamination process easier and less expensive (Kim et al. 2011). Nevertheless recently, most of the cold plasma devices available commercially was developed for research to aim at biomedical applications. Therefore, for food applications, these devices may need to be customized or tailor made. The barrier glow discharge generated between two parallel electrodes is a widely employed NTP system.

Food may be conveyed through the discharge to achieve microbial decontamination in most of the industrial scale. Another configuration is the plasma pen or jet, in which a stream of gases can be directed at the object to be treated. Biozone, a Scientist has developed the new process for the generation of the cold oxygen plasma (COP) by using air to high- energy deep UV light with a effective radiation spectrum between 180 nm & 270 nm. This cold gas plasma, composed of several species of negative and positive ions, free radical molecules, electron, UV-photons and ozone (Terrier et al. 2009). Duo-Plasma line is linearly extended plasma source excited using microwaves of 2.45 GHz at a pressure <1000 Pa (Petasch et al. 1997) and several other plasma treatment systems have evolved based on this principle. The

Plasmodul is a microwave sustained low pressure plasma reactor with a modular concept based on the Duo-Plasmaline principle which provides an easy up scaling for industrial applications (Schulz et al.).

This type of microwave excited plasma sources are well suited for large area plasma treatment (Petasch et al. 1997) and can probably be employed for surface treatment of foods or processing surfaces at industrial scale. More recently, Kim et al. (2010) developed a cold plasma jet operating at 20 kHz Alternating Current (AC) under atmospheric pressure. The most changable feature of most plasma systems is the freedom to select a gas or gas mixture. Improvements in the existing plasma systems and newer equipment directed for treatment of real food systems are likely to draw attention of researchers and engineers in near future.

Recently a novel approach which shows significant potential for the treatment of various foods has been reported. The approach is based on a dielectric barrier discharge with the food package in contact with high voltage electrodes. Only 40-50 W of power is needed to ionize air inside a 4 L re-sealable plastic (LDPE) bag (Klockow and Keener 2009). The high voltage process ionizes any gas within the electric field contained within the package. Ionization can generate significant amounts of reactive molecules with little increase in product surface temperature. Particular treatment times for targeted spore or bacterial reductions are dependent on product loading, packaging material, gas composition and package/electrode configuration. The in-package ionization process has been demonstrated in a number of common packaging materials including cardboard, glass, LDPE, HDPE, PETE, polystyrene, rubber, tygon, and others. Scale-up of the system has facilitated treatment of air filled packages with an electrode gap of up to 10 cm with rapid processing times (Keener et al. 2010).

Types of Cold Plasma Systems

There is a rapidly expanding array of technologies used to generate cold plasma. These can operate at atmospheric pressure or at some degree of partial vacuum. The gas being ionized may be as simple as air or nitrogen, or it may be a more exotic mixture containing some proportion of noble gases, such as helium, argon, or neon. The driving energy may be electricity, microwaves, or lasers. This wide array of design elements is an indication of the flexibility of cold plasma systems and the extent to which new forms of cold plasma systems continue to be built and evaluated.

However, all cold plasma systems intended for use in food processing fall generally into one of three categories. These categories are defined by where the food to be treated is positioned with respect to the cold plasma being generated: at some significant distance from the point of generation, relatively close to the point of generation, or within the plasma generation field itself. Conceptually, these categories are derived from the nature of cold plasma chemistry, with delineations having to do with the half-life and reactivity of charged, active species within the plasma (Niemira & Gutsol 2010).

The first category is remote treatment cold plasma systems. The plasma is generated using one of a variety of methods and moved onto the surface to be treated. The plasma may be driven by a flow of the feed gas or (less commonly) manipulated through the use of magnetic fields. This type of system has the advantage of placing the surface to be treated at a physically separate point of generation (Chirokov et al. 2005). This simplifies the design and operation of the device, and increases the flexibility with respect to the shapes and sizes of objects to be treated. However, the most reactive chemical species are also those that have the shortest half-life. During the time of flight, free electrons may recombine with other plasma products, such as heavy ions or atomic species. By the time the quenched plasma reaches the target surface, the composition is secondary chemical species, i.e. lower activity, long-living chemical species resulting from chemical recombination within the plasma (Gadri et al. 2000).

The lower concentration of ions that exist in this afterglow plasma generate UV light and activate chemical species upon reaction with the target, but their concentration is much lower than in active plasma (i.e., plasma supported by electric field) (Fridman & Kennedy 2004). These Cond category is known as direct treatment cold plasma systems. In this systems, plasma generation instrument supplies active plasma directly to the object to be treated. As with the first category, the plasma is moved via the flow of the feed gas or by a comparable means. So the target is relatively close to the site of cold plasma generation & exposed to the plasma before active species recombine and are lost, these systems provide higher concentrations of active agents (Laroussi&Lu2005). Systems of this type can operate in pulsed mode, with plasma generated at pulse frequencies of hundreds or thousands of times per second.

Cold-Plasma Generators

In this section, several methods that have been used to generate relatively large volumes of non-equilibrium cold plasmas, at or near atmospheric pressure (sometimes referred to as "high" pressure) are presented. This is not a comprehensive list of all existing methods. The methods presented here were chosen for two main reasons.

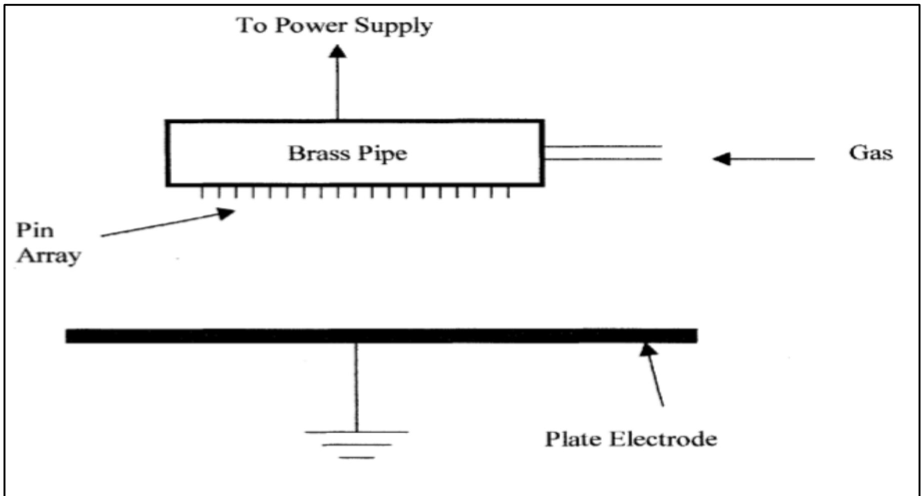
1. They have been used extensively to study the germicidal effects of cold, high-pressure plasmas; and

their potential use in various other industrial plasma processing applications (lighting, surface modification, etching, deposition).

The Corona Discharge

Siemens (2005) was the first to suggest the use of a corona discharge to generate ozone in order to disinfect water supplies. This was the first recorded use of plasma toward the inactivation of micro-organisms. Menashi(1972) used a pulsed RF-driven corona discharge to create a plasma at atmospheric pressure. He reported that up to microbial spores could be inactivated in less than 1 s. Garate(1978) et al. used an "Enhanced Corona Discharge" to destroy concentrations of up to 4.10^6/ml of Escherichia coli, and spores of Bacillus subtilis in less than 15 min. A schematic of the enhanced corona discharge is shown in Fig. This discharge consists of a line of pins fastened to a hollow pipe at one end and protruding from the other end through tiny holes.

The feed gas escapes through the holes and provides a local atmosphere around the corona points. The feed gas, a non-electronegative gas such as helium or argon, replaces the air around the corona points and therefore enhances the discharge by removing the electron-attaching electronegative-oxygen molecules. The pin array can be biased by a dc or ac high-voltage supply, or by a pulsed power supply.



Basic configuration of the enhanced corona discharge.

Fig:1:The Glow Discharge at Atmospheric Pressure

One of the early developments of diffuse glow discharge plasma at atmospheric pressure was reported by Donohoe. Donohoe(1979) used a large gap (cm) pulsed-barrier discharge in a mixture of helium and ethylene to polymerize ethylene. Later, Kanazawa et al(1979). reported their development of a stable glow discharge at atmospheric pressure by using a dielectric-barrier discharge (DBD) configuration. They claimed that to obtain a diffuse discharge (as opposed to a filamentary discharge, which is traditionally produced by DBDs), helium had to be the major constituent of the gas mixture, and the frequency of the applied voltage had to be in the kilohertz range. Schematic of the DBD-based glow discharge at atmospheric pressure. At least one of the two electrodes must be covered by a dielectric material. After the ignition of the discharge, charged particles are collected on the surface of the dielectric.

This charge build-up creates a voltage drop, which counteracts the applied voltage, and therefore chokes the discharge current. The discharge subsequently extinguishes. As the applied voltage increases again (at the second half cycle of the applied voltage) the discharge reignites. This process is repeated over and over during each full cycle of the applied voltage. Laroussi(2003), reported the use of the glow discharge at atmospheric pressure to destroy cells of Pseudomonas fluorecens. He used suspensions of the bacteria in Petri dishes placed on a dielectric-covered lower electrode. The electrodes were placed within a chamber containing mostly helium with an admixture of air. He obtained full destruction of concentrations of 4.10^6/ml in less than 10 min. Using a similar discharge, Kelly-Win tenberg(2000). reported the inactivation of B. subtilis spores using an air gap. E. coli, B. subtilis, and a variety of other gram-negative as well as gram-positive bacteria were inactivated successfully by many researchers using the DBD-based diffuse-glow discharge.

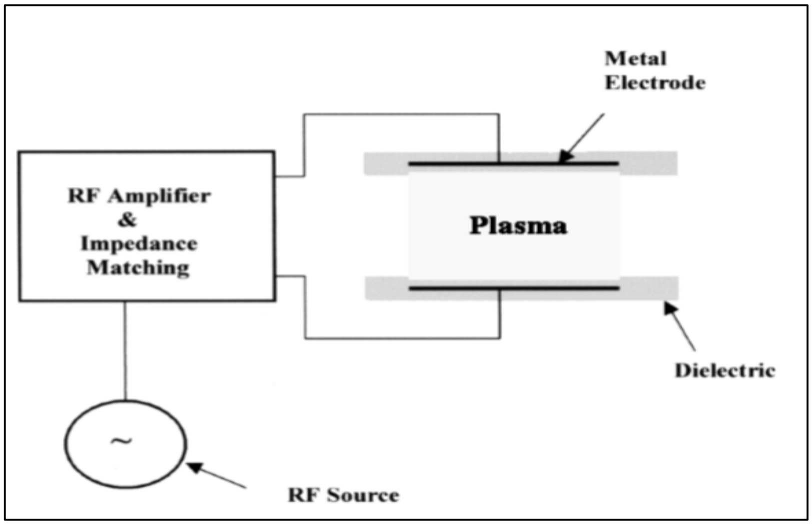


Fig:2:Configuration of the DBD-based diffuse glow discharge at atmospheric pressure.

The Atmospheric-Pressure Plasma Jet

The atmospheric-pressure plasma jet (APPJ) is a capacitively coupled device consisting of two coaxial electrodes between which a gas flows at high rates. The outer electrode is grounded, while the central electrode is excited by RF power at 13.56 MHz The free electrons are accelerated by the RF field and enter into collisions with the molecules of the background gas. These inelastic collisions produce various reactive species (excited atoms and molecules, free radicals) that exit the nozzle at high velocity. The reactive species can therefore react with a contaminated surface placed in proximity (cm) of the nozzle.

As in the case of the diffuse DBD, the stability of the APPJ plasma (as well as its nonthermal characteristic) depend on using helium as a carrier gas. Herrmann et al(1999). used the APPJ to inactivate spores of Bacillus globigii, a simulant to Anthrax (Bacillus anthracis). Herrmann et al(1999). reported the reduction of seven orders of magnitude of the original concentration of B. globigii in about 30 s.

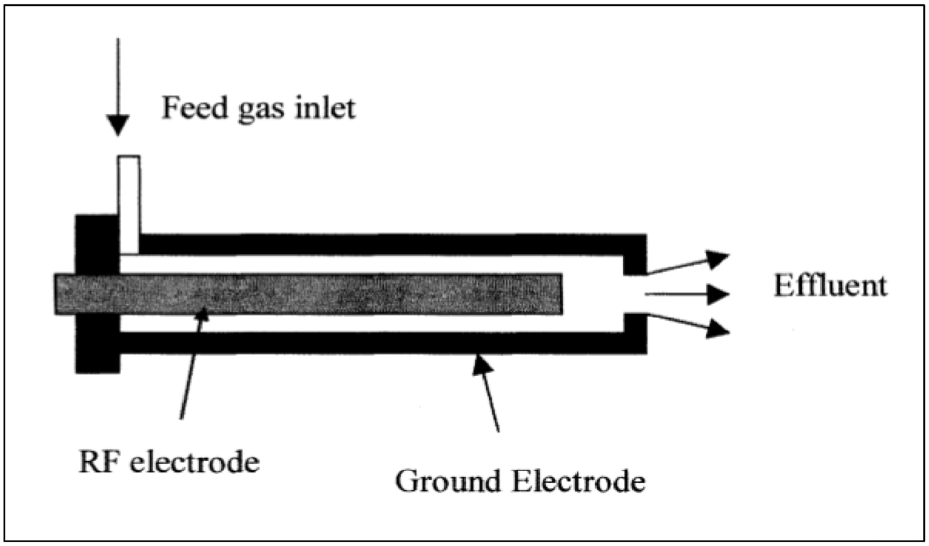


Fig:3: Configuration of the atmospheric-pressure plasma jet (APPJ)

The Resistive Barrier Discharge

The concept of the resistive-barrier discharge (RBD) is based on the DBD configuration. However, instead of a dielectric, a high-resistivity material is used to cover at least one of the electrodes. The high-resistivity layer plays the role of a distributed ballast which limits the discharge current and therefore prevents arcing. The advantage of the RBD over the DBD is the possibility to use dc power (or low-frequency ac, 60 Hz) to drive the discharge.

Using helium, large-volume diffuse cold plasma at atmospheric pressure can be generated. Richardson et al. and Laroussi et al. reported a fourorders-of-magnitude reduction in the original concentration of vegetative B. subtilis cells in about 10 min. They also reported that the RBD-inactivated endospores of B. subtilis, but not as effectively as the vegetative cells. In these experiments, they used a gas mixture of 97%–3% helium-oxygen, respectively.

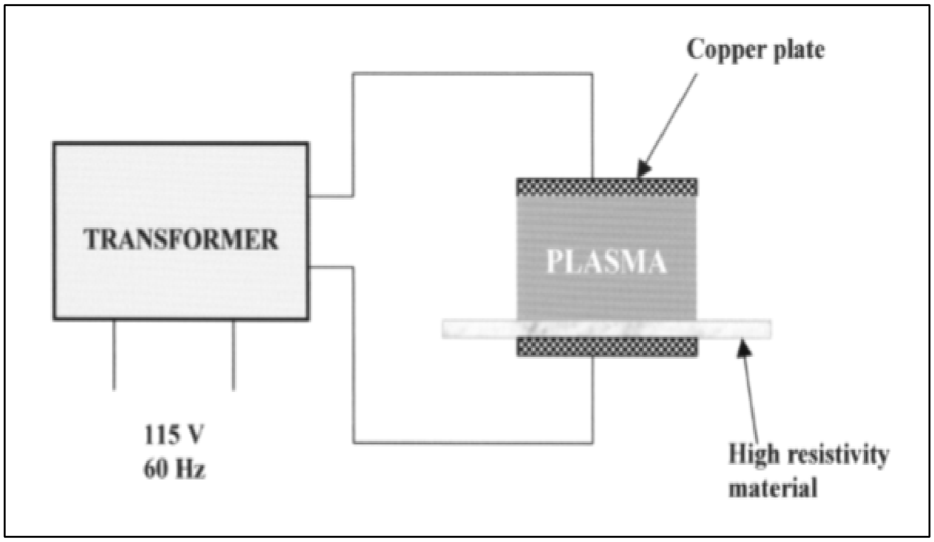


Fig4 : Configuration of the resistive-barrier discharge (RBD)

Action of Plasma on microorganisms

Action on cell components and functions

The use of sterilizing properties of plasma was first introduced towards the end of 60s, patented in 1968 (Menashi 1968) and first works with plasma made from oxygen were proposed in 1989. Thereafter, considerable research has been performed on the mechanism of microbial inactivation by plasma agents. The plasma agents contribute to the lethal action by interacting with the biological material. Nelson and Berger (1989) have shown that O2 plasma could be a very efficient biocidal against bacteria.

Plasma treatment can effectively inactivate a wide range of micro-organisms including spores (Kelly-Wintenberg et al. 1999; Feichtinger et al. 2003; Lee et al. 2006) and viruses (Terrier et al. 2009). Effect of plasma can be quite selective, meaning tuneable between damage to pathogenic organisms without damage to the host, or activation of different pathways in different organisms (Dobrynin et al. 2009).

Low-pressure oxygen plasma has been shown to degrade lipids, proteins and DNA of cells (Mogul et al. 2003). The reactive species in plasma have been widely associated to the direct oxidative effects on the outer surface of microbial cells. As an example, commonly used oxygen and nitrogen gas plasma are excellent sources of reactive oxygen-based and nitrogen-based species, such as O•, O2, O3, OH•, NO•, NO2 etc. Atomic oxygen is potentially a very effective sterilizing agent, with a chemical rate constant for oxidation at room temperature of about 106 times that of molecular oxygen (Critzer et al. 2007).

These act on the unsaturated fatty acids of the lipid bilayer of the cell membrane, thereby impeding the transport of bio-molecules across it. The double bonds of unsaturated lipids are particularly vulnerable to ozone attack (Guzel-Seydim et al. 2004). Membrane lipids are assumed to be more significantly affected by the reactive oxygen species (ROS) due to their location along the surface of bacterial cell, which allows them to be bombarded by these strong oxidizing agents (Montie et al. 2002). The proteins cells and the spores are equally vulnerable to the action of these species, causing denaturation and cell leakage. Oxidation of amino acids and nucleic acids may also cause changes that result in microbial death or injury (Critzer et al. 2007).

Micro-organisms in coldplasma are exposed to an intense bombardment by radicals, most likely provoking surface lesions that the living cell cannot repair sufficiently faster. This may partially explain the observations where in cells are in many cases destroyed very quickly. This process is termed “etching” (Pelletier 1992). The cell wall rupture has been additionally attributed by Laroussi et al., (2003) and Mendis et al., (2002) to electrostatic forces due to accumulation of charges at the outer surface of cell membranes. The morphological changes in E. coli cells treated with atmospheric plasma at 75W for 2 min as observed under an electron microscope by (Hong et al. 2009), clearly revealed that the treated cells had severe cytoplasmic deformations and leakage of bacterial chromosome.

These observations demonstrate the loss of viability of bacterial cells after plasma treatment. An analogy between plasma and pulsed electric field has also been drawn to explain the action of plasma on the membranes (Pothakamury et al. 1995; Spilimbergo et al. 2003). It is well established that electroporation of membranes is induced by pulsed electric fields and it appears that plasma acts on similar lines inducing perforations in the membranes of micro-organisms (Sale and Hamilton 1967; Pothakamury et al. 1995; Wouters and Smelt 1997). In addition to generating pores, humid air plasma additionally provokes a marked acidification of the medium (Moreau et al. 2005; Moreau et al. 2007).

Role of UV photons and charged particles

The production of UV photons of different wavelengths has been proposed to be involved in dimerizing the thymine bases of DNA including that of spores (Munakata et al. 1991).

The role of UV photons in bacterial death when they are submitted to a plasma treatment was reviewed in detail by (Boudam et al. 2006). More recently, by exclusion of the reactive particles and spectral fractions of UV radiation from access to the spores Roth et al., (2010) revealed that UV-C radiation is the most effective inactivation agent in the plasma. Ultraviolet (UV) photons play a less important role in atmospheric pressure glow discharge (APGD) because they are easily absorbed by gas atoms and molecules at atmospheric pressure (Vleugels et al. 2005).

The role of the charged particles in the bacterial inactivation process was recently investigated by Lu et al. (2009). Their work revealed that the charged particles play a minor role in the inactivation process when He/N2 (3%) is used as working gas than when He/O2 (3%) is used. Also, they concluded that heat and UV play no or minor roles in the inactivation process. Similar results were earlier obtained by (Perni et al. 2007) who interplayed bacterial inactivation kinetics with optical emission spectroscopy, and identified oxygen atoms as major contributor in plasma inactivation with minor contributions from UV photons, OH radicals, singlet oxygen metastables and nitric oxide. Thus, a contradiction over the role of UV photons in plasma exists and future studies must be directed to get a clear picture.

Effect of process parameters

The concentrations in which the plasma agents occur in plasma depend greatly on the device set-up (reactor geometry), operating conditions (gas pressure, type, flow, frequency and power of plasma excitation) and gas composition which affect their efficacy in a process when employed. To cite an example, the destructive efficiency of various gas plasma sources and temperatures on Bacillus spp. spores were compared by (Hury et al. 1998).

This group demonstrated that oxygen-based plasma is more efficient than pure argon plasma. Another deciding criterion is whether the substrate to be sterilized is in direct contact with the plasma (Direct Exposure) or located remote from it (Remote Exposure) (Moisan et al. 2001; Laroussi 2005; Boudam et al. 2006). If exposed remotely, the quantum of heat transmitted to a sample is reduced, the charged particles do not play a role since they recombine before reaching the sample, and many of the short-lived neutral reactive species also do not reach the sample.

Since, the components of the plasma are reactive and self-quenching, with a relatively short half-life, decreased time of flight would be expected to be one of the major factors in antimicrobial efficacy in this case (Niemira and Sites 2008).

By varying the process parameters involved in plasma generation, a multitude of mechanisms can be actuated which may act individually or synergistically. Nevertheless, the details of interaction of the different plasma agents with the different components of bacterial cells or spores are currently very limited. The interactions which occur between plasma agents and biological materials, ultimately leading to sterilization are still under investigation.

Potential Applications:

Results of action of cold plasma on packing materials

Packing materials	**Treatment plasma**	**Applied voltage (dosage)**	**Results**	**References**
Polypropylene	Air Corona	30 kHz, 1.7 j/cm2	Decrease in contact angle, increase in adhesion	D. Dixon, B.J. et.al. (2012)
PET films	Glow discharge	10 W	Decrease in contact angle, increase in roughness, crystallinity and degradation yield	K.N. Pandiyaraj, et.al. (2008)
Potato starch film	Air plasma	15W	Decrease in hydrophilic nature, Increase in tensile strength	F.Starzyk, et.al. (2001)
PP film	Diode plasma discharge	8.3 W	Decrease in contact angle, increase in surface energy	P. Slepicka, et. Al.(2010)
PET film	Jet plasma DCdischarge	35 W	Increase in hydrophobic nature	Y. Akishev, et al., (2008)
PET	Jet plasma	285 V	Increase in weight, decrease in contact angle and wettability	K. Gotoh, et.al.,(2011)
HDPE film	RFAr:O2 Plasma	150 W	Decrease in crystallinity, contact angle, increase in roughness	I. Banik,K.S. Kim, et al., (2002)

LDPE	RFArgon plasma	25–100 W	Decrease in contact angle and ageing effect, increase in crystallinity	M. Ataeefard, et.al.,(2009)
BOPP film	RFAir plasma	10–50 W	Decrease in contact angle, increase, roughness ageing effect	S.M. Mirabedini, et. Al.,(2007)

Recent findings in the area of non-thermal plasmas for inactivation of microorganisms and spores.

Organism	**Plasma conditions**	**Treatment surface/ medium**	**Salient result**	**Reference**
Escherichia coli, Staphylococcus aureus	Atmospheric plasma corona discharge, with high voltage (20kV) DC power supply	On agar plates	Changes of pH levels from alkaline to acid, upon plasma application to bacteria in water, does not play a predominant role in cell death.	Korachi et al. (2010)
Escherichia coli, Bacillus subtilis, Candida albicans, and Staphylococcus aureus	High-frequency capacitive discharge (0.4 torr) and barrier discharge (0.4-0.5 torr) in air excited at commercial frequency of 5.28 MHz	Glass plate and petri dish	The most probable sterilization agents of the plasma generated were established to be "hot" and "cold" OH radicals, the excited electrically neutral N2 and O2 molecules, and the UV plasma radiation	Azharonok et al. (2009)
Escherichia coli KCTC1039 *Bacillus subtilis*	Helium and Oxygen based electric discharge plasma produced at a radio frequency (RF) of 13.56 MHz	Dried cells and endospore suspension on a cover-glass	Treated cells had severe cytoplasmic deformations and leakage of bacterial chromosome. UV from the plasma only slightly affected the viability of the spores.	Hong et al. (2009)
Escherichia coli type 1 *Saccharomyces cerevisiae Gluconobacter liquefaciens Listeria monocytogenes*	Cold atmospheric plasma plume generated by an AC voltage of 8 kV at 30 kHz	Inoculated membrane filters and inoculated fruit surfaces	Efficacy of inactivation was markedly reduced for microorganisms on the cut surfaces than on filters due to the migration of microorganisms from the exterior of the fruit tissue to its interior and not	Perni et al. (2008a)

			quenching of reactive plasma species.	
Escherichia coli Saccharomyces cerevisiae Pantoea agglomerans Gluconacetobacter liquefaciens	Cold atmospheric plasma generated by an AC voltage (variable12kV and 16kV)	Pericarps of mangoes and melons	S. cerevisiae was the most resistant amongst all test organisms. An increase in the applied voltage led to more efficient production of reactive plasma species (oxygen atoms) which was attributed for better inactivation.	Perni et al. (2008b)
Escherichia coli O157:H7 Salmonella sp. Listeria monocytogenes	One atmosphere uniform glow discharge plasma (OAUGDP) operated at 9 kV power and 6 kHz frequency	Apples, Cantaloupe and Lettuce	Inactivation was observed in all the cases. Extent of log reduction varied with the organisms	Critzer et al. (2007)
Biofilms produced by *Chromobacterium violaceum*	RF high pressure cold plasma jet using Atomflo 250 reactor with 100 W RF power supply using He and N2 gas	Biofilms produced in 96-well polystyrene microplates	A 10 min plasma treatment was able to kill almost 100% of the cells. A complex, biphasic model of inactivation was observed.	Abramzon et al. (2006)

Recent finding of microbial inactivation using cold plasma

Microorganism	**Substrate**	**Plasma source**	**Exposure time and dosage**	**Results**	**Reference**
S.enteritidis (01) *L. monocytogenes*	Table Egg	RBD prototype	90 min & 30kV	4–5 Log reduction	N. Rowan, et. Al.,(2007)
E. coli 12955 & Salmonella spp	almonds	Dielectric discharge	30 s & 30 kV and 2000 Hz.	4 log CFU/ml	Deng S, et. Al.,2007
E.coli, C.jejuni	Chicken skin	Pulsed gas plasma discharge	24 s at 45kV	up to 8 Log reduction	E. Noriega, et.al.,(2011)
E. coli *Saccharomyces cerevisiae Pantoea agglomerans Gluconobacter liquefaciens*	Mango & Melon (honeydew)	AC voltage	2.5 s, 5 s and 10s,at 12 to 16 kV	P. agglomerans and G. liquefaciens > 3 log reductions after 2.5 s.E. coli > 3 log reductions after 5 s S. cerevisiae > 3 log reductions after 10s	Perni et al. (2008)

E. coli O157:H7	apple juice	corona discharges	40 s 100 Hz with 4000 pulses of 9000 V peak voltage	5 log CFU/g	Montenegro et al.
E. coli O157:H7 Salmonella Stanley	Red Apples	Gliding arc	3 min & 18kV	up to 3.7 Log reduction	Niemira and Sites (2008)
Yeast/mouls	Strawberris	DBD	5 min at 16 t0 18kV	up to 3 Log reduction	N.N. Misra, et.al.,(2014)
A. hydrophila	Lettuce	COP	5 min at 20kV	5 Log reduction	I.K. Jahid, et.al.,(2014)
S. typhimurium	Tomatoes	DBD	300s at 18kV	3.8 Log reduction	D. Ziuzina, et.al.,(2014)

Associated benefits and concerns:

Cold plasma treatment to the foods is a promising technology in that which acts rapidly, does not leave toxic residuals on processed parts or in the exhaust gas and the temperature rise can be kept to an acceptable level. The viability of grains and legumes had shown to be preserved post plasma treatment with air and SF6 gases (Selcuk et al. 2008). Moreover, unlike pulse light and gamma radiation, the shadow effect is minimised considerably using gas plasma method as reactive species are produced in the whole chamber (Lassen et al. 2003; Goldman and Pruitt 1998). Contact angle (CA) measurements for nonthermal oxygen plasma treated lamb’s lettuce have shown increased wettability of adaxial leaf surfaces after plasma exposure (Grzegorzewski et al. 2010a).

Further, in this case a successive degradation of epicuticular waxes and cutin of the plant’s epidermis was indicated by means of FTIR (ATR) and scanning electron microscopy (SEM). Above all, it can be conveniently operated in either batch or continuous mode. An aspect of the future of plasma technology is the possibility of pairing it with other decontamination processes such as pulsed-light treatment where synergistic effects may be more appreciable.

Studies on effect of nonthermal plasma on food components are scarce in literature. Based on the experiments using low-pressure oxygen plasma it have been observed that time and structure-dependent degradation can be seen for different selected model flavonoids adsorbed on solid surfaces, which was attributed to plasmaimmanent reactive species such as

O (3P), O_2 ($1\Delta g$ and $1\Sigma g+$), O_3, or OH radicals (Grzegorzewski et al. 2010b). It has been observed in lamb's lettuce that pure compounds show a time-dependent degradation (flavonoids) or remain unchanged (phenolic acids) after exposure to oxygen plasma (Grzegorzewski et al. 2010a).

Also, for the same model plant based food, a significant increase of protocatechuic acid, luteolin, and disometin has been recorded after 120 s treatment time, independent of the applied plasma driving voltage. The effect of the UV and radical species of plasma on the lipids and other sensitive constituents of the foods such as vitamins C and E (which are naturally occurring in most fruits and vegetables and many foods) still remains ambiguous. Suitability of plasma technology for treatment of high fat/ lipid containing and other sensitive foods (where chemical changes may be induced) is doubted.

Products that have high lipid content would likely be affected by oxidation, resulting in formation of hydroxyl acids, keto acids, short-chain fatty acids and aldehydes etc. that cause off-flavours and odours. For these reasons meat products may not be ideal substrates for treatment with plasma (Critzer et al. 2007). For a full evaluation, additional issues concerning food quality must be considered and these include changes in nutrient content colour and textural qualities, toxic residues and other chemical changes (Vleugels et al. 2005). Research efforts must be undertaken to evaluate the projected cost of the treatment for large quantities of food commodities and also the safety of gases used before direct plasma techniques will become common in the food industry (Basaran et al. 2008).

Therefore, cold plasma technology is an emerging disinfection method that offers an exciting complementary or alternative, novel non thermal approach for reducing the microbial populations on the raw or fresh produce surface and packaging materials. There may be several other applications in relation to food systems, which still remain unexplored. Various reactive species of plasma interact with the biological cells to cause permanent changes in them at cellular level and morphology, leading to inactivation.

Although cold plasma technology is not yet used commercially on a large scale, the equipment should be readily scalable. Systems for large scale cold plasma treatment of food and related products using various energy sources and methods (like a multiplicity of microwave magnetrons) are already under development. This technology is increasingly finding acceptance among food processors for the surface sterilization and combating biofilm formation. The effect of cold plasma on the sensitive constituents of foods, mainly lipids, vitamins etc. have still some issues that need to be addressed and once this is achieved the technology will find wider applications and adaptation in food industries.

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, optimization 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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