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COLD PLASMA TECHNOLOGY IN AGRICULTURE AND FOOD INDUSTRY - A REVIEW

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Abstract. Cold plasma is an emerging non-thermal technology which has shown potential applications at different interfaces of life sciences. Cold plasma treatment plays a crucial role in a broad spectrum of plant development and physiological processes in plants in promotion of seed germination and seedling growth, activation of photosynthesis, regulation of carbon and nitrogen metabolism in reducing the bacterial bearing rate of seeds, changing seed coat structures, increasing the wettability and permeability of seed coats. It is an effective substitute for the traditional presowing seed treatment with chemical agents. In food industry, its application is mostly focused on food decontamination, improving food quality, degradation of toxins and surface modification of packaging materials. The impact of Cold Plasma on food quality is very important for its acceptance as an alternative food processing technology. This review paper aims at focusing the potential applications of cold plasma in agriculture and food industry highlighting its current research and trends besides providing a summary of plasma chemistry and its sources. Cold Plasma processing is still in its nascent form and needs further research to reach its potential.

Keywords: Cold plasma technology, agriculture, seed germination, food industry, food decontamination.

1. Introduction

Cold plasma (CP) is an emerging technology, which has attracted the attention of scientists globally. Plasma, often referred to as the fourth state of matter, is an quasi-neutral ionized gas, containing an array of active species of electrons, photons, free radicals and ions exhibiting unique properties. (Ekezie, F.C *et al*., 2017 and Dasan *et al*., 2017). Cold plasma is obtained at atmospheric or reduced pressures (vacuum) via partial ionization, and requires relatively lower power inputs. It was originally developed for ameliorating the printing and adhesion properties of polymers by surface modification and functionalization of polymers and it has a variety of usage domains in polymer and electronic industry. However, in recent years, its applications have rapidly expanded into food industry and treatment of biomedical devices as a powerful tool for non-thermal processing, with diverse forms for utilization.

The application of cold plasma in food industry include food decontamination, food quality improvement, degradation of mycotoxins and pesticides in the agricultural produce, functionality modification of food materials, and surface modifications of packaging materials. (Pankaj. S.K., and Kevin M Keener., 2017). Its application has also been expanded in to areas, such as hydrogenation of edible oils to yield trans-free edible oils, control of food allergens in mitigation of food allergy, inactivation of anti-nutritional factors, enhancement of seed germination performance and improved physiochemical properties of grains. (Mir, Shah and Mir,2016; Sivachandiran and Khacef, 2017). It has proven to be effective for inactivation of various pathogens and spoilage organisms without adversely affecting the food quality. Current plasma research analyzes its effectiveness against various pathogens with emphasis on the inactivation mechanisms at a molecular level. Cold Plasma is a trending area of research with a significant number of research output in the past two years and as the technology is new and has not been fully exploited, it is essential that consistent generalization should be made to drive the technology's awareness, focus its future research needs and promote its acceptability.

2. Plasma Physics and chemistry of Plasma generation

The term 'plasma' refers to a quasi-neutral ionized gas, primarily composed of photons, ions and free electrons as well as atoms in their fundamental or excited states with a net neutral charge. A plasma state is achieved when sufficient energy such as heat or electricity is applied to a gas. It is estimated that 99.9% of the universe is in a plasma state. The northern aurora, southern aurora and lightning were recognized as naturally-occurring plasmas and have been described and studied since the 17^{th} and 18^{th} centuries. Electrical energy is always used for the generation of manmade plasmas. Cold plasmas are composed of a cocktail of different chemical species such as positive ions, negative electrons, excited atoms, UV photons, radicals and reactive neutral species such as reactive oxygen (ROS) and Nitrogen species (RNS). Application of a voltage to a gas generates an electric field that can accelerate any free electrons in the gas. Accelerated electrons will collide with neutral gas atoms, resulting in excitation or ionisation. Ionisation releases more free electrons to be accelerated, causing an 'avalanche effect' generating a rich abundance of highly reactive, short-lived chemical species that are capable of inactivating a wide range of microorganisms, including foodborne pathogens and spoilage organisms.

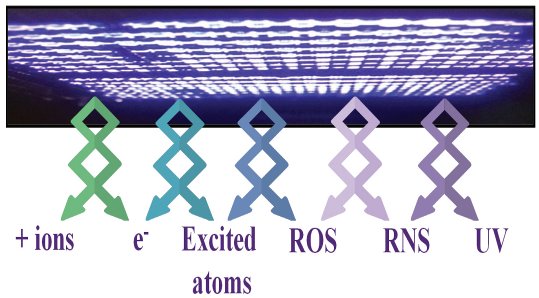

FIGURE 1. Reactive chemical species generated in plasma discharges

Plasma is generally categorized as 'thermal' when electrons and other gas species are in thermodynamic equilibrium and 'non-thermal' when they exist in non-equilibrium. Thermal plasma requires extreme pressure levels (105 Pa) and up to 50 MW of power for its propagation, which is also distinguished by a thermodynamic equilibrium between the electrons and heavier species due to uniform gas temperature for all constituents (Scholtz, *et al.,* 2015). The non-thermal plasma is produced at low levels of pressures and power, without a localized thermodynamic equilibrium, thus designated as non-equilibrium plasma. Non-thermal plasma induced by electrical discharges is of primary interest for the applications in the food industry, due to its potential relevance in processing foods at low temperatures. Earlier, cold plasma was generated under low-pressure conditions limiting its applications. However, recent advances in plasma engineering have allowed cold plasma generation at atmospheric pressure leading to increased research on cold plasma at various interfaces of life sciences.

Cold atmospheric plasmas can be generated using direct current (DC) or alternating current (AC) power supplies. A range of different frequencies for AC power supplies from low kHz frequencies to Radio frequencies (MHz) and Microwave (GHz) frequencies have been used to generate plasma discharges. Typically, noble gases such as helium or argon are used to generate a plasma because lower voltages are required to break down the gas and sustain a discharge. Other gases can be added either oxygen or nitrogen to provide the type of reaction chemistries required. Plasmas have also been generated using only nitrogen as the operating gas and with the correct electrode configuration and power supply, plasmas can also be generated using air. For example, air plasma consists of over 75 unique species and more than 500 chemical reactions happening at nano, micro, milli and seconds time scales [Gordillo-Va zquez FJ, 2008].

There are various types of cold plasma generation systems used for different applications in the industry. They could range from corona discharges, microwave plasma, radio frequency

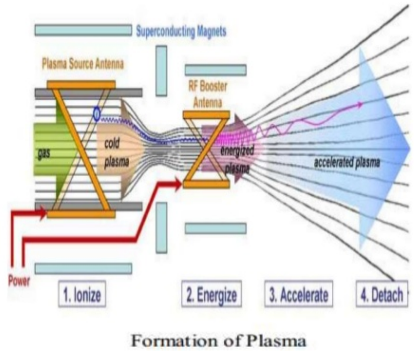

FIGURE 2. Formation of Plasma

plasma, inductively coupled plasma, capacity coupled plasma, electron cyclotron resonance plasma and dielectric barrier discharge plasma. Among these, dielectric barrier discharge and jet plasma are the most used for food research owing to their simple, versatile and adaptive designs and working.

There is a continuing research in the field of plasma chemistry for identification of reactive species generated in the plasma and their interaction with the biological and chemical components of food products.

When the electrical supply is switched off, all the reactive plasma species return to their neutral ground state. The dominating reactive gas species can be significantly altered depending on the type of power supply, how the power is applied (continuous vs. pulsed), the configuration of the electrodes and the type of gases used. This means the technology has the potential ability to tailor reaction chemistries for specific applications. The diffuse nature of plasma allows a greater chance that the reactive chemical species can inactivate bacteria in pores, crevices or harder-to-reach areas of equipment and surfaces. This offers significant advantages over alternative techniques, such as UV light where microbes can be protected by ‘shadowing’ effects.

2.1. **Cold Plasma in Seed germination:** Drought, salinity and seed dormancy are the problems encounter across the world adversely affecting crop production, germination, growth and yield. Sustaining the quality of seeds is a major task in attempting to supply nutrition to the growing world population. The most commonly used methods including agrochemicals, fungicides, insecticides and hormones to enhance the seed germination are the chemical methods. But these types of treatments leave chemical residues which are harmful to the human health and environment. Cold plasma treatment is a fast, economic and pollution-free method to improve seed performance, plant growth and ultimately plant yield (Ling *et al.* 2014). This treatment plays a crucial role in a broad spectrum of plant development and physiological processes in plants, including the promotion of seed germination and seedling growth, activation of photosynthesis, regulation of carbon and nitrogen metabolism, reducing the bacterial bearing rate of seeds, changing seed coat structures, increasing the wettability and permeability of seed coats. (Selcuk *et al.*, 2008, Ling *et al.* 2014). This phenomenon has been demonstrated in several plants such as *Chenopodium album*, *Oryza sativa, Triticum aestivum*, *Lycopersicon esculentum* and *Solanum melongena* L. In addition, plasma treatment also could improve the physiological metabolism of the plant, such as dehydrogenase activity, superoxide dismutase and peroxidase activities, photosynthetic pigments, photosynthetic efficiency and nitrate reductase activity. Plasma treatment could significantly increase crop yields.

Jiang *et al.*2014 reported that an 80 W cold plasma treatment significantly increased wheat yields, and Selcuk *et al.* 2008 have reported that grain and legume yields were significantly increased by cold plasma. It is an effective substitute for the traditional pre-sowing seed treatment with chemical agents.

The plasma etching or scratching effect on the seed coat increased the hydrophilicity of seeds. Reactive species formed in the plasma has the ability to breakdown the seed dormancy and increase the seed germination rate. The seed germination rate can be increased on application of cold plasma by both direct and indirect treatments. In direct treatment method the seeds are directly placed in between the electrodes or placed under the plasma regime like in plasma jets. Recently, the indirect treatment through the application of plasma activated water (PAW) has gained importance. The formation of reactive oxygen species and reactive nitrogen species in the plasma are mainly responsible for increase in seed germination rate. Of all those reactive species formed in the PAW, the nitrate ions serves as the fertilizer and NO radical breakdown dormancy which enhanced the seed germination rate. The synergistic effect of cold plasma can replace the traditional seed disinfection solutions and chemical seed germination enhancers. (Thirumdas *et al.,* 2017)

The difference was observed between optical emission spectra generated by plasma without seeds and during their treatment. The species identified in the spectra are neutral molecular nitrogen $N_{2,}$ ionized molecular nitrogen N_2. Appearance of CO molecules and ionized O_2^+ molecules in spectra during seeds treatment confirmed that the plasma chemical etching of seed surface plays an important role in stimulation of biochemical processes that influence on seed germination.

The impact of cold radiofrequency air plasma on the wetting properties and water imbibition of beans (*Phaseolus vulgaris*) was elucidated by Bormashenko *et al*., 2015. He established that cold plasma treatment leads to hydrophilization of the cotyledon and tissues constituting the testa when they are separately exposed to plasma. By contrast, when the entire bean is exposed to plasma treatment, only the external surface of the bean is hydrophilized by the cold plasma. In a study on water imbibition by plasma-treated beans, plasma treatment accelerates the water absorption. However, the speed of germination was markedly higher for the plasma-treated samples. Cold plasma treatment markedly hydrophilized the external (exotesta) surface of the seed coat, whereas the mesotesta and cotyledon kept their hydrophobicity when the entire bean was exposed to plasma treatment. It was found that plasma ions lose their energy when penetrating through the coat, and these ions are incapable of modifying the wetting regime of the biological surface.

Hydrophilization of biological tissues by cold plasma resembles the similar effect observed and researched in synthetic polymers. PT usually strengthens the hydrophilicity of treated synthetic polymer surfaces. However, the surface hydrophilicity created by plasma treatment is often lost over time. This effect of decreasing hydrophilicity is called ‘hydrophobic recovery’ (Mortazavi *et al.*, 2012). By contrast, hydrophobic recovery was not observed in plasma-treated seeds (Bormashenko *et al.*, 2012). It should be emphasized that cold plasma treatment only influences the nano-scaled external layer of a tissue (Mortazavi and Nosonovsky, 2012; Bormashenko *et al.*, 2013). This fact may be crucial for the biological applications of cold plasma treatment.

Ji *et al.* 2016, has proved that NO formed in the plasma is responsible for seed germination enhancement of coriander seeds. The NO formed in the plasma serve as a signaling pathway, triggers activation of several biological processes and a crucial regulator for cellular activation. Zhang *et al.* 2017 reported the synergistic effect of formation of endogenous NO radicals and breakdown of seed dormancy for increase in higher germination rates.

An investigation carried out by Bormashenko *et al.* 2012 on the wettability of seeds using cold air plasma decreased the contact angle from $115\circ$ to $0\circ$. The plasma treatment resulted in complete hydrophilicity of seeds. The water imbibition of plasma treated seeds was increased by 30% after the 12 h of germination.

2.2. **Cold Plasma in improving drought tolerance:** Plasma treatments are thought to enhance the ability of plants to cope with biotic and abiotic stress, such as drought stress and disease stress. Water is one of the major limiting factors for the agricultural production in arid and semiarid areas. Drought is the main environmental constraint, which often having devastating effects on crop productivity. Hence, improved tolerance to drought has been an important goal in crop improvement programs. Therefore, selection based on the phenotype would be difficult for such traits.

Positive influence of the cold plasma treatment on the germination rate of bean seeds and on the kinetics of germination was recorded for the alfalfa and trifolium seeds under the conditions of drought stress. Under mild drought stress and harsh drought stress, the germination rate of non-treated seeds was significantly reduced by 6% and by 10%, respectively, compared with the well-watered seeds. The cold plasma treatment significantly increased the germination rate by 10% in beans compared to the mild drought-stressed and harsh drought-stressed non-treated seeds. (Ling *et al.,* 2015)

Seeds	**Findings**	**Reference**
Chick pea	On exposure to atmospheric cold air plasma for 5 min, seed germination increased by 89.2%.	Mitra .A, *et al.*, 2014
Soy, honey clover and catgut	Germination of seeds increased by 10–20 %	Filatova *et al*., 2011
Corn seeds	Increased the germination rate and higher yields	Violleau *et al*.2008
Rapeseeds	Increased the germination rate by 7.7%	Puligundla *et al*. 2017
Oat, corns and wheat	Did not affect the germination of oats but there is slight increase in germination rate in wheat	Sera *et al.,* 2010
Rice	Increase in seed germination potential by 6.0% and the germination rate by 6.7%	Jiang *et al.,* 2014
Mung beans	Increased the germination rate by 36.2%, radical root length by 20% and conductivity of seeds by 102%. Increased the seeds coat conductivity and there is increase in soluble sugars and proteins after the treatment. Apparent decrease in contact angle; decrease in anti-nutritional properties like trypsin inhibition activity and phytic acid	Sadhu *et al.*, 2016
Brown rice	Increased the hydrophilicity of seeds	Thirumdas *et al.,* 2017
Coriander seeds	91-97% germination of seeds	Zhang *et al*., 2017
Beans	Improved seed germination (89.2 %), speed of germination (7.1??0.1 seeds/day), and increased seed vigor, besides a decrease in the mean germination time (2.7 days)	Bormashenko *et al*., 2015
Soybean	Germination and vigor indices significantly increased by 14.66% and 63.33%, respectively. Seed's water uptake improved by 14.03%, and apparent contact angle decreased by 26.19%. Characteristics of seedling growth, including shoot length, shoot dry weight, root length and root dry weight significantly increased by 13.77%, 21.95%,21.42% and 27.51%, respectively	Ling *et al.*, 2014

TABLE 1. **Reports on the different seeds germination enhancement caused by cold plasma.**

Effects of cold plasma treatment on seed germination, seedling growth, antioxidant enzymes, lipid peroxidation levels and osmotic-adjustment products of oilseed rape under drought stress were investigated in a drought-sensitive (Zhongshuang 7) and drought-tolerant cultivar (Zhongshuang 11). It was observed that cold plasma treatment significantly improved the germination rate by 6.25% in Zhongshuang 7, and 4.44% in Zhongshuang 11 under drought stress. Seedling growth characteristics, including shoot and root dry weights, shoot and root lengths, and lateral root number, significantly increased after cold plasma treatment. The apparent contact angle was reduced by 30.38% in Zhongshuang 7 and 16.91% in Zhongshuang 11. Cold plasma treatment markedly raised superoxide dismutase and catalase activities by 17.71% and 16.52% in Zhongshuang 7, and by 13.00% and 13.21% in Zhongshuang 11. Moreover, cold plasma treatment significantly increased the soluble sugar and protein contents, but reduced the malondialdehyde content in seedlings suggesting that cold plasma treatment improved oilseed rape drought tolerance by improving antioxidant enzyme activities, increasing osmotic-adjustment products, and reducing lipid peroxidation, especially in the drought-sensitive cultivar (Zhongshuang 7). Cold plasma treatment could be thus used in an ameliorative way to improve germination and protect oilseed rape seedlings against damage caused by drought stress.

2.3. **Cold Plasma techniques in food industry:** The food industry is faced with the challenge of delivering nutritious, safe and shelf-stable food products to consumers by preventing microbial contamination. Innovation with regards to food production and processing has become essential to meet the emerging challenges of global food security and the complexities of the modern food chain. Novel preservation technologies are an interesting option to produce high quality food products with an extended shelf life. Cold plasma is an upcoming nonthermal technology with potential applications in food industry. The use of cold plasma has not yet been fully realised in the food industry but the antimicrobial properties of plasma systems make it an attractive tool for food manufacturers in the fight against cross-contamination, microbiological spoilage and reduced shelf-life. The most important application is the disinfection of surfaces in processing equipment, packaging, food contact surfaces and, potentially, food itself.

The non-thermal properties of plasma make it potentially suitable for treating the surface of delicate raw and fresh produce as well as other foods. Cold plasma can inactivate spoilage organisms and significantly extend the shelf-life of samples in strawberries. Shelf-life extension of fresh produce could reduce product waste and therefore increase manufacturers' and retailers' overall profitability and generating greater convenience for consumers. Over the past decade, research has been focused on the potential of cold atmospheric pressure plasma to be used for inactivating pathogens and spoilage organisms on the surface of food products. To date varying log reductions have been achieved on the surfaces of melons, mangoes, apples, strawberries, tomatoes, lettuce, potatoes, cheese, almonds, nuts, seeds, egg shells, ready-to-eat meats, bacon, chicken and pork.

2.3.1. **Cold Plasma in control of bacterial Pathogens:** Cold plasma is known for its excellent antimicrobial properties in a range of fields, including the biomedical, textile, and polymer industries. The non-thermal technology is highly advantageous for microbial decontamination of food products including sporulating and pathogenic organisms due to ample amount of reactive oxygen species (ROS) contained in the quasi-neutral plasma gas. During processing or post-harvest operations, microbial invasion can occur at any step including human handling, equipment operation, transportation or the actual processing.

The antimicrobial efficacy of Cold Plasma treatment has been established by a significant number of studies. CP treatment destroys microorganisms in chicken meat. It is also capable of killing *Listeria innocua* in ready-to-eat meat by up to 1.6 0.5 log cfu/g, depending on the composition of the charged species. Inoculated samples were treated at 15.5, 31, and 62 W for 2-60 s inside sealed linear-low-density-polyethylene bags containing 30% oxygen and 70% argon while multiple treatments at 15.5 and 62 W of 20 s with a 10 min interval increased reduction of *L. innocua* with increasing number of treatments. (Rød, Hansen, Leipold, and Knøchel, 2012).

Atmospheric plasma at 18 kV can kill *Salmonella* spp. on strawberry samples by 1.7-3 log CFU/sample (Ma *et al*., 2015). Cold plasma generated in a gliding arc was applied to outbreak strains of *Escherichia coli* O_{157}:H_7 and *Salmonella* Stanley on agar plates and inoculated onto the surfaces of Golden Delicious apples. This novel sanitizing technology inactivated both pathogens on agar plates, with higher flow rate (40 liters/min) more efficacious than lower flow rates (20 liters/min), irrespective of treatment time (1 or 2 min). Golden Delicious apples treated with various flow rates (10, 20, 30, or 40 liters/min) of cold plasma for various times (1, 2, or 3 min), are applied to dried spot inoculations. All treatments resulted in significant reductions from the untreated control, with 40 liters/min more effective than lower flow rates. Inactivation of *Salmonella* Stanley followed a time-dependent reduction for all flow rates. Reductions after 3 min ranged from 2.9 to 3.7 log CFU/ml, close to the limit of detection. For *E. coli* O157:H7, 40 liters/min gave similar reductions for all treatment times, 3.4 to 3.6 log CFU/ml. At lower flow rates, inactivation was related to exposure time, with 3 min resulting in reductions of 2.6 to 3 log CFU/ml. Temperature increase of the treated apples was related to exposure time for all flow rates. The maximum temperature of any plasma-treated apple was 50.8$\circ$C after 20 liters/min for 3 min, indicating that antimicrobial effects were not the result of heat.

Likewise, using dielectric barrier discharge (DBD) plasma at 80 kV for 5 min successfully decontaminated cherry tomatoes containing *Escherichia coli, Salmonella typhimurium,* and *Listeria monocytogenes* by up to 3.5, 3.8, and 4.2 log CFU/tomato, respectively.

Lee *et al.,* 2016 evaluated the microbial and physicochemical characteristics of brown rice (BR) treated with cold plasma. Cold plasma was generated in a plastic container (250 W, 15 kHz, ambient air) and the cold plasma was applied to BR samples for periods of 5, 10 and 20 min. When BR samples were inoculated with *Bacillus cereus, Bacillus subtilis, and Escherichia* *coli O157:H7,* a 20min plasma treatment resulted in a reduction in bacterial counts by approximately 2.3 log CFU/g. The pH of the BR decreased slightly after the 5 min plasma treatment. The α-amylase activity and water uptake rate increased significantly while hardness decreased significantly indicating that cold plasma treatments can improve the microbial quality of BR and produce slight changes in the physicochemical quality of BR. Cold plasma has been described as a possible decontamination technology on fruits and vegetables including cucumber, carrot and pear slices experimentally contaminated by *Salmonella*. (Wang *et al.,* 2012). The seeds of *Cicer arietinum* exposed to cold atmospheric plasma (CAP) resulted in significant reduction of the natural microbiota attached to the seed surface and observed that increasing CAP treatment times 2 and 5 min was sufficient to achieve a 1 and 2 log reductions, respectively. Reductions of E. coli O157: H7, *Salmonella, and Listeria monocytogenes* counts have also been reported for apples and lettuce (Misra N.N. *et al*., 2011).

2.3.2. **Mechanism of Bacterial Inactivation by Cold Plasma:** The mechanisms of bacterial inactivation via plasma are not yet fully understood and research is ongoing. This is partly due to the complex dependence of cellular mechanisms and signaling on RONS. Reactive oxygen species play the most crucial role in microbial inactivation leading to strong oxidative stress conditions, causing cell damage by lipid peroxidation, enzyme inactivation and DNA cleavage . (Han *et al*., 2014) . Damage to the cell wall, capsule, or plasma membrane occurs either due to restructuring of surface polysaccharides via lipid peroxidation in water or direct etching due to ion bombardment. Peroxidation of the phospholipids in the plasma membrane is one such disruption mechanism. This occurs in several steps beginning with radicals abstracting hydrogen from the side chain of an unsaturated fatty acid to produce a lipid-radical and water. Propagation occurs in a chain reaction mechanism, and the process is only terminated when two radicals react with one another or antioxidants scavenge radicals. This results in shorter lipids with an altered ability to rotate within the membrane. The loss of membrane integrity leads to permeabilization and possible lysis. Once the outer structures are significantly damaged, cell contents vital to cellular function, such as adenosine triphosphate (ATP) leak out of the cell, resulting in cell death. Additionally, if any reactive species diffuse into the interior of the cell, they cause oxidative damage to DNA and proteins . If this damage is significant enough, the cell may no longer be able to function.

There are two theories as to how plasma can disrupt the bacterial capsule. An accumulation of electric charge on the membrane may result in electrostatic disruption, where the outward electrostatic stress exceeds the material tensile strength of the capsule. This mechanism seems to have a greater effect against gram-negative bacteria due to the irregular nature of the capsule and thinner peptidoglycan layer, allowing for greater charge buildup. Additionally, oxidation of membrane components can occur, in which active radicals diffuse to the membrane and react with the bio macromolecules at the surface, creating structural instabilities.

Recently, Han *et al.,* 2016 have reported different inactivation mechanisms for Gram positive and Gram negative bacteria by cold plasma. They showed that cold plasma inactivation of Gram positive bacteria (*Staphylococcus aureus*) was mainly due to intracellular damage and envelope damage whereas Gram negative bacteria (*Escherichia coli*) was inactivated mainly by cell leakage and low-level DNA damage. These studies clearly indicated the selective and differential interactions of reactive gas species in the plasma systems emphasizing the need for future mechanistic studies for better understanding. Cold plasma systems can produce these chemical species simultaneously and directly at the point of need, thus maximising the antimicrobial potential of this technology.

2.3.3. **Cold Plasma in control of fungal pathogens:** Phytopathogenic fungi infect crops in the field either during pre-harvest spoilage while spoilage fungi colonize harvested commodities during storage in post-harvest spoilage. Besides the reduction of yield and quality, infection with fungal pathogens often leads to contamination with mycotoxins. Plasma processing had a significant fungicidal effect on seeds (Dasan, *et al*., 2016). Fungal infection (*Fusarium*, *Ascochyta*, of treated crops was lowered by 3–15 % compared with untreated ones. At the same time, no effect on the treatment was observed during seed infection with *Anthracnose*. The difference was observed between optical emission spectra generated by plasma without seeds and during their treatment. The species identified in the spectra are neutral molecular nitrogen N_2 (bands of the first and the second positive systems, ionized molecular nitrogen N_2+ (bands of the first negative system). Atomic oxygen and OH radicals generated in plasma could be the most probable sterilizing agents, while the nitrogen presented in air plasma plays an important role in the intensification of the biological processes in seeds. The radio-frequency electromagnetic field and rf cold plasma processing of seeds of grains and legumes promoted increase in their germination energy as well as in laboratory and field germinating capacity due to both change in transport properties of the cellular plasmatic membranes accompanying with an enhancement of humidity permeability of seed surface and the reduction of seed contamination with pathogenic fungi. Pre-sowing radio-wave treatment increased grain and green mass productivity of legumes by 14–24 % . (Choi, S.*et al.,* 2016)

2.4. **Cold Plasma in toxin degradation:** Natural and synthetic toxins are ubiquitously present in our ecosystem. Toxic compounds like trypsin inhibitors, saponins, goitrogens, lectins might be inherently present in various food products. Other toxins like pesticides, endocrine disruptors, mycotoxins are also present in our food and water system raising concern for consumer health and safety. Currently, there are very limited non-thermal technologies available for control and degradation of toxic compounds present in food products.

Cold plasma has shown promising potential for degradation of various food toxins gaining increased interest from food researchers in past few years. Most of the current research in food products is focused on cold plasma degradation of mycotoxins and pesticides. Mycotoxins are toxic secondary metabolites produced by filamentous fungi contaminating various food products posing serious health risks problems. Many of the mycotoxins have been classified as carcinogenic, mutagenic and genotoxic. The resistance for degradation of mycotoxins by thermal processing and inability to completely inactivate them by conventional processing has led to active research for alternative processing technologies. Cold plasma has shown significant degradation of mycotoxins in various food products. (Tenbosch L. *et al.,*2017)

Aflatoxins, produced by *Aspergillus flavus* and A. parasiticus, can contaminate different foodstuffs, such as nuts. (Ji SH, *et al*., 2016). Cold atmospheric pressure plasma has the potential to be used for mycotoxin detoxification. In the study by Siciliano *et al.,* 2016, the operating parameters of cold atmospheric pressure plasma were optimized to reduce the presence of aflatoxins on dehulled hazelnuts. First, the effect of different gases was tested (N_2, 0.1% O_2 and 1% O_2, 21% O_2), then power (400, 700, 1000, 1150 W) and exposure time (1, 2, 4, and 12 min) were optimized. In preliminary tests on aflatoxin standard solutions, this method allowed to obtain a complete detoxification using a high power for a few minutes. On hazelnuts, in similar conditions (1000 W, 12min), a reduction in the concentration of total aflatoxins and AFB1 of over 70% was obtained. Aflatoxins B1 and G1 were more sensitive to plasma treatments compared to aflatoxins B2 and G2, respectively. Under plasma treatment, aflatoxin B1 was more sensitive compared to aflatoxin G1. At the highest power, and for the longest time, the maximum temperature increment was 28.9 ^{0}C. Cold atmospheric plasma has the potential to be a promising method for aflatoxin detoxification on food, because it is effective and it could help to maintain the organoleptic characteristics.

2.5. **Cold plasma in degradation of Pesticides:** The use of pesticides is a common agricultural practice which has significantly decreased the crop losses by insects and in turn increasing the yield at a global scale. However, pesticides are also toxic for humans and thus have to be stringently regulated below the residual toxicity limit in all the food products. The use of cold plasma has proven efficient in degradation of many pesticides in various food products (Bai Y, *et al.,* 2009, Misra, N. 2015 and Misra N.*et al*., 2016). O_2 plasma has the potential to reduce pesticide residues in agricultural products.

The effectiveness of cold plasma for pesticide degradation has been attributed to the generation of different reactive gas species like ions, molecular species and reactive radicals. An advanced oxidation process for the mineralization of a model pesticide endosulfan from aqueous medium has been developed by non-thermal plasma combined with cerium oxide catalysts. Plasma was generated in a dielectric barrier discharge reactor, whereas ceria was prepared by combustion synthesis and characterized by various physico-chemical techniques. Typical results indicated the synergy between plasma excitation of endosulfan followed by the catalytic action of cerium oxide, which not only improved the conversion, but also increased the mineralization efficiency, which was confirmed by a total organic carbon content analyzer and an infrared CO_x analyzer. Catalytic plasma approach showed a threefold increase in mineralization. (Reddy.M.K., *et al*.,2014)

Mousavi, S, M., 2017 investigated the potential effect of cold plasma on reducing residues of pesticides diazinon and chlorpyrifos in apples and cucumbers and its effects on quality of products. Two separate concentrations of each pesticide with 500 and 1,000ppm were prepared and the samples were inoculated by dipping them into the solutions. All samples treated with pesticides were exposed to cold plasma in a monopole cold plasma apparatus (DBD) run at 10 and 13 kV voltages. Liquid-liquid extraction (LLE) was used to remove pesticide residues from the samples. Eventually, high-performance liquid chromatography (HPLC) was used to measure the amount of pesticides in the samples. Also, to investigate generated metabolites, extracts were injected into a GC/MS apparatus and the results revealed that treatment of samples with cold plasma considerably reduced pesticide residues without leaving any traces of harmful or toxic substances. Furthermore, it did not have any undesirable effects on the color and texture of the samples. The efficiency of this method increased with higher voltage and longer exposure time. In general, the best results were obtained by the combination of 500 ppm concentration, 10 min exposure and 13 kV voltages. The residues of diazinon were reduced better than the residues of chlorpyrifos. Apples were detoxified much better than cucumbers. Also, cold plasma treatment transformed diazinon and chlorpyrifos pesticides into their less toxic metabolites.

Researchers are now focusing on optimizing the process parameters to increase the degradation efficiency of different classes of pesticides along with insights into the degradation mechanism. Although, cold plasma has been presented as a viable alternative, further studies are required to understand the reaction mechanism in presence of different reactive species along with the toxicity study of degradation products.

2.6. **Cold Plasma in food packaging:** CP have also been employed for the processing of packaging materials in order to improve barrier properties and bestow antimicrobial activity (Oh, Roh, & Min, 2016; Puligundla, Lee, and Mok, 2016). Cold plasma is being used in the packaging industry for many decades. It has been extensively used for packaging material sterilization, surface etching, surface functionalization, surface activation and surface deposition. Along with its use for conventional polymers, recently it has been used for surface modification of bio-based films and coatings. (Oh Y.A *et al*., 2016). X-ray photoelectron spectroscopy and Fourier transform Infrared spectra confirmed the increase in surface oxygen content and appearance of new O=C–O groups on the film surface after plasma treatment at 70 and 80 kV for 5 min. X-ray diffraction showed the A-type crystal structure which was not affected by plasma treatment. Some recent studies have also shown the potential of cold plasma to be used for active and intelligent packaging materials. Pankaj *et al*., 2017 analyzed the effects of cold atmospheric air plasma treatment of antimicrobial chitosan film with different levels of thymol. Optical characterization of the dielectric barrier discharge showed the generation of reactive nitrogen and oxygen species by the system. A significant increase in the surface roughness was observed after cold plasma treatment of the films. No significant difference was observed in the thermal profile of the plasma-treated films. A significant increase in the thymol diffusion coefficient was observed after the plasma treatment for all the active films. Similiarly, the inactivating effect of plasma jet operating at 50W against biofilms of *E.coli* O157:H7, S. *Typhimurium* and *L.monocytogenes* present on selected food container materials collagen casing, polypropylene and polyethylene terephthalate portrayed a 3-4 log CFU/cm^2 reduction in the biofilms after 10 min (Kim *et al.,* 2015).

Recently DBDs have been employed for generation of plasma inside sealed packages containing bacterial samples. The in-package plasma decontamination of foods and biomaterials relies on use of the polymeric package itself as a dielectric and has been studied using several packaging materials such as LDPE, HDPE, polystyrene (PS), Tyvek. Cold plasma systems can produce these chemical species simultaneously and directly at the point of need, thus maximising the antimicrobial potential of this technology. All these works have demonstrated significant reduction in microbial population on food products. Moreover, this approach is easy to scale up to continuous industrial processing and could prevent post-packaging contamination(Misra N.N, 2011).

2.7. **Impact of Cold Plasma techniques on food quality:** Apart from microbial inactivation, effects of cold plasma on the food quality has been another important aspect drawing attention of food researchers. Cold plasma inactivation of food enzymes have received significant attention. A review by Misra *et al.* 2016 reported that plasma gas species causes loss of secondary enzyme structure due to breakdown of specific bonds and chemical modifications of the side chains and it depends on power input, degree of exposure, mass transfer between the plasma-liquid phases, structural complexity and stability of the enzymes in their local environment.

Cold plasma processing has been shown to affect the quality attributes of the food products during treatment as well as in storage. For instance, CP treatment of cashew apple juice resulted in the degradation of all the reducing sugars, such as fructose and glucose and non-reducing sucrose. (Kim *et al,* 2015). They also reported an increase in sucrose content after long exposure to CP, which they attributed to the degradation of the oligosaccharides with a high degree of polymerization. A similar decrease in the fructose, increase in the sucrose and degradation of oligosaccharides with a high degree of polymerization was also reported after CP treatment of prebiotic orange juice (Almeida, F.D.L *et al,* 2015). The studies suggest ozonolysis to be the main route of degradation causing the cleavage of glycoside bonds, leading to depolymerization of the macromolecule and the oxidation of functional groups to form carbonyl and carboxyl compounds, lactones, hydroperoxides and CO_2 (Almeida, F.D.L *et al*, 2015 and Ben'ko, E.M *et al.,*2013). Overall, CP treatment lead to de-polymerization and cross-linking of starch affecting its structural, functional and rheological properties. Most of the studies on CP treatment of whole fruits and vegetables have reported no significant reduction in ascorbic acid content after plasma treatment. No significant changes in the antioxidant capacity after CP treatment were reported in radish sprouts, kiwifruits, red chicory and onion powder (Kim, J.E.et al 2017; Ramazzina, *et al,* 2016). Some studies have shown a reduction in antioxidant activity after CP treatments in apples, white grape juice, and cashew apple juice on an extended exposure (Rodríguez, Ó *et al.*,2017). Almeida, Cavalcante, Cullen, Frias, Bourke, Fernandes and

Rodrigues reported a reduction in the antioxidant capacity of prebiotic orange juice after direct mode of plasma treatment. Yepez and Keener, 2017 reported a novel application of CP treatment recently. They showed the potential of hydrogen plasma to be used for the manufacturing of partially hydrogenated soybean oil without any trans-fatty acid. CP technology has shown unique advantages over the current hydrogenation processes as it can be performed at room temperature, under atmospheric pressure without any catalyst. No significant effect on lipid oxidation were observed after CP treatment in fresh and frozen pork , beef jerky (Kim *et al,* 2014) and raw pork. It presents a research opportunity to further explore the effects of cold plasma on the physico-chemical and sensory properties of the food products at the molecular level. The differences in the reported studies demonstrate the need for mechanistic studies to understand the interaction of plasma reactive species with food components. Optimization studies are also required to avoid the negative impacts on quality, such as accelerated lipid oxidation, loss of vitamins and sensory characteristics (Shashi K. *et al.,* 2018). The precise understanding of the mechanisms and control over the quality attributes will be required for cold plasma technology to realize its full potential at commercial scale.

3. Conclusion

Some plasma systems may not actually be suitable for certain foods. It is therefore essential to determine the best plasma system for treating a specific food product. For the process to become commercialised and more widely used, it is important to characterise the reactive chemistry of the plasma system in question. Defining the plasma chemistry is essential to understand how it interacts with the food and whether the treatment impacts on nutritional quality. Cold plasma technology is still in its nascent stage and requires further work in system designing, scalability studies, eco-toxicity studies and mechanistic insights before it could be fully utilized at a commercial scale. The recent studies conducted on the interaction of reactive species with food contact surfaces establish plasma processing as an eco-friendly technique with minimal changes to food products, making it a befitting alternative to traditional techniques. The current review will encourage early adoption of this eco-friendly technology by the food industry and food regulatory agencies so that its full potential for industrial applications could be established. Active researches focused on up-scaling for commercial applications are urgently required.

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