

RECENT ADVANCEMENTS IN COLD PLASMA TECHNOLOGY

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. The data provided in this book chapter highlights the effects of cold plasma technology on enzyme inactivation, bioactive components, minerals, antioxidants, the mechanism of action of cold plasma on microbial cells inactivation and various application and limitation of cold plasma technology.

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

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I. 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 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 meat 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 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 advancements 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]. Research on new non-conventional processing technologies has significantly increased the processes that ensure the products' safety and quality. All of these prior and ongoing efforts have led to the widespread acceptance and establishment of "processing" operations such as application of cold plasma technology for the production of food products [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.

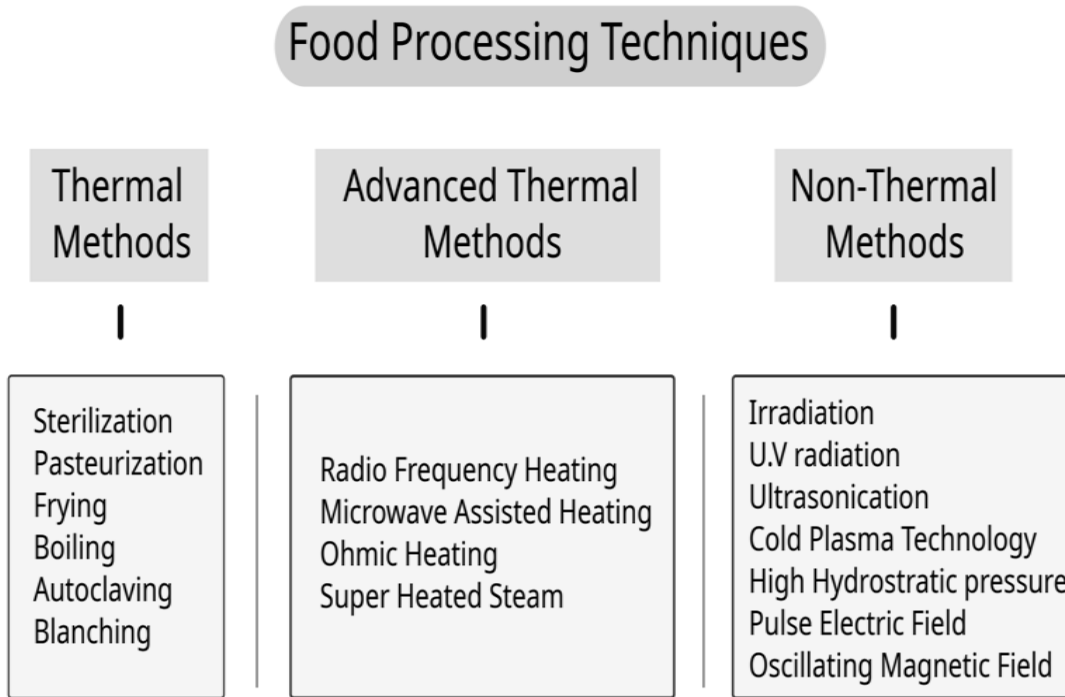


Figure 1: Food processing techniques

II. COLD PLASMA TECHNOLOGY

The fourth state of matter present in the universe is plasma, created by ionized particles 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 like collision, ultraviolet radiation and the chemical reactions among the samples and the energetic species during interaction with the samples distinguish plasma as a special type of matter [9]. Plasma applications are determined by temperature, and it can be classified as thermal or non-thermal, depending on temperature. 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 special benefits in industrial applications of food. On food surfaces, it is used to destroy microbes like *Listeria monocytogenes* [11]. Food containers and surfaces made of stainless steel and polyethene can be sterilized using cold plasma technology in an efficient and safe manner. Antimicrobial compounds like chitosan, triclosan, and silver that were immobilized into active packaging application by cold plasma technology demonstrated strong antibacterial 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, Yopez and Keener (2016) discovered extreme high plasma using hydrogen (H) as the gas source might be a novel

potential method for hydrogenating botanical extracts without causing trans-fatty acids [12]. Also, it was reported that following plasma treatment, the peanut protein isolates and zein powder, cold plasma technology could be used to improve the physical and biological characteristics of proteins, such as their ability to foam and emulsify [13,14]. According to Chaiwat et al., (2016) plasma treatment is a novel method for changing starch that can be scaled up for use in industry [15].

Sterilization of fresh produce without compromising its sensory and nutritional characteristics is valuable. Conventional heat and chemistry-based techniques will lower the sensory and nutritional qualities. Non-conventional 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 and though mild processing technique for perishable food products due to the unique properties of cold plasma and the effective purification at nearly standard temperature and atmospheric pressure [10].

- 1. 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 cold plasma technique is ideal for inhibiting the microbes [18].
- 2. 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. A high bacterial density clusters more cells, which decreases the ability of reactive species to penetrate the bacteria. Also, because of the complex resistance imbued 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].

III. EFFECTS OF COLD PLASMA TECHNOLOGY ON ENZYME INACTIVATION

- 1. 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 adheres to the enzyme and modulates its activity. 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].
- 2. Enzyme Inactivation and Cold Plasma:** 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 catalytic activity of enzymes along with proteins, structural properties, and cofactors 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].

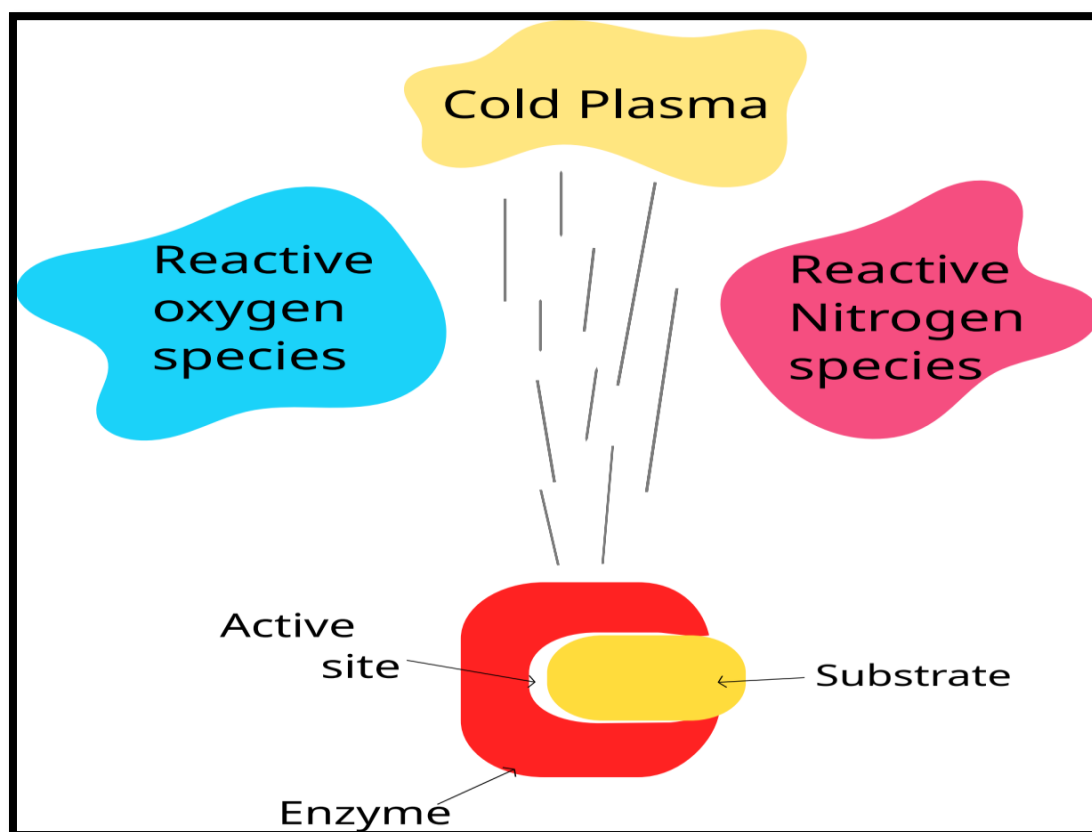


Figure 2: Effect of Cold plasma on Enzyme Inactivation

The function of an enzyme might be changed by even a single oxidised amino acid in a protein [27]. RNase A activity was irreversibly down regulated within 60 seconds of direct treatment with a Dielectric Barrier Discharge (DBD) plasma by oxidising sulfur-containing proteins and rupturing the configuration of peptide bond [28]. And 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 Sadhu et al., (2017), changes in protein structures cause an increase in protease activity [29]. The well-organized secondary structures of lactate dehydrogenase (LDH) were influenced mainly by α -helix structures and twists [27]. And, he also confirmed the connection between α -helical structures and enzyme active sites, stating that certain amino acids found in the catalytic areas were all engaged in the formation of α -helical configurations. Peroxidase (POD) seemed to be more susceptible than polyphenol oxidase (PPO) to plasma treatment at the α -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) [30]. Peptide chains were organised in β -sheet structures in a pleated fashion, which was thought to make them significantly more resistant to heat and chemical agents. Also, secondary structures' random areas and turn modifications were simultaneously experienced. While Zhang et al., (2015) demonstrated the reverse tendency among turns and arbitrary regions such as significant decline in turns and rise in unexpected areas, Surowsky et al., (2013) found that the number of turns in polyphenol oxidase and peroxidase didn't alter following treatment with cold plasma technology (by using pure argon) [30].

The availability of enzyme active sites affects enzymatic activity. Apart from heme destruction, Choi S et al., (2017) reported different variants of catalytic region after 12-mins cold plasma treatment, demonstrating that small changes of 0.22, 0.2, and 0.25 Å occurred to W62, D101, and W108 following and-dielectric barrier discharge plasma treatment, respectively, while significant variations of 0.54, 0.41, and 0.24 Å were noted in the same catalytic region following Atmospheric pressure plasma jet (N₂-APPJ) plasma treatment. Metal ions also procatalyse the active site as a cofactor [31]. Ke Z et al., (2013) found that plasma treatment dramatically reduced horseradish peroxidase (HRP) activity and its iron (Fe) concentration [32]. Lackmann et al., (2013) 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 [33]. When assessing the effects of cold plasma on enzyme inactivation, extrinsic factors such as the treatment environment can be taken into account. Lackmann et al., (2015) said that RNase A actively participated even at extreme heat and acidic pH value of 3, excluding the effects of temperature and acidic values on downregulating the RNase by dielectric barrier discharge treatment [28]. According to Zhang et al., (2015) 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 heat did not contribute to the inactivation of lactate dehydrogenase activity [27]. Following plasma treatment, Bubler et al., (2017) noted that the pH values on the surface of freshly sliced apple and potato tissue decreased to 1.5, while Segat et al., (2016) also noted a significant pH fall after plasma treatment for 15 min [34, 35]. 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 polyphenol oxidase and peroxidase within exposure times of 0–360 s from diverse plasma generations, which was followed by a slight decrease. Furthermore, POD was less impacted by plasma than PPO. Similar findings demonstrating the inactivation of lactate dehydrogenase, horseradish activity and RNase A 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 configuration is enough 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. 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 specified proportion of protein stability among all the intrinsic components. Using average B factor analysis, Choi et al., (2017) showed that the thermal consistency 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 although not in associated concentrations [31]. 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 plasma 2. Dielectric Barrier Discharge	120sec	Low activity confirmed by low helical contents	[35]
2.	Lipase	1. Dielectric Barrier Discharge 2. 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-temperature atmospheric pressure 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	After 10 minutes of treatment, there was low activity, about 62 percent and 77 percent in newly 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 pressure plasma 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]

Sr No	Enzyme	Plasma Sources	Treatment Time	Effect of plasma on enzyme	Reference
12.	LDH	Helium-oxygen non-thermal DBD	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 atmospheric pressure plasma	1 min	After being exposed to the entire effluent for 10 min, only 20%, 40%, and 75% of the enzymatic activity were still present by entire jet, particle-jet, and UV-jet, respectively.	[33]
15.	Protease	RF- Driven Plasma	12 hours, 24 hours	High activity	[29]

IV. 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-HPP, PEF, UV irradiation (UV-C), ozone, plasma-activated water (PAW), and cold atmospheric plasma have been shown to increase the preservation of important nutrients, characteristics, and functional attributes [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^- , H_2O^+ , electrons), excited molecules (excited O_2 , N_2), UV photons, and positive and negative ions. It also contains reactive oxygen species (ROS), reactive nitrogen species (RNS), and reactive oxygen species. Examples of ROS include oxygen radicals, oxidants, atomic oxygen, oxides of nitrogen, hydroperoxides, nitrogen, nuclear ammonia, reactive nitrogen species, UV photons, charged particles, and positively and negatively charged ions [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 Meinlschmidt et al., (2016) utilizing 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% [44]. There has been a fair amount of research done on the use of cold plasma on food and how it affects various food ingredients. There is still some ambiguity about the interaction between plasma reactive species and small substances. 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].

- 1. 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 defense 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]. Almeida et al., (2018) found that functional foods exposed to ambient cold plasma preserved 76% of the original phenolic content following an indirect exposure of plasma for 60 s [48]. In particular, the phenolic compounds were vulnerable to ozone assault, and a noticeable alteration only developed after 60 s of exposure to plasma. Power intensity, time of exposure and fluid velocity 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].
- 2. 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⁺, Grzegorzewski et al., (2011) 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 reactive species 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 biologically synthesized quercetin, catechins, phenylpropanoid, and other metabolic products 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 speed and output volume 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].

- 3. 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 phenolic acids, catechins, and flavones found within food, even though it is not a precise indicator of quality in the food sectors [51]. Illera et al., (2019) 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 [52]. 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 anti-oxidant activity, free radicle scavenging activity and transition metal-chelating activity, are what provide them their potential for antioxidant action. The ferric reducing antioxidant power (FRAP) assay, 1,1-diphenyl-2-picrylhydrazyl (DPPH) scavenging activity, 2,2'-azino-bis-3-ethylbenzothiazoline-6 sulfonic acid (ABTS) radical scavenging activity and oxygen radical absorbance capacity (ORAC) are the main methods used to assess the antioxidant potentials of food products. The results of the testimonies about the effect of cold plasma 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].
- 4. 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 amount [54]. Here, the treatment duration and plasma flow rate both had a beneficial impact on the vitamin A content. Because acerola is a significant source of ascorbic acid, the treatment had the advantage of not significantly changing the vitamin C content, which would have diminished the juice's high nutrient content. The interaction of ascorbic acid during processing with ozone and other oxidising plasma species may be accountable for its deterioration [51]. The key operating variables that can influence the deterioration

of vitamin content are exposure period, power density, process gas, nutritional matrix, and fluid velocity. 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 different cold plasma sources on various bioactive compounds.

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

Sl 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 chlorogenic acid, ferulic acid, punicalagin 1, ellagic acid, catechin, and ferulic acid, have been found. Punicalagin 2 protocatechuic acid, caffeic acid, and protocatechuic acid 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), and anthocyanin	Blue Berry	Atmospheric cold plasma	0, 2, and 5 min 80kv, 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 min 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 -N ₂ plasma	5-15min, 80KHZ	Vitamin C levels drop at greater flow rates Sucrose content rose whereas glucose	[61].

Sl no	Bioactive compounds	Food source	Plasma type	Treatment condition	Observation	Reference
					and fructose levels fell. Increased polyphenol and total flavonoid concentration were facilitated by longer treatment.	

V. 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]. Han et al., (2016) 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 Misra et al., (2016) plasma gas species exhibit secondary enzymatic configuration loss due to the collapse of specific bonds or 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.	<i>Escherichia coli</i> , <i>Listeria monocytogenes</i> and <i>Staphylococcus aureus</i>	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 <i>Listeria</i> populations.	[68].
2	Aerobic Mesophiles Yeast 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 log ₁₀ 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	<i>Listeria innocua</i>	Cold atmospheric plasma pen	10 sec to 8 min	On membrane filters, a 10 s cold plasma treatment resulted in > 3 log reductions of <i>L. innocua</i> , 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, <i>Staphylococcus aureus</i>	Corona discharge plasma jet	0-3 minutes	After being exposed to a corona discharge plasma jet for 0–3 minutes, the contaminants <i>Staphylococcus aureus</i> , marine bacteria, and aerobic bacteria were all inactivated by 2.0, 1.6, and 0.9 log units, respectively.	[71].
6	<i>B. cereus</i> , <i>A. brasiliensis</i> , and <i>E. coli O157:H7</i>	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 <i>B. cereus</i> , <i>A. brasiliensis</i> , and <i>E. coli O157:H7</i> by 2.1, 1.6, and 1.9 log CFU/cm ² , respectively, without influencing the sample's quercetin content, colour, or	[72].

Sr No	Microbial Type/ Species	Plasma Sources	Treatment Time	Mechanism of cold plasma on microbial cells	Reference
				capacity to scavenge free radicals.	
7	<i>Salmonella</i> 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 <i>Salmonella</i> 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 <i>Salmonella</i> levels in packaged chicken eggs.	[73].
8	Mesophiles. Psychrophiles and <i>Pseudomonas</i> 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	<i>E. coli</i>	Microwave-induced cold plasma	–	About 90% of <i>E. coli</i> 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	<i>Salmonella typhimurium</i>	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 <i>S. typhimurium</i> 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].

VI. 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, dielectric barrier discharge (DBD), pulsed power discharge, radio-frequency (RF) discharge, microwave discharge, and atmospheric-pressure plasma jet (APPJ) are a few of the methods used to create plasma discharges which facilitate it to be applied in different areas of food industry [76]. A neutral gas can be made into cold plasma by providing it with enough energy. The gas is ionised during this process, resulting in a variety of chemically active residues, such as energetic particles, free radicals like oxidative stress and superoxide anion, excited or non-excited ions, and UV radiation. Although radio frequency (RF) discharges typically have a frequency of 13.56 MHz and corona discharges typically have an operating voltage of less than 10 mbar, both types of discharges produce plasmas at the tip of the needle electrode. Gas mixtures can include noble gases like helium (He) and argon as well as common atmospheric gases like oxygen (O₂), nitrogen (N₂), and carbon dioxide (CO₂) (Ar). Plasma composition is significantly influenced by the use of equipment, such as the generator, operational conditions, such as the power of plasma excitation, treatment duration, flow rate, gas pressure, and exposure modes, such as direct or distant mode [25].

1. 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. Also, 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 Wongsagonsup et al., (2014) 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 [81].

- 2. Processing of Milk and Dairy Product:** 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]. Atmospheric cold plasma (ACP) was applied to ALP in solution at periods ranging from 15 seconds to five minutes at 3 distinct extreme 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].
- 3. 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]. Sarangapani et al., (2017) 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) [84]. Doubla 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 [85]. The efficacy of the method can be increased by combining plasma with biological therapy [84, 85]. 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 [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].

- 4. 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 [89]. Similar to this, Wang et al., (2016) 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 [74].
- 5. 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 Bai et al found that the effectiveness of the decomposition was influenced by the operating conditions and the chemical makeup of the pesticide [90]. 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].
- 6. 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 polyethylene (LDPE), high-density polyethylene (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].

VII. LIMITATIONS OF COLD PLASMA TECHNOLOGY

Although the impacts of plasma atoms 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 based on the principal mechanism of action, and the research 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. Cold plasma applications are also constrained by the uncertainty of the processing and packaging, the relatively early stage of technological development, and, in the majority of cases, the unknown impacts on the food's composition. Due to the low amount of readily available data on food substances related to other well-established new therapeutic 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 hydroxyl radicals, reactive oxygen species and reactive nitrogen species induced oxidative stress by removing H 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]. Sarangapani et al., (2017) 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 [95]. In light of the extensive work put into developing and exploring the cold plasma treatment method on a laboratory gage, a careful assessment of its industrial use is needed, taking into account the plasma algorithms, operational efficiencies, legal issues, and power prices.

VIII. 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 plasma-induced modifications of food's functional and bioactive components are greatly influenced by the reaction's chemistry. One of the main mechanisms of interaction that was discovered was the rupture of the cell membrane. Other mechanisms included surface etching, subsequent oxidation, cross-linking of starch granules, increased surface unevenness, starch depolymerization hydroxylation of benzene rings and active contact area. It was realized that the induced alterations are mostly linked to the oxidative breakdown that results in the creation of certain chemicals. The food's physical, chemical,

biological, and sensory properties, as well as its proteins, proteases, anticancer activity, irritants, and essential micronutrients, 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. This chapter is majorly focused on the effects of cold plasma on various bioactive components, microbial cells, enzyme inactivation, and its various application in the food industry.

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