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# COLD PLASMA – A NOVEL TECHNIQUE IN FOOD PRESERVATION

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**ABSTRACT**

Food is anything edible and provide nutrients to human growth. Food spoilage is the major problem where microorganisms enter from surrounding environment. Spoilage microflora reduce the shelf life of foods by causing defects whereas pathogenic microflora cause diseases. This has led to the evolution of food preservation methods to inhibit the microflora. Heat treatments normally reduce the nutritive value of foods thus reduce the quality. Now a day's consumers focus on non-thermal based foods. One such method catching up in present day is cold plasma technology. Cold plasma is a novel non-thermal food processing technology that uses energetic, reactive gases to inactivate contaminating microbes on vegetables, fruits, poultry and meat. This method uses electricity and a carrier gas, such as oxygen, nitrogen or helium. A wide array of cold plasma systems that operate at atmospheric pressures are under development. Reduction of more than 5 logs can be obtained for pathogenic bacteria such as *Salmonella* spp., *Escherichia coli* O157:H7, *Listeria monocytogenes* and *Staphylococcus aureus*. Effective treatment times can range from 120 sec to as less as 3 sec, depending on the food treated. The primary modes of action of cold plasma on microflora of food are due to UV light and reactive chemical products of cold plasma ionization process that denatured microbial cell proteins and mutated nucleic acids. Relatively early state of technology development, complexity of necessary equipment and largely unexplored impacts of cold plasma treatment on sensory and nutritional qualities of treated foods may be considered as limitations. Besides limitations, this area of technology is promising and subject of active research to enhance efficacy.

Keywords: Cold plasma; ionization; pathogenic microflora; inactivation; emerging; non-thermal plasma.

## INTRODUCTION

Food is anything edible and provide nutrients to human growth. Food spoilage is the major problem that occur due to growth of microorganisms which enter from various sources like soil, handler, air, storage environment etc. Spoilage microflora reduce the shelf life of foods by causing defects whereas pathogens cause diseases making the food unsafe for consumption. Food preservation principles are based on inhibiting or killing the microflora of foods in order to enhance the storage stability. Heat treatments normally decrease the nutritive value of foods thus reduce the quality. Now a day's consumers focus on such foods preserved through non-thermal ways. Plasma technology is an emerging non-thermal preservation technique. This food preservation method, is in demand due to convenience, high nutritional and sensorial quality, long shelf life, freshness, additive-free status, environment-friendly processing and low production costs.

The term Plasma is used to designate the state of an ionized gas. Plasma is considered the fourth state of matter that comes after solid, liquid and gas and is the predominant state across the universe [1]. In order to produce plasma it is necessary to supply energy to a gas to cause its ionization.

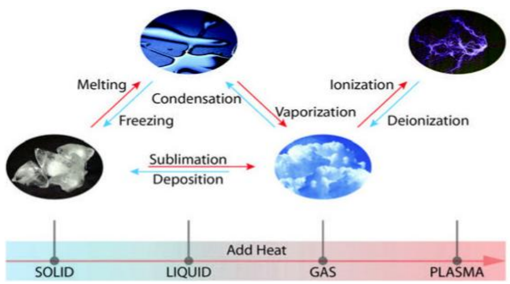


**Fig. 1. The Four State of Matter**

## HISTORY

Plasma derived from Ancient Greek means mouldable substance is one of the four fundamental states of matter. Plasma was first identified in laboratory by Sir William Crookes. Crookes presented a lecture on what he called "radiant matter" to the British Association for the Advancement of Science, in Sheffield, on Friday, 22 August 1879. However, systematically studies of plasma began with the research of Irving Langmuir and his colleagues in 1920's. Langmuir also introduced the term "plasma" as a description of ionized gas in 1928 [2].

## PLASMA FORMS

Plasmas occur naturally but can also be artificially made. Naturally occurring plasmas can be Earth-based (terrestrial – Lighting, auroras, ionosphere) or space-based (astrophysical – stars, solar wind). Artificial plasmas (plasma torch, fluorescent lighting) have been developed to service the needs of a wide range of fabricating, manufacturing and food industries.

## Classification of Plasma

Plasmas can be classified according to their temperature into two large groups: thermal plasmas and cold plasmas. Thermal plasmas can reach temperatures of up to several thousand Celsius degrees and are used in applications where high temperatures are required, in metallurgy or in chemical synthesis processes. Cold plasmas, with temperatures close to ambient temperature, are on the contrary suitable for the treatment of heat sensitive materials. These cold or non-thermal plasmas (NTAP) are generated by the application of an electric or electromagnetic field to a gas [3]. The field energy causes the free electrons to accelerate and ionizes the gas atoms and molecules, which release more free electrons that in turn provoke new ionizations. In addition, excited electrons produce molecular dissociations, with the formation of new atoms and free radicals, also able to excite atoms and molecules to higher energy levels. Excited atoms and molecules, when returning to the more stable state, emit excess energy in the form of broad-spectrum electromagnetic radiation, including ultraviolet (UV) radiation. Consequently, the plasma is constituted basically by molecules and atoms in an excited state, positive and negative ions, free radicals, electrons, UV radiation and reactive oxygen and nitrogen species, such as ozone, superoxide, hydroxyl radicals, singlet oxygen, atomic oxygen, nitric oxide or nitrogen dioxide. Interestingly, all these agents show antimicrobial activity against a wide range of microorganisms, including bacteria, molds, yeasts, and even bacterial and fungal spores.

## PLASMA PROPERTIES

Plasma is the highest energy state of matter. It consists of a collection of free-moving electrons, positive ions and neutral particles. Although it is closely related to the gas phase in that it has no definite shape or volume, it does differ in a number of ways:

- Plasma has a very high electrical conductivity.
- Plasma is more readily influenced by electric and magnetic fields than by gravity
- The motion of electrons and ions in plasma produces its own electric and magnetic fields.
- Because of the totally chaotic and highly energetic state of the constituent particles of plasma, it produces its own electromagnetic radiation [4].

## GENERATION OF PLASMA

Plasma can be artificially generated by heating a neutral gas or subjecting it to a strong electromagnetic field. The presence of free charged particles makes plasma electrically conductive, with the dynamics of individual particles and macroscopic plasma motion governed by collective electromagnetic fields and very sensitive to externally applied fields. Depending on temperature and density, a certain amount of neutral particles may also be present, in which case plasma is called partially ionized.

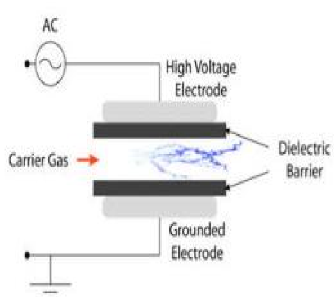


**Fig. 2. Formation of Plasma by Dielectric Barrier Discharge (DBD)**

Energy is needed to produce and maintain plasma. Usually, the discharge needed to produce CAP is induced electrically. Some methods used to produce CAP include: Dielectric Barrier Discharge (DBD), Atmospheric Pressure Plasma Jet (APPJ) and Plasma Pencil [5].

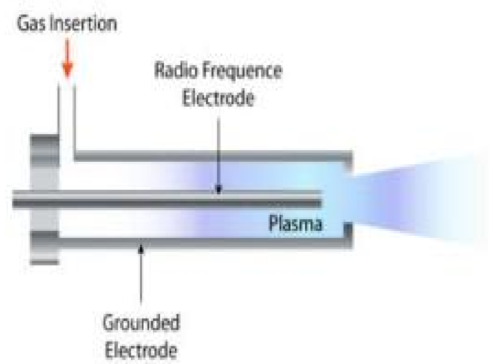


**Fig. 3. Formation of plasma by Atmospheric Pressure Plasma Jet**

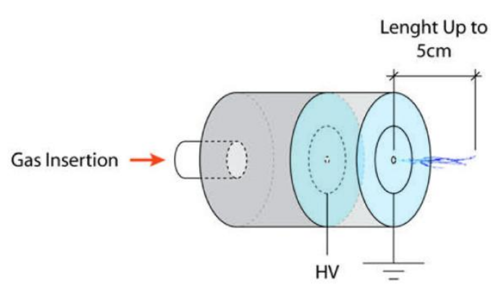


**Fig. 4. Formation of plasma by plasma pencil**

## COLD PLASMA

Cold plasma is a novel non-thermal food processing technology that uses energetic, reactive gases to inactivate contaminating microbes on meats, poultry, fruits, and vegetables. This flexible sanitizing method uses electricity and a carrier gas, such as air, oxygen, nitrogen, or helium; antimicrobial chemical agents are not required. Among all the existing plasma sources, DBD and plasma jets are the most widely explored configurations in food research, due to their easiness of construction and adoption

## COLD PLASMA PROCESS

Non-thermal plasmas, also known as cold plasmas, are characterized by the fact that the temperature of heavy species (neutral particles and ions) is close to room temperature (25°C–100°C). Cold plasmas usually occur at a low pressure (p < 133 mbar) in reactors with very different geometries. Such reactors generate plasmas through direct current, radio frequency, microwave or pulsed discharge systems. There are special types of cold plasmas, produced in the so-called corona and dielectric barrier discharges, that are generated at atmospheric pressure by using pulses between 10–6 s and 10–9 s. In these types of discharges, referred to as CAP (cold atmospheric plasma), the highly energetic electrons are produced that, due to the shortness of the pulses used, have little time to exchange energy with their surroundings. This kind of plasmas have found many applications in the food industry [6].

In cold plasma treatment, energy among the constituent particles is distinctly non-uniform (a non-equilibrium), in which electrons are likely to transfer heat *via* collisions with heavier particles, thus controls the electron component in a given matrix. Also, the temperature within the media remains constant (35°C), which is a unique characteristic feature suitable for heat-sensitive foods. The inactivation of microorganisms in non-thermal plasma is based upon the reactive species anddo not rely on the thermal killing of pathogens [7]. Various non-thermalplasma discharge methods depending on the mechanism and desired target reaction are used. Forinstance, dielectric barrier discharge (DBD) plasma is generated by placing an insulating ordielectric material between the electrodes, which is responsible for a self-pulsing operation. Thisprocess was initially used to produce ozone gases, but now have broader applications in the food industry [8]. In case of glow discharge plasma, the plasma is formed by the passage of alternating current (AC) through a gas, generated by applying a high voltage input of 4.4 kV (60 Hz) between two electrodes (an inter-electrode distance of 13 cm). The samples to betreated are placed inside a vacuum chamber, and the plasma is generated once the pressure in the vacuum chamber is stabilized at 2 Pa [8].

## MODE OF ACTION OF COLD PLASMA ON MICROORGANISMS

The primary modes of action are due to UV light and reactive chemical products of the cold plasma ionization process. Reductions of greater than 5 logs can be obtained for pathogens such as *Salmonella*, *Escherichia coli* O157:H7, *Listeria monocytogenes* and *Staphylococcus aureus*. Effective treatment times can range from 120 s to as little as 3 s, depending on the food treated and the processing conditions [9]. Reineke et al. [10] compared the effectiveness of different plasmas for the inactivation of *Bacillus atrophaeus* and *Bacillus subtilis* spores and found that, although plasmas containing oxygen and nitrogen emitted four times more UV radiation than pure argon plasmas, the greatest lethal effect was achieved when pure argon was used as the working gas. These authors suggested that the antimicrobial effect was determined by reactive species of oxygen and nitrogen generated in the pure gas, and especially by hydroxyl radicals. These studies demonstrated that the contribution of UV light to microbial inactivation was negligible, compared to that observed for direct exposition to plasma. In addition, exposure of *B. cereus* vegetative cells to nitrogen plasma has been shown to result in a transcriptional response in which the expression of genes involved in UV damage repair (*uvrA, uvrB*) was unaffected, while various genes involved in the response to oxidative stress, such as those encoding nitric oxide dioxygenase, as well as membrane-associated enzymes that catalyze oxidation-reduction reactions, were overexpressed [11]. Finally, it has also been shown that microorganisms exhibiting variability in resistance to UV light exhibit a similar tolerance against NTAP. For instance, although *B. cereus* spores were more sensitive to UV light than spores of *Geobacillus stearothermophilus* and *B. atrophaeus*, a given NTAP treatment produced a similar degree of inactivation for the three species [12], suggesting that the mechanism of inactivation through both technologies was different.There exists on the contrary a general agreement in that reactive chemical species generated through gas ionization exert an antimicrobial effect through a direct and non-specific attack on various microbial structures and components, including cellular envelopes, DNA and proteins [13].

## FACTORS AFFECTING THE PROCESS

The values of density and electronic temperature, two of the main parameters that characterize plasmas, cover a wide spectrum. Thus, the electron density varies between 1 electron/$cm^3$ and 1025 electrons/$cm^3$ ie., it even exceeds the concentration of electrons in metals. On the other hand, the average free path of the particles in a plasma, that is, the average distance covered before a particle collides with another particle in the plasma can range from tens of millions of kilometres to just a few microns

## APPLICATION OF COLD PLASMA IN FOOD INDUSTRY

Lee et al. [14] compared the effectiveness against *Listeria monocytogenes* of plasmas obtained with helium or nitrogen and observed a higher inactivation level for nitrogen-based plasmas. Other studies have described that air-based plasmas are most effective in inactivating *E. coli* O157:H7, *Bacillus cereus, Staphylococcus aureus, L. monocytogenes, L. innocua* and various serovars of *Salmonella enterica*, such as *S. anatum, S. stanley, S. typhimurium and S. enteritidis*, than plasmas generated with nitrogen [15,16]. Rowan et al. [17] concluded that oxygen is the gas of choice, over $CO_2$ or nitrogen, when generating plasmas for the inactivation of *E. coli, Campylobacter jejuni, C. coli, L. monocytogenes, S. typhimurium, S. enteritidis* and *B. cereus* spores. The addition of small amounts of oxygen to noble gases, such as helium [18] and argon [19], or nitrogen (Lee et al., 2012b) improves the antimicrobial effectiveness of NTAP against vegetative cells and spore-forming bacteria). This effect is mainly attributed to a higher formation of reactive oxygen species, such as hydroxyl and hydroperoxyl radicals, atomic oxygen, hydrogen peroxide, singlet oxygen and ozone, all of them with a high antibacterial activity.

Another processing parameter determining the antimicrobial effectiveness of NTAP is the gas moisture content. In fact, it has been occasionally observed [20] that the use of completely dry gases is ineffective for *E. coli* inactivation, and there are several studies which show that an increase in the water content of the gas improves its effectiveness Thus, Ragni et al. [21] reported that an increase in the air relative humidity from 35 to 65% increased the inactivation of *Salmonella enteritidis* and *S. typhimurium* from 2.5 to 4.5 log cycles, and attributed this effect to a higher concentration of hydroxyl radicals in the plasma. Similar results were found by Patil et al. [22] for *B. atrophaeus* spores. These authors used plasmas with different moisture content (3, 10, 30, 50, and 70%), and obtained a 5 to 6 log reduction at humidities of 3 and 10 %, while a complete inactivation was observed at higher humidities. These authors related this higher antimicrobial activity to the increased generation of numerous reactive species, such as $N_2O_5$, $H_2O_2$, $HNO_4$, or hydroxyl radicals, and, especially, to the decomposition of ozone in the presence of water, with the consequent formation of highly oxidizing species, such as hydroxyl and hydroperoxyl radicals, superoxide anion and $H_2O_2$.

**Table 1. Application of cold plasma on various foods**

| Food matrices | Cold plasma treatment and experimental condition | Impact of cold plasma on microflora | Reference |
|---|---|---|---|
| **Rice** | DBD-CAP at 15 khz input power of 250 W for 20 min | TBC decreased the population 2.68–2.84 log CFU/g to | Lee et al. [23] |
| **Fresh cut carrot** | Cap-direct exposure at 0- 120 kv, 60 hz | Reduction of mesophiles and yeast up to 2.1 $\log_{10}$ CFU/g after 5 min treatment at 100 kv<br>Shelf-life improved at 80 to 100 kv and treatment times between 4 to 5 min | Mahnot et al. [24] |
| **Fresh cut apples** | Plasma activated water at 7 khz with amplitudes of 10 kv for 10 min | Aerobic bacteria reduced by 1.05 $\log_{10}$ CFU/g. | Liu et al. [25] |
| **Egg** | Arc plasma consists of atmospheric air<br>With a pressure of 101 kpa and plasma voltage of 12,000 V exposed for 40 s at a temperature of 27°C | Bacteria population was reduced from 8.58 ± 0.24 to 4.49 ± 0.15CFU $cm^2$<br>Salmonellas count reduced by 4.09 log cycle<br>No change on albumen-, yolk- pH & colour | Gavahian et al. [26] |
| **Chicken patties** | Direct exposure of DBD-CAP consists<br>of 65% $O_2$ + 30% $CO_2$ at 70 kv for180 s | Microbial populations decreased by1.70 log cycles | Gao et al. [27] |

For liquids, only those plasma-generated reactive species with a relatively long life, such as ozone, atomic oxygen, nitric oxide or hydrogen peroxide, would have the capacity to diffuse through the medium and interact with microbial cells. For example, the coexistence of ozone and hydrogen peroxide in water results in highly reactive species such as hydroperoxides and hydroxyl radicals. In addition, atomic oxygen, upon reaction with water, can generate hydrogen peroxide and singlet oxygen and the interaction of nitric oxide and superoxide produces peroxynitrite, a highly reactive compound that can readily diffuse through cell membranes [28–32].

## LIMITATIONS OF COLD PLASMA

Key limitations for cold plasma are the relatively early state of technology development, the variety and complexity of the necessary equipment, and the largely unexplored impacts of cold plasma treatment on the sensory and nutritional qualities of treated foods. Also, the antimicrobial modes of action for various cold plasma systems vary depending on the type of cold plasma generated. Optimization and scale up to commercial treatment levels require a more complete understanding of these chemical processes. Nevertheless, this area of technology shows promise and is the subject of active research to enhance efficacy [33].

## CONCLUSION

Cold plasma is a promising food decontamination technology capable of inactivating bacteria, yeasts, molds, fungal and bacterial spores both on abiotic surfaces (e.g., packaging materials, food processing environments and equipment) and on foods through reactive oxygen species leading to damage caused to nucleic acids and proteins of microbial cells. In addition, the method has also other different innovative applications of great interest for food quality and safety improvement. Designing fit-for-purpose equipment susceptible to be easily adopted in a processing line, being compact, energetically efficient and cost-effective are the need for the proper application of cold plasma.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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