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Nonthermal Plasma Inactivation of Food-Borne Pathogens

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Abstract

Non-thermal plasma (NTP) is electrically energized matter, composed of highly reactive species including gas molecules, charged particles in the form of positive ions, negative ions, free radicals, electrons and quanta of electromagnetic radiation (photons) at near-room temperature. NTP is an emerging nonthermal technology with potential applications for decontamination in the food industries. An upsurge in the research activities for plasma based inactivation of food borne pathogens is evident in the recent years. These studies have shown that NTP can be used for the surface decontamination of raw produce, dried nuts and the packaging materials etc. This paper reviews the action of plasma agents on the microbial classes and describes proven and potential applications in food processing. Novel developments in the technology and a future outlook for the application to foods are discussed.

Keywords

Nonthermal, plasma, sterilization, decontamination, food

Introduction

Ensuring safe and quality food has become more complex task today, than at any point of time in the history. New risks are being encountered because of changing characteristics of the relevant micro-organisms, changing production methodologies, changes in the environment and the ecology, and an increase of the global trade of food stuffs (Havelaar et al. 2010). As consumer demands and food safety issues have changed, so have the food processing technologies (Gould 2001) to ensure food safety. Moreover, there are multiple issues relating to quality of thermally processed foods such as nutritional losses and adverse effects on organoleptic quality. This has led to the emergence of so called “Nonthermal Technologies” (Sampedro et al. 2005). Nonthermal technologies are preservation treatments that are effective at ambient or sub-lethal temperatures, thereby minimizing negative thermal effects on nutritional and quality parameters of food (Tiwari et al. 2009). These include the application of gamma irradiation, beta irradiation (electron beam), power ultrasound, ozonation, pulsed light, UV treatment, pulsed electric field (PEF), high hydrostatic pressure etc. Nevertheless, even some of these commercially viable inactivation methods are limited in practice due to adverse perceptions associated (like with treatments of gamma irradiation and high energy electron beams) or high initial investments required and/or other constraints. Purely physical techniques, such as high hydrostatic pressure, are chemically safe but require complex or expensive equipment (Rastogi et al. 2007), affect the quality of the product (Kruk et al. 2009) and are generally incompatible with online treatments. Very few approaches are suitable for treatment of solid foods, in particular fruit and vegetables. Very recently NTP has been added to the existing list of non-thermal processes for the decontamination of fresh produce (Critzler et al. 2007; Niemira and Sites 2008; Lee et al. 2006).

Technologies like UV treatment, ozonation, power ultrasound, pulsed light, electric discharge and nonthermal plasma are commonly designated as Advanced Oxidation Processes (AOP). The use of pulsed UV light as a means of microbial inactivation is a mature technology that has commercial application in surface disinfection of packaging materials, but demonstrates limitations due to shadowing effects in food products (Gómez-López et al. 2007). Indeed, there is currently no perfect method to achieve sterilization at ambient temperature. NTP or cold plasma, which has recently

drawn considerable attention of the food scientists and researchers (Vleugels et al. 2005; Basaran et al. 2008; Selcuk et al. 2008), forms the subject of this paper. This paper reviews the applications of nonthermal plasma techniques for inactivation of food borne pathogens, proposed mechanisms underlying microbial inactivation and spore destruction, while discussing limitations and scope for future studies.

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 2006), 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.

Types of Plasma

Two classes of plasma, namely thermal and 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°C) is obtained at atmospheric or reduced pressures

(vacuum) and requires less power. NTPs are characterised by an electron temperature much above that of the gas (macroscopic temperature) and consequently do not present a local thermodynamic equilibrium. NTP can be generated by an electric discharge in a 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. In contrast, thermal plasmas are generated at higher pressures, require high power, and an almost thermal equilibrium exists 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.

Plasma Sources

Formerly, plasma treatments were carried out under vacuum conditions, but researchers have now developed atmospheric pressure plasma system, resulting in 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). However, until very recently, most of the cold plasma devices available commercially were developed for research and aimed 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. In a possible industrial scale set-up, food may be conveyed through the discharge to achieve microbial decontamination. Another configuration is the plasma pen or jet, in which a stream of gases can be directed at the object to be treated. *Biozone Scientific* has developed a new process for the generation of cold oxygen plasma (COP) by subjecting air to high- energy deep-UV light with an effective radiation spectrum between 180 nm and 270 nm. This cold gas plasma is composed of several species like negative and positive ions, free radical molecules, electron, UV-photons and ozone (Terrier et al. 2009). *Duo-Plasmaline* is a 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 upscaling 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 versatile feature of most of the 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. Specific 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).

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 of the 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 plasma are exposed to an intense bombardment by the radicals most likely provoking surface lesions that the living cell cannot repair sufficiently faster. This may partially explain the observations wherein 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). Recently, by exclusion of 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

The combination of highly energetic plasma species with a nonthermal treatment mode makes NTP particularly suited for decontamination in food processing settings (Yu et al. 2006). NTP has a myriad of potential applications for the food industry including the dry disinfection of food surfaces (like meat, poultry, fish and freshly harvested horticultural produce), granular and particulate foods (dried milk, herbs and spices) and sprouted seeds. There is a significant scope for NTP sterilization of the particulate foods, particularly after the ban of ethylene oxide gases. This technology

has also been successfully applied for the surface sterilization of packaging material (Deilmann et al. 2008) and also their functional modification for imparting desired properties (Ozdemir et al. 1999; Güleç et al. 2006). A considerable body of data has already accumulated in recent years addressing the efficacy of NTP in inactivating microorganisms on the surfaces of abiotic materials such as glass and synthetic membranes.

Treatment of raw and dried produce

Escherichia coli, *Salmonella typhimurium*, *Staphylococcus aureus*, *Listeria monocytogenes* and *Enterococcus faecalis* are general food-borne pathogens that cause severe diseases and in some cases even death (Yun et al. 2010). Raw agricultural produce has frequently been implicated in disease outbreaks. Any treatment applied to ensure the microbiological safety of a food must be selected so as to minimize changes to its sensory, nutritional and functional properties (Manas and Pagán 2005). Conventionally, sterilization methods such as heat, chemical solutions and gases (for example ethylene oxide, hydrogen peroxide) are used for the surface disinfection of fruits, spices, nuts etc., which are often time consuming, damaging or have toxic residues (Muranyi et al. 2007). The pathogen inactivation effects of cold plasma potentially offer a treatment step for fresh produce to reduce the microbial load without adversely affecting the nutritional and other key characteristics. Recent, important and selected findings on plasma based inactivation of microorganisms have been summarized in Table 1.

Recent regulations by the US Food and Drug Administration (FDA) have required processors to achieve a 5 log reduction in the numbers of the most resistant pathogens in their finished products. Interestingly, investigations at Commonwealth Scientific and Industrial Research Organization (CSIRO), Food and Nutritional Sciences, Australia, have also demonstrated up to 5 log₁₀ reductions of microorganisms after only a few seconds of exposure to cold plasma (Anonymous 2010). Similar results have also been published by many other researchers (Table 1). The ions of cold plasma can penetrate the cracks and crevices of even complex shaped bodies unlike other potential surface treatments such as UV light. Therefore the technology may act

more effectively and efficiently over undulated or cracked surfaces such as those found on many foods like seeds, meat etc.

Selcuk *et al.* (2008) successfully decontaminated the seeds of tomato, wheat (*Triticum durum*), bean, chickpea, soybean, barley, oat, rye, lentil (*Lens culinaris*) and corn, contaminated with *Aspergillus parasiticus* 798 and *Penicillium sp.* to less than 1% of initial count depending on treatment times. The treatment times varied from 30 s to 30 min. This group employed a custom designed batch type low pressure cold plasma (LPCP) prototype unit operating under vacuum, using air and SF₆ gases. The results suggest that in practical terms, after plasma treatment food qualities of wheat and beans were not affected or only marginally affected. It is worth mentioning that the seeds were found to be viable post plasma processing.

In a study of *E. coli* 12955, a non-pathogenic surrogate for *Salmonella spp.* inoculated onto almonds, Deng *et al.*, (2006) reported a reduction of more than 4 log CFU/ml. In this study sterilization was achieved by placing the almonds in a 10-mm gap between two plasma discharge electrodes and treating for 30 s (25 kV, 2 kHz). Similarly, Niemira & Sites (2008) significantly reduced the viable populations of *Salmonella* and *E. coli* 0157:H7 inoculated on apple surfaces using cold plasma generated in a gliding arc. A 2.9 to 3.7 log CFU/ml and 3.4 to 3.6 log CFU/ml reduction of *Salmonella* Stanley and *E. coli*, respectively was observed. The authors reported the highest flow rate of the air (discharge medium) i.e. 40 litres/min, to be most effective.

Control of biofilms and decontamination of processing surfaces

Microorganisms are often embedded in the nutrient-rich environment of biofilms where they proliferate and are largely protected from external stresses such as direct attack of plasma species (Vleugels *et al.* 2005). The mechanisms involved in formation and behaviour of biofilms, deleterious effects associated with their presence and control strategies are reviewed in Simões *et al.* (2010). Biofilms are problematic in particular food industry sectors such as brewing, dairy processing, fresh produce, poultry processing and red meat processing (Simões *et al.* 2010; Chen *et al.* 2007; Frank *et al.* 2003; Jessen and Lammert 2003; Somers and Wong 2004).

Plasma technology can also be exploited for possible applications in combating with the menace of biofilms that form on processing surfaces (Critzler et al. 2007). The US patent (# 6096564, 2000) describes the invention by Denes et al. (2000) for the passivation of bacterial biofilm surfaces subjected to cold-plasma treatments. The patent claims that the exposure of the food processing surface with biological contamination thereon to an oxygen plasma results in sterilisation and cross-linked the biological contamination into a form which is resistant to further adhesion of bacteria and other biomaterials while cleansing and sterilizing areas of the substrate that are not covered by the biofilm. The inventors further suggested that a second step may be carried out to plasma mediate the deposit of an anti-fouling film using components that will provide macromolecular networks on the substrate with a desired structure which is resistant to bacterial adhesion. Vleugels and co-workers (2005) successfully inactivated biofilms forming *Pantoea agglomerans* (commonly associated with plant tissues) grown on synthetic membranes by two orders of log reduction in 10 min. They reported insignificant color change of the substrates viz. red, green and yellow bell pepper samples on exposure to glow discharge plasma. Abramzon *et al.* (2006) have reported almost 100% kill of *Chromobacterium violaceum* cells embedded in a four day old biofilms using a 100W RF high-pressure cold plasma jet. Distinct kinetic stages of inactivation have been reported in all the investigations suggesting different inactivation mechanisms, each of which might have been triggered by different plasma species or their different combinational/synergistic effects.

Survival of food-borne pathogens has been detected at a level of 10^5 CFU/cm² on stainless steel surfaces in a study by Kusumaningrum *et al.* (2003). Deng *et al.* (2009) showed that cold gas plasmas have the potential to denaturize proteins attached to stainless steel. Gas plasmas could also be used to remove allergens from the surface of food processing equipment (Shama et al. 2009). Leipold et al. (2010) investigated the decontamination of a rotating cutting tool used for slicing in the meat industry by means of atmospheric pressure dielectric barrier discharge operated in air. Targeting for *Listeria monocytogenes*, this group inoculated the knife with *Listeria innocua*. The knife itself was used as a ground electrode in the experiment and a 5 log reduction of *L. innocua* was obtained after 340 s of plasma operation. It was also reported that the temperature of the knife after treatment remained below 30⁰C. Because the decontamination is achieved while the knife is in operation and this method allows

reduced risk of cross contamination between separate batches of meat. Food manufacturers are increasingly investing in the cold plasma technology in order to kill the pathogens in the air and on the surfaces of processing plants (Brown 2010).

Surface decontamination of eggs

Salmonella spp. has been largely reported as a potential hazard for egg consumers and a need for alternative methods of decontaminations has been highlighted by Davies and Breslin (2003). The scientific literature has already evidenced several nonthermal approaches for the superficial decontamination of egg shells, including advanced oxidation procedures (AOP's) such as pulsed light technology (Hierro et al. 2009), ozone, UV radiation (Rodriguez-Romoand and Yousef 2005; Fuhrmann et al. 2010), slightly acidic electrolyzed water (Cao et al. 2009). These attempts are in extension to the failure of conventional techniques in efficient cleaning of the surface of eggs. Ragni and co-workers (2010) investigated the efficacy of resistive barrier discharge (RBD) plasma for decontamination of shell egg surfaces. Their work revealed a maximum reduction of 2.2–2.5 Log CFU/eggshell in *Salmonella enteritidis* levels following a 60–90 min of treatment at 35% RH. Further, the effectiveness of the treatments enhanced at a higher RH level of 65%, where maximum declines of 3.8 and 4.5 Log CFU/eggshell were achieved after 90 min of exposure. Similar observations were made for *Salmonella typhimurium*, with an overall reduction of 3.5 Log CFU/eggshell, after 90 min treatment. The enhanced effects of increased RH on the efficiency of the treatments were explained in this study on the basis of the presence of oxygen species as detected in the discharge emission spectra. These results are at par with the UV and ozone treatments of eggs reported earlier (Rodriguez-Romoand and Yousef 2005). An important aspect of this study is that plasma treatment does not lead to any compromise with the cuticle quality, generally considered as the first mode of defence against microbial invasion. This was evidenced from the scanning electron microscopy (SEM) studies and dye uptake method.

Sterilization of packaging materials

Food packaging materials are aimed at serving the functions of both preserving food and protecting it from deterioration, outside contamination or damage during distribution and storage. When not stored in proper conditions, packaging materials can get contaminated with microorganisms. These contaminants are transferred to food via packages, and their growth on food can result in economic losses because of spoilage (Turtoi and Nicolau 2007). In addition, they may cause public health concerns. In general, sterilization in bottling lines is achieved by means of oxidizing chemical germicidal liquids such as hydrogen peroxide, peracetic acid, ozonated water etc. (with or without mild heat treatment). The bottle is dipped or internally sprayed, optionally heated, rinsed and dried before being filled. Despite the effectiveness, this method generates liquid effluents, for which the cost of treatment gets added to that of the process. Moreover, in general, the management of water circuits always incurs a risk of development of inadvertent microbial contamination. Cold plasma has the potential to take over or complement the current chemical based sterilization methods for food packaging materials.

Current food-related applications of plasma are mostly limited to packaging industry and include sterilization of anti-fouling and printable surfaces and permeability reduction of polymers for carbon dioxide and oxygen (Schneider et al. 2005; Basaran et al. 2008). Low temperature gas plasma sterilization allows fast and safe sterilization of packaging materials such as plastic bottles, lids and films without adversely affecting the properties of the material or leaving any residues. This is also evident from the work of Muranyi et al. (2007) where Polyethylene terphthalate (PET) was used as the treatment medium with reported inactivation of several micro-organisms (Fig. 1). However, the type of materials is crucial and appropriate treatment conditions should be considered for achieving satisfactory inactivation levels (Yun et al. 2010). For example, no viable count has been detected after 90 and 120 s plasma treatments on plastic trays and only three decimal reductions on paper cups and aluminium foils under identical process parameters (Yun et al. 2010). Rostaing (2007) has patented an invention regarding a method for cold non-germicidal gas based plasma treatment of plastic bottles. This invention has added advantage of simultaneous deposition of diffusion barrier layer, thereby allowing integration of the sterilization and optionally impermeabilisation operations in the bottling line which extend from the moulding of the bottles till filling. Cold plasma for such applications

can be generated either by distributed propagation of non-pulsed microwaves to an inside surface of the bottle or by a hollow cathode system adapted to the bottle and supplied with pulsed DC or radiofrequency voltage. Prior to this, Schmidt (2003) patented a system and method of applying energetic ions for sterilization of a container in which cold plasma is caused to get disposed near a surface to be sterilized, and the cold plasma is then subjected to a pulsed voltage differential for producing energized ions in the plasma.

Plasma deposition of heat sensitive materials such as vitamins, antioxidants and antimicrobials into the packaging material may be sought as potential alternative in the emerging field of antimicrobial and active packaging. Partial success has been achieved in depositing a plasma-processed vanillin film over red delicious apples by Fernandez-Gutierrez et al. (2010).

Waste water treatment

The surface water rules by the United States Environmental Protection Agency relating to chlorine and chlorine-derived products have stimulated operators (especially of poultry and meat industries, which are consumers of large volumes of water) to seek technologies that will assure discharge compliance (Kim et al. 2003). A potential method for generating plasma in liquids is by the application of high-voltage pulses to the gas-injected or sparged liquid and has opportunities in treating waste water from food industries, like poultry wash water (Rowan et al. 2007). This can be visualized as a combination of pulsed electric field and cold plasma and causes the generation of free radicals, free electrons, UV light, acoustic and shock waves, and electric fields at levels of 10-40 kV/cm (Espie et al. 2001). The application of high-voltage pulses to gas-sparged test liquids results in partial discharge activity and ionization of the gas, which leads to complete breakdown of the gas in the liquid medium (Rowan et al. 2007).

Associated benefits and concerns:

Cold plasma treatment of foods is a promising technology in that it 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 has been shown to be preserved post plasma treatment with air and SF₆ gases (Selcuk et al. 2008). Moreover, unlike pulsed light and gamma radiation, the shadow effect is minimized considerably using gas plasma methods 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 experiments using low-pressure oxygen plasma it has been observed that a time- and structure-dependent degradation can be observed for different selected model flavonoids adsorbed on solid surfaces, which was attributed to plasma-immanent reactive species such as O (³P), O₂ (¹Δ_g and ¹Σ_g⁺), O₃, 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 (Critzler 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).

Conclusions

The cold plasma technology is an emerging disinfection method that offers an exciting complementary or alternative, novel nonthermal 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.

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Table 1. Recent findings in the area of nonthermal plasmas for inactivation of microorganisms and spores.

Organism	Plasma conditions	Treatment surface/ medium	Salient result	References
<i>Escherichia coli</i> , <i>Staphylococcus aureus</i>	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)
<i>Staphylococcus aureus</i>	DC cold- atmospheric-pressure plasma microjet with compressed air as the working gas	Aqueous suspensions of the organism	First 10 min treatment led to insignificant inactivation. After 16 min <i>S. aureus</i> was completely inactivated. Effective inactivation of <i>S. aureus</i> was found to start after the pH values decreased to about 4.5.	Liu et al. (2010)
<i>Bacillus atrophaeus</i> , <i>Geobacillus stearothermophilus</i> , <i>Clostridium sporogenes</i> , <i>Kocuria rhizophila</i> , <i>Staphylococcus aureus</i> , <i>Aspergillus niger</i>	low- pressure inductively-coupled plasma (ICP) with different mixtures of gases	Glass substrates and silicon wafers coated with the organism by spraying.	All the organisms were found to be reduced by at least 4 orders of magnitude under optimized low-pressure argon plasma. Efficiency of inactivation was variable for different strains of a given species.	Von Keudell et al. (2010)
<i>Bacillus subtilis</i>	Oxygen and nitrogen treated using 8 Duo-Plasmalines driven by microwaves power	Microscopic slides stacked with spores	Plasma treatment of the spores caused release of DPA, generation of auxotrophic mutants, reduction in Kat X activity and damage to DNA. A biphasic model for the inactivation kinetics was proposed.	Roth et al. (2010)

<i>Escherichia coli</i> , <i>Bacillus subtilis</i> , <i>Candida albicans</i> , and <i>Staphylococcus aureus</i>	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 N ₂ and O ₂ molecules, and the UV plasma radiation.	Azharonok et al. (2009)
<i>Influenza viruses</i> (RSV, hPIV-3 and A (H5N2))	Air subjected to high-energy deep-UV light using Biozone scientific COP generator	Air	More than 99.8% reduction of influenza virus A (H5N2).	Terrier et al. (2009)
<i>Escherichia coli</i> KCTC1039 <i>Bacillus subtilis</i>	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)
<i>Deinococcus radiodurans</i>	Dielectric Barrier Discharge (DBD)	Cells dried in laminar flow hood and cells suspended in distilled water	4 log reduction of CFU count in 15 s of the extremophile organism suspended in distilled water. This was attributed to the fact that plasma compromises the integrity of the cell membrane of the organism.	Cooper et al. (2009)
<i>Escherichia coli</i> type 1 <i>Saccharomyces cerevisiae</i> <i>Gluconobacter liquefaciens</i> <i>Listeria monocytogenes</i>	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 quenching of reactive plasma species.	Perni et al. (2008a)

<i>Escherichia coli</i> O157:H7 <i>Salmonella</i> Stanley	Gliding Arc plasma	On agar plates and inoculated onto surfaces of Golden Delicious apples	Bacterial inactivation was shown to be a function of flow rate and duration of exposure.	Niemira and Sites (2008)
<i>Aspergillus parasiticus</i> and Aflatoxins	Air gases and SF ₆ plasma using total applied power of approximately 300 W	Hazelnuts, Peanuts, and Pistachio nuts	SF ₆ plasma application was more effective with a 5-log decrease in fungal population for the same duration as air gases plasma. 20 min air gases plasma treatment resulted in a 50% reduction in total aflatoxins (AFB1, AFB2, AFG1, and AFG2), while only a 20% with SF ₆ plasma treatment. No significant organoleptic changes were observed.	Basaran et al. (2008)
<i>Escherichia coli</i> <i>Saccharomyces cerevisiae</i> <i>Pantoea agglomerans</i> <i>Gluconacetobacter liquefaciens</i>	Cold atmospheric plasma generated by an AC voltage (variable 12kV and 16kV)	Pericarps of mangoes and melons	<i>S. cerevisiae</i> 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)
<i>Escherichia coli</i> O157:H7 <i>Salmonella</i> sp. <i>Listeria monocytogenes</i>	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)
<i>Escherichia coli</i>	Air in a Dielectric discharge chamber	Raw almonds	Up to 5 log reduction observed	Deng et al. (2009)

<p><i>Escherichia coli</i> NCTC 9001, <i>Campylobacter jejuni</i> ATCC 33560, <i>Campylobacter coli</i> ATCC 33559, <i>Listeria monocytogenes</i> NCTC 9863, <i>Salmonella enterica</i> serovar Enteritidis ATCC 4931, <i>Salmonella enterica</i> serovar Typhimurium ATCC 14028, and <i>Bacillus cereus</i> NCTC 11145 endospores.</p>	<p>Pulsed plasma gas discharge (PPGD) using high-voltage pulses (with 40-kV DC capacitor) in a coaxial treatment chamber. Different treatment gases viz. nitrogen, carbon dioxide, oxygen and air as sparging</p>	<p>Poultry wash water at 4 °C</p>	<p>Rapid reductions in microbial numbers (by ≤ 8 log CFU/ml). Use of oxygen alone produced the greatest reductions. In general gram negative test bacteria were more susceptible.</p>	<p>Rowan et al. (2007)</p>
<p><i>Aspergillus niger</i>, <i>Bacillus atrophaeus</i>, <i>Bacillus pumilus</i>, <i>Clostridium botulinum</i> type A, <i>Clostridium sporogenes</i>, <i>Deinococcus radiodurans</i>, <i>Escherichia coli</i>, <i>Staphylococcus aureus</i>, <i>Salmonella mons</i></p>	<p>Cascaded dielectric barrier discharge with air as the plasma gas</p>	<p>PET foils</p>	<p>Highest count reduction was observed for the vegetative cells with at least 6.6 log₁₀ with in 1 s. <i>Aspergillus niger</i> was the most resistant test strain with an inactivation rate of about 5 log₁₀ in 5 s.</p>	<p>Muranyi et al. (2007)</p>
<p>Biofilms produced by <i>Chromobacterium violaceum</i></p>	<p>RF high pressure cold plasma jet using Atomflo 250 reactor with 100 W RF power supply using He and N₂ gas</p>	<p>Biofilms produced in 96-well polystyrene micro-plates</p>	<p>A 10 min plasma treatment was able to kill almost 100% of the cells. A complex, biphasic model of inactivation was observed.</p>	<p>Abramzon et al. (2006)</p>

<i>Bacillus subtilis</i> spores	Dielectric-barrier discharge (DBD) with pure helium or helium–oxygen mixture.	Polycarbonate membranes, supported by a layer of technical no.3 agar in a petridish.	Spore inactivation was mostly induced by the reactive oxygen species with the heat, UV photons, electric field and charged particles; all making minor contributions. Atmospheric-helium plasma plume was more effective than atmospheric-helium–oxygen plasma plume.	Deng et al. (2006)
<i>Escherichia coli</i> K12	Atmospheric plasma plume generated using He gas with a peak voltage of 6 kV	<i>E. coli</i> cells deposited on surface of membrane filters	SEM revealed severe loss in structural integrity of plasma-treated cells. Survival of <i>E. coli</i> cells was found to depend on the cell surface density, as it affects plasma penetration depth. Physiological state of cells (i.e. phase of growth) affects their resistance to plasma inactivation.	Yu et al. (2006)

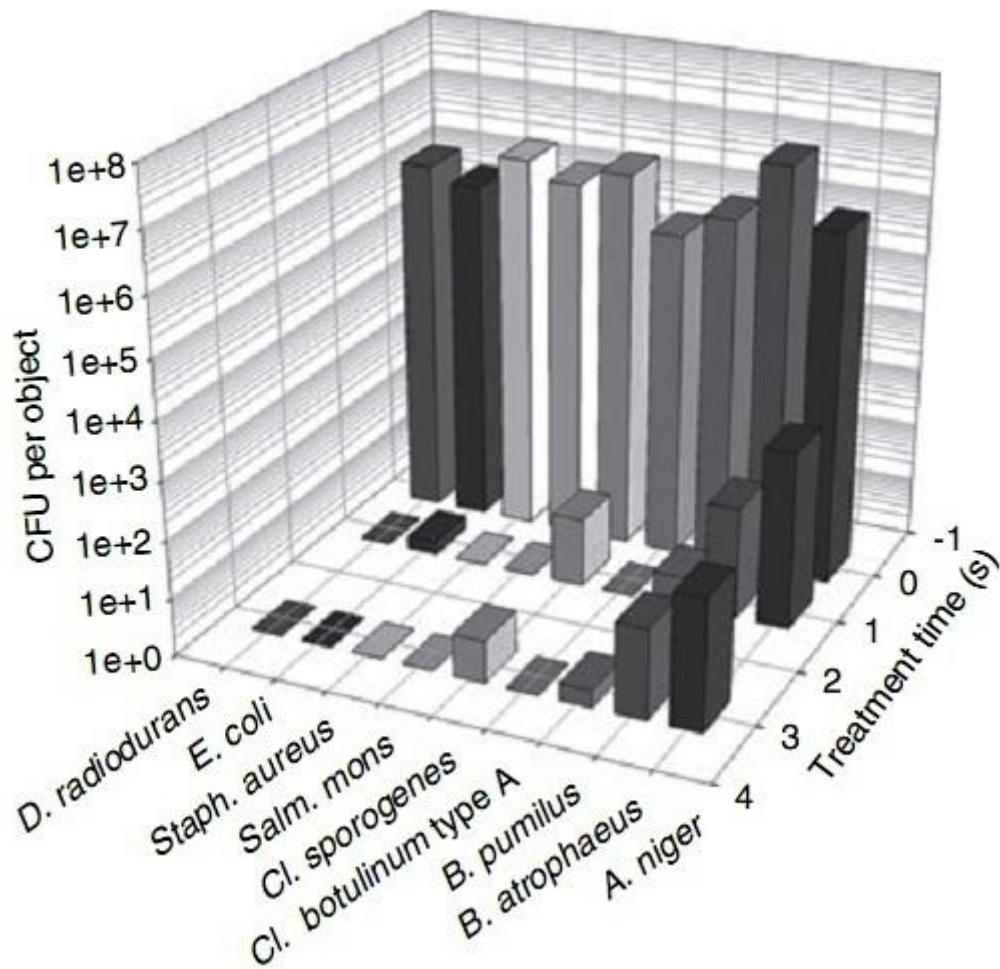


Fig. 1. Time-dependent inactivation of different test strains on PET foils with the CDBD equipped with a 282 nm excimer flat lamp. For comparison, the initial bacterial count should be in the range of approx. 10^6 CFU per sample. Process gas was laboratory air and input power was kept at about 130W (Muranyi et al. 2007).