
# The Freezing of Some Nutrients Using Plasma

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ABSTRACT

Certain foodborne germs produce resistant spores and poisons, reducing the efficacy of most green technology. Formerly, plasma's primary usage was in surface disinfection; today, it is also widely employed in the food processing business. They are removing hazardous bacteria from food and food items like fruits, vegetables, dairy, and meat using plasma, cutting-edge green technology, or environmentally friendly techniques. Inactivating spores and deactivating enzymes and toxins, respectively, are areas where the modern technology of cold plasma has proven to be vastly superior. Cold plasma consists of free electrons, ions, reactive atomic and molecular species, and ultraviolet (UV) radiation created when a gas is ionized nonthermal. However, the specific mechanism by which cold plasma is effective has remained unknown. Optimal optimization and widespread use of cruel plasma treatment in food processing require understanding these mechanisms and the likely elements that can restrict or boost their effectiveness and effects. For example, there is no thermal damage or limited output variation when using cold plasma to modify the surface of solid or liquid foods (because of the wide variety of gas, power, plasma type, and other input parameters).

**Keywords:** Plasma at room temperature; microbiological spores; food safety; food quality; food quality

## Introduction

The food business is facing difficulties due to consumers' shifting preferences away from chemically and thermally processed food and toward less processed options that they view as healthy. More than ever, reliable methods are available to ensure that processed food has not been tampered with, keeping consumers as safe as possible **[1]**. As a consequence of technological improvements, we can now give nutritious and delicious solutions with minimum loss of quality. Recent findings were presented the most weight when producing this review on plasma therapy. Cereals, dairy, meat, fish, eggs, poultry products, nuts, seeds, fruits, vegetables, spices, and herbs were all discussed about the consequences of cold plasma treatment **[2]**. This study aims to determine how different types of food are affected by complicated plasma interaction and to assess the decontamination effects of this process. Alternative, effective, and safe methods for preserving food products and agricultural produce have arisen because of food science and technology breakthroughs. These advancements were made to accommodate the taste preferences of trending consumers and to ensure the safety of consumers **[3–5]**.

Most emerging environmentally friendly technologies used to preserve food block the growth of microorganisms and, at higher intensities, kill off the organisms themselves. The presence of microbial spores in food constitutes a significant hazard to food safety. However, most food preservation and storage methods, such as pasteurization and freezing, may efficiently suppress or inactivate pathogenic bacteria. Spore-forming microorganisms are notorious for contaminating final products due to their resistance to the treatments employed in food processing and preservation. To begin with, any food preservation technique worth its salt needs to involve some treatment that prevents the reproduction of microbial spores. Although they can successfully deactivate vegetative cells of microbes, several developing technologies still need to be capable of removing spores from the environment. Spores of microorganisms can endure harsh conditions and treatments, including thawing, heating **[6]**, freezing, and UV radiation **[7–9]**. Recently, Laroque et al. **[10]** released another review that goes into great detail about the most promising cold plasma sources and operation modes, the effects of operating circumstances on plasma properties, and the effectiveness of this technology in food processing. In this review, we analyzed the development of cold plasma procedures that kill pathogens without negatively impacting the taste or safety of the food. Previous literature reviews looked at the molecular-level impacts of cold plasma species on food components and developed methods to mitigate these interactions. A study by Saremnezhad et al. **[11]** covers the effects of cold plasma on the chemical structure of several food components and the influence these changes have on the qualities of the meal. In contrast, this article aims to feature the cold plasma approach as a contemporary perfect green preservation method by explaining its merits, superior features at spore inactivation, the process of microbial inactivation, and aspects that should be regarded to guarantee the best outcomes. However, biofilm formation on food contact surfaces is a significant safety problem in the food business due to the possibility of contamination; thus, the most original contribution of this paper is an examination of how biofilm can be disturbed through the use of plasma treatment.

## 2. Plasma Technology

Several environmentally friendly methods have been implemented in the food sector to eliminate harmful bacteria that can cause poor product quality and, in extreme cases, recalls and outbreaks of foodborne illness. As a result, discussions on using environmentally friendly techniques to preserve and store food for longer have heated up. Plasma has emerged as an exciting new nonthermal decontamination solution in the food sector over the past decade. Plasma is a promising new nonthermal decontamination method that has been widely used in the food sector over the past decade. Decontamination of medical equipment and settings **[12-15]**, destruction of heat-sensitive biological besides chemical agents **[16]**, degradation of trash also toxic residues **[17]**, in addition textiles **[18-20]** are just some of the various applications of this technology beyond the food business. Plasma, including ionized or partially ionized gases, is used in this technique to neutralize poisonous microbes, enzymes, and toxins in food. The major plasma components are free radicals, which either heating gas can generate to high enough temperatures in a sealed container with a vacuum strong enough or by zapping the gas molecules with radio or microwave radiation.

According to some definitions, plasma is the fourth state of matter (i.e., solid, liquid, gas, and plasma). Reactive species include both neutral and charged particles. Molecules that lose electrons also gain an advanced energy state as they disintegrate into their atomic elements and enter a plasma state. Ionized or partially ionized gases are used in this method to destroy harmful microorganisms, enzymes, and poisons in food. Free radicals are the primary building blocks of plasma, and either heating gas can produce them in a vacuum or by using radiofrequency or microwave energy to excite the gas molecules. In general, reactive species include both neutral and charged components.

Although in the plasma state, molecules break apart into their constituent atoms, shedding electrons and gaining a more excited energy level **[21]**. Since advances in plasma physics have allowed the generation of "cool plasma" at room temperature and pressure, inducing plasma no longer necessitates highly high energy levels. This produced cold plasma has medical uses for sterilization **[13]**.

However, during sterilisation, cold plasma has revolutionized food preservation by offering a safer solution with minimal effect on the food's

nutritional and organoleptic properties. The partial ionization of gas molecules results in cold plasma **[22]**.
Several cold plasma designs are set up in Fig. 1 to ensure food safety. To generate plasma, a dielectric barrier discharge (DBD) is used to spread current across dielectric materials between electrodes (see Fig. 1a). There are five primary plasma-generating processes employed in the MW food processing sector, including dielectric barrier discharge (DBD), plasma jet (PJ), corona discharge (CD), radio frequency (RF), and microwave **[23, 24]**. Figure 1b depicts the structure of a plasma jet, with an intake gas flowing between electrodes while the outer electrode is grounded. The free electrons produced by the middle electrode, driven by the high-voltage power source, collide with the gas molecules to generate a wide range of reactive species. Current flowing from a high-potential electrode into a room full of air or other gases causes a region of plasma to form around the electrode, as depicted in Fig. 1c. However, as shown in Fig. 1d, RF plasma is generated by directing a gas flow through an RF field. Helicon wave sources, capacitively coupled plasma, and inductively coupled plasma can all be classified as RF plasmas. Two parallel electrodes are placed side by side in a vacuum chamber, with a small gap separating them **[23-24]**. It can be used methodologically between 1 and 100 MHz **[25]**. On the other hand, a magnetron generates a microwave discharge with a frequency of about 2.45 GHz, which is typical of electromagnetic waves (Fig. 1e).

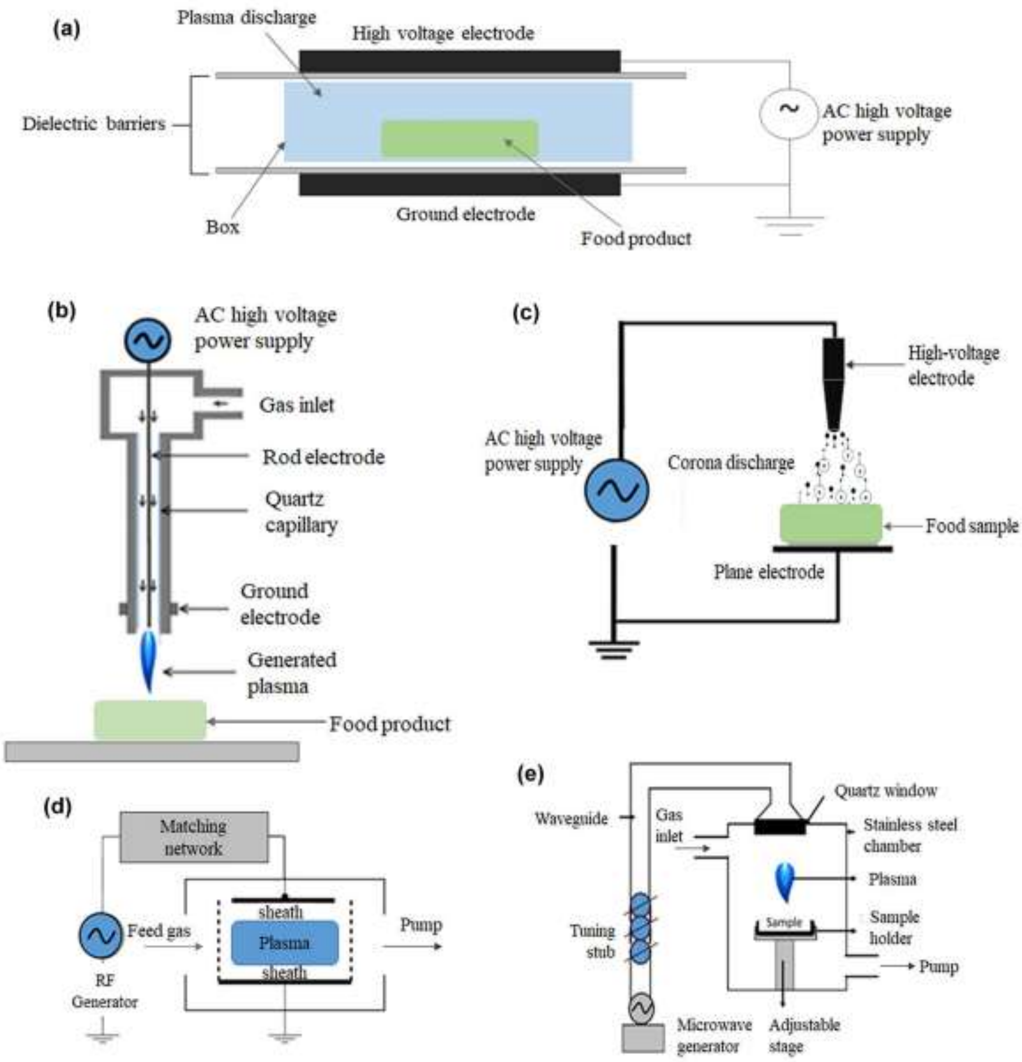


**Fig1.** A dielectric barrier discharge, b plasma jet, c corona discharge, d radiofrequency, and e microwave plasma system is all examples of cold plasma system designs used to guarantee food safety.

## 3. Constraints on Cold Plasma's Efficacy

Several variables affect how well cold plasma treatment works, from the microbes to the foods used and the plasma's operating conditions. Adjusting the instrument's voltage, frequency, treatment time, and working gas composition will affect the outcome of the therapy. The inactivation of Bacillus atrophaeus spores in a hermetically sealed packing **[26]** was shown to be extremely sensitive to the selection of process parameters for dielectric barrier discharge high voltage atmospheric cold plasma. After being exposed to plasma for 60 seconds, spore counts in every gas examined dropped by more than six log10 cycles. However, 60 seconds of indirect exposure reduced spore counts by either 2.1 or 6.3 log10 processes depending on the gas type. Researchers found that relative humidity played a significant role in the effectiveness of high-voltage atmospheric cold plasma in killing bacterial spores. The antibacterial effects of cold plasma therapies are controlled by several parameters **[27, 28]**. Similar results were seen when cold plasma was applied to almonds for 30 seconds at increasing voltages **[29]**. The success of plasma-mediated microbial inactivation depends on the gas utilized to form the plasma; many different gases can be excited to produce plasma. For the killing of Campylobacter jejuni, using nitrogen gas in a dielectric barrier discharge plasma was shown to have no effect ($p > 0.05$) on the number of viable cells compared to air. However, when the air was added to the nitrogen gas at a 2% (vol/vol) concentration, the number of CFUs decreased by 0.8 logs (p 0.05) **[30].** Both internal and external factors significantly impact how long perishable foods last and what kinds of microorganisms they contain. In a similar vein, the success of a food preservation routine depends on some elements **[31]**. Treatment with cold plasma is beneficial against several infectious agents. However, the composition of food components may affect their antibacterial efficiency (e.g., fat, protein, carbohydrate content). Microscopic study has been done to clarify the implications of food composition and nature on the efficacy of cold plasma treatment, even though many reviews have attracted attention to the problem. Growth under osmotic stress or at sub-optimal pH promotes microbial cell adaptability and resistance to cold plasma therapy, as shown in studies of the effects of cold plasma inactivation on Salmonella Typhimurium and Listeria monocytogenes on varied meal structures **[31].** The spore development and treatment-resistance of foodborne bacteria, including Clostridium and Bacillus species, are well-documented. The limited permeability of the inner spore membrane to hydrophilic molecules, the saturation of spore DNA by a group of acid-soluble proteins, the amount of water in the central area (core), and so on all have a role **[32–34]** all contribute to the spore's resistance to preservation treatments. The exosporium coating of a microbe shields its inner workings from the deteriorating effects of preservation. As a result, several investigations have shown that cold plasma and other preservation methods are additionally effective in contradiction to vegetative cells. The inactivation of vegetative cells of G. stearothermophilus treated with indirect and direct cold plasma and vegetative cells and spores of B. cereus exhibited a statistically significant difference. However, there was no significant difference in the inactivation rates of G. stearothermophilus spores exposed either indirectly or directly **[35]**.

## 4. The Cold Plasma Treatment: Pros and Cons Compared to Other Methods

As a consequence of the transition away from processing based on chemical preservatives to processing mediated by green technology, there has been a proliferation of technologies that are looking for use in the food processing industry. This transition has been orchestrated by shifting consumer preferences, safety concerns, and food regulations. Sadly, the majority of the eco-friendly technologies that have been presented cannot be implemented due to prohibitively expensive equipment, negative impacts on product quality, incompatibility with certain food kinds, or an inability to provide adequate protection for food products. The use of cold plasma has demonstrated many benefits and applicability for treating various food types. This is important because some bacteria, like microbial spores, enzymes, and poisons, can survive other types of disinfection and still contaminate food. The cold plasma method has several possible uses in the food business, as shown in Figure 2. Cold atmospheric plasma's versatility makes it useful in many fields, such as medical and food. As a bonus, there is very little danger involved when working with cold atmospheric plasma; however, without uniformity about the treatment parameters and the biological effects of those parameters **[36].** Decontamination of food products with uneven surfaces may increase the risk of internalization of microorganisms, leading to a decline in food quality. Cold plasma therapy is often touted to preserve food quality; however, even little modifications can harm the meal's aesthetics, leading to a drop in sales. Lipid oxidation is one example of a chemical reaction that might cause problems. Cold plasma treatment of mackerel was found to promote lipid oxidation. Mackerel slices had a similar reduction in oleic acid and eicosapentaenoic acid concentration **[37].**

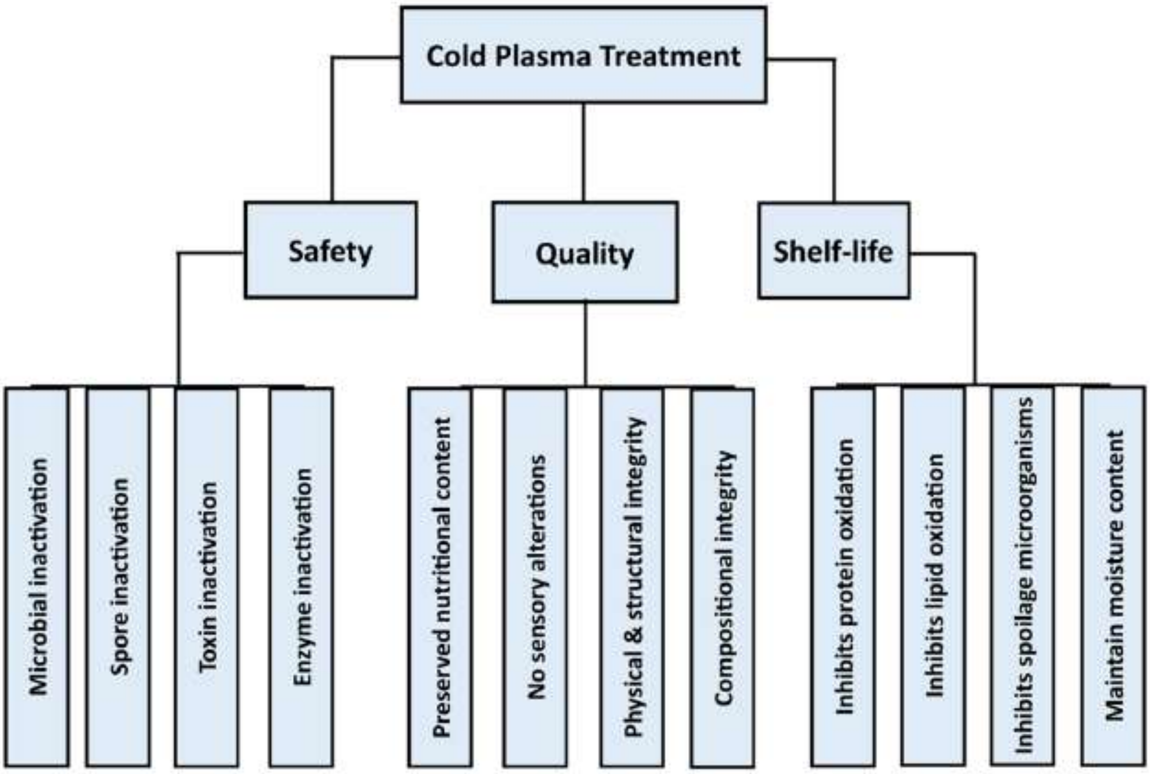


**Fig2.** Cold plasma processing benefits food preservation.

## 5. Industrial Use of Cold Plasma

Reactive oxygen species (ROS) in cold plasma is problematic because it leads to lipid oxidation, particularly in meat tissue and fish, and the creation of fatty acids, aldehydes, hydroxyl acids, and keto acid, all of which contribute to the development of off-flavours and odours during storage **[38]**. The presence of these substances decreases the product's appeal and its longevity on the shelf. Extremely polymerized oligosaccharides (such as those found in juices) are degraded by ozonolysis when applying cold plasma technology **[39]**. Especially in industrial settings, the technology's significant gas consumption is often considered a disadvantage **[40].** Another significant barrier is the hefty initial investment required to implement cold plasma technology. In addition, cold plasma therapy is linked to unfavourable changes in the treated foods' textural qualities, acidity, and decolourization. Cold plasma has a deleterious effect on the sensory attributes of products of animal origin due to the acceleration of lipid oxidation. The application of cold plasma technology presents several promising prospects. The effectiveness and feasibility of cold plasma technology for wastewater purification have been established in some studies. Decontaminating wastewater with cold plasma reduces contaminant levels **[40]**. After treating water with cold plasma, many dangerous bacteria were eliminated, and it was found that the spike protein in the water was significantly inactivated, making it less likely that coronavirus would be transmitted **[41-45]**. Therefore, CP's potential for use in wastewater remediation is enhanced when combined with other acceptable treatments.

## 6. Inhibition of Biofilm Formation by Cold Plasma Technology on Food-Contact Surfaces

Cross-contamination can occur when biofilm forms on food contact surfaces. Biofilms occur on food contact surfaces and allow microorganisms to thrive despite exposure to high temperatures, disinfectants, and other stresses. Biofilms protect cells against food-grade sanitisers, antibacterial compounds, and food treatment processes using their exopolymeric matrix. Quorum sensing helps biofilm cells resist antimicrobial chemicals. Quorum sensing allows cells to respond collectively to environmental changes, producing neutralizing enzymes and poisons. Several chemical and technical ways inactivate planktonic cells but not sessile biofilm cells. The exopolymeric matrix is a barrier to the antibacterial agent, increasing the required dose or concentration to kill the bacterium. Convective transport and diffusion are both hampered by biofilm architecture and water channels.

Biofilms are destroyed by plasma. Staphylococcus aureus and Escherichia coli biofilms were disturbed by 70% and 85%, respectively, after 4 minutes of exposure to an atmospheric-pressure dielectric-barrier discharge plasma **[46]**. Atmospheric air plasma inactivated E. coli also Listeria innocua biofilms. Air plasma destroyed biofilm cells and structure. Scanning electron microscopy showed plasma treatment disrupted biofilms and formed bacterial cell pores **[47]**. Bacterial inactivation was caused by atmospheric air plasma-induced reactive oxygen and nitrogen species. Plasma type, delivery method, and gas mix affect plasma species penetration into samples. Through water routes, active plasma species reach bacterial biofilm cells. Figure 3 shows the destruction of biofilms caused by plasma. After ten days, plasma could break through a 30-layer, 15-micrometre-thick biofilm of Porphyromonas gingivalis and kill all bacteria inside **[48]**. Penetration of cold plasma is affected by the gas used to generate it and the intensity, voltage, and free radical species created in the process. Despite $O_3$'s chemical stability and lower gas phase concentration than $H_2O_2$, Liu et al. **[49]** found that $H_2O_2$ penetrates better than nitrous/nitric acid and $O_3$(aq). Additionally, active plasma species oxidatively destroy the exopolymeric matrix, exposing the cells to plasma directly. The use of atmospheric cold plasma technology has been shown to reduce quorum sensing-regulated components of Pseudomonas aeruginosa **[50]** and has the potential to eliminate biofilms.

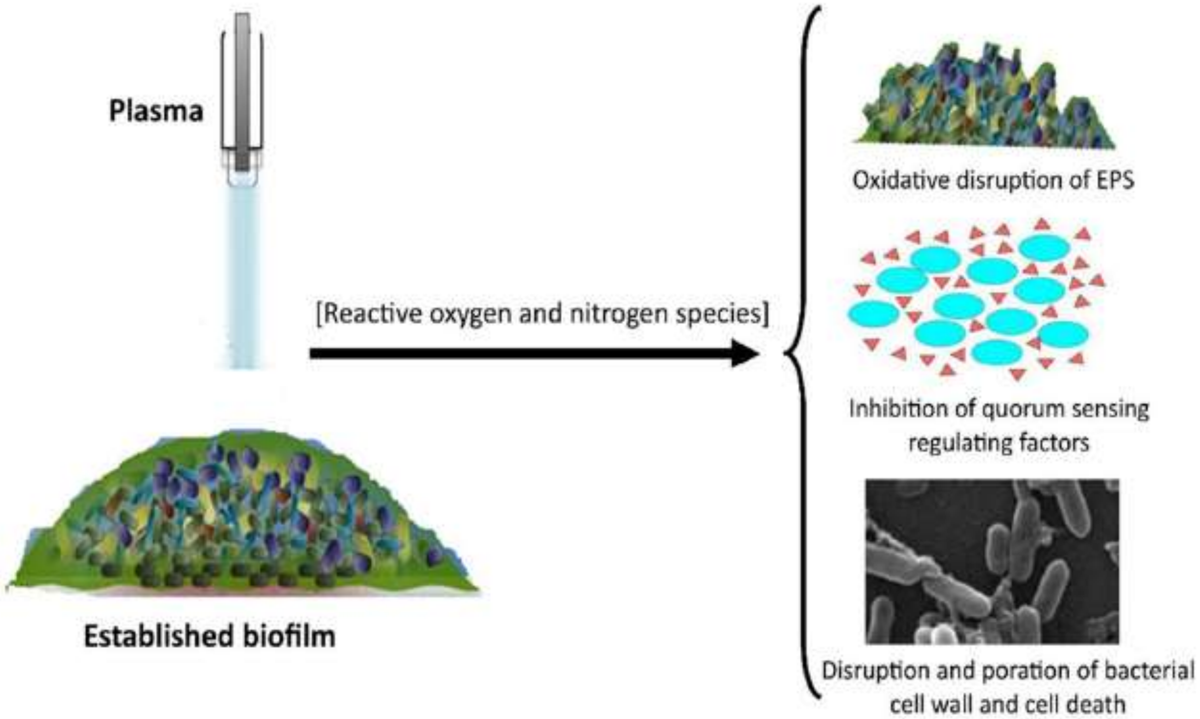


**Fig4.** Plasma-induced biofilm breakdown.

## 7. Conclusion

In recent years, cold plasma treatment has been gaining favour in the food sector. This is owing to the treatment's purported efficacy in the inactivation of microorganisms, the destruction of toxins and enzymes, and a mild to nonexistent impact on the qualities of the food. Many studies and reviews have been conducted to shed light on the mechanism by which the cold plasma approach inactivates microbial spores. Despite this, the specific mechanism of cold plasma has yet to be completely understood. In this article, the many different hypothesized mechanisms described in research articles have been examined, which should serve as a guide for future research. More technical change is expected in the food production and processing industries as they continually adjust to the changing demands and limits imposed by customers. Cold plasma is one of the most recent advancements in nonthermal technologies that have shown to be helpful to the food business. These technologies have a lower impact on food's sensory, flavorful, and textural characteristics. Several types of research on a wide variety of foods have each provided evidence that supports the conclusion that plasma therapy is a beneficial component of the food processing industry. However, it is essential to examine the efficacy of this treatment across a range of physiological and microbiological conditions and to have a thorough understanding of the mechanism of action of the cold plasma treatment and the role of the produced radical species.

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