Recent Advancements in Cold Plasma Technology

M Mounika S Reddya,b, c; Krishna Bhaskar Ta

aPlant Cell Biotechnology Department, CSIR- Central Food Technological Research Institute (CFTRI), Mysuru, Karnataka, India

bAcademy of Scientific and Innovative Research (AcSIR), Ghazibad – 201002, India

cEmail Id: monikareddy041196@gmail.com

G.V Swarnalatha

Department of Biochemistry, Rayalaseema University, Kurnool- 518007

Corresponding Author: venkata85722@gmail.com

ABSTRACT

A unique non-thermal technology called cold plasma has demonstrated tremendous potential for use in the food industry. The polymer and electronic industries were the principal users of cold plasma in the past for surface modification and functionalization of various polymers. However, in recent years, the uses of cold plasma have expanded rapidly into biological materials including the food sector. Thermal and non-thermal are the two food processing techniques used in the food industry. However, due to the intricate interactions between food components, numerous studies have shown that thermal processing can negatively impact the nutritional and functional qualities of foods. Non-Thermal processing methods such as cold plasma technology are becoming more prevalent as chemical processing technology undergoes the current paradigm shift from using processes that involve high temperatures and high pressure to processes that are mild and sustainable for the environment. In this book chapter, we will be focusing on the effects of cold plasma on various bioactive components, microbial cells, enzyme inactivation, and its various application in the food industry.

Keywords— Cold Plasma Technology; Non-thermal; food processing; enzyme inactivation; microbial cell inactivation

#  INTRODUCTION

 Thermal processing is the most widely used method to preserve food by limiting pathogenic and contaminant microorganisms, despite several disadvantages such as overheating, compositional damage, alteration in flavour and textural properties, and a significant reduction in nutritional value caused by increased temperature, etc. [1]. The current food sector is looking for ways to meet the growing demand for hygienic, nutritious, and quality food with "fresh-like" qualities attributed to more excellent consumer knowledge [2]. A different processing strategy has been deemed necessary to improve food quality while keeping technology costs within reachable bounds. This has created a huge need for non-conventional food processing research to increase. Researchers have looked into a variety of novel processing techniques over the past few years to produce food that is healthy, shelf-stable, and of high functional and nutritional value. In recent years, research has focused on a number of alternative thermal and non-thermal technologies (see figure 1), such as dielectric heating (radiofrequency heating and microwave heating), Cold plasma technology (CPT), Infrared heating, High-pressure processing (HPP), Ozone processing (OP), Pulsed electric field (PEF), ultrasound, Ohmic heating (OH), etc. These procedures are performed at temperatures that are close to the ambient level, which eliminates one or more negative effects related to traditional thermal processing. The food industry is increasingly adopting these technologies due to this benefit. Cold plasma technology (CPT) is a comparatively new biotechnological initiative for ensuring the safety and quality of food among all developing non-thermal technologies [1]. This technique has been extensively applied in a variety of applications in other industries, such as the surface disinfection of medical devices, enzyme inactivation, and microbial inactivation [3]. Food processing has advanced over time, starting with the application of fire for roasting meats about 1.8 million years ago and continuing with the development of numerous techniques such as cooking, heat preservation, fractionation, pickling, drying, fermentation, and freezing over time to the most recent advancement in food processing that is 3D printing [4]. It has been demonstrated clearly that food processing is essential for turning frequently inedible raw materials into edible, safe, and nutritious foods as well as for food preservation and bioconversion [5]. The last three decades have seen a rise in interest in a healthy lifestyle as consumers prefer foods that are fresh, delicious, sustainable, and produced with minimal environmental impact. Research and development in the field of food processing have focused heavily on cleaner and greener non-conventional processing methods. While conventional food processing generally destroys a variety of heat-sensitive nutrients (such as vitamins) to varying degrees, it has been demonstrated that sensible processing treatments, particularly emerging non-thermal processes (such as cold plasma technology, pulsed electric field, high hydrostatic pressure) and novel thermal processes (such as the use of superheated steam, microwave-assisted processes, and ohmic heating) can improve nutrient availability (See figure1). The bio-accessibility of certain nutrients is influenced by processing, which modifies the structure of the food [6,7]. Processes that guarantee the safety and quality of the products have been markedly increased by research on emerging non-conventional processing technologies. Due to all of these previous and ongoing efforts, "processing" operations for creating food products are now widely recognized and established [8]. This chapter provides a concise summary of several findings found in the literature to convey pertinent aspects and recent advancements of Cold plasma technology, focusing on its applicability to food processing.



**Figure 1: Food processing techniques**

# COLD PLASMA TECHNOLOGY

 The fourth known state of matter in the universe is plasma, created by ionizing a gas with a lot of energy. Plasma is a high-energy system comprising various reaction products such as ions, molecules, electrons, atoms and free radicals. Ultraviolet radiation and numerous chemical interactions take place in the plasma. Besides the physical interactions with samples, such as collision and ultraviolet radiation, the chemical reactions between the samples and the active species during interaction with the samples distinguish plasma as a special type of matter. [9] Plasma applications are determined by temperature, and depending on temperature, plasma can be divided into thermal plasma and nonthermal plasma. The electrons' temperature in thermal plasma is the same as the ambient temperature (equilibrium plasma). Thermal plasma is typically used in the aerospace industry because it can only be produced when exceptionally high operating power is used [10]. In non-thermal plasma, the background heating rate is lesser than the temperature of the electrons. As a result, the gas's overall temperature stays low, which is necessary for polymer modifications in medicines, food industry, textile, and electronics [10]. Cold plasma, which is close to room temperature, has until recently demonstrated unique advantages in applications in the food industry. On food surfaces, it can be used to destroy microorganisms like Listeria monocytogenes [11]. Cold plasma technology can be used as an effective and environmentally friendly method of sterilization for food containers and surfaces made of stainless steel and polyethene. Antimicrobial compounds like chitosan, triclosan, and silver that were immobilized into packaging films by cold plasma technology demonstrated good antimicrobial activity. Additionally, it was developed to use plasma technology to resist biofilm on materials that come into contact with food. In addition, the efficient degradation of allergens, anti-nutrients, agrochemicals and toxins by cold plasma can enhance the quality and safety of food [10]. Intriguingly, Yepez and Keener (2016) discovered that high-voltage atmospheric plasma using H as the gas source might be a new alternative for hydrogenating plant oils without causing trans-fatty acids [12]. Additionally, it was reported that following plasma treatment, the peanut protein isolate [13] and zein powder [14]. This suggested that cold plasma technology could be used to improve the physical and biological characteristics of proteins, such as their ability to foam and emulsify. According to [15], altering starch by plasma treatment was a unique process that could be scaled up for commercial use [15].

 Sterilization of fresh produce without compromising its sensory and nutritional characteristics is valuable. Conventional thermal and chemical techniques are most likely to lower the sensory and nutrient values. Nonthermal processing technique like cold plasma is a new, emerging trend [16]. Recent years have seen an increase in the use of cold plasma as an effective but moderate processing method for fresh produce due to the unique properties of cold plasma and the efficient sterilization at nearly ambient temperature and pressure [10]

## **Generation of Cold Plasma**

 Cold Plasma is commonly generated at normal atmospheric pressure with electron temperatures typically between 1 and 10 eV. Several plasma generation parameters, including gas composition, frequency, plasma reactor configuration and structure, plasma energy, pulse form, modulation, and input energy period, are essential to generate a well-defined process catered to particular plasma chemical requirements [17]. Cold Plasma is appropriate for food decontamination since it lacks demanding system requirements. Systems for cold plasma discharge can be divided into three categories. The first type of plasma is a non-thermal glow discharge, which is created by passing a voltage across two electrodes inside a glass tube that is filled with a low-pressure gas. The second type uses an RF (radio frequency) discharge to create cold plasma in the core of an electric coil using pulsed electricity. To generate plasma, the 3rd type of plasma generation distributes current flow between electrodes using an insulating (dielectric) material. In packages containing fresh produce where reactive oxygen and nitrogen species can be produced immediately, the discharge wall is ideal for inhibiting the microbes [18]

## **Factors Influencing Cold Plasma Efficiency**

 Internal traits of the microbe are also crucial criteria that affect cold plasma effectiveness. For instance, the essential characteristics of the microorganisms are crucial for an efficient process during the microbial decontamination of foods because sensitivity can vary within species. Gram-positive bacteria were less sensitive to plasma than gram-negative bacteria because of the external characteristics of their lipopolysaccharide membranes and peptidoglycan thickness. Generally, bacteria in the stationary phase are more susceptible to several inactivation treatments than those in the exponential phase [19]. Like other inactivation procedures, sporulated bacteria are more resistant to plasma treatment than vegetative cells. Additionally, a high bacterial density clusters more cells, which decreases the ability of reactive species to penetrate the bacteria. Additionally, because of the complex resistance imbibed by the chitin, the cell walls of fungi show greater resilience to plasma treatment than bacteria [20]. The environment, including variables like pH, moisture content, and sample type, has a significant impact on the efficiency of cold plasma treatment. For instance, because most liquids can vaporize during treatment and participate in succeeding reactions, solid and liquid food mixtures interact with active species such as reactive oxygen in different ways [20]

# EFFECTS OF COLD PLASMA TECHNOLOGY ON ENZYME INACTIVATION

## **Enzyme Inactivation**

 Enzyme inactivation, in simple terms, refers to the point at which the enzyme stops working. The active site of an enzyme loses functionality when it is denatured. The active site of an enzyme is the site at which the substrate binds to the enzyme and activates the enzyme. The enzyme may be denatured or become inactive when its pH is altered, or its temperature is raised. Every enzyme has some optimum conditions at which it shows maximum activity. Beyond or less than these conditions activity of the enzyme will be undetectable. To increase the rates of biochemical reactions, enzymes are frequently used as biocatalysts in the food industry. During food preparation or preservation, endogenous enzymes that are naturally present in food affect the texture, colour, and flavour of food, which can be either favourable or unfavourable [21]. To increase the shelf life of the product, high temperatures may be applied, which can be above the optimal condition of the enzyme, for which it gets either denatured or complicates the binding of the substrate at the active site. Native enzymes are structurally organized on three levels; however, many have four. The specific amino acid sequence along the covalent polypeptide chain is the primary structure. The secondary structure outlines standard configurations for the hydrogen-bonded α-helices, β-pleated sheets, and turn structures that make up the polypeptide backbone. Tertiary structure describes how helices and sheets are arranged into globular units or domains that are physically apart from one another. Some enzymes also have a quaternary structure, which is the specialized connection of monomeric subunits into oligomers, which are defined by the aforementioned degrees of structural organization [21]. Enzyme molecules are typically tightly packed, although they frequently have cavities that enable flexibility. They may also have apertures that contain prosthetic groups that are used in catalytic processes to change substrate into the product, such as metal ions, heme, nicotinamide adenine dinucleotide (NAD), flavin adenine dinucleotide (FAD), or pyrroloquinoline quinone. Certain enzymes also contain covalent bonds between the amino acid backbone and carbohydrate molecules. Food heating often results in irreversible loss of enzyme activity, making equilibrium thermodynamic relationships irrelevant. At high temperatures, native enzymes unfold in a highly cooperative manner to produce randomly coiled structures devoid of catalytic activity. This process is entirely reversible for some enzymes, and differential scanning calorimetry makes it possible to calculate the proportion of calorimetric to Van't Hoff enthalpies [22]. An enzyme can become inactive in various ways, ranging from a simple, single-molecule process to a more complicated one involving many enzyme molecules. Therefore, the reaction's order may be one (first order), larger, or less than one. The principal cause of the inactivation of enzymes by ultrasound is protein denaturation, either by shear pressures caused by the development and collapse of cavitating bubbles or by the free radicals generated during the sonolysis of water molecules [23].

## **Cold Plasma and Enzyme Inactivation**

 The low temperature of the cold plasma is crucial in preventing the loss of food nutrients and the high energy that can change biomacromolecules, boosting the applications of cold plasma in the food sector [24]. Since cold plasma is a non-thermal technology, its typical temperature is below 40 C, which means that it works by using UV light, charged particles, and an amplified electric field instead of heat. A gas that has been ionised and has atoms or molecules in a metastable state with essentially no electrical charge is called plasma. Cold plasma is produced when the neutral gas is given enough energy and becomes ionised to produce a variety of chemically active byproducts, including charged particles, free radicals like ROS and RNS, excited or non-excited molecules, and ultraviolet (UV) radiations [25]. Corona discharge, microwaves, radiofrequency waves, capacitive or inductive coupling methods, or, more frequently, dielectric barrier discharges (DBDs) at relatively lower frequencies are some of the technologies used to create cold plasmas. Another method is the one-atmosphere uniform glow discharge plasma [24]. The majority of dietary enzymes are proteins, which are polymers of amino acids organised into intricate three-dimensional structures (divided into primary, secondary, and tertiary), which are linked to their functioning. Any action that alters an enzyme's supramolecular structure can inactivate the enzyme. By using X-ray photoelectron spectroscopy, it has been confirmed that L-direct alanine's exposure to argon plasma causes the COOH group and CNH2 group to degrade [26]. Cold plasma also induces transformation in the active sites of enzymes along with amino acids, secondary structures, and prosthetic groups leading to enzyme inactivation (see figure 2). Conformational alterations of enzymatic proteins, which include fundamental systems and spatial structure, can also result in low activity [21].



**Figure 2: Effect of Cold plasma on Enzyme Inactivation**

 The function of an enzyme might be changed by even a single oxidised amino acid in a protein [27]. Within minutes of direct treatment with a Dielectric Barrier Discharge (DBD) plasma, RNase A activity was irreversibly inactivated by oxidising sulfur-containing amino acids and rupturing the structure of disulfide bonds according to [28]. Additionally, they noted that while RNase A was inactive for 600 seconds following plasma treatment, the protein backbone remained stable. Additionally, they saw more significant levels of methionine sulfoxidation and quicker oxidation of methionine than cysteine residues, demonstrating that methionine oxidation was adequate for the inactivation of RNase A. Enzyme activity and the spatial organisation of enzymatic proteins have a distinctive connection. According to reference [29], changes in protein structures cause an increase in protease activity. The well-organized secondary structures of lactate dehydrogenase (LDH) were influenced mainly by a-helix structures and twists, according to an explanation by [27]. Additionally, he also confirmed the connection between a-helical structures and enzyme active sites, stating that certain amino acids found in active areas were all engaged in the formation of a-helical structures. Peroxidase (POD) seemed to be more susceptible than polyphenol oxidase (PPO) to plasma treatment at the a-helix content however, POD lost less activity than PPO, probably as a result of the various amounts of samples applied to the Circular Dichroism (CD), according to [30]. Peptide chains were organised in b-sheet structures in a pleated fashion, which was thought to make them significantly more resistant to heat and chemical agents. Additionally, secondary structures' random areas and turn modifications were simultaneously experienced. While [27] Zhang et al. 2015 demonstrated the reverse tendency between turns and random regions, i.e., a decrease in turns and an increase in unexpected areas, reference [30] found that the number of turns in PPO and POD did not alter following treatment with cold plasma (120 and 360 seconds, pure argon).

 The availability of enzyme active sites affects enzymatic activity. Additionally, to heme destruction, reference [31] reported variations of active sites after 12-minute plasma treatment, demonstrating that small changes of 0.22, 0.2, and 0.25 Å occurred to W62, D101, and W108 following and-DBD plasma treatment, respectively, while significant changes of 0.54, 0.41, and 0.24 Å were noted in the same active sites following Atmospheric pressure plasma jet (N2-APPJ) plasma treatment. Metal ions also procatalyse the active site as a cofactor. Reference [32] found that plasma treatment dramatically reduced horseradish peroxidase (HRP) activity and its iron (Fe) concentration. Reference [33] revealed that no apparent change in Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) molecular weight was seen following cold plasma treatment using Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE) analysis, which is relevant to the structure of enzymes. When assessing the effects of cold plasma on enzyme inactivation, extrinsic factors such as the treatment environment should be considered. Reference [28] said that RNase A remained active even at high temperatures and a low pH value of 3, excluding the effects of heat and low pH on inactivating the RNase by DBD treatment. According to reference [27], even after 300 seconds of plasma exposure at 24° C and a pH of roughly 7.5, enzymes did not become inactive. This demonstrated that pH and temperature did not contribute to the inactivation of lactate dehydrogenase (LDH) activity. Following plasma treatment, reference [34] noted that the pH values on the surface of freshly sliced apple and potato tissue decreased to 1.5, while reference [35] also noted a significant pH fall after plasma treatment for 15 min. Time and voltage are the most critical parameters affecting plasma inactivation of enzyme activity out of all the extrinsic influences. The first 60 s saw a dramatic decline in enzyme activity of PPO and POD within exposure times of 0–360 s from different plasma generations, which was followed by a slight decrease. Furthermore, POD was less impacted by plasma than PPO. Similar findings demonstrating the inactivation of LDH, RNase A, and HRP activity as a treatment duration was also reported [32, 28, 27]. Due to the intricacy and mutability of enzyme structures, the breakage of a single bond or structure is sufficient to render an enzyme inactive, making the first-order kinetic model unsuitable in scenarios where the situation is very unfavourable. During the preparation of fruit and vegetable beverages, a more extended exposure period is required if a plasma method aims to accomplish total enzyme inactivation. Additionally, by precisely regulating treatment parameters, such as treatment duration and applied voltage, it is possible to maximise energy utilisation [21]. The thermodynamic parameter Gibbs free energy change (DGU) is particularly significant in the quantitative description of protein conformation among all the intrinsic components. Using average B factor analysis, reference [31] showed that the thermodynamic stability of lysozyme was decreased following cold plasma treatment. It was inferred that plasma feeding N2 had a more significant impact on the thermodynamic stability of lysozyme because of a similar ability to vary in melting temperatures but not in their corresponding intensities. Table 1 shows some of the enzymes and plasma types along with treatment time commonly studied for enzyme inactivation.

### **Table 1: Effects of different Cold plasma sources on Enzyme Inactivation**

| **Sr****No** | Enzyme | Plasma Sources | Treatment Time | Effect of plasma on enzyme | Reference |
| --- | --- | --- | --- | --- | --- |
| 1. | Alkaline phosphatase | 1. Cold Atmospheric pressure plasma2. Dielectric Barrier Discharge | 120sec | Low activity confirmed by low helical contents | [35]  |
| 2. | Lipase | 1. Dielectric Barrier Discharge2. RadioFrequency (RF) atmospheric pressure glow discharge (APGD) | 50sec | After 1 min, the activity of lipase significantly increased, confirmed by changes in secondary and tertiary structures | [36] |
| 3. | Lipoxygenase | Low-pressure Barrier Discharge | 30min | The voltage-dependent decline in activity; over a storage time, activity is lower than control | [37] |
| 4. | Lysozyme | Low-temperatureatmosphericpressure plasma | 8-10min | Low activity confirmed by low α helix and high β helix and also change in substrate binding site | [31] |
| 5. | Peroxidase | Dielectric barrier Discharge | 30 & 60min | Very low activity | [30] |
| 6. | Polyphenol oxidase | Microwave Driven Plasma | 2.5, 5, 7 and 10 min | Low activity after 10 minutes of treatment, with values of 62% and 77% in freshly cut apple and potato tissue. | [34] |
| 7. | Pectin methyl esterase | Dielectric barrier Discharge | 30 & 60 min | Till 15 min no change and then gradually low activity observed | [25] |
| 8. | Superoxide Dismutase | Atmospheric pressure discharge plasma activated water | 20min | High Activity of plasma on the enzyme was found | [27] |
| 9. | α- Chymotrypsin | Cold atmospheric pressureplasma jet | 5 min | Low activity  | [31] |
| 10. | Laccase | Cold plasma Jet | 60sec | Low activity confirmed by degradation of protein molecule | [38] |
| 11.  | RNase | Dielectric barrier Discharge | 1-600sec | Enzyme activity is permanently suppressed in a short period. | [28] |
| 12. | Lactate dehydrogenase | Helium-oxygennon-thermaldielectric barrier discharge | 300sec | Low activity was confirmed by modification of the secondary structure | [27] |
| 13. | Phytase | RF-driven plasma | 10, 15 and 20 min | High activity of plasma on phytase was found  | [29] |
| 14. | GAPDH | Cold atmosphericpressure plasma | 1 min | By entire jet, particle-jet, and UV-jet, respectively, only 20%, 40%, and 75% of the enzymatic activity remained after exposure to the complete effluent for 10 min. | [33] |
| 15. | Protease | RF- Driven Plasma | 12 hours, 24 hours | High activity | [29] |

# EFFECTS OF COLD PLASMA TECHNOLOGY ON BIOACTIVE COMPONENTS, MINERALS AND ANTIOXIDANTS

 Extra nutritional components, in simple terms known as "bioactive compounds", are usually present in food in trace amounts. In-depth research is being done on them to see how they affect health. Diverse bioactive compounds have been isolated and discovered. These substances are categorised based on how differently they behave and are chemically structured [39]. Most foods may naturally contain bioactive components. Antioxidant, anticarcinogenic, anti-inflammatory, and antibacterial activities are present in most bioactive substances. As a result, several epidemiologic studies claim that certain of them protect against cardiovascular illnesses. Carotenoids, flavonoids, carnitine, choline, coenzyme Q, dithiolthiones, phytosterols, phytoestrogens, glucosinolates, polyphenols, and taurine are a few examples of bioactive components. Vitamins and minerals can also be considered bioactive substances since they have pharmacological effects. During thermal food processing, these bioactive compounds may get degraded or lost due to an increased demand for non-thermal food processing technologies. Innovative food processing techniques such as ultrasound, gamma irradiation, high-hydrostatic pressure processing, pulsed electric field, ultraviolet irradiation (UV-C), ozone, plasma-activated water (PAW), and cold atmospheric plasma have been shown to increase the preservation of essential nutrients, quality, and functional qualities [40].

 As an alternative to conventional thermal processing methods for maintaining food quality attributes, Cold plasma (CP) treatment is a well-researched non-thermal processing technology used to sterilise food. Plasma is a mixture of charged particles (OH-, H2O+, electrons), excited molecules (excited O2, N2), UV photons, and positive and negative ions. It also contains reactive oxygen species (ROS), reactive nitrogen species (RNS), and reactive oxygen species. Superoxide anion, atomic oxygen, singlet oxygen, hydroxyl radical, ozone, and RNS, including nitrogen, nuclear nitrogen, nitric oxide, UV photons, free electrons, and positive and negative ions, are examples of ROS [41]. One of the most significant and influential factors in the quality of treated food is the length of time that food is exposed to CP. By prolonging the exposure period, reactive species would have ample time to interact with the chemical constituents of the food to produce new compounds or take part in degradation processes [42]. Tropomyosin antigenicity was only slightly affected by 3 to 9 min of CP treatment, whereas the protein's surface hydrophobicity and total sulfhydryl content were altered by longer treatment durations [43]. Another aspect influencing the effectiveness of the plasma process is the distance between the food and the plasma-producing source. According to reference [44], utilising direct CP increased the immunoreactivity in the soluble protein fraction of soy protein isolate to 91–100%, whereas using the indirect method of plasma lowered it to 89%. There has been a fair amount of research done on the use of cold plasma on food and how it affects various food ingredients. Regarding how low-molecular-weight organic molecules and plasma reactive species interact, there is still some ambiguity. This exists because the plasma species is highly dynamic and reactive. Using plasma as a food processing aid depends on accurately interpreting this mechanism [41].

## **Effect on Polyphenols**

 One of the key secondary bioactive metabolites in plants that can prevent oxidative stress and the associated metabolic disorders is phenolic compounds. They are most frequently found in conjugated forms with mono and polysaccharides and are distinguished by having at least one aromatic ring with one or more hydroxyl substituents. In plants, the pentose phosphate, shikimate, and phenylpropanoid pathways generate phenolic compounds [42]. As a plant's stress defence mechanism, applying CP to plants can operate as an abiotic activator and lead to the production of secondary metabolites, including phenolic compounds. CP may increase the amount of ATP present and speed up the use of carbohydrates, stimulating the formation of phenolics in fruits [45]. The effect of treatment duration on the phenolic contents of uncut blueberries was assessed in the research [46]. Longer treatment times resulted in lower phenolic levels, which the scientists also found correlated with higher fruit temperatures (over 45 °C). This circumstance implies that phenolic compounds degrade over prolonged exposure to radical species. Recent investigations have found that fresh-cut sample preservation results in high polyphenol contents. Freshly cut pitaya was exposed to plasma produced by dielectric barrier discharge equipment, and the results showed that the phenolic content increased during storage (from 12 to 36 hours at 15 °C), especially for gallic, protocatechuic, and p-coumaric acids [45]. Similar stimulatory effects were seen during storage in an experiment with freshly cut strawberries. During storage (4 °C), a considerable rise in the total phenolic, anthocyanin, and flavonoid contents was seen, especially on days 1, 3, and 5 [47]. Reference [48] found that prebiotic orange juice exposed to ambient cold plasma preserved 76% of the original phenolic content following an indirect exposure of plasma for 60 s. In particular, the phenolic compounds were vulnerable to ozone assault, and a noticeable alteration only developed after 60 s of exposure to plasma. Exposure time, power intensity, and flow rate are the key factors that affect the decrease in phenolic compounds. The overall phenol concentration decreased when time, flow rate, and power were increased [41]

## **Effect on Flavonoids**.

 Foods include chemicals called flavonoids that have a distinct phenolic structure. The anti-mutagenic, anti-oxidative, anti-inflammatory and anti-carcinogenic properties of flavonoids make them significant. After food processing, this substance must be conserved in order to ensure the food's chemical stability [41]. As a result of the etching of the top epidermis of lamb's lettuce brought on by plasma ROS such OH-and Ar+, Reference [49] theorised, flavonoids and other chemicals were released from the central vacuoles of the guard cells and were then degraded [49]. They also hypothesised that under NTP exposure, flavonoids broke down considerably more quickly than phenolic acids. Their study was underpinned by polyphenols' ability to scavenge radicals and neutralise RS produced by the plasma. This has made it possible for phenolic compounds to withstand degradation more than flavonoids. In contrast after plasma treatment, flavonoids were shown to increase in strawberry, blueberry, and lotus petal powder. This increase may be the result of cellular components being leached due to the powder's surface modification [45]. It was discovered that biosynthesized flavonol, flavones, phenylpropanoid, and some other metabolites produced by phenolic substances were plasma-activated and the accumulation of these metabolites increased the flavonoid concentration. The release of flavonoid chemicals from the bound membranes needed less energy than the release of polyphenols. It was observed that increasing processing time and flow rate improved the lowering of flavonoids. A fraction of the flavonoids and phenolics found in some foods are bonded to the cell membranes. They need a certain amount of energy to become liberated and available, increasing their entire content in the food matrix [50].

## **Effect on Antioxidants**

 One of the most significant bioactive chemicals, antioxidants, are affected by the CP process depending on a variety of variables, including the type and reactivity of plasma species and their permeability into the food matrix [42]. Antioxidants are crucial substances that control free radicals, bind oxygen, and stop oxidation, maintaining the nutritional value of food. Phenolic chemicals, vitamin C, and vitamin E are the main antioxidants and free radical scavengers found in fruits and vegetables. Antioxidant activity closely relates to the numerous polyphenols, flavanols, and flavonoids contained in foods, even though it is not a precise indicator of quality in the food sectors [51]. Reference [52] found that spark discharge plasma treatment increased the antioxidant capacity of turbid apple juice, while a declining trend was seen after storage for up to seven days. The capacity of antioxidants to scavenge plasma-generated free radicals and their subsequent drop in concentration in the juice were connected to the overall reduction in antioxidant potency. The redox properties of phenolic compounds, which include possible mechanisms such as free-radical scavenging activity, singlet oxygen quenching capacity, and transition metal-chelating activity, are what provide them their potential for antioxidant action. The 1,1-diphenyl-2-picrylhydrazyl (DPPH) scavenging activity, oxygen radical absorbance capacity (ORAC), 2,2′ -azino-bis-3-ethylbenzothiazoline-6 sulfonic acid (ABTS) radical scavenging activity, and ferric reducing antioxidant power (FRAP) assay are the main methods used to assess the antioxidant potentials of food products. The results of the testimonies about the effect of CP processing on the phenolic composition of foods have shown a wide range of heterogeneity. The major causes of the decline in antioxidant activity are the decrease in total phenols brought on by the interaction of phenol molecules with reactive oxygen species and the decrease in ascorbic acid [41].

## **Effect on Vitamins**

 The nutritional value of food items must be preserved, which depends on how sensitive vitamins are to different processing methods. While certain vitamins, like biotin, pyridoxine, and riboflavin (B2) are often stable, others, including thiamin (B1) and vitamins A, C, and E, are very unstable and unpredictable. The stability of vitamin C (ascorbic acid) has been the exclusive focus of the majority of the reported experiments on CP treatment of food items [53]. An increase in the amount of nitrogen plasma that was transferred over the treatment cavity helped the acerola juice's vitamin A content [54]. Here, the treatment duration and plasma flow rate both had a beneficial impact on the vitamin A content. Ascorbic acid content did not change considerably as a result of the treatment, which was favorable because acerola is a large source of vitamin C and a decrease would reduce the juice's high nutritional value. The interaction of ascorbic acid during processing with ozone and other oxidising plasma species may be accountable for its deterioration [51]. The exposure period, process gas, input power, food matrix, and flow rate are the crucial process factors that can influence the deterioration of vitamin content. When compared to foods that have been thermally treated, the bioactive components can be preserved to some extent by optimising these process settings to provide a moderate treatment [41]. Table 2 shows effects of cold plasma treatment on various bioactive compounds.

### **Table 2: Effects of different Cold plasma sources on Bioactive Compounds**

| **Sr no** | Bioactive compounds | Food source | Plasma type | Treatment condition | Observation  | Reference |
| --- | --- | --- | --- | --- | --- | --- |
| 1. | Phenolic compounds | pomegranate | Cold atmospheric gas phase plasma | Argon gas; 3, 5, and 7 min; 25 kHz; | Elevated levels of ellagic acid, chlorogenic acid, ferulic acid, catechin, and punicalagin 1 have been found. Protocatechuic acid, caffeic acid, and punicalagin 2 content reduction | [55].  |
| 2. | Flavonoid glycosides | Pea | Cold Atmospheric pressure plasma | 0, 2.5, 5 and 10 min; 3 kHz | A decrease in quercetin glycoside concentration. The amounts of kaempferol glycosides reduced. | [56].  |
| 3. | Flavonoids | Lamb lettuce | Atmospheric pressure plasma jet | 0.20, 40, 80, and 120 s; 12 MHz; | lower concentrations of phenolic acids. a reduction in caffeic acids enhancement of diosmetin | [49].  |
| 4. | Total Phenolic content (TPC) | Apple | Dielectric barrier discharge atmospheric cold plasma | 30, 40, and 50 W; 0, 5,10, 15, 20, 30, and 40S | A decrease in TPC with an increase in treatment duration and intensity. | [57]. |
| 5. | TPC, TFC (Total flavonoid content), andanthocyanin | Blue Berry | Atmospheric cold plasma |  0, 2, and 5 min80kv, 50Hz | A notable rise in TPC and TFC following one minute of plasma exposure. With prolonged therapy, anthocyanin levels significantly decline. | [58]. |
| 6. | TPC | Orange  | Atmospheric cold plasma | 15, 30, 45, and 60 s; 70 kV; 50 Hz; | Regardless of direct or indirect exposure, a decrease in TPC. | [48]. |
| 7. | Total phenolics content, Carotenoids | Kiwi fruit | Atmospheric double barrier discharge plasma | 10+10 and 20+20 min | No noticeable shift in the overall phenolic content. a reduction in the sum of the carotenoids | [59]. |
| 8. | Phenols, flavonoids, flavonols | White grape | High voltage atmospheric cold plasma | 0, 1, 2, 3, and 4 min; 80 kV; | A reduction in all phenolics. a depletion of flavonoids. increased level of all flavonols | [51]. |
| 9. | Vitamin C | Orange | Dielectric barrier discharge | 30-120sec 90kv | 22% vitamin C got reduced in air | [60].  |
| 10. | Vitamin C | Cashew apple Juice | PE 100 -N2 plasma | 5-15min,80KHZ | Vitamin C levels drop at greater flow rates Sucrose content rose whereas glucose and fructose levels fell. Increased polyphenol and total flavonoid concentration was facilitated by longer treatment. | [61]. |

# MECHANISM OF MICROBIAL CELLS INACTIVATION BY COLD PLASMA TECHNOLOGY

 Currently, the food industry must resolve the formidable challenge of providing consumers with nutrient-dense, secure, and shelf-stable foods while restricting bacterial growth. Microbial invasion can happen at any stage of handling or post-harvest processes, including transportation, processing, equipment handling, or the actual processing. Thermal processing is commonly employed to produce foods that are safe and secure from microorganisms. Moreover, because nutritional and sensory qualities are lost, it is not a primary approach. Additionally, the majority of consumers now look critically at food items that contain chemical substances. Gentle non-thermal decontamination techniques, like PEF, irradiation, HPP, and non-thermal plasma, have emerged as a result of the above [20]. An efficient non-conventional processing method for inactivating a variety of spoilage bacteria present in food products is cold plasma. There are numerous kinds of literature on cold plasma applications in the literature that give an overview of the development of the cold plasma technique in food processing. There is a significant amount of curiosity in learning how different microorganisms are suppressed by plasma, despite the fact that most recent findings are still primarily focused on researching and improving the plasma decontamination conditions for food ingredients targeting various microorganisms [62]. ROS have been said to be the most important factor in inactivation of microorganisms, which results in severe oxidative stress ailments and damages cells by causing oxidative damage, protein degradation, and DNA cleavage [63]. Additionally, reference [64] reported various cold plasma inactivation mechanisms for both Gram positive and Gram-negative bacteria. They demonstrated that Gram negative bacteria like Escherichia coli were primarily suppressed by cell contamination and low-level DNA damage, whereas Gram positive bacteria such as Staphylococcus aureus were primarily inactivated by intracellular damage [64]. The associations of plasma gas with a variety of microbial contamination targets as well as the advancements in the metabolic observations have been briefly reviewed [65]. These experiments unequivocally demonstrate the specific and heterogeneous associations of reactive gas species in plasma systems, highlighting the necessity of further mechanistic research for a deeper comprehension. Other than inactivation of microorganisms, influence of cold plasma on food standards have also been a significant factor attracting food researchers' attention. Food enzyme inactivation by cold plasma has attracted a lot of interest in this regard. According to reference [66], plasma gas species exhibit secondary enzymatic configuration loss due to the collapse of specific bonds o structural changes of the side chains that were reliant on energy input, level of exposure, transport phenomena among the plasma-liquid stages, secondary structure, and stability of the enzymes in their surrounding ecology [66]. With varying degrees of success, a variety of cold plasma sources are also used to inactivate a large class of pathogenic microorganisms in meat and meat products [67]. Table 3 shows effects of different cold plasma sources on microbial cells.

### **Table 3: Effects of different Cold plasma sources on Microbial Cells**

| **Sr****No** | Microbial Type/ Species | Plasma Sources | Treatment Time | Mechanism of cold plasma on microbial cells | Reference |
| --- | --- | --- | --- | --- | --- |
| 1. | *Escherichia coli, Listeria monocytogenes* and *Staphylococcus aureus* | Dielectric barrier discharge | 15, 60 and 300 sec | The oxygen level of applied gases, in combination with exposure period and post-treatment storage conditions, determines the bactericidal effect of atmospheric cold plasma. Following a 15 second treatment with a high oxygen modified atmospheric pressure mix and a 24-hour storage period, there were no detectable *Listeria* populations. | [68]. |
| 2 | Aerobic MesophilesYeast and molds | Dielectric barrier discharge | 5 minutes | In 300 s of in-package atmospheric cold plasma discharge, micro-flora of the strawberries was lowered on average by 3.0 log cycles from the starting levels of 5 log10 Colony forming units (CFU/g). Similar effects on the levels of microbial reduction were seen after plasma treatments with the two gas mixtures. | [69]. |
| 3 | *Listeria innocua* | Cold atmospheric plasma pen | 10 sec to 8 min | On membrane filters, a 10 s cold plasma treatment resulted in > 3 log reductions of *L. innocua*, an 8 min treatment resulted in 1 log reduction on skin, and a 4 min treatment resulted in > 3 log reductions on muscle. These findings demonstrate how the topography of the treated surface has a significant impact on the gas plasma treatment's effectiveness. | [70]. |
| 4 | Gram positive and Gram negative strains | Non-thermal helium plasma | 15, 30, and 60 sec | To create efficient therapeutic approaches, relations between plasma and living cells must be thoroughly characterised. In fact, our study reveals an important interaction between mechanical damage and induction of apoptosis brought on by non-thermal plasma in addition to various scenarios of plasma-induced bacterial death. | [17]. |
| 5 | Aerobic bacteria, marine bacteria, *Staphylococcus aureus* | Corona discharge plasma jet | 0-3 minutes | After being exposed to a corona discharge plasma jet for 0–3 minutes, the contaminants Staphylococcus aureus, marine bacteria, and aerobic bacteria were all inactivated by 2.0, 1.6, and 0.9 log units, respectively. | [71]. |
| 6 | *B. cereus, A. brasiliensis, and E. coli O157:H7* | Microwave assisted cold plasma | 40 minutes | We investigated the sporadic activity of microwave-induced CP against onion powder stored at two different temperatures (4°C and 25° C). The study found that after 21 days, a 40-minute burst of high microwave density cold plasma could reduce the numbers of *B. cereus, A. brasiliensis*, and *E. coli* O157:H7 by 2.1, 1.6, and 1.9 log CFU/cm2, respectively, without influencing the sample's quercetin content, colour, or capacity to scavenge free radicals. | [72].  |
| 7 | *Salmonella* Enteritidis | High voltage atmospheric cold plasma | 15 minutes | The length of treatment, the kind of gas used, and the way the eggs were exposed to the plasma all had an impact on *Salmonella* reductions. After cold plasma treatment, no discernible gap between direct and indirect modes of exposure was found in the quality of the eggs. These findings show that high voltage atmospheric cold plasma has the potential to be used as an effective non-thermal treatment to lower *Salmonella* levels in packaged chicken eggs. | [73]. |
| 8 | Mesophiles. Psychrophiles and *Pseudomonas* species | Dielectric barrier discharge | 180 sec | The rapid killing effect of plasma gas is explained by the severe reactive species bombardment that cold plasma treated microorganisms experience, which results in surface lesions on the living cells. Regarding the quality of food products, exposure to cold plasma did not appear to affect the fresh chicken meat's appearance or surface lightness. | [74]. |
| 9 | *E .coli* | Microwave-induced cold plasma |  \_ | About 90% of *E. coli* O157:H7 on lettuce was inactivated by microwave-induced cold plasma, and no significant changes to the product's organoleptic and quality characteristics, such as colour, weight loss, ascorbic acid concentration, or antioxidant activity, were noted. | [20] |
| 10 | *Salmonella typhimurium* | Nitrogen cold plasma | 10 min | During storage for 12 days at 4 and 10 °C, the effects of nitrogen cold plasma treatment for 10 min on microbial activity and the quality characteristics of the radish sprouts were assessed. The amount of *S. typhimurium* was decreased by 2.6 0.4 log CFU/g by nitrogen cold plasma treatment at 900 W as well as 667 Pa for 20 min. | [75].  |

# APPLICATION OF COLD PLASMA TECHNIQUE

 Plasma technology offers a special mix of reactive species, which is one of its main benefits for the food industry. Many of these species are quite reactive, and plasma is frequently mentioned for its many antimicrobial action mechanisms. Inherent resistance to plasma therapy is therefore seldom recorded. The growing knowledge of the longer-term function of cold plasma reactive species and follow-on impacts across a range of systems will provide guidance on how cold plasma may be used most effectively with biological systems in the agricultural and food industries [76]. A key aspect of plasma technology is the ability to discharge the reactive species that form the afterglow at atmospheric pressure and without the use of heat. High-temperature electrons may be present in nonthermal plasmas, although neutrals, ions, and radicals often remain at or near normal temperatures. Glow discharge, radio-frequency (RF) discharge, dielectric barrier discharge (DBD), atmospheric-pressure plasma jet (APPJ), microwave discharge, and pulsed power discharge are a few of the methods used to create plasma discharges which facilitate it to be applied in different areas of food industry [76].

## **Functional Modifications**

 The unique technique of plasma therapy for starch modification has recently been demonstrated. When oxygen-containing groups like hydroxyl, carboxyl, and carbonyl groups interact chemically with the starch polymer and plasma species, they transform smooth hydrophobic surfaces into rough hydrophilic surfaces, which is the primary cause of functionalization of starch. The type of starch, gas and treatment exposure are plasma characteristics that can affect the phase transition [77]. O2 plasma can introduce functional groups such as carboxylic acid, peroxides, and hydroxyl groups, whereas CO2 gas plasma contains hydroxyls, ketones, aldehydes, and esters. Additionally, primary, secondary, and tertiary amines are introduced by nitrogen and ammonia plasmas via the transformation processes of these functional groups [78]. When compared to amylopectin, amylose is shown to be less vulnerable to depolymerization after plasma treatment. Different functionality linked to gelatinization, thickening, and gelling is provided by atmospheric plasma treatment, which modifies the granular structure somewhat on a variety of scales, including the molecular, mesoscopic, and macroscopic levels (79). Starch viscosity, having a significant characteristic, may be altered by plasma. The dough strength and ideal mixing time for both strong and weak wheat flour improved as a result of the plasma treatment of the flour, according to its rheological characteristics. With applied voltage and treatment duration, the strong wheat flour's elastic and viscous moduli gradually rose [80]. According to reference [81], cross-linking caused samples that had been exposed to plasma to produce a stronger gel structure, whereas a depolymerization effect is responsible for samples that developed a weaker gel structure.

## **Processing of Milk and Dairy Products**

 Due to the reduced Maillard browning, production of off flavours, and loss of nutritional content, cold plasma may one day replace conventional thermal pasteurization procedures for food. Similar to how bacteria function, plasma also affects natural milk enzymes. Proteins' altered shape causes oxidation reactions of peptides to inactivate enzymes, which reduces their enzymatic activity [82]. Alkaline phosphatase (ALP), a naturally occurring milk enzyme, was tested for its impact on the activity and structure in recent research by [35]. ACP was applied to ALP in solution at periods ranging from 15 seconds to five minutes at three distinct high voltages (40, 50, and 60 kV). The outcomes showed that the enzyme may be turned inactive within a few seconds using plasma technology based on dielectric barrier discharge. The enzyme appeared to have a mostly -helix structure based on the dichroic spectra, and the helical content tended to decrease with longer treatment times and higher voltages. The most intensive treatments only reached a maximum temperature of about 30 °C with no pH alterations. Bacteria, bacterial spores, fungus, and biofilms may all be effectively inactivated by cold plasma. Plasma causes cell death by three fundamental processes, including etching of cell surfaces brought on by reactive species created during plasma production, compound volatilization and intrinsic UV photodesorption, and genetic material damage [35]. The electrical input (voltage, frequency, and power) employed in the process has an impact on the reactive species produced in the discharge as well. Higher process efficiency results from longer treatment times and more electrical input. To choose the appropriate electrical input settings, food quality characteristics must be considered. The short lifespan of the active species prevents coverage of greater sample regions, even with longer treatment durations, which further contributes to the enhancement of efficacy with time until saturation [83].

## **Food Waste Processing**

 Because of the high concentrations of carbohydrates, lipids, proteins, and mineral salts in food manufacturing waste, the complete breakdown is frequently challenging. High organic loads in the effluent from the processing of dairy, meat, poultry, and shellfish can severely pollute the water supply. These effluents are often treated using physical, chemical, or biological techniques that are ineffective in removing the organics. These organics can promote bacteria to multiply quickly, which lowers the quantity of dissolved oxygen in the water [77]. Reference [84] reported on a case study using cold plasma technology to quickly remove contaminants from model dairy effluents (In 15-minute treatments at 80 kV, about 90% of the pollutants were removed, and the amount of total organic carbon (TOC) was reduced by 50% for a model dairy effluent). Reference [85] published an early paper on the use of plasma in effluent processing. 98% less biological oxygen was required after atmospheric-pressure plasma treatment of commercial brewery wastewater (BOD). The efficacy of the method can be increased by combining plasma with biological therapy, according to these authors. The environment is worried about the odours (emissions) that the agriculture and food processing sectors emit. Reduced carbon, nitrogen, and sulphur compounds make up the majority of the substances created during food processing. Odours can occasionally be caused by the generation of volatile organic compounds (VOCs) [77]. A nonthermal plasma pilot-scale device for odour elimination of ventilation air from a pig home was recently studied by [86]. For all trials, reductions of more than 90% were attained at flows of 135 m3/h and voltages ranging from 15 to 45 kV. The development of plasma as a special pretreatment technique for anaerobic digestion of food waste has become the best solution to manage food waste and to use this waste as a substrate for Biomethanation. The ethanol output was increased by up to 52% when atmospheric plasma pretreatment of wheat straw was followed by fermentation. The release of glucose from the cellulose caused by plasma pretreatment enhanced the generation of ethanol [87].

## **Shelf-Life Extension**

 The factor that drives efforts toward shelf-life extension, particularly for fresh produce and meat products, are mounting pressure to reduce food waste and improve sustainability, as well as the globalisation of the food market with growing distances between the point of production/processing and consumption [76]. CP's potential to inactivate microbes allows it to prevent the bacterial and fungal development that leads to food spoiling. Studies on increasing food shelf life by atmospheric cold plasma have taken ready-to-eat goods including fresh fruit, vegetables, and meat into consideration [88]. Salmonella, E. coli, and L. monocytogenes on cherry tomatoes were reduced to undetectable levels in samples treated with an in-package plasma technique for 10, 60, and 120 seconds, according to research [89]. Similar to this, [74] demonstrated that in-package plasma treatment with MAP (Modified atmospheric packaging) caused a 4-log decrease during storage and might increase the shelf life of fresh chicken meat without degrading the quality of the final product.

## **Degradation of Toxins and Allergens**

 Around 10% of the world's population suffers from food allergies, which are caused by the Big 8 food protein sources: milk, eggs, fish, crustaceans and shellfish, tree nuts, peanuts, wheat, and soy. Total avoidance of the food allergen with individually adjustable threshold dosages is the only available preventive measure. Direct CP may unfold whey protein molecules and alter their 3D architectures [35]. These new findings show the potential of CP as a method to minimise food allergen immunoreactivity in foods and processing environments and may be especially useful for those allergens that prove resistant to normal processing because of their thermostability. Pesticide degradation is also a major factor of concern. Dichlorvos and omethoate were shown to be degraded by O2-plasma on samples of maize, and reference [90] found that the effectiveness of the decomposition was influenced by the operating conditions and the chemical makeup of the pesticide. The fact that the intermediates generated were less harmful than the parent pesticides was also validated by the authors. Mycotoxin-contaminated food consumption can cause illnesses in people that damage important systems including the neurological and immunological systems. After plasma treatment for 30 minutes at 60 W, it was found reductions of 99.9% and 99.5% of Aspergillus flavus and Aspergillus parasiticus spores, respectively, were inoculated on ground nuts [91]. The degradation of organic material by etching and photodesorption, which are connected to chemical bond breaking and result in the generation of volatile chemicals, was explained by SEM analysis as the cause of the membrane rupture in the spore [92].

## **Food Packaging**

 For many years, the packaging industry has employed cold plasma. It has been widely utilized for surface etching, surface functionalization, surface activation, and surface deposition as well as for sterilizing packaging material [62]. DBDs have recently been used to create a plasma within sealed packaging holding meat and bacterial samples. It has been researched utilising a variety of packaging materials, including low-density polyethene (LDPE), high-density polyethene (HDPE), and polystyrene, for the in-package plasma decontamination of foods and biomaterials. This method depends on employing the polymeric package itself as a dielectric [92]. Recently, it has also been utilized to modify the surface of bio-based films and coatings in addition to its application for conventional polymers. Cold plasma has the potential to be exploited to create active and intelligent packaging materials, according to certain recent research. Plasma treatment of various packaging materials is still a popular trend in this field for enhancing packaging qualities [62].

#  LIMITATIONS OF COLD PLASMA TECHNOLOGY

 Although the impacts of plasma species on the antimicrobial and other induced effects are obviously effective, such complicated chemistry is expected to provide difficulties for method validation and regulatory approval. The regulatory approval procedure is currently ambiguous due to the evaluation criteria's lack of specificity. Due to the intricacy of plasma chemistry, the process largely depends on the principal mechanism of action, and the literature now in circulation explains a wide range of chemical effects that support various applications [41]. For both direct and indirect food applications, the approval of plasma procedures necessitates a significant amount of data collecting, data analysis, and time. It might be difficult to understand not just the chemical processes occurring inside food matrices but also the mechanisms of antibacterial activity. The other limitations of cold plasma processing are the complexity of the processing equipment, the relatively early stage of technological development, and, in the majority of cases, the unknown effects of the treatment on the food contents. Due to the low amount of readily available information on food sustenance compared to other established novel treatments, optimising the process parameters of cold plasma also offers a significant problem. Another challenge is the lack of a measurable dose for food products. The majority of research on food describe the plasma discharge by identifying neutral atomic spectral lines and molecular bands using optical emission spectroscopy [77].  The prospect of standardizing the so-called plasma dosage would be made possible by the availability of absolute calibrated emission spectra [93]. The costly instruments, operating concerns, and maintaining process control restrict the practical application as well. This furthers the uncertainty because the mode of action might differ based on the sort of generating mechanism. A few instances where OH radicals and superoxide anions induced lipid oxidation by removing hydrogen atoms from lipids have also been linked to detrimental impacts on food quality. For eukaryotic cell lines, the cytotoxic action of plasma-treated liquids like Plasma activated water (PAW) or more sophisticated solutions like plasma-activated medium (PAM) has been observed [94]. Reference [95] used FTIR, proton nuclear magnetic resonance, and chromatographic methods to assess plasma-induced lipid oxidation of dairy and animal fats. Such early investigations suggest a secure food processing technique, however there is still a limited knowledge in this field. In light of the extensive work put into developing and exploring the cold plasma treatment method on a lab scale, a careful assessment of its industrial use is needed, taking into account the plasma analytics, process optimization, legal issues, and energy costs.

#  CONCLUSION

 Cold plasma is a very efficient, reliable, emerging non-thermal food processing technology for preservation, decontamination and sterilization of the food. The main attractive characteristic of cold plasma technology is that it works under a minimal temperature zone due to which the nutritional and dietary compounds of the food are not lost during processing. The chemistry of the reaction plays a significant role in the plasma-induced changes of food's functional and bioactive components. Cell membrane rupture, surface etching, increased surface roughness, effective contact area, oxidative degradation, starch depolymerization, cross-linking of starch granules, and hydroxylation of benzene rings were among the main interaction mechanisms discovered. It was realized that the induced alterations are mostly linked to the oxidative breakdown that results in the creation of certain chemicals. The physicochemical, functional, bioactive, and sensory qualities of the food, as well as the enzymes, proteins, cytotoxicity, and presence of allergens and antinutrients, all underwent substantial alterations as a result of these interactions. To know the chemical interaction and mechanism of plasma and its secondary products on food, more study on the genotoxic/cytotoxic effects of cold atmospheric plasma therapy is recommended. By causing changes to the secondary structure of enzyme proteins, cold plasma therapy has also been shown to inactivate enzymes. It was also shown that the protein changes reduced the potency and inhibitory actions of food allergens and antinutrients. Its applications in the food industry are mainly concentrated on food decontamination, food quality improvement, toxin breakdown, and surface modification of packaging materials. The data provided in this chapter highlights the effects of cold plasma technology on enzyme inactivation, bioactive components, minerals, anti-oxidants, the mechanism of action of cold plasma on microbial cells inactivation and various application and limitation of cold plasma technology.

##### REFERENCES

1. Mandal R, Singh A, Singh AP. Recent developments in cold plasma decontamination technology in the food industry. Trends in Food Science & Technology. 2018 Oct 1;80:93-103.
2. Vanga SK, Singh A, Raghavan V. Review of conventional and novel food processing methods on food allergens. Critical reviews in food science and nutrition. 2017 Jul 3;57(10):2077-94.
3. Ucar Y, Ceylan Z, Durmus M, Tomar O, Cetinkaya T. Application of cold plasma technology in the food industry and its combination with other emerging technologies. Trends in Food Science & Technology. 2021 Aug 1;114:355-71.
4. Knorr D, Augustin MA. Food processing needs, advantages and misconceptions. Trends in Food Science & Technology. 2021 Feb 1;108:103-10.
5. Augustin MA, Riley M, Stockmann R, Bennett L, Kahl A, Lockett T, Osmond M, Sanguansri P, Stonehouse W, Zajac I, Cobiac L. Role of food processing in food and nutrition security. Trends in Food Science & Technology. 2016 Oct 1;56:115-25.
6. Fardet A, Richonnet C. Nutrient density and bioaccessibility, and the antioxidant, satiety, glycemic, and alkalinizing potentials of fruit-based foods according to the degree of processing: a narrative review. Critical Reviews in Food Science and Nutrition. 2020 Oct 27;60(19):3233-58.
7. Deng LZ, Mujumdar AS, Zhang Q, Yang XH, Wang J, Zheng ZA, Gao ZJ, Xiao HW. Chemical and physical pretreatments of fruits and vegetables: Effects on drying characteristics and quality attributes–a comprehensive review. Critical reviews in food science and nutrition. 2019 May 15;59(9):1408-32.
8. Morales-de la Peña M, Welti-Chanes J, Martín-Belloso O. Novel technologies to improve food safety and quality. Current opinion in food science. 2019 Dec 1;30:1-7.
9. Muhammad AI, Xiang Q, Liao X, Liu D, Ding T. Understanding the impact of nonthermal plasma on food constituents and microstructure—A review. Food and bioprocess technology. 2018 Mar;11(3):463-86.
10. Chen YQ, Cheng JH, Sun DW. Chemical, physical and physiological quality attributes of fruit and vegetables induced by cold plasma treatment: Mechanisms and application advances. Critical Reviews in Food Science and Nutrition. 2020 Sep 7;60(16):2676-90.
11. Pan Y, Cheng JH, Lv X, Sun DW. Assessing the inactivation efficiency of Ar/O2 plasma treatment against Listeria monocytogenes cells: Sublethal injury and inactivation kinetics. Lwt. 2019 Aug 1;111:318-27.
12. Yepez XV, Keener KM. High-voltage atmospheric cold plasma (HVACP) hydrogenation of soybean oil without trans-fatty acids. Innovative Food Science & Emerging Technologies. 2016 Dec 1;38:169-74.
13. Ji H, Dong S, Han F, Li Y, Chen G, Li L, Chen Y. Effects of dielectric barrier discharge (DBD) cold plasma treatment on physicochemical and functional properties of peanut protein. Food and Bioprocess Technology. 2018 Feb;11(2):344-54.
14. Dong S, Gao A, Xu H, Chen Y. Effects of dielectric barrier discharges (DBD) cold plasma treatment on physicochemical and structural properties of zein powders. Food and Bioprocess Technology. 2017 Mar;10(3):434-44.
15. Chaiwat W, Wongsagonsup R, Tangpanichyanon N, Jariyaporn T, Deeyai P, Suphantharika M, Fuongfuchat A, Nisoa M, Dangtip S. Argon plasma treatment of tapioca starch using a semi-continuous downer reactor. Food and Bioprocess Technology. 2016 Jul;9(7):1125-34.
16. Bevilacqua A, Petruzzi L, Perricone M, Speranza B, Campaniello D, Sinigaglia M, Corbo MR. Nonthermal technologies for fruit and vegetable juices and beverages: Overview and advances. Comprehensive Reviews in Food Science and Food Safety. 2018 Jan;17(1):2-62.
17. Whitehead, J. C. "Chapter 3—The Chemistry of Cold Plasma. Cold Plasma in Food and Agriculture." (2016): 53-81.
18. Varilla C, Marcone M, Annor GA. Potential of cold plasma technology in ensuring the safety of foods and agricultural produce: a review. Foods. 2020 Oct 11;9(10):1435.
19. Lunov O, Zablotskii V, Churpita O, Jäger A, Polívka L, Syková E, Dejneka A, Kubinová Š. The interplay between biological and physical scenarios of bacterial death induced by non-thermal plasma. Biomaterials. 2016 Mar 1;82:71-83.
20. Ekezie FG, Sun DW, Cheng JH. A review on recent advances in cold plasma technology for the food industry: Current applications and future trends. Trends in food science & technology. 2017 Nov 1;69:46-58.
21. Han Y, Cheng JH, Sun DW. Activities and conformation changes of food enzymes induced by cold plasma: A review. Critical reviews in food science and nutrition. 2019 Mar 9;59(5):794-811.
22. Adams JB. Enzyme inactivation during heat processing of food‐stuffs. International journal of food science & technology. 1991 Feb;26(1):1-20.
23. Islam MN, Zhang M, Adhikari B. The inactivation of enzymes by ultrasound—a review of potential mechanisms. Food Reviews International. 2014 Jan 2;30(1):1-21.
24. Misra, N. N., H. I. Yong, R. Phalak, and C. Jo. 2018. Atmospheric pressure cold plasma improves viscosifying and emulsion stabilizing properties of xanthan gum. Food Hydrocolloids 82:29–33.
25. Tappi S, Gozzi G, Vannini L, Berardinelli A, Romani S, Ragni L, Rocculi P. Cold plasma treatment for fresh-cut melon stabilization. Innovative Food Science & Emerging Technologies. 2016 Feb 1;33:225-33.
26. Setsuhara Y, Cho K, Shiratani M, Sekine M, Hori M. Plasma interactions with aminoacid (l-alanine) as a basis of fundamental processes in plasma medicine. Current Applied Physics. 2013 Mar 20;13:S59-63.
27. Zhang H, Xu Z, Shen J, Li X, Ding L, Ma J, Lan Y, Xia W, Cheng C, Sun Q, Zhang Z. Effects and mechanism of atmospheric-pressure dielectric barrier discharge cold plasmaon Lactate Dehydrogenase (LDH) Enzyme. Scientific Reports. 2015 May 20;5(1):1-2.
28. Lackmann JW, Baldus S, Steinborn E, Edengeiser E, Kogelheide F, Langklotz S, Schneider S, Leichert LI, Benedikt J, Awakowicz P, Bandow JE. A dielectric barrier discharge terminally inactivates RNase A by oxidizing sulfur-containing amino acids and breaking structural disulfide bonds. Journal of Physics D: Applied Physics. 2015 Nov 16;48(49):494003.
29. Sadhu S, Thirumdas R, Deshmukh RR, Annapure US. Influence of cold plasma on the enzymatic activity in germinating mung beans (Vigna radiate). Lwt. 2017 May 1;78:97-104.
30. Surowsky B, Fischer A, Schlueter O, Knorr D. Cold plasma effects on enzyme activity in a model food system. Innovative Food Science & Emerging Technologies. 2013 Jul 1;19:146-52.
31. Choi S, Attri P, Lee I, Oh J, Yun JH, Park JH, Choi EH, Lee W. Structural and functional analysis of lysozyme after treatment with dielectric barrier discharge plasma and atmospheric pressure plasma jet. Scientific reports. 2017 Apr 21;7(1):1-0.
32. Ke Z, Huang Q. Inactivation and heme degradation of horseradish peroxidase induced by discharge plasma. Plasma Processes and Polymers. 2013 Aug;10(8):731-9.
33. Lackmann JW, Schneider S, Edengeiser E, Jarzina F, Brinckmann S, Steinborn E, Havenith M, Benedikt J, Bandow JE. Photons and particles emitted from cold atmospheric-pressure plasma inactivate bacteria and biomolecules independently and synergistically. Journal of The Royal Society Interface. 2013 Dec 6;10(89):20130591.
34. Bußler S, Ehlbeck J, Schlüter OK. Pre-drying treatment of plant related tissues using plasma processed air: Impact on enzyme activity and quality attributes of cut apple and potato. Innovative Food Science & Emerging Technologies. 2017 Apr 1;40:78-86.
35. Segat A, Misra NN, Cullen PJ, Innocente N. Effect of atmospheric pressure cold plasma (ACP) on activity and structure of alkaline phosphatase. Food and Bioproducts Processing. 2016 Apr 1;98:181-8.
36. Tolouie H, Mohammadifar MA, Ghomi H, Yaghoubi AS, Hashemi M. The impact of atmospheric cold plasma treatment on inactivation of lipase and lipoxygenase of wheat germs. Innovative Food Science & Emerging Technologies. 2018 Jun 1;47:346-52.
37. Chen HH, Hung CL, Lin SY, Liou GJ. Effect of low-pressure plasma exposure on the storage characteristics of brown rice. Food and bioprocess technology. 2015 Feb;8(2):471-7.
38. Malinowski S, Herbert PA, Rogalski J, Jaroszyńska-Wolińska J. Laccase enzyme polymerization by soft plasma jet for durable bioactive coatings. Polymers. 2018 May 16;10(5):532.
39. Kris-Etherton PM, Hecker KD, Bonanome A, Coval SM, Binkoski AE, Hilpert KF, Griel AE, Etherton TD. Bioactive compounds in foods: their role in the prevention of cardiovascular disease and cancer. Am J Med. 2002 Dec 30;113 Suppl 9B:71S-88S. doi: 10.1016/s0002-9343(01)00995-0. PMID: 12566142.
40. Muhammad AI, Liao X, Cullen PJ, Liu D, Xiang Q, Wang J, Chen S, Ye X, Ding T. Effects of nonthermal plasma technology on functional food components. Comprehensive Reviews in Food Science and Food Safety. 2018 Sep;17(5):1379-94.
41. Sruthi NU, Josna K, Pandiselvam R, Kothakota A, Gavahian M, Khaneghah AM. Impacts of cold plasma treatment on physicochemical, functional, bioactive, textural, and sensory attributes of food: A comprehensive review. Food Chemistry. 2022 Jan 30;368:130809.
42. Saremnezhad S, Soltani M, Faraji A, Hayaloglu AA. Chemical changes of food constituents during cold plasma processing: A review. Food Research International. 2021 Sep 1;147:110552.
43. Ekezie FG, Sun DW, Cheng JH. Altering the IgE binding capacity of king prawn (Litopenaeus Vannamei) tropomyosin through conformational changes induced by cold argon-plasma jet. Food Chemistry. 2019 Dec 1;300:125143.
44. Meinlschmidt P, Ueberham E, Lehmann J, Reineke K, Schlüter O, Schweiggert-Weisz U, Eisner P. The effects of pulsed ultraviolet light, cold atmospheric pressure plasma, and gamma-irradiation on the immunoreactivity of soy protein isolate. Innovative Food Science & Emerging Technologies. 2016 Dec 1;38:374-83.
45. Li X, Li M, Ji N, Jin P, Zhang J, Zheng Y, Zhang X, Li F. Cold plasma treatment induces phenolic accumulation and enhances antioxidant activity in fresh-cut pitaya (Hylocereus undatus) fruit. Lwt. 2019 Nov 1;115:108447.
46. Lacombe A, Niemira BA, Gurtler JB, Fan X, Sites J, Boyd G, Chen H. Atmospheric cold plasma inactivation of aerobic microorganisms on blueberries and effects on quality attributes. Food microbiology. 2015 Apr 1;46:479-84.
47. Li M, Li X, Han C, Ji N, Jin P, Zheng Y. Physiological and metabolomic analysis of cold plasma treated fresh-cut strawberries. Journal of agricultural and food chemistry. 2019 Mar 18;67(14):4043-53.
48. Almeida FD, Cavalcante RS, Cullen PJ, Frias JM, Bourke P, Fernandes FA, Rodrigues S. Effects of atmospheric cold plasma and ozone on prebiotic orange juice. Innovative Food Science & Emerging Technologies. 2015 Dec 1;32:127-35.
49. Grzegorzewski F, Ehlbeck J, Schlüter O, Kroh LW, Rohn S. Treating lamb’s lettuce with a cold plasma–Influence of atmospheric pressure Ar plasma immanent species on the phenolic profile of Valerianella locusta. LWT-Food Science and Technology. 2011 Dec 1;44(10):2285-9
50. Alves Filho EG, Almeida FD, Cavalcante RS, de Brito ES, Cullen PJ, Frias JM, Bourke P, Fernandes FA, Rodrigues S. 1H NMR spectroscopy and chemometrics evaluation of non-thermal processing of orange juice. Food Chemistry. 2016 Aug 1;204:102-7.
51. Pankaj SK, Wan Z, Keener KM. Effects of cold plasma on food quality: A review. Foods. 2018 Jan 1;7(1):4.
52. Illera AE, Chaple S, Sanz MT, Ng S, Lu P, Jones J, Carey E, Bourke P. Effect of cold plasma on polyphenol oxidase inactivation in cloudy apple juice and on the quality parameters of the juice during storage. Food chemistry: X. 2019 Sep 30;3:100049.
53. Dionísio AP, Gomes RT, Oetterer M. Ionizing radiation effects on food vitamins: a review. Brazilian Archives of Biology and Technology. 2009;52:1267-78.
54. Fernandes FA, Santos VO, Rodrigues S. Effects of glow plasma technology on some bioactive compounds of acerola juice. Food Research International. 2019 Jan 1;115:16-22.
55. Herceg Z, Kovačević DB, Kljusurić JG, Jambrak AR, Zorić Z, Dragović-Uzelac V. Gas phase plasma impact on phenolic compounds in pomegranate juice. Food Chemistry. 2016 Jan 1;190:665-72.
56. Bußler S, Herppich WB, Neugart S, Schreiner M, Ehlbeck J, Rohn S, Schlüter O. Impact of cold atmospheric pressure plasma on physiology and flavonol glycoside profile of peas (Pisum sativum ‘Salamanca’). Food Research International. 2015 Oct 1;76:132-41.
57. Liao X, Li J, Muhammad AI, Suo Y, Chen S, Ye X, Liu D, Ding T. Application of a dielectric barrier discharge atmospheric cold plasma (Dbd‐Acp) for Eshcerichia coli inactivation in apple juice. Journal of food science. 2018 Feb;83(2):401-8.
58. Sarangapani C, O'Toole G, Cullen PJ, Bourke P. Atmospheric cold plasma dissipation efficiency of agrochemicals on blueberries. Innovative Food Science & Emerging Technologies. 2017 Dec 1;44:235-41.
59. Ramazzina I, Berardinelli A, Rizzi F, Tappi S, Ragni L, Sacchetti G, Rocculi P. Effect of cold plasma treatment on physico-chemical parameters and antioxidant activity of minimally processed kiwifruit. Postharvest Biology and Technology. 2015 Sep 1;107:55-65.
60. Xu L, Garner AL, Tao B, Keener KM. Microbial inactivation and quality changes in orange juice treated by high voltage atmospheric cold plasma. Food and Bioprocess Technology. 2017 Oct;10(10):1778-91.
61. Rodríguez Ó, Gomes WF, Rodrigues S, Fernandes FA. Effect of indirect cold plasma treatment on cashew apple juice (Anacardium occidentale L.). Lwt. 2017 Oct 1;84:457-63.
62. Pankaj SK, Keener KM. Cold plasma: Background, applications and current trends. Current Opinion in Food Science. 2017 Aug 1;16:49-52.
63. Lu H, Patil S, Keener KM, Cullen PJ, Bourke P. Bacterial inactivation by high‐voltage atmospheric cold plasma: influence of process parameters and effects on cell leakage and DNA. Journal of applied microbiology. 2014 Apr;116(4):784-94.
64. Han L, Patil S, Boehm D, Milosavljević V, Cullen PJ, Bourke P. Mechanisms of inactivation by high-voltage atmospheric cold plasma differ for Escherichia coli and Staphylococcus aureus. Applied and environmental microbiology. 2016 Jan 15;82(2):450-8.
65. Bourke P, Ziuzina D, Han L, Cullen PJ, Gilmore BF. Microbiological interactions with cold plasma. Journal of applied microbiology. 2017 Aug;123(2):308-24.
66. Misra NN, Pankaj SK, Segat A, Ishikawa K. Cold plasma interactions with enzymes in foods and model systems. Trends in Food Science & Technology. 2016 Sep 1;55:39-47.
67. Misra NN, Jo C. Applications of cold plasma technology for microbiological safety in meat industry. Trends in Food Science & Technology. 2017 Jun 1;64:74-86.
68. Han L, Boehm D, Amias E, Milosavljević V, Cullen PJ, Bourke P. Atmospheric cold plasma interactions with modified atmosphere packaging inducer gases for safe food preservation. Innovative Food Science & Emerging Technologies. 2016 Dec 1;38:384-92.
69. Misra NN, Moiseev T, Patil S, Pankaj SK, Bourke P, Mosnier JP, Keener KM, Cullen PJ. Cold plasma in modified atmospheres for post-harvest treatment of strawberries. Food and bioprocess technology. 2014 Oct;7(10):3045-54.
70. Noriega E, Shama G, Laca A, Díaz M, Kong MG. Cold atmospheric gas plasma disinfection of chicken meat and chicken skin contaminated with Listeria innocua. Food microbiology. 2011 Oct 1;28(7):1293-300.
71. Choi S, Puligundla P, Mok C. Effect of corona discharge plasma on microbial decontamination of dried squid shreds including physico-chemical and sensory evaluation. Lwt. 2017 Jan 1;75:323-8.
72. Kim JE, Oh YJ, Won MY, Lee KS, Min SC. Microbial decontamination of onion powder using microwave-powered cold plasma treatments. Food Microbiology. 2017 Apr 1;62:112-23.
73. Wan Z, Chen Y, Pankaj SK, Keener KM. High voltage atmospheric cold plasma treatment of refrigerated chicken eggs for control of Salmonella Enteritidis contamination on egg shell. LWT-Food Science and Technology. 2017 Mar 1;76:124-30.
74. Wang J, Zhuang H, Hinton Jr A, Zhang J. Influence of in-package cold plasma treatment on microbiological shelf life and appearance of fresh chicken breast fillets. Food microbiology. 2016 Dec 1;60:142-6.
75. Oh YJ, Song AY, Min SC. Inhibition of Salmonella typhimurium on radish sprouts using nitrogen-cold plasma. International journal of food microbiology. 2017 May 16;249:66-71.
76. Bourke P, Ziuzina D, Boehm D, Cullen PJ, Keener K. The potential of cold plasma for safe and sustainable food production. Trends in biotechnology. 2018 Jun 1;36(6):615-26.
77. Sarangapani C, Patange A, Bourke P, Keener K, Cullen PJ. Recent advances in the application of cold plasma technology in foods. Annual review of food science and technology. 2018 Mar 25;9:609-29.
78. Desmet T, Morent R, De Geyter N, Leys C, Schacht E, Dubruel P. Nonthermal plasma technology as a versatile strategy for polymeric biomaterials surface modification: a review. Biomacromolecules. 2009 Sep 14;10(9):2351-78.
79. Zhang B, Chen L, Li X, Li L, Zhang H. Understanding the multi-scale structure and functional properties of starch modulated by glow-plasma: A structure-functionality relationship. Food Hydrocolloids. 2015 Aug 1;50:228-36.
80. Misra NN, Keener KM, Bourke P, Cullen PJ. Generation of in-package cold plasma and efficacy assessment using methylene blue. Plasma Chemistry and Plasma Processing. 2015 Nov;35(6):1043-56.
81. Wongsagonsup R, Deeyai P, Chaiwat W, Horrungsiwat S, Leejariensuk K, Suphantharika M, Fuongfuchat A, Dangtip S. Modification of tapioca starch by non-chemical route using jet atmospheric argon plasma. Carbohydrate polymers. 2014 Feb 15;102:790-8.
82. Coutinho NM, Silveira MR, Rocha RS, Moraes J, Ferreira MV, Pimentel TC, Freitas MQ, Silva MC, Raices RS, Ranadheera CS, Borges FO. Cold plasma processing of milk and dairy products. Trends in Food Science & Technology. 2018 Apr 1;74:56-68.
83. Nishime TM, Borges AC, Koga-Ito CY, Machida M, Hein LR, Kostov KG. Non-thermal atmospheric pressure plasma jet applied to inactivation of different microorganisms. Surface and Coatings Technology. 2017 Feb 25;312:19-24.
84. Sarangapani C, Dixit Y, Milosavljevic V, Bourke P, Sullivan C, Cullen PJ. Optimization of atmospheric air plasma for degradation of organic dyes in wastewater. Water Science and Technology. 2017 Jan 9;75(1):207-19.
85. Doubla A, Laminsi S, Nzali S, Njoyim E, Kamsu-Kom J, Brisset JL. Organic pollutants abatement and biodecontamination of brewery effluents by a non-thermal quenched plasma at atmospheric pressure. Chemosphere. 2007 Sep 1;69(2):332-7.
86. Andersen KB, Beukes JA, Feilberg A. Non-thermal plasma for odour reduction from pig houses–A pilot scale investigation. Chemical engineering journal. 2013 May 1;223:638-46.
87. Schultz-Jensen N, Kádár Z, Thomsen AB, Bindslev H, Leipold F. Plasma-assisted pretreatment of wheat straw for ethanol production. Applied biochemistry and biotechnology. 2011 Oct;165(3):1010-23.
88. Moon AY, Noh S, Moon SY, You S. Feasibility study of atmospheric-pressure plasma treated air gas package for grape's shelf-life improvement. Current Applied Physics. 2016 Apr 1;16(4):440-5.
89. Ziuzina D, Patil S, Cullen PJ, Keener KM, Bourke P. Atmospheric cold plasma inactivation of Escherichia coli, Salmonella enterica serovar Typhimurium and Listeria monocytogenes inoculated on fresh produce. Food microbiology. 2014 Sep 1;42:109-16.
90. Bai Y, Chen J, Mu H, Zhang C, Li B. Reduction of dichlorvos and omethoate residues by O2 plasma treatment. Journal of agricultural and food chemistry. 2009 Jul 22;57(14):6238-45.
91. Devi Y, Thirumdas R, Sarangapani C, Deshmukh RR, Annapure US. Influence of cold plasma on fungal growth and aflatoxins production on groundnuts. Food Control. 2017 Jul 1;77:187-91.
92. Keener KM, Jensen JL, Valdramidis VP, Byrne E, Connolly J, Mosnier JP, Cullen PJ. Decontamination of Bacillus subtilis spores in a sealed package using a non-thermal plasma system. InPlasma for bio-decontamination, medicine and food security 2012 (pp. 445-455). Springer, Dordrecht.
93. Cullen PJ, Milosavljević V. Spectroscopic characterization of a radio-frequency argon plasma jet discharge in ambient air. Progress of Theoretical and Experimental Physics. 2015 Jun 1;2015(6).
94. Boehm D, Heslin C, Cullen PJ, Bourke P. Cytotoxic and mutagenic potential of solutions exposed to cold atmospheric plasma. Scientific reports. 2016 Feb 24;6(1):1-4.
95. Sarangapani C, O'Toole G, Cullen PJ, Bourke P. Atmospheric cold plasma dissipation efficiency of agrochemicals on blueberries. Innovative Food Science & Emerging Technologies. 2017 Dec 1;44:235-41.