A Study on Cold Plasma for Food Preservation

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Authors’ contributions
This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.

ABSTRACT
Cold plasma is an electrically energized matter composed of highly reactive species which includes molecules of charged and gas with minute particle in the form of negative and positive 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 is employed to inactivate the food borne pathogens seen in the recent years. The present study recollects the activity of the plasma agents on the microbe population, surface decontamination of the raw produce in the food processing and future novelty in food technology.

Keywords: Cold plasma; food; preservation; sterilization.

1. INTRODUCTION
"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. Matter changes 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 to the plasma state. D’Agostino et al. reported that plasma could be obtained either at 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. Application of plasma technology in biomedical sector, for cold sterilization of instruments and prostheses many thermolabile materials for its particular advantages including moderate or negligible effect on substrate materials and non toxic compounds. Traditionally various sterilized methods used for disinfection of fruits, vegetables, seeds etc. such as heat, chemical solutions and surface disinfectants, which are often time-consuming and damaging or have toxic residues. Van et al. [1] outlined the result of cold plasma on bacterial spores is more than the conventional techniques like heat, and chemicals and UV treatment. One of the important challenges associated with cold plasma technology is ensuring high microbial inactivation while maintaining sensory qualities and that ensure their fresh appearance.

The three conventional states of matter are solids, liquids, and gases; plasma has been described as next stage of matter with an unfamiliar designation that warrants explanation. As materials absorb energy (i.e. by heating) which effect to change from solid state (lowest energy) to liquid followed by 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 and ultimately breakdown entirely [2]. Gases are collection of molecules such as nitrogen, oxygen, carbon dioxide etc. or single atoms such as helium, argon etc. without large scale structure.

At still higher energies, the intra molecular and intra-atomic structures separates, liberating free and electrons. The plasma may be though the fast ionized gas consisting of ions namely electrons, protons and neutrons. 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 [3]. Within these hot plasmas, all species are extremely reactive. Within cooler (i.e., non-thermal) plasmas, such as those found in neon signs and plasma display screens and some chemical species which are more reactive than others. Thereupon, chemical composition of the feed gas, becomes a determining factor in the types of reactions that the plasma can initiate [4,5]. 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. When the active particles recombine with each other, energy was released as visible and UV light in the process of recombination [4,6] Of more interest to food processors, the active particles present in plasma may react with 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.

2. PLASMA SCIENCE (PLASMA-DEFINITION, PHYSICS, CHEMISTRY)

In 1922, Irving Langmuir, an American Scientist suggested that electrons, neutrons and ions in an ionized gas may be considered as corpuscular matter entrained in a kind of fluid phase known as entraining medium “plasma”. Resembling, to the plasma initiation, by Czech physiologist Jan Evangelista Purkinje denoted clear fluid which, thereafter left as corpuscular material in the blood. Although, it transpire that there was no “fluid medium” compelling neutrons, electrons and ions in a ionized gas [7].

plasma, a ionized gas consisting of photons, free electrons, atoms and ions along with electrons in the excited state and possessing a neutral charge. Hence, net neutral charge consists of equal amount of positive and negative carrier charges [8]. Light species (i.e. electrons and photons) named as heavy constituents in contrast with other species because of its unique nature, often referred as the fourth state of matter as off scheme of expression increasing the energy the level from the solid to liquid to gas and finally to the plasma.

3. DEFINITION OF TECHNOLOGY

A thermal plasma, an operating tool above hundreds and thousands of degrees above ambient, would be immediately detrimental to the
quality of food products. A nonthermal plasma is therefore the focus. For the sake of clarity, however, a distinction must be made between what nonthermal 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 [3,5]. 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 regarded as those that cause little or no thermal damage to the food product being treated.

- There are three major process by which the cold plasma inactivates the microorganisms [9].
- The foremost chemical interaction of radicals reactive species or charged particles is with cell membranes.
- This is followed by damage to cell membranes and intercellular components by ultraviolet radiation. Finally, DNA strands may be broken by UV generation by recombination of these plasma species.

But in given commodity, one way of action may be highly significant than another, the greatest sanitizing efficacy answer from plasma in multiple antimicrobial mechanisms [9,10]. As a food processing technology, cold plasma is new enough that the terminology is still evolving. The terms cold plasma [11], cool plasma [12], atmospheric pressure plasma [3], cold atmospheric gas plasma [13], 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 [14], uniform glow discharge plasma [15] gliding arc discharge [16], etc.

### 3.1 Types of Plasma

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 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 [5].

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 [3]. 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 [15].

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 & Lu 2005). Systems of this type can operate in pulsed mode, with plasma generated at pulse frequencies of hundreds or thousands of times per second.

4. COLD PLASMA GENERATOR

Though, diverse methods that had been utilized to generate, high amount non-equilibrium cold plasmas, near or at atmospheric pressure (sometimes referred as high pressure) were presented. It doesn’t mean the comprehensive list of the available methods. The methods selected were mainly for two reasons, germicidal effects; high pressures and potential industrial applications of plasmas (i.e. lighting, surface modification, sterilization, etching and deposition).

4.1 The Corona Discharge

The advantage of the corona discharge was to build ozone to disinfect where the water received recently (Menashi, 1972). They used a pulsed RF-driven corona to generate a plasma at atmospheric pressure and utilization of Helium was reported for the inactivation microbes at less than 1 second. The discharge would contain systematic arrangement of line of pins fastened to a hollow pipe at one end and protruding from the other end through tiny holes. Accordingly, local atmosphere was created around the corona points when the feed gas escapes. Therefore, feed gas referred as non-electronegative gas such as helium, argon replaces the air around corona points and helps in removing electron attaching electro-negative oxygen molecules.

The glow discharge is one of the beginning development reported by Donohoe [17], at atmospheric pressure. Hence, he polymerized ethylene using a large gap (cm) pulsed barrier discharge with mixture of helium and ethylene. Later, Kanazawa et al. (1988) reported that utilization of dielectric barrier discharge(DBD) configuration revealed development of stable grow pressure at atmospheric pressure. Further they insisted that to obtain DBD (as opposed to a filamentary discharge, which was traditionally produced by DBDs), Helium would have the major constituent of gas mixture, along with the other parameters such as frequency of the voltage (Khz), atleast one among the two electrodes must be coated with dielectric material, charged particles could be collected on the surface of the dielectric point after ignition of the discharge.

The voltagedrop created during charge buildup, counteracts the applied voltage and results to choke of discharge current. Further discharge extinguishes. As the process was repeated, accordingly the applied voltage increases (at the second half of applied cycle) and discharge reignites. Laurossi [10] noted the adoption of glow discharge at atmospheric pressure would eliminate cells of pseudomonas fluorescence. He passed-down suspensions of bacteria in petridishes placed on dielectric-covered lower electrode. The electrodes were implanted within the chamber consisting of helium with commixture of air. He accomplish full annihilation of concentrations of $4.10^6$/ml in less than 10 min. Using identical discharge, Kelly-Win tenberg (2000) noted the inactivation of *B. subtilis* spores

![Fig. 1. The glow discharge at atmospheric pressure](image-url)
using an air gap. *E. coli*, *B. subtilis* and a variety of other gram-negative, gram-positive bacteria were inactivated successfully by the most researchers using the DBD-based diffuse glow discharge.

### 4.2 The Atmospheric-pressure Plasma Jet

Atmospheric-pressure plasma jet (Shown in Fig. 3) was capacitively coupled subsists of two coaxial electrodes between which gas passes at high rates. The outer electrode was grounded, while the central electrode was excited by RF power at 13.56MHZ. The free electrons were accelerated by RF field and enter into collisions with molecules of the background gas. These inelastic collisions expel various reactive species (excited atoms, molecules and free radicals) that prevail the nozzle at high velocity, therefore react with a contaminated surface placed in proximity (cm) of the nozzle.

Herrmann et al. [18], reported diffuse DBD, the stability of the APPJ plasma depend on using a carrier gas (helium) to inactivate spores of *bacillus globigii*, a simulant to Anthrax (*Bacillus anthracis*). The attrition of seven orders of magnitude of the original concentration of *B.glonigii* in about 30s.
4.3 Resistive Barrier Discharge

The approach of resistive-barrier discharge (RBD) is stationed on DBD configuration (shown in Fig. 4). In spite of dielectric, high resistivity material was utilized to cover at least one of the electrodes. Thereby, high resistivity layer plays the role of distributed ballast which inhibits the discharge current and therefore prevents arcing. The benefit of RBD over DBD was possibly the use of DC power (low frequency, Hz) to drive the discharge.

Richardson et al., Laroussi et al., reported a four orders-of-magnitude reduction in the authentic concentration of vegetative B. subtilis cells in about 10 min. they have also reported RBD-inactivates endospores of B. subtilis, but not as effectively as vegetative cells. Hence the experiment consists of gas mixture of 97%-3% helium-oxygen, respectively.

4.4 Action on Cell Components and Functions

In 1960’s menashi reported sterilizing properties of plasma and patented in 1968 and first works with plasma invention from oxygen were proposed in 1989. Thereafter, ample research had been done on the structure of microbial inactivation by plasma agents. These plasma agents continue to the lethal action by interacting with biological material. Nelson and Berger [19] have laid out O₂ plasma could be very efficient towards bacteria.

A wide range of microorganisms such as spores (Kelly-Wintenberg et al. [20], Lee et al. 2006) and viruses [21] can be completely inactivate using plasma treatment. The consequence of plasma could be quite selective, significantly tunable between damage to pathogenic organisms after without damage to host, or activation of different pathways in various organisms (Dobynin et al. 2009).

Mogul et al. 2003 reported that the use of low pressure oxygen plasma had shown to degrade lipids, proteins and DNA cells. The active species in the plasma had been widely associated to direct oxidative effects on the outer surface of the microbial cells. E.g O₂ and N₂ gas plasma were excellent sources of active oxygen based and nitrogen based species, such as O•, O2, O3, OH•, NO•, NO2, etc Atomic oxygen was a very competent sterilizing agent, with a chemical rate constant for oxidation at room temperature of about 106 times that of the molecular oxygen [22].

These play a role on unsaturated fatty acids of lipid bilayer of the cell membrane, so that impeding the movement of bio-molecules across it. This double bond was due to the ozone attack (Guzel-Seydim et al. 2004). Membrane lipids were expected to be more significantly influenced by the active species due to the location with the surface bacterial cell, which allowed to destroy the oxidizing agents [23]. The protein cells and spores were equally vulnerable to the action of the reactive species, causing cell leakage and

![Fig. 4. Configuration of the resistive-barrier discharge (RBD) Action of Plasma on microorganisms](image-url)
denaturation. The cause of microbial death may be also a result for oxidation of amino acids and nucleic acids [22].

Microorganisms present in the coldplasma are exposed to an intense bombardment by radicals, most provoking surface lesions that living cell could not repair faster. This may eventually explain that the observations where in cells, may destroy quickly, which is termed as “etching” [24]. The cell wall may damage had been additionally attributed by Laroussi et al. [10] and Mendis et al. [25] to electrostatics forces, which cause accumulation of charges at the outer surface of the cell membranes. The morphological changes in E.coli cells treated with the atmospheric plasma (75W/2min) as observed under an electron microscope [26,27], clearly revealed, treated cells have severe cytoplasmic deformations and leakage of bacterial chromosome.

These investigations demonstrate the damage of viability to bacterial cells after plasma treatment. The correlation between plasma and pulsed electric field had been starved to explain the action of the plasma towards membranes (Pothakamury et al. 1995) [27] It was well positioned that electroporation of membranes is induced by PEF and it come out to be plasma acts on similar lines happen, perforations in the membranes of the microorganisms [28] (Pothakamury et al. 1995) [29]. In addition to generating pores, humid air plasma additionally provokes marked acidification of the medium [30,31].

4.5 Role of UV Photons and Charged

The formation of UV photons at different wavelengths had been proposed to be tangled in dimerizing the thymine bases of DNA including that of spores (Munakata et al. 1991). The action of UV photons in the bacterial death when they were submitted to the plasma treatment was reviewed in detail by [32]. Moreover, the exclusion of the reactive particles and spectral fractions of UV radiations from the access to the spores Roth et al., 2010 disclosed that UV-c radiation was the most efficient inactivation agent in the plasma. UV photons play minor role in the atmospheric pressure glow discharge (APGD) because they are easily absorbed by gas atoms and molecules at atmospheric pressure [33].

The performance of the charged particles in the bacterial inactivation process was recently investigated by Lu et al. 2009. Hence, worked revealed that charged play a minute act in the inactivation process when He/N2 (3%) was used as working gas than when He/O2 (3%) was used. Also, it was concluded that heat and UV played a less role in the inactivation process. Resembling results were earlier obtained by [34] who interplayed bacterial inactivation kinetics with optical emission spectroscopy, and identified oxygen atoms as a major contributor in plasma inactivation with less contributions from UV photons, singlet oxygen metastable and nitric oxide. Thus, a contradiction over the role of UV photons in the plasma prevails and further studies must be directed to get a clear picture.

4.6 Effect of Process Parameters

The concentrations in which the plasma gents present in plasma depend mostly on the device parameters such as set up (reactorgeomtry), operating conditions (gas pressure, type, flow, frequency and power of plasma extraction) and gas composition which effect their efficiency 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 [35].

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 [13]; (Laroussi 2005) [32]. 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 [36].

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 the 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.
5. POTENTIAL APPLICATIONS

Table 1 shows the variability in chemical nature of cold plasma depends upon the material with which it is packed. Treatment with disparate electric discharges, cold plasma tends to amend its chemical properties all together. Recent findings in the area of non-thermal plasmas for inactivation of microorganisms and spores have been described in Table 2, whereas microbial inactivation using cold plasma have been tabulated in Table 3.

6. 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 using 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 the effect of nonthermal plasma on food components are scarce in the literature. Based on the experiments using low-pressure oxygen plasma it has been observed that time and structure-dependent degradation can be seen for differently selected model flavonoids adsorbed on solid surfaces, which was attributed to plasmaimmanent reactive species such as O (3P), O2 (1Δg and 1Σg+), 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).

Table 1. Results of action of cold plasma on packing materials

<table>
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<tr>
<th>Packing materials</th>
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<th>Applied voltage (dosage)</th>
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<td>Polypropylene</td>
<td>Air Corona</td>
<td>30 kHz, 1.7 j/cm²</td>
<td>Decrease in contact angle, increase in adhesion</td>
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<td>PET films</td>
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<td>Decrease in hydrophilic nature, Increase in tensile strength</td>
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<td>Diode plasma discharge</td>
<td>8.3 W</td>
<td>Decrease in contact angle, increase in surface energy</td>
<td>P. Slepicka, et al. [40]</td>
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<tr>
<td>PET film</td>
<td>Jet plasma DC discharge</td>
<td>35 W</td>
<td>Increase in hydrophobic nature</td>
<td>Y. Akshev, et al. [41]</td>
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<tr>
<td>PET</td>
<td>Jet plasma</td>
<td>285 V</td>
<td>Increase in weight, decrease in contact angle and wettability</td>
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<td>150 W</td>
<td>Decrease in crystallinity, contact angle, increase in roughness</td>
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<td>LDPE</td>
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<td>25–100 W</td>
<td>Decrease in contact angle and ageing effect, increase in crystallinity</td>
<td>M. Aasaeefard, et al. [44]</td>
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<td><em>Escherichia coli</em>, <em>Staphylococcus aureus</em></td>
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<td><em>Escherichia coli</em> KCTC1039 <em>Bacillus subtilis</em></td>
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<td><em>Escherichia coli</em> type 1 <em>Saccharomyces cerevisiae</em>, <em>Gluconobacter liquefaciens</em>, <em>Listeria monocytogenes</em></td>
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<td>Perni et al. [49]</td>
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<td><em>Escherichia coli</em> <em>Saccharomyces cerevisiae</em>, <em>Pantoeaagglomerans</em> <em>Gluconacetobacter liquefaciens</em></td>
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### Table 3 Recent finding of microbial inactivation using cold plasma

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<td>4 log CFU/ml</td>
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<td>Perni et al. [49,50]</td>
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</tbody>
</table>
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 [22]. 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 [33]. 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.

7. CONCLUSION AND RECOMMENDATION

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 particular temperature, humidity or pressure conditions. The application of 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 commercial applicability for the food industry.

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.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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