Universitatea de Științe Agricole și Medicină Veterinară a Banatului "Regele Mihai I al României" din Timișoara
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Food Quality versus Oxidative Stress Effect Related with Food-Borne Pathogens Inactivation by Cold Plasma

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Abstract

Cold plasma (CP) is an emerging non-thermal technology for food applications, which plays an important role in microbial decontamination of food, packaging materials processing, dissipation of agrochemical residues and modification of food materials. Cold plasma is an ideal antimicrobial agent for a wide range of chemically reactive species, which can be obtained through electrical discharges within the atmospheric gases. Reactive species are effective against a wide range of microorganisms, including bacteria, fungi, spores and viruses, as well as pesticides and mycotoxins. Over the past decade, its applications have been extended to the food industry as a powerful tool for non-thermal processing with various forms of use. This review presents an overview of the latest studies on cold plasma applications in the food industry, emerging issues, regulatory context and cold plasma opportunities in the broad stages of primary and secondary food production. In addition, key areas for future research are highlighted and the most important advantages and disadvantages of cold plasma are presented.

Keywords: cold plasma, antimicrobial resistance, oxidative stress, food technology

Introduction

Cold plasma is a relatively new and emerging technology in the agri-food processing sector and attract widespread interest in the food industry due to its economic and environmental characteristics [1].

Plasma is an assembly of several exciting atomic, molecular, ionic and radiant species coexisting with many reactive species including electrons, positive and negative ions, free radicals, gaseous atoms, soil molecules and electromagnetic radiation quantum (UV photons and visible light). These active chemicals in cold plasma are able to quickly and efficiently inactivate microorganisms in their native environments or lead to functional changes in food [2].

Cold plasma can be produced at both low pressure and atmospheric pressure, communicating electromagnetic energy to a volume of gas [3].

Cold plasma can be obtained at atmospheric or sub-atmospheric pressures by means of an electrical discharge or strong ultraviolet radiation in a gas. Plasma sources commonly used in the direct or indirect treatment of foods include: corona discharge, dielectric barrier discharge (DBD), luminescent discharge, sliding electrical discharges (Glidarc or ramping spring) [4], [5].

Cold plasma sterilization is a new technology that has gained interest due to its unique features such as low or ambient temperature treatment for a short period of time, which helps maintain the integrity and quality of food. Cold plasma has been shown to be effective for disinfecting, for inactivating pathogens in fresh food and packaging. It also helps to catalyse certain manufacturing processes, acts as active packaging and delays the oxidation reaction to fruit and vegetables. Being a cold treatment, it is effective in keeping the texture, sensory and functional properties of food. Thus, the use of cold plasma sterilization is a successful technique promoted for food processing in the near future [5].

Cold plasma is an ideal antimicrobial agent with a wide range of reactive chemical species that can be obtained by electrical discharge into atmospheric gases. Reactive species are effective against a wide range of microorganisms, including bacteria, fungi, spores and viruses, as well as pesticides and mycotoxins [6]. The antimicrobial effect of plasma is associated with the synergistic action of electrically charged or neutral particles, photons and discharge-derived radicals. The simultaneous action of these factors induces physical and chemical processes. The latter is predominant in the case of reactive plasma, produced in pure gases or mixtures containing oxygen, nitrogen, hydrogen or another reactive gas. In addition to the ability to destroy microorganisms, cold plasma is of great interest in applications aimed at inactivating and removing contaminants from surfaces at a molecular level, more precisely biomolecules. Such infectious agents are represented by toxic enzymes that can remain on a surface after the destruction of the bacterial charge, prions, responsible for transmissible spongiform encephalopathies or other protein molecules [7].

Cold plasma applications in various fields of science and technology

In addition to the thermal techniques such as cooling, freezing and drying, which are commonly used in food processing, nonthermal processing techniques have gained popularity in recent years [8]. Cold plasma technology (CP) is an emerging technique of non-thermal food processing that has attracted the attention of many researchers across the globe. A new trend in research suggests that CP technology is a powerful and profitable technology for the food industry. Non-thermal technology is extremely advantageous for microbial decontamination of food, including sporadic pathogenic organisms and degradation; due to a large number of reactive oxygen species (ROS) contained in the quasi-neutral plasma gas.

Cold plasma technology has demonstrated potential multi-stage applications in primary and secondary food production, including treating raw materials, intermediate or finished products, and treating equipment, facilities, and processing environment due to their abundance of benefits. These advantages relate to the low-temperature operation, short processing time, energy efficiency and high antimicrobial efficacy with minimal impact on food quality and the environment [9].

It is also noted that cold plasma induces oxidative processes in some food systems, so this problem has to be addressed with great care [10].

It is obvious that the range of cold plasma applications covers many aspects of everyday life and almost all major industries. A critical analysis of plasma science and technology developments over the last decade clearly demonstrates plasma biology applications as one of the most exciting and multidisciplinary fields. This marks the transition from the treatment of inanimate objects to living or cellular objects. Such applications include the treatment of food, plant material and applications in human and animal medicine. With the emergence of plasma in medicine, a great emphasis has been put on research into the use of cold plasma for wound healing, skin treatment, cancer treatment and bone development [11].

Cold plasma in food technology is a novelty. To assess the potential opportunities that cold plasma presents to the food industry, it is possible to compare the limits of non-thermal technologies and the benefits of cold plasma. Based on scientific, non-scientific and patent literature, the described benefits of cold plasma treatment for food preservation can be summarized as follows:

- Cold plasma delivers high microbial inactivation efficiency at low temperatures (generally <50 °C), allowing for extended shelf life, thus improving the efficiency of the supply chain.
- Almost all plasma sources available so far allow in-situ production of agents acting on demand only and in a certain range of gases. Therefore, cold plasma is compatible with most existing and modified atmosphere packaging.
- The active chemical species of the plasma are characterized by high diffusion and therefore act quickly and access the entire surface of the food in most cases.
- Cold plasma is apparently benign to many foods, if not all, and generally has a negligible impact on the product matrix. In addition, it could also reduce the use of preservatives.
- Cold plasma technology does not use water or solvents, so it is considered environmentally friendly.
- Generally, cold plasma does not leave any residue, with enough time for the recombination reactions to continue. However, this cannot be universally true and requires comprehensive validation studies.
- Most cold plasma sources require only low energy input; therefore, cold plasma technology is energy efficient
- Another important aspect to be mentioned here is that the technology is applicable to both solid foods and liquids [5].

Cold plasma as an alternative to commercial antibacterial

Eradication of multi-resistant supermicroorganisms is one of the clinical challenges of the 21st century [12]. In addition, the situation will be exacerbated even more, because the production of new antibiotics is not increasing. The Society of Infectious Diseases in America (IDSA) points out that in recent years, the number of new approved antibacterial drugs continues to decline [13, 14]. Only three of the eight approved antibiotics in the last decade act through a new mechanism of action, while all of them are just modified variants of already known antibiotics.

In parallel, the resistance of *S. aureus* has increased substantially: a total of ~ 21% of *S. aureus* in Germany is resistant to methicillin/oxacillin [15]. The overall increase in methicillin resistance of S. aureus (MRSA) was 3.1% per year in the USA based on a linear model from 1992-2003 [16]. Thus, there is a great interest in new antimicrobial strategies. Recently, research on the use of cold plasma has demonstrated both bactericidal, virucidal and fungicidal properties due to the generation of reactive species and charged particles [17, 18, 19]. Numerous studies have demonstrated the efficacy of cold plasma for eradicating especially planktonic microorganisms [20, 21]. Furthermore, successful disinfection of biofilms generated by *Staphylococcus aureus* and *Staphylococcus epidermidis* resistant to methicillin was demonstrated using a non-thermal gaseous plasma [22].

In the context of the decrease in the number of new antibacterial drugs and the increased resistance of bacteria, effective disinfection of hospital surfaces is of major interest as a factor in the prevention of hospital-acquired infections. The first attempts were demonstrated by Burts *et al.*, [23] that were able to demonstrate that disinfection of hospital pagers experimentally coated with MRSA could be accomplished within 30 seconds of plasma treatment. Generally, cold atmospheric plasma, which means less than 40 °C at the point of application, can be obtained in the air to produce reactive molecules such as atoms, ions and radicals.

This chemical "cocktail" of reactive species can be used for various biomedical applications. Plasma efficacy was in the same range as standard antimicrobial agents up to 3-5 log10. However, to our knowledge, there are only a few reports on the use of cold atmospheric plasma for decolonization of bacteria applied to vital skin surfaces [24, 25].

Lademann *et al*., [25], for example, used a plasma tissue tolerable plasma jet and achieved a 94% reduction in bacterial load using an ex vivo model for skin located on a pig's ear. However, this plasma jet is capable of disinfecting only small areas of skin (2 mm inhibition area) and was moved at an average speed of 10 mm/sec on the skin surface.

Cold plasma is an ideal antimicrobial agent for a wide range of reactive chemical species that could be obtained from electrical discharge into atmospheric gases. Reactive species are effective against a range of microorganisms, including bacteria, fungi, spores and viruses, as well as pesticides and mycotoxins. Cold plasma generation inside sealed packets allows locating and extending reaction time for reactive species over microorganisms while preventing any postprocessing contamination. Here we present an examination of the design aspects of the plasma model used in packaging, packing requirements and discussion of their efficacy with respect to microbiological and chemical safety of food [6].

Cold plasma at atmospheric temperature (ACP) is a promising non-thermal technology effective against a wide range of pathogens. The reactive oxygen species (ROS) play a crucial role in inactivation when using air or other oxygen-containing gases. With strong oxidative stress, cells can be damaged by lipid peroxidation, enzyme inactivation and DNA cleavage.

Identifying ROSs and understanding their role is important for advancing ACP applications for a number of complex microbiological problems.

Food processing technologies have evolved greatly with the evolution of heat application/removal, the use of micro-organisms, natural and chemical preservatives and the application of electromagnetic fields for preservation. A notable recent development in the evolution of non-thermal food processing technologies is the application of cold plasma for agro-food decontamination and the improvement of food properties [5]. Cold plasma processing is not limited to food applications but also to agriculture [26] and the medical environment [27]. Numerous studies have been conducted to decontaminate bacteria, yeasts, fungi, spores and toxins grown on microbiological media using “in-package” plasma sources.

In many cases, these systems allowed a reduction in the number of *E. coli, L. monocytogenes* and *Staphylococcus aureus* to an undetectable number, starting from an initial bacterial burden of 6=8 $\log_{10}$ [28].

Fresh products remain the main cause of outbreaks of food-borne diseases involving pathogens such as *E. coli* producing Shiga, *Salmonella and L. monocytogenes*, among others.

Olaimat and Holley, 2012 [29] claim that fresh products contaminated with *E. coli, Salmonella* and *L. monocytogenes* such as spinach, lettuce, radishes, lucerne, red peppers, peppers, melons, strawberries and fruit and vegetable salad caused outbreaks of food-borne diseases in the first decades of the 21st century.

Between 1982 and 2002, food outbreaks of *E. coli* have become more common, with more than 350 such outbreaks being reported in the United States [30]. In 2006, a multi-state outbreak of *E. coli* 0157: H7 transmitted through spinach with 276 cases of illness and three deaths [31] occurred in the United States. In 2011, a major outbreak associated with *E. coli* occurred in Germany, involving 3911 cases of illness followed by 47 deaths [29].

As with fresh herbal products, the richness of nutrients in the meat makes it an ideal environment for the growth and proliferation of microorganisms of degradation, especially human food-borne pathogens. Of particular importance in this context are *E. coli* and *L. monocytogenes*, which, although inactivated by traditional processing, are often transmitted through ready-to-eat foods such as soft cheese, poultry meat products, RTE meat products, smoked fish and seafood [32].

Decontamination of fruits and vegetables with cold plasma

In recent years, cold plasma treatment applied to fresh fruit and vegetables has been the subject of many researches, which has shown that this technology offers a good alternative to conventional methods used in food production [5].

In one of his works, Critzer *et al*., [33] investigated the efficacy of plasma discharge in a uniform atmosphere at 9 kV resulting in a reduction in *E. coli* O157: H7 inoculated on apples after 2 minutes of treatment. Similar results were recorded by Niemira and Sites (2008) [34] after 3 minutes of treatment on apples with cold atmospheric plasma generated in gliding arc discharge. Also, Klockow and Keener [35] applied cold plasma treatment on spinach leaves pre-packaged and inoculated with *E. coli* O157:H7, which were exposed for 5 minutes to cold plasma subsequently stored for 0.5-24 hours. In this case, the largest CFU/leaf (3-5 log) reductions were obtained after 24 hours of storage. In this study, prolongation of the post-treatment storage maturity appeared as a critical treatment parameter for the maximum efficiency of bacterial inactivation with this type of system.

Generally, researchers studying the effects of cold plasma decontamination have demonstrated that plasma antimicrobial efficacy is influenced by the amount and type of reactive species generated during plasma processing that are influenced by experimental parameters such as working gas, frequency, plasma emitter, electrodes geometry and treatment time [5].

Fernandez *et al*., [36] reported that intrinsic parameters, such as the bacterial growth phase and growth temperature, play a minor role in the antimicrobial efficacy of cold plasma.

In contrast, Yu *et al*., [37] found that the inactivation efficiency of plasma treatment was a function of the concentration of E. coli cells and the cell growth phase. After 2.5 minutes of treatment, there was a 7-log decrease in the number of cells deposited on the surface of the membrane filters at a cell concentration lower than 107 CFU/cm^2 compared to a 1 log reduction value obtained at cell density greater than 1011 CFU/cm^2.

However, unexpected results have been reported regarding the effect of the bacterial growth phase on the antimicrobial efficacy of the treatment; the exponential phase cells are not more sensitive than those in the stationary phase. Similarly, trends in decreasing plasma inactivation efficiency at higher baseline cell concentrations were reported by Baier *et al*., [38]. Direct treatment for 2 minutes and at a shorter distance (5 mm) between plasma discharging discs and *E. coli* inoculated gel disks resulted in the most effective and complete inactivation at initial concentrations less than 105 CFU/cm^2 compared to concentrations of 107 CFU/cm^2 in combination with a 4-minute treatment.

An improved plasma processing time was recorded by Wang *et al*., [39] with 90-100% inactivation of *Salmonella* inoculated on carrots, cucumbers and sliced pears obtained after application of 4 s of treatment. The population of *E. coli* O157:H7 inoculated on radicchio leaves was significantly reduced after 15 minutes of 15 kV (1.35 log MPN/cm^2) aerogenic plasma treatment [40]. However, despite the range of intrinsic target parameters, high-dielectric high-voltage plasma treatment (DBD) used for the decontamination of cherry tomatoes and strawberries demonstrates high efficiency against key pathogens. In this study, short treatments for 10, 60 and 120 s together with 24 hours of posttreatment storage reduced the population of *Salmonella, E. coli* and *L. monocytogenes* on cherry tomatoes to undetectable levels (6.3, 6.7 and 3.1 $\log_{10}$ CFU/sample), while the background microflora of cherry tomatoes was not detected after 120-300 s. The effect of the complexity of the substrate on cold plasma treatment was evident in the treatment of inoculated strawberries where extended treatment was required for 5 minutes to obtain considerable reductions in *E. coli, Salmonella* and *L. monocytogenes* by 3.5, 3, 8 and 4.2 $\log_{10}$ CFU/sample [41]. The study by Misra *et al*., [42] showed that the 5-minute high-dose plasma DBD treatment in a pack was highly effective against natural microflora of strawberries, resulting in a 2-log reduction in 24 hours after post-plasma treatment without affecting the quality of the product.

Bacterial pathogens can develop rapidly and irreversibly in fruits and vegetables and persist for long periods of time. For example, in 30 seconds of exposure, 30% of the *Salmonella inoculum* can attach firmly to the slices of fat peppers, and cannot be easily removed by washing [43]. Warning and Datta [44] have described that cuts in plants, lenticules, trichomes, venous stomata and stomata are preferential places for bacterial cell development. In one of the first studies, Perni *et al.*, [45] evaluated the efficacy of treatments using a cold plasma pen for decontamination of various microorganisms on the melon peel and mango skin.

They reported a 3-log reduction of *E. coli*. at the exposure of mangled and melon cuts to the same plasma source, also observing a 2.5 log reduction in the population of *E. coli* and *L. monocytogenes* on mango and 1.5 and 2 logs on the melon [46]. Reduced inactivation of cut fruits as compared to whole fruit was attributed to the migration of bacteria inside the tissues (ie internalization). Jahid *et al.*, [47] reported increased resistance to plasma treatment of *Salmonella typhimurium* biofilms on salad leaves due to internalization and extensive colonization in stomal wells production.

In a recent study, Cui, H. *et al.*, [48] studied the sequential treatment of CNP and phage techniques on the biofilm in vegetables. Compared to the treatment performed separately, sequential treatment was not only performed under lighter conditions compared to treatment with 400 W CNP for 2 minutes and 5% treatment for 30 minutes but showed a marked effect on biofilm eradication *E. coli* O157: H7 in vitro and on vegetables. The population of *E. coli* O157: H7 was reduced by approximately 2 log CFU/cm^2 after individual treatment of 5% phages for 30 minutes or 500W CNP for 3 minutes. Following the sequential treatment of CNP (400 W, 2 min) and phage (5%, 30 min) (sequential treatment of CNP (400 W, 2 min) and phages (5%, 30 min) *E. coli* O157: H7 biofilm via 5.71 log CFU/cm^2. Therefore, the sequencing treatment has a great promise to improve the current steams for treating bacterial contamination on different vegetable surfaces.

Kim, *et al.*, [49] investigated the effects of high microwave (CP) (CP) treatment (HMCPT) on *E. coli* O157: H7 onion powder. An increase in the number of *E. coli* O157: H7 in the onion powder treated during storage at 4 and 25 °C, as well as the physicochemical and sensory properties of the powder, was evaluated. HMCPT at 400 W for 40 minutes, representing optimal conditions for inhibition of *B. cereus* spore, initially reduced the number of *E. coli* O157: H7 by 1.9 CFU/cm^2. The number of *E. coli* in the treated powder fell below the detection level after day 21 at both temperatures.

HMCPT did not affect the colour, antioxidant activity or quercetin concentration of the powder during storage at both temperatures.

Bacterial inactivation was evaluated on cherry tomatoes that were inoculated with multiple strains of *Salmonella enterica* (Se), *E. coli* producing *Shiga toxins* (STEC) or *Listeria monocytogenes* (Lm). Inactivation of the pathogen has been observed at all distances and at all treatment times but with decreased efficiency at increased distances and shorter treatment times. A reduction of approximately 1 and 2 log CFU/ml in cherry tomatoes after 4 and 10 min respectively was observed [50].

While regular consumption of fresh fruits and fruit juices helps prevent many degenerative diseases such as cardiovascular problems, diabetes and cancer, they could pose a danger to consumers by the possible presence of pathogenic microorganisms or microbial toxins [51].

The presence of *E. coli, Salmonella* and *S. aureus* in fruit juices is of prime interest because these pathogens have been associated with a series of outbreaks associated with fruit juices [52]. In a recent study, 25 microbial species, including 9 bacteria, were detected including *E. coli*, 5 yeasts and 11 moulds from a total of 30 juice samples [52]. Montenegro *et* *al*., [53] in its study, used steady-state corona discharge obtained from a 0-15 kV DC pulsed power supply which allowed the reduction of the number of *E. coli* O157: H7 in apple juice with more of 5 log CFU/ml in 40 seconds.

An effective means to control the contamination of fresh products is post-harvest decontamination interventions that can replace or complement the washing operation. The package decontamination process is particularly important for ready-to-eat fruits, vegetables and foods [54, 55]; these categories of food require the use of ambient temperature processes to ensure safety with minimal impact on product quality. The latest study of plasma-based treatment in DBD-based packaging involved the treatment of inoculated spinach with *E. coli* O157: H7 in a flexible package [35].

The results were quite promising with a reduction of 1.5 log10-2 $\log_{10}$ after 0.5 hours of treatment and down to 3-5 $\log_{10}$ reduction over 24 hours of treatment at 12 kV for 5 minutes.

Later, it has been demonstrated that cold plasma treatment of fresh products reduces the total number of aerobic microbes by up to 5 $\log_{10}$ in products such as tomatoes and strawberries when using a DBD volumetric feed at the frequency of 60 kV and 50 Hz [42].

The microbial inoculated *Salmonella* and native load in cherry or salad tomatoes were reduced by 0.75 $\log_{10}$ and 0.34 $\log_{10}$ CFU/g, indicating the poor effects of this configuration in Salmonella decontamination in mixed lettuce. In another study by Min *et al*., [56] salad treatment with a 42.6 kV volumetric DBD system for 10 minutes resulted in a reduction of 1.1 log10 CFU/g of *E. coli* O157: H7 [56]. Overall, the increase in treatment time (plasma generation time) and tension have been reported to have a positive impact on the reduction of the bacterial population.

Two inactivation mechanisms have been observed in which it was found that the reactive species either react first with the cell membrane or destroy the intracellular components. *E. coli* was primarily inactivated by cell damage and DNA damage. On the contrary, *Staphylococcus aureus* was mainly inactivated by intracellular lesions, with significantly higher intracellular levels of ROS observed and small membrane damage. However, for both bacteria studied, the increase in treatment time had a positive effect on the levels of generated intracellular ROS [5].

Decontamination of animal products with cold plasma

A particularly important problem is that in frozen meat products, pathogens such as enterohemorrhagic *E. coli* (EHEC) can survive even 180 days [57]. In order to meet consumer requirements for high-quality meat products without the use of artificial preservatives and without compromising safety, it is necessary to develop and apply new intervention technologies in the meat industry. In expanding the success of cold plasma treatment to decontaminate fresh products, researchers have found good results for meat products as well.

The potential of cold plasma as a new decontamination technology in the meat processing sector can be justified by the wide range of microorganisms that it can inactivate [5].

Indirect use of plasma in combination with the use of closed rooms for the decontamination of meat products has been highlighted in recent studies by [58].

Jayasena *et al*., [59] treated fresh pork and beef packaged in plastic and sealed plastic using flexible thin film DBD at an average power of 2 W of a bipolar square-square voltage at 15 kHz. They noted that a 10-minute treatment led to the reduction of *L. monocytogenes, E. coli* O157: H7 and *Salmonella typhimurium* by 2.04, 2.54 and 2.68 log CFU/g in pig samples and 1, 90, 2.57 and 2.58 log CFU/g in beef. The key advantage of cold plasma treatment is that bactericidal molecules are generated and contained in the package, allowing for prolonged exposure to pathogenic microbes, returning to initial gas within a few hours of storage [54].

Unlike most conventional food technologies, antimicrobial treatment inside a sealed packaging ensures the prevention of further contamination.

It is known that N_2 inactivation efficacy is greater than He because He compared with N_2 produces more active species, especially N_2 + and N + groups [60]. Moreover, when He or N_2 is mixed with O_2, the total bacterial inactivation of plasma increases. Thus a 2-3-fold decrease in the population of L. monocytogenes (KCTC 3596), *E. coli* (KCTC 1682) and *Salmonella typhimurium* (KCTC 1925) using the He + O_2 mixture, compared to a reduction of the 1-2 log when only He [24] was used. Increasing antimicrobial action in the presence of oxygen results from the action of singlet oxygen and ozone. Using a corona discharge plasma stream (20 kV, 58 kHz) for 2 min. reductions of 1.5 log and >1.0 logarithmic units were observed in *E. coli* O157:H7 and *L. monocytogenes*, respectively, in pork loins [61]. When investigating plasma-based inactivation of inoculated microorganisms on pork, Kim *et al*., [62] found that the populations of *E. coli* and *L. monocytogenes* decreased significantly in the pig loins from 0.5 to 0.55 log CFU/g and from 0.43 to 0.59 log CFU/g, to prolong the treatment time from 5 to 10 minutes. Kim *et al*., [63] used an array of atmospheric RF pressure systems operating in Ar (flow: 20,000 sc cm, 200 watts power) to treat the beef carcase contaminated with *S. aureus* ATCC 12600. They noticed a reduction by approx. 3-4 log in beef after 10 minutes of treatment. However, the rate of inactivation was much faster when the cells were inoculated onto a polystyrene surface, indicating that the surface morphology of the product was a decisive factor for the effectiveness of the process.

Dirks *et al*., [64] reported that airborne DBD plasma resulted in 0.84 and 0.85 log reduction of microflora in a chicken breast sample after exposure for 15 and 30 seconds, respectively.

Salmonella spp. poses a major risk to food safety, proving to be present in eggs and foods containing eggs [64]. To address the safety problems of *Salmonella*-related outbreaks, several non-thermal approaches to egg surface decontamination have been developed since the 1990s, including pulsed light, ozone, ultraviolet and electrolysis [66] but they have shown only a limited degree of success.

Cold plasma technologies have also been explored for decontamination of eggs. Davies and Breslin [67] in their study reported that gaseous air plasma is ineffective against *Salmonella enteritidis* PT4 inoculated on eggs, not showing sufficient details of the plasma source and process parameters. Donner and Keener [67] in their study treated the eggs in closed plastic bags, followed by 24-hour storage. They observed a reduction of *S. enteritidis* by 3 $\log_{10}$.

The use of a radiofrequency (RF) plasma jet has synergistically influenced the inhibitory effects of plant essential oils (garlic, sweet basil oil and lime oil) against *E. coli, S. typhimurium*, and *S. aureus* found on chicken eggs [69]. The study found that after exposure to plasma at 40 W, total inhibition of the three bacteria on the eggshell mixed with strawberry oil (10 µL/ml) or the key chemical constituent of the oil (eugenol at 5 µL/ml) is completely inhibited. Overall, cold plasma treatments have been shown to be effective in reducing pathogenic microorganisms in eggs and egg-containing products, as shown by recent literature. The efficacy of a corona discharge generated using a 9 kV AC (AC) in decontaminant milk inoculated with E. coli was evaluated by Gurol *et al*., [70].

In another study, the milk was subjected to plasma treatment at 0, 3, 6, 9, 12, 15 and 20 min. A significant reduction in the *E. coli* cell population was observed by up to 54% after only 3 minutes regardless of the milk fat content. The baseline count of whole milk bacteria at 7.78 log CFU/ml decreased to 3.63 log CFU/ml after 20 minutes of plasma treatment. In this case, LTP did not cause any significant change in the pH and colour values of the raw milk samples. Also, viable cells were not detected after one week of treatment in whole milk samples, thus remaining for the entire 6-week storage period [70].

Significant reductions in the *E. coli* and *S. aureus* population inoculated on cheese were recorded after treatment with a DBD plasma that functions in a mixture of He and O_2 [71].

However, after 10 minutes of plasma treatment, deterioration of cheese slices was observed.

Following the air-encoded DBD plasma treatment, decimate reductions of 2.67, 3.10 and 1.65 were observed in the *E. coli, S. typhimurium* and *L. monocytogenes* populations at 60 s, 45 s and 7 min treatment [72]. This study also revealed further decreases in bacterial populations during post-treatment storage. After posttreatment storage for 5 minutes, the populations of *E. coli, S. typhimurium* and *L. monocytogenes* on cheese (5 log CFU/g) decreased by 1.75, 1.97 and 1.65 log CFU/g. In a similar work, Yong *et al*., [73] developed a flexible barrier discharge in the form of a thin barrier, with the idea that it could be part of the food package itself. Using this configuration, the researchers observed that the population of *E. coli* O157: H7, *L. monocytogenes* and *S. typhimurium* in sliced cheddar cheese decreased by 3.2, 2.1 and 5.8 log CFU/g respectively after 10 minutes of plasma treatment.

Conclusions

In conclusion, the literature has shown successful inactivation of bacteria, bacterial spores and biofilms using volumetric and surface DBD plasma in packs. For DBD plasma at atmospheric pressure, the ability to inactivate microorganisms for bacteria (gram positive and gram negative), as well as fungi, was demonstrated. Low-pressure cold plasma was found to be even more effective in obtaining sterilization of microorganisms but also in modifying biomolecules. Research on cold plasma applications in the food industry is far from complete, and the interest in this method is increasing every day. Although cold plasma techniques are still in the early stages of the marketing process, the literature shows the effectiveness of this process, and the variety of systems using cold plasma is considered for further research and eventual global developments.

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REFERENCES

1. Pankaj S.K., Wan Z., Keener K. M. (2018). Effects of cold plasma on food quality: A review, Foods, 7(1).
2. Misra N.N., Koubaa M., Roohinejad S., Juliano P., Alpas H., Inacio R.S. (2017). Landmarks in the historical development of twenty first century food processing technologies, Food Research International, 97, pp. 318-339.
3. Motrescu I. (2011). Studiul oxigenului atomic din plasma de temperature joasă produsă în amestecuri de gaze. Aplicaţii. PhD thesis. Universitatea "Alexandru Ioan Cuza", Iaşi, România.
4. Fridman A. (2008). Plasma chemistry, New York: Cambridge University Press.
5. Misra N.N., Schlüter O., Cullen P.J., (2016). Cold Plasma in Food and Agriculture, Fundamentals and Applications, Ed AP Elsevier.
6. Misra N.N., Yepez X., Xu Lei, Keener K. (2018). In-package cold plasma technologies, Journal of Food Engineering, 244, pp. 21-31.
7. Motrescu I., Poiata A., Nastuta A.V., Creanga D., Popa G. (2015). Bioeffects of atmospheric plasma discharge on gram-positive and gram-negative bacteria, Journal of Science and Arts, 3(32), pp. 249-256.
8. Mandal R., Singh A., Singh A.P., (2018). Recent developments in cold plasma decontamination technology in the food industry, Trends in Food Science & Technology, 80, pp. 93-103.
9. Bourke P., Ziuzina D., Boehm D., Cullen P.J., Keener K. (2018). The Potential of Cold Plasma for Safe and Sustainable Food Production, Trends in Biotechnology, 36(6), 615-626.
10. Gavahian M., Chu Y.-H. Khaneghah A.M., Barba F.J., Misra N.N. (2018). A critical analysis of the cold plasma induced lipid oxidation in foods, Trends in Food Science Technology, 77, pp. 32-41.

11. Woedtke von T., Reuter S., Masur K., Weltmann K.D., (2013). Plasmas for medicine, Physics Reports, 530(4), pp. 291-320.
12. Arias C.A., Murray B.E. (2009). Antibiotic-resistant bugs in the 21st century a clinical super-challenge, N Engl J Med, 360(5), pp. 439-443.
13. Rice L.B. (2008). Federal funding for the study of antimicrobial resistance in nosocomial pathogens: no ESKAPE. J Infect Dis., 197(8), pp. 1079-81.
14. Spellberg B., Guidos R., Gilbert D., Bradley J., Boucher H.W. (2008). The epidemic of antibiotic-resistant infections: a call to action for the medical community from the Infectious Diseases Society of America, Clin Infect Dis., 46(2), pp. 155-64.
15. EARSS MT (2009). EARSS Annual Report 2008, pp. 55-57, http://wwwrivmnl/en/.
16. Klevens R.M., Edwards J.R., Tenover F.C., McDonald L.C., Horan T. (2006). Changes in the epidemiology of methicillin-resistant Staphylococcus aureus in intensive care units in US hospitals, 1992–2003, Clin Infect Dis., 42(3), pp. 389-91.
17. Zimmermann J., Dumler K., Shimizu T., Morfill G., Wolf A. (2011). Effects of cold atmospheric plasmas on adenoviruses in solution, J Phys D: Appl Phys., 44(50), pp. 1-9.
18. Shimizu T., Zimmermann J.L., Morfill G.E. (2011). The bactericidal effect of surface micro-discharge plasma under different ambient conditions, New Journal of Physics, 13, pp. 1-7.
19. Kong M.G., Kroesen G., Morfill G.E., Nosenko T., Shimizu T. (2009). Plasma medicine: an introductory review, New Journal of Physics, 11, pp. 1-35.
20. Ermolaeva S.A., Varfolomeev A.F., Chernukha M.Y., Yurov D.S., Vasiliev M.M. (2011). Bactericidal effects of non-thermal argon plasma in vitro, in biofilms and in the animal model of infected wounds, J Med Microbiol., 60(Pt 1), pp. 75-83.
21. Joshi S.G., Paff M., Friedman G., Fridman G., Fridman A. (2010). Control of methicillin-resistant Staphylococcus aureus in planktonic form and biofilms: a biocidal efficacy study of nonthermal dielectric-barrier discharge plasma, Am J Infect Control., 38(4), pp. 293-301.
22. Cotter J.J., Maguire P., Soberon F., Daniels S., O'Gara J.P. (2011). Disinfection of meticillin-resistant Staphylococcus aureus and Staphylococcus epidermidis biofilms using a remote non-thermal gas plasma, J Hosp Infect, 78(3), pp. 204-207.
23. Burts M.L., Alexeff I., Meek E.T., McCullers J.A. (2009). Use of atmospheric non-thermal plasma as a disinfectant for objects contaminated with methicillin-resistant Staphylococcus aureus, Am J Infect Control, 37(9), pp. 729-33.
24. Kim P.Y., Kim Y.S., Koo I.G., Jung J.C., Kim G.J. (2011). Bacterial inactivation of wound infection in a human skin model by liquid-phase discharge plasma, PLoS One, 6(8).
25. Lademann O., Kramer A., Richter H., Patzelt A., Meinke M.C. (2011). Skin disinfection by plasma-tissue interaction: comparison of the effectivity of tissue-tolerable plasma and a standard antiseptic, Skin Pharmacol Physiol, 24(5), pp. 284-288.
26. Puač N., Gherardi M., Shiratani M. (2018). Plasma agriculture: A rapidly emerging field, Plasma Processes and Polymers, 15(2), pp. 170-174.
27. Bobkova E., Rybkin V. (2015). Peculiarities of Energy Efficiency Comparison of Plasma Chemical Reactors for Water Purification from Organic Substances, Plasma Chemistry and Plasma Processing, 35(1), pp. 133-142.
28. Han L., Patil S., Boehm D., Milosavljevic V., Cullen P.J., Bourke P. (2016). Mechanisms of Inactivation by High-Voltage Atmospheric Cold Plasma Differ for Escherichia coli and Staphylococcus aureus, Applied and Environment Microbiology, 82(2), pp. 450-458.
29. Olaimat A.N., Holley R.A. (2012). Factors influencing the microbial safety of fresh produce: a review, Food Microbiol., 32(1), pp. 1-19.
30. Rangel J.M., Sparling P.H., Crowe C., Griffin P.M., Swerdlow D.L. (2005). Epidemiology of Escherichia coli O157:H7 outbreaks, United States, 1982-2002, Emerg. Infect. Dis., 11(4), pp. 603-609.
31. Goodburn C., Wallace C.A. (2013). The microbiological efficacy of decontamination methodologies for fresh produce: a review, Food Control, 32(2), pp. 418-427.
32. Martin L., Ognier S., Gasthauer E., Cavadias S., Dresvin S., Amouroux J. (2007). Destruction of highly diluted volatile organic components (VOCs) in air by dielectric barrier discharge and mineral bed adsorption, Energy Fuel, 22(1), pp. 576-582.
33. Critzer F., Kelly-Wintenberg K., South S., Golden D. (2007). Atmospheric plasma inactivation of foodborne pathogens on fresh produce surfaces, J. Food Prot., 70(10), pp. 2290-2296.
34. Niemira B.A., Sites J. (2008). Cold plasma inactivates Salmonella Stanley and Escherichia coli O157: H7 inoculated on golden delicious apples., J. Food Prot., 71(7), pp. 1357-1365.
35. Klockow P.A., Keener K.M. (2009). Safety and quality assessment of packaged spinach treated with a novel ozone-generation system, LWT – Food Sci. Technol., 42(6), pp. 1047-1053.

36. Fernandez A., Noriega E., Thompson A. (2013). Inactivation of Salmonella enterica serovar Typhimurium on fresh produce by cold atmospheric gas plasma technology, Food Microbiol., 33(1), pp. 24-9.
37. Yu H., Perni S., Shi J.J., Wang D.Z., Kong M.G., Shama G. (2006). Effects of cell surface loading and phase of growth in cold atmospheric gas plasma inactivation of Escherichia coli K12, J. Appl. Microbiol., 101(6), pp. 1323-1330.
38. Baier M., Ehlbeck J., Knorr D., Herppich W.B., Schleuter O. (2015). Impact of plasma processed air (PPA) on quality parameters of fresh produce, Postharvest Biol. Technol., 100, pp. 120-126.
39. Wang R.X., Nian W.F., Wu H.Y., Feng H.Q., Zhang K., Zhang J., Zhu W.D., Becker K.H., Fang J. (2012). Atmospheric-pressure cold plasma treatment of contaminated fresh fruit and vegetable slices: inactivation and physiochemical properties evaluation, Eur. Phys. J. D., 66, pp. 1-7.
40. Pasquali F., Stratakos A.C., Koidis A., Berardinelli A., Cevoli C., Ragni L., Mancusi R., Manfreda G., Trevisani M. (2016). Atmospheric cold plasma process for vegetable leaf decontamination: a feasibility study on radicchio (red chicory, Cichorium intybus L.), Food Control, 60, pp. 552-559.
41. Ziuzina D., Patil S., Cullen P.J., Keener K.M., Bourke P. (2014). Atmospheric cold plasma inactivation of Escherichia coli, Salmonella enterica serovar Typhimurium and Listeria monocytogenes inoculated on fresh produce, Food Microbiol., 42, pp. 109-116.
42. Misra N.N., Keener K.M., Bourke P., Mosnier J.P., Cullen P.J. (2014). In-package atmospheric pressure cold plasma treatment of cherry tomatoes, J. Biosci. Bioeng. 118(2), pp. 177-182.
43. Solomon E.B., Sharma M. (2009). Microbial attachment and limitations of decontamination methodologies, Sapers, G.M., Solomon, E.B., Matthews, K.R. (Eds.), Produce Contamination Problem: Causes and Solutions. Elsevier Inc., San Diego, CA, pp. 21-45.
44. Warning A., Datta A.K. (2013). Interdisciplinary engineering approaches to study how pathogenic bacteria interact with fresh produce, J. Food Eng., 114(4), pp. 426-448.
45. Perni S., Liu D.W., Shama G., Kong M.G., (2008). Cold atmospheric plasma decontamination of the pericarps of fruit, J. Food Prot., 71(2), pp. 302-308.
46. Perni S., Shama G., Kong M.G., (2008). Cold atmospheric plasma disinfection of cut fruit surfaces contaminated with migrating microorganisms, J. Food Prot., 71(8), pp. 1619-1625.
47. Jahid I.K., Han N., Zhang C.Y., Ha S.D. (2015). Mixed culture biofilms of Salmonella typhimurium and cultivable indigenous microorganisms on lettuce show enhanced resistance of their sessile cells to cold oxygen plasma, Food Microbiol., 46, pp. 383-394.
48. Cui H., Bai M., Yuan L., Surendhiran D., Lin, L. (2018). Sequential effect of phages and cold nitrogen plasma against Escherichia coli O157:H7 biofilms on different vegetables, International Journal of Food Microbiology, 268, pp. 1-9.
49. Kim J.E., Ji Oh Y., Won M.Y., Lee K.S., Min S.C. (2017). Microbial decontamination of onion powder using microwave-powered cold plasma treatments, Food Microbiology, 62, pp. 112-123.
50. Timmons C., Pai K., Jacob J., Zhang G., Ma L.M. (2018). Inactivation of Salmonella enterica, Shiga toxin-producing Escherichia coli, and Listeria monocytogenes by a novel surface discharge cold plasma design, Food Control, 84, pp. 455-462.
51. Raybaudi-Massilia R.M., Mosqueda-Melgar J., Soliva-Fortuny R., Martin-Belloso O. (2009). Control of pathogenic and spoilage microorganisms in fresh-cut fruits and fruit juices by traditional and alternative natural antimicrobials, Compr. Rev. Food Sci. Food Saf., 8(3), pp. 157-180.
52. Aneja K.R., Dhiman R., Aggarwal N.K., Kumar V., Kaur M. (2014). Microbes associated with freshly prepared juices of citrus and carrots, International Journal of Food Science, pp. 1-7.
53. Montenegro J., Ruan R., Ma H., Chen P. (2002). Inactivation of E-coli O157:H7 using a pulsed nonthermal plasma system, J. Food Sci. 67(2), pp. 646-648.
54. Misra N.N., Ziuzina D., Cullen P.J., Keener K.M. (2013). Characterization of a novel atmospheric air cold plasma system for treatment of packaged biomaterials, Trans. ASABE, 56(3), pp. 1011-1016.
55. Xu L., Garner A.L., Tao B., Keener K.M. (2017). Microbial inactivation and quality changes in orange juice treated by high voltage atmospheric cold plasma, Food Bioprocess Technol., 10(10), pp. 1778-1791.
56. Min S.C., Roh S.H., Boyd G., Sites J., Uknalis J., Fan X., Niemira B.A. (2017). Inactivation of Escherichia coli O157:H7 and aerobic microorganisms in romaine lettuce packaged in a commercial polyethylene terephthalate container using atmospheric cold plasma, J. Food Protect. 80(1), pp. 35-43.
57. Ro E.Y., Ko Y.M., Yoon K.S. (2015). Survival of pathogenic enterohemorrhagic Escherichia coli (EHEC) and control with calcium oxide in frozen meat products, Food Microbiol., 49, pp. 203-10.
58. Rød S.K., Hansen F., Leipold F., Knøchel S. (2012). Cold atmospheric pressure plasma treatment of ready-to-eat meat: inactivation of Listeria innocua and changes in product quality, Food Microbiol., 30(1), pp. 233-8.

59. Jayasena D.D., Kim H.J., Yong H.I., Park S., Kim K., Jo C. (2015). Flexible thin-layer dielectric barrier discharge plasma treatment of pork butt and beef loin: effects on pathogens inactivation and meatquality attributes, Food Microbiol., 46, pp. 51-57.
60. Lee H.J., Jung H., Choe W., Ham J.S., Lee J.H., Jo C. (2011). Inactivation of *Listeria monocytogenes* on agar and processed meat surfaces by atmospheric pressure plasma jets, Food Microbiology, 28(8), pp. 1468-1471.
61. Choi S., Puligundla P., Mok C. (2016). Corona discharge plasma jet for inactivation of Escherichia coli O157:H7 and Listeria monocytogenes on inoculated pork and its impact on meat quality attributes, Ann. Microbiol, 66(2), pp. 685-694.
62. Kim H.-J., Yong H.I., Park S., Choe W., Jo C. (2013). Effects of dielectric barrier discharge plasma on pathogen inactivation and the physicochemical and sensory characteristics of pork loin, Curr. Appl. Phys., 13(7), pp. 1420-1425.
63. Kim J.E., Lee D.U., Min S.C. (2014). Microbial decontamination of red pepper powder by cold plasma, Food Microbiol., 38, pp. 128-136.
64. Dirks B.P., Dobrynin D., Fridman G., Mukhin Y., Fridman A., Quinlan J.J. (2012). Treatment of raw poultry with nonthermal dielectric barrier discharge plasma to reduce *Campylobacter jejuni* and *Salmonella enterica*, J. Food Prot., 75(1), pp. 22-28.
65. Berchieri, Jr. A., Wigley P., Page K., Murphy C., Barrow P. (2001). Further studies on vertical transmission and persistence of Salmonella enterica serovar Enteritidis phage type 4 in chickens, Avian Pathol., 30, pp. 297-310.
66. Bermudez-Aguirre D., Corradini M.G. (2012). Inactivation kinetics of Salmonella spp. under thermal and emerging treatments: a review, Food Res. Int., 45(2), pp. 700-712.
67. Davies R., Breslin M. (2003). Investigations into possible alternative decontamination methods for Salmonella enteritidis on the surface of table eggs, J. Veterinary Med. Ser. B, 50(1), pp. 38-41.
68. Donner A., Keener K.M. (2011). Investigation of in-package ionization, J. Purdue Undergrad. Res., 1, pp. 10-15.
69. Matan N., Nisoa M., Matan N. (2013). Antibacterial activity of essential oils and their main components enhanced by atmospheric RF plasma, Food Control, 39, pp. 97-99.
70. Gurol C., Ekinci F.Y., Aslan N., Korachi M. (2012). Low Temperature Plasma for decontamination of E. coli in milk, International Journal of Food Microbiology, 157(1), pp. 1-5.
71. Lee H.J., Jung S., Jung H., Park S., Choe W., Ham J.S., Jo C. (2012). Evaluation of a dielectric barrier discharge plasma system for inactivating pathogens on cheese slices, J. Anim. Sci. Technol., 54, pp. 191-198.
72. Yong H.I., Kim H.J., Park S., Alahakoon A.U., Kim K., Choe W., Jo C. (2015). Evaluation of pathogen inactivation on sliced cheese induced by encapsulated atmospheric pressure dielectric barrier discharge plasma, Food Microbiol., 46, pp. 46-50.
73. Yong H.I., Kim H.J., Park S., Kim K., Choe W., Yoo S.J., Jo C. (2015). Pathogen inactivation and quality changes in sliced cheddar cheese treated using flexible thin-layer dielectric barrier discharge plasma, Food Res. Int., 69, pp. 57-63.