Cold Plasma Decontamination of Foods*

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nonthermal plasma, food processing, food safety, pathogen, ozone, antimicrobial treatment

Abstract

Cold plasma is a novel nonthermal food processing technology that uses energetic, reactive gases to inactivate contaminating microbes on meats, poultry, fruits, and vegetables. This flexible sanitizing method uses electricity and a carrier gas, such as air, oxygen, nitrogen, or helium; antimicrobial chemical agents are not required. The primary modes of action are due to UV light and reactive chemical products of the cold plasma ionization process. A wide array of cold plasma systems that operate at atmospheric pressures or in low pressure treatment chambers are under development. Reductions of greater than 5 logs can be obtained for pathogens such as *Salmonella*, *Escherichia coli* O157:H7, *Listeria monocytogenes*, and *Staphylococcus aureus*. Effective treatment times can range from 120 s to as little as 3 s, depending on the food treated and the processing conditions. Key limitations for cold plasma are the relatively early state of technology development, the variety and complexity of the necessary equipment, and the largely unexplored impacts of cold plasma treatment on the sensory and nutritional qualities of treated foods. Also, the antimicrobial modes of action for various cold plasma systems vary depending on the type of cold plasma generated. Optimization and scale up to commercial treatment levels require a more complete understanding of these chemical processes. Nevertheless, this area of technology shows promise and is the subject of active research to enhance efficacy.

THE NEED FOR NONTHERMAL FOOD PROCESSING TECHNOLOGIES

Foodborne illness associated with contaminated food continues to be a significant concern (Sivapalasingam et al. 2004, U.S. FDA 2008). Associated costs have been estimated to be between \$34 and \$39 billion (IFT 2004, Scharff 2010). From 1990 to 2005, produce accounted for 713 of 3,204 outbreaks, or 22% of the most common outbreaks, a significant increase in recent years (CSPI 2010). Chief among the pathogens of concern are *Salmonella*, *Escherichia coli* O157:H7 and other shigatoxigenic *E. coli*, *Listeria monocytogenes*, and *Shigella* spp. (Sivapalasingam et al. 2004). In 2011, a foodborne outbreak of *E. coli* O104:H4, a relatively rare strain, was responsible for at least 30 deaths and at least 2,400 illnesses, including 720 cases of kidney failure (Chapman & Powell 2011). These outbreaks reduce confidence in the microbial safety of the food supply (Calvin 2007).

In addition to concerns related to raw meats and poultry, the occurrence of human pathogens on fresh produce has been documented and recently reviewed (Gombas et al. 2003, Sivapalasingam et al. 2004, Mandrell 2009). Challenge studies have demonstrated that potentially life-threatening bacteria are able to grow on a wide variety of fresh and fresh-cut produce surfaces (Annous et al. 2005, Gomez-Lopez et al. 2009). Despite these investigations, significant knowledge gaps remain as to how to prevent fruits and vegetables from becoming contaminated and how pathogens can best be removed or inactivated from at-risk produce.

Conventional postharvest washing and sanitizing treatments are not highly effective for produce, often resulting in less than 2 log unit reductions of pathogens (Annous et al. 2001, U.S. FDA 2009). Despite considerable research effort on the development of antimicrobial intervention technologies for meats, poultry, and produce, there remains a need for new, safe, affordable, and more effective interventions.

Cold plasma is one such nonthermal food processing technology. Plasma plumes have been widely used in materials processing industries to treat textiles, glasses, electronics, paper, and other products (Niemira & Gutsol 2010). However, cold plasma is a relatively new intervention with respect to improving the safety of foods. Therefore, the technical aspects of cold plasma are unfamilar to food producers, processors, and researchers. This review provides a technical overview of plasma chemistry and explains how cold plasmas have been used to inactivate foodborne pathogens and how this technology may be developed for commercial application.

INTRODUCTION TO COLD PLASMA

Conventional chemical treatments are familiar to food processors, as are conventional energy-based processes, such as heating. The three conventional states of matter are solids, liquids, and gases; plasma has been described as the fourth state of matter, an unfamiliar designation that warrants explanation. As materials acquire energy (such as by heating), they change state, from solid (lowest energy) to liquid and then ultimately to gas. The melting points and boiling points of materials widely vary. For all materials, however, at each phase transition, the interactions and structures between molecules become looser and ultimately break down entirely (Niemira & Gutsol 2010). Gases are collections of molecules (e.g., N_2, O_2, CO_2) or single atoms (e.g., He, Ne, Ar) without large-scale structure. At still higher energies, the intramolecular and intra-atomic structures break down, liberating free electrons and ions. Plasma may be thought of as an ionized gas consisting of neutral molecules, electrons, and positive and negative ions.

Plasmas generated in conventional devices do not ionize all of the atoms in a gas, even for hot (i.e., thermal) plasmas, such as welding arcs and spark plugs (Fridman et al. 2005). Within these hot plasmas, all species are extremely reactive. Within cooler (i.e., nonthermal) plasmas, such as those found in neon signs and plasma display screens, some of the chemical species are more reactive than others. For this reason, the chemical composition of the feed gas becomes a determining factor in the types of reactions that the plasma can initiate (Lieberman & Lichtenberg 2005, Niemira & Gutsol 2010).

The energy required to ionize gases into plasma can come from a variety of sources, such as heat, electricity, laser light, radiation, and extremely rapid compression. As a cloud of active particles, the plasma retains the imparted energy for a period of time. When the active particles recombine with each other, the energy is released as visible and UV light in the process of recombination (Lieberman & Lichtenberg 2005, Niemira & Gutsol 2010). Of more interest to food processors, the active particles in the plasma can react with the food substrate, releasing the stored energy into the bacteria or viruses to be targeted. How much energy a plasma has to impart will depend on its chemical composition, density, and temperature.

Thermal plasma: energy is sufficiently high that all particles are in equilibrium and do not transfer energy among themselves

Nonthermal plasma: electrons have higher average energy than heavier particles and transfer energy with every collision

Cold plasma: plasma generated at or near room temperature that does not cause thermal damage to foods or associated bacteria

Definition of Technology

Thermal plasma, operating at many hundreds or thousands of degrees above ambient, would be immediately detrimental to the quality of food products. Nonthermal plasma is therefore the focus of this chapter. For the sake of clarity, however, a distinction must be made between what nonthermal means to a plasma physicist and what the same term means to a food processor. To the physicist, nonthermal means that the plasma has a distinctly nonuniform distribution of energy (a nonequilibrium) among the constituent particles. Electrons are likely to transfer energy via collisions with heavier particles, exciting the larger particle into a state of reactivity (Fridman et al. 2005, Niemira & Gutsol 2010). To a food processor, nonthermal means that the mode of action of the antimicrobial process does not rely on thermal kill for inactivation of associated pathogens. As a practical matter, nonthermal processes are generally regarded as those that cause little or no thermal damage to the food product being treated.

There are three primary mechanisms by which cold plasma inactivates microbes (Moisan et al. 2002). The first is the chemical interaction of radicals, reactive species, or charged particles with cell membranes. The second is by damage to membranes and internal cellular components by UV radiation. Finally, DNA strands may be broken by UV generated during recombination of the plasma species. While on a given commodity, one mode of action may be more significant than another, the greatest sanitizing efficacy results from plasma with multiple antimicrobial mechanisms (Moisan et al. 2002, Laroussi 2003).

As a food processing technology, cold plasma is new enough that the terminology is still evolving. The terms cold plasma (Noriega et al. 2011), cool plasma (Tran et al. 2008), atmospheric pressure plasma (Chirokov et al. 2005), cold atmospheric gas plasma (Moisan et al. 2001), and other comparable terms have been used in recent publications. In other cases, the plasma is described by the generative technology, e.g., dielectric barrier discharge (Fridman et al. 2006), plasma jet (Lu et al. 2009), uniform glow discharge plasma (Gadri et al. 2000), gliding arc discharge (Burlica et al. 2010), etc. As the technology matures, it is expected that terminology and experimental methodologies will become more standardized (Fernandez et al. 2011). For the purposes of consistency, the general term cold plasma is used in this review to mean reactive nonequilibrium plasma, generated at or near room temperature, that has antimicrobial modes of action that do not rely on heat to kill or inactivate target organisms.

General Technical Parameters, Including Commodity Considerations

Cold plasmas have, until recently, only been possible at low pressures and very small scales (Lieberman & Lichtenberg 2005). In recent years, however, different cold plasma technologies have been developed that operate in the range of environmental conditions suitable for food processing. These will be discussed in later sections. With respect to the commodities that may be treated with cold plasma, certain key factors dictate the suitability of the product and/or the specific engineering challenges to be overcome. Water activity (both of the product and of the food processing environment), protein and fat content and general physical conformation of the product are gross parameters to be considered. For example, skin-on poultry pieces versus skinless poultry pieces represent distinctly different challenges for sanitation with cold plasma (Noriega et al. 2011). Impacts on color, taste, and aroma may have a notable significance on the potential use of cold plasma processing with respect to one kind of commodity vs. another. Particularly with fragile commodities such as fresh produce, subtle changes as a result of antimicrobial processing can prove detrimental (Annous et al. 2005, U.S. FDA 2008).

Quenched plasma: cold plasma at a distance from source of generation, with a high proportion of secondary chemical species

Types of Cold Plasma Systems

There is a rapidly expanding array of technologies used to generate cold plasma. These can operate at atmospheric pressure or at some degree of partial vacuum. The gas being ionized may be as simple as air or nitrogen, or it may be a more exotic mixture containing some proportion of noble gases, such as helium, argon, or neon. The driving energy may be electricity, microwaves, or lasers. This wide array of design elements is an indication of the flexibility of cold plasma systems and the extent to which new forms of cold plasma systems continue to be built and evaluated.

However, all cold plasma systems intended for use in food processing fall generally into one of three categories. These categories are defined by where the food to be treated is positioned with respect to the cold plasma being generated: at some significant distance from the point of generation, relatively close to the point of generation, or within the plasma generation field itself. Conceptually, these categories are derived from the nature of cold plasma chemistry, with delineations having to do with the half-life and reactivity of charged, active species within the plasma (Niemira & Gutsol 2010).

The first category is remote treatment cold plasma systems. The plasma is generated using one of a variety of methods and moved onto the surface to be treated. The plasma may be driven by a flow of the feed gas or (less commonly) manipulated through the use of magnetic fields. This type of system has the advantage of placing the surface to be treated at a physically separate point of generation (Chirokov et al. 2005). This simplifies the design and operation of the device, and increases the flexibility with respect to the shapes and sizes of objects to be treated. However, the most reactive chemical species are also those that have the shortest half-life. During the time of flight, free electrons may recombine with other plasma products, such as heavy ions or atomic species. By the time the quenched plasma reaches the target surface, the composition is secondary chemical species, i.e. lower activity, long-living chemical species resulting from chemical recombination within the plasma (Gadri et al. 2000). The lower concentration of ions that exist in this afterglow plasma generate UV light and activate chemical species upon reaction with the target, but their concentration is much lower than in active plasma (i.e., plasma supported by electric field) (Fridman & Kennedy 2004).

The second category is known as direct treatment cold plasma systems. In these systems, plasma generation equipment supplies active plasma directly to the object to be treated. As with the first category, the plasma is moved via the flow of the feed gas or by a comparable means. Because the target is relatively close to the site of cold plasma generation and is exposed to the plasma before active species recombine and are lost, these systems provide higher concentrations of active agents (Laroussi & Lu 2005). Systems of this type can operate in pulsed mode, with plasma generated at pulse frequencies of hundreds or thousands of times per second. As there is little or no intervening normal atmosphere between the plasma generation apparatus and the target object, the level of UV radiation generated by recombination is relatively high at the treated surface. An important factor is that commodities with high water activity and internal moisture content can be made to conduct electricity at sufficiently high voltages. As electricity is conducted through meat or plant tissues, localized heating can cause overt sensory damage, such as burn marks or protein coagulation, as well as more subtle changes in aroma, texture, and appearance. Therefore, direct treatment cold plasma systems must be designed to exacting specifications to avoid concentrated electrical conduction through the product to be treated. Point-source electrical discharges concentrate the current flow, thereby causing heat damage. Direct treatment systems can be somewhat more challenging to build and operate than remote treatment systems. Depending on the design of the cold plasma emitter, systems of this type may be as flexible with respect to target shape and size. However, the potential for electrical conduction through the commodity may pose limits on the types of products for which direct treatment cold plasma systems may be suitable.

In the final category, the electrode contact systems, the surface to be sterilized is between one electrode and the other electrode or the neutral ground connection. The surface to be sterilized is physically within the cold plasma generation field. In these systems, the product is exposed to the broadest combination of active antimicrobial agents, at the highest possible intensity of free electrons, radicals, ions, and UV radiation (Fridman et al. 2006). The shape and composition of the electrodes must be carefully controlled to match the commodity being treated to avoid point discharges and the associated heat buildup (Niemira & Gutsol 2010). Electrode contact cold plasma systems have a physical constraint based on the spacing between the electrodes. Although changes in the feed gas composition or the design of one or both electrodes can provide some flexibility, these systems are limited to commodities that physically fit between the electrodes (Gadri et al. 2000). Systems of this type may be best suited for smaller or flatter commodities, such as nuts, berries, seeds or, potentially, shell eggs. Other potential products could include flattened products, such as chicken breasts. Therefore, although electrode contact cold plasma systems are among the most technically challenging to design and operate, they may be the most suitable for certain commodity classes.

Active plasma: cold plasma near the source of generation, with a high proportion of ions, radicals, and active species

Direct treatment system: product is close to cold plasma generator and is treated with active plasma

Remote treatment system: product is at some distance from the cold plasma generator and is treated with quenched plasma

Electrode contact system: product is within the zone of plasma generation and is treated with highly active plasma

Overview of Methods of Generation

Cold plasma discharges can be produced by a variety of means, some of which have been the subject of research since the earliest years of inquiry into electrical phenomena (Becker et al. 2005). **Figure 1** describes basic forms of three types of cold plasma discharge systems. The glow discharge has electrodes at either end of a separating space, which may be partially evacuated or filled with a specific gas. The radio frequency discharge uses pulsed electricity to generate cold plasma within the center of the electrical coil. The barrier discharge uses an intervening material with high electrical resistance (the dielectric material) to distribute the flow of current and generate the plasma. A simple form of the barrier discharge systems is shown; these systems may use one or two layers of dielectric material, arranged in various configurations. These may also be arranged in an annular or tubular form, with one electrode entirely within the other. In those designs, the cold plasma is generated in the space between the electrodes. These designs allow for gas movement across the zone of plasma generation and delivery of the cold plasma to the target. Another basic type of discharge is the arc discharge, shown in **Figure 2**. In this example, a stream of gas moves the arc along the electrodes, expanding and cooling it until it ultimately discharges upward.

Non-Food Applications with Applicability to Foods

The applications of cold plasma are many and varied (Becker et al. 2005, Niemira & Gutsol 2010). The reactive species generated in a cold plasma discharge are used for electrostatic precipitation

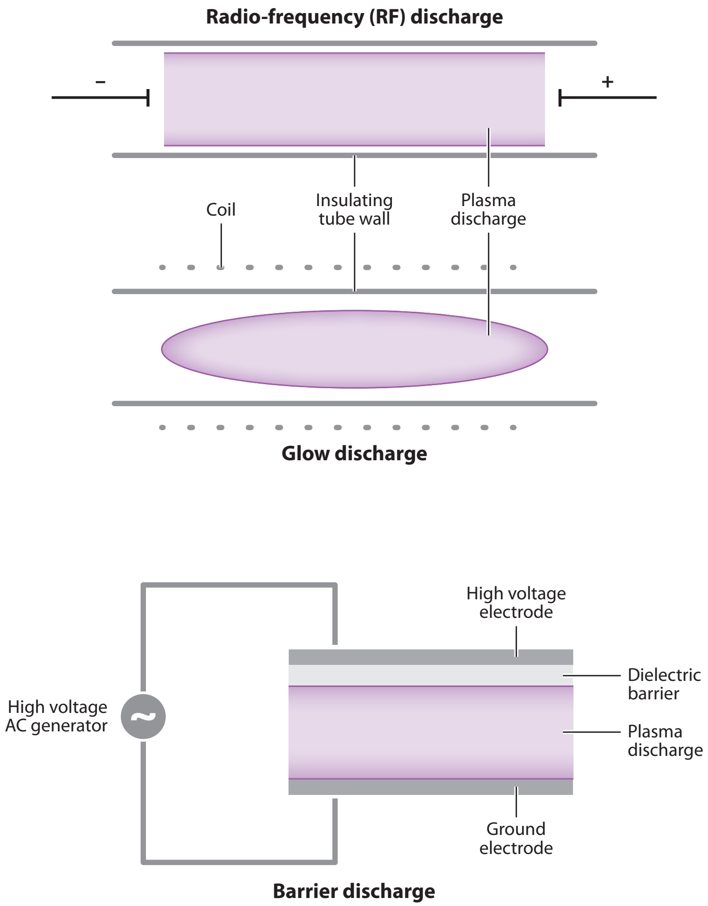

Figure 1

Diagrammatic representation of three basic types of discharges: radio-frequency discharge (*top*), glow discharge (*middle*), barrier discharge (*bottom*). The physical conformations of the various elements can be varied according to need and design requirements. Cold plasma discharges are indicated by the purple-colored zones.

of fine particulates, ozone generation, and chemical cleaning and/or decontamination. Surface treatments can include oxidation, etching, or ashing. Treatment with cold plasma can modify hydrophobic surfaces to render them hydrophilic, or can be used to deposit a hydrophilic coating on various surfaces (Kim et al. 2006). Plastic packaging films and papers treated with cold plasma had improved performance, including surface charge and ink adhesion (Tuominen et al. 2010). Biological decontamination of inert surfaces is also an application of cold plasma; applications to food surfaces are the subject of the bulk of this review.

LOW-PRESSURE COLD PLASMAS

The ionization voltage for any gas mixture is determined by the configuration of the distance between the electrodes (the gap width) and the gas pressure between them (Becker et al. 2005, Niemira & Gutsol 2010). **Figure 3** shows the effect of this relationship for various gases. Lowering the pressure of the gas also lowers the voltage required to ionize it. Cold plasma systems that

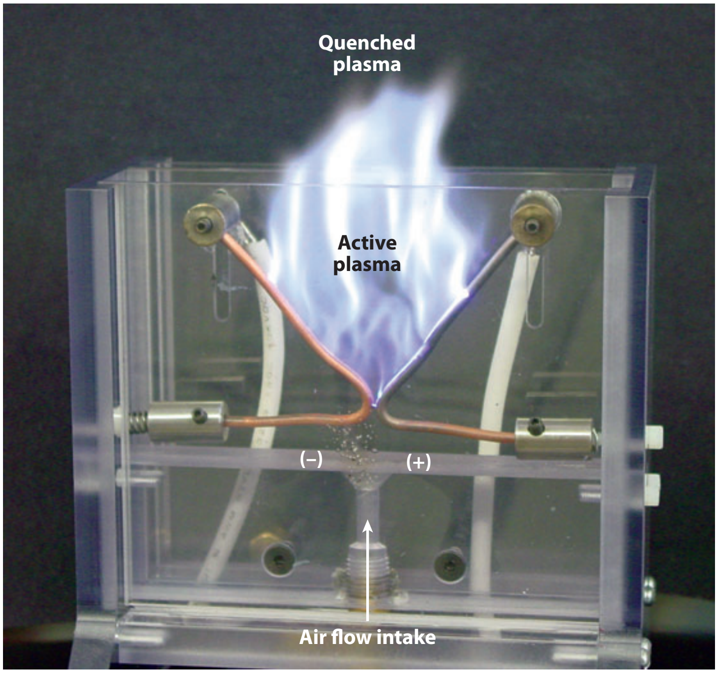

Figure 2

Gliding arc discharge. Direct current system, 320 mA at 10 kV, feed gas is air, delivered from below. Labels indicate regions of active plasma (*within the plume*) and quenched plasma (*above the plume*).

operate under reduced pressure therefore require less power to generate antimicrobially active plasmas. This fundamental aspect of ionization potentials has led to a set of cold plasma generation technologies that use reduced pressure treatment chambers to generate plasma and distribute it to the food surface to be treated.

Microwave-Pumped Cold Plasma

Cold plasma systems that use microwaves to ionize the treatment chamber gas have the advantage of using technology similar to that found in conventional microwave ovens. This allows for a more rapid technology development curve (Tran et al. 2008). In a study using microwave-pumped cold plasma within a one meter long unit, glass slides treated with *Bacillus subtilis* or *Staphylococcus aureus* were treated for very short times. Reductions of 5.2 and 3.7 log were obtained after six seconds of treatment in air at 1.6 mBar (160 Pa) (Tran et al. 2008). A study comparing air, argon, and ammonia as feed gases treated *Aspergillus niger*, *B. subtilis*, *Bacillus stearothermophilus*, and *Saccharomyces cerevisiae* in a microwave-pumped cold plasma chamber, evacuated to between 10 Pa and 50 Pa (Feichtinger et al. 2003). Although all three types of gases were ultimately effective in this cold plasma system, air yielded greater log reductions than did ammonia or argon. The microorganisms were reduced by 6 logs within 30 seconds (air), 60 seconds (ammonia), and 180 seconds (argon). The authors concluded that UV production by oxygen species significantly contributed to the antimicrobial efficacy.

Another study found similar efficacy. Using an 80%:20% N_2:O_2 mixture at 10 Pa, the inactivation kinetics of *B. subtilis* (Roth et al. 2010a) or *Deinococcus radiodurans* (Roth et al. 2010b) on glass slides were determined following treatment in a microwave-pumped cold plasma chamber.

Ionization potential: the energy level at which gas molecules come apart and form a plasma

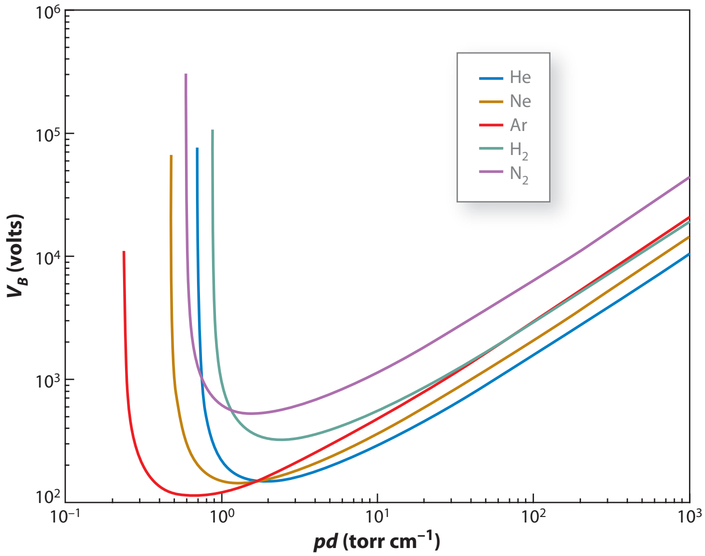

Figure 3

Paschen ionization curves obtained for helium (He), neon (Ne), argon (Ar), hydrogen (H_2), and nitrogen (N_2). V_B (breakdown voltage, in volts) as a function of *pd* (pressure × distance, in torr cm^{-1}). Assumes parallel plate electrodes. Redrawn from Lieberman & Lichtenberg 2005.

Depending on the strain tested, 20 seconds of treatment reduced *B. subtilis* by 4–6.5 logs. *Deinococcus radiodurans* showed a reduction of 6 logs after 60 s of treatment. In these studies, genes related to DNA repair, oxidative stress response, and cell wall synthesis were activated in the surviving populations. The conclusion was that, for this form of cold plasma, oxidative damage and UV damage to DNA were primary mechanisms of inactivation (Roth et al. 2010a, 2010b).

Radio Frequency–Driven Cold Plasma

Radio frequency (RF) cold plasma systems ionize gases through the use of rapidly cycling electrical impulses, operating at various power and voltage settings. As with microwave-based systems, the technologies associated with RF electronics have been in use for many years. This class of cold plasma systems has a similar advantage of leveraging existing expertise. Frequencies for these systems can range from several Hz to high MHz. Lassen et al. (2005) used an RF system operating at (13.56 MHz) to treat *B. stearothermophilus* spores. Various atmospheres were tested: O_2, $Ar:H_2$ (50%:50%, 15%:85%, 25%:75%, and 5%:95%), $O_2:H_2$ (50%:50% and 95%:5%) and $O_2:CF_4$ (88%:12%). The authors also varied the power levels, testing 100 W and 400 W. A gas mixture of $Ar:H_2$ at 5%:95% at 20 Pa gave the best antimicrobial result, with a reduction of 2 log after a three minute treatment. In this experiment, as with the oxygen-containing mixtures, the mode of action was identified as UV effects.

As the study by Lassen et al. (2005) shows, the composition of the initial gas has a significant impact on the antimicrobial power of the resultant plasma. This phenomenon was explored in a series of experiments by another team of researchers. An argon-based RF (13.56 MHz) cold plasma system operating at 0.1 Pa was able to reduce *S. aureus* by 3 logs following a five minute exposure at 100 W (Sureshkumar & Neogi 2009). In that study, the antimicrobial effect was increased by increasing the exposure time or input power. The effect was lessened by increased flow rates. Argon free radicals and UV were suggested as the primary modes of action, based on spectroscopy of the cold plasma. A subsequent study using the same equipment (Sureshkumar et al. 2010) demonstrated that N_2 plasma had a significant effect on sterilization of *S. aureus* because of generation of UV radiation. Addition of 2% O_2 to the gas mixture enhanced the antimicrobial effect, leading to a 6 log reduction. The presence of oxygen led to the formation of nitric oxide and other reactive species. Bacterial cells treated by 98%:2% N_2:O_2 mixture plasma were severely damaged after 5 min treatment with 100 W power, suffering extensive cellular disruption and shredding of the cell membrane, with loss of cytoplasmic fluid and cellular contents into the suspending medium (Sureshkumar et al. 2010).

These studies demonstrate the ability of low-pressure cold plasmas to inactivate pathogens. A key consideration for systems of this type is that the necessity of drawing a partial vacuum prior to plasma treatment effectively prevents these systems from being run as a continuous flow, conveyor belt process. As with high pressure processing (HPP), these low-pressure cold plasma systems would have to be used as a batch-processing method. In the scale-up to pilot-scale and commercial-scale processing, batch systems face technical hurdles related to the speed and volume of throughput. After a number of years of technological development, these challenges have been successfully addressed in commercial high-pressure processing systems. The lessons learned therein are valuable for low-pressure cold plasma processing systems.

ATMOSPHERIC PRESSURE COLD PLASMAS

The preceding section discussed cold plasma systems that rely on reduced partial pressure to facilitate ionization and plasma generation. It is important to note that, aside from the limitations on throughput imposed by batch processing, not all food commodities can tolerate vacuum conditions. Cold plasma systems that operate at one atmosphere (1 bar, 100 kPa) do not require air-tight vacuum chambers and are therefore simpler to build from that standpoint. Without the need for vacuum-tight chamber doors or gaskets, material to be processed can be moved through a treatment zone via conveyor. However, ambient pressure processing imposes new challenges for cold plasma systems because of the difficulty of ionization. As can be seen in **Figure 3**, operating at one atmosphere requires significantly greater voltage between electrodes of the same spacing. Thus, the main cold plasma generation apparatus has a substantially different set of performance criteria to meet. For example, the higher ionization voltage means a greater challenge in maintaining a uniform plasma field, i.e., one that is free of higher temperature point discharges. This is particularly important in circumstances where active plasma is being blown onto the food surface. As is shown below, one of the options that has been explored is to use a gas mixture with a lower ionization potential.

Microwave-Pumped Cold Plasma

In a study examining the effect of microwave-pumped plasmas on two human pathogens, *Escherichia coli* and MRSA (methicillin-resistant *S. aureus*) were mounted on glass slides and subjected to an argon-based microwave cold plasma system (1 kW, 2.45 GHz) (Lee et al. 2005). The samples were oriented directly under the high velocity (100 L min^{-1}) plasma outflow. The authors reported UV-254 intensity of 65–94 W cm^{2-1} at the point of treatment. This system yielded a greater than 7 log_{10} reduction in viable populations after a treatment as brief as 1 s. Scanning electron microscopy analysis of the glass-mounted samples showed cellular disruption that increased with treatment time, up to 5 s (Lee et al. 2005). These results show notably greater kill than the microwave-pumped cold plasma systems operating under low pressure (see above).

Radio Frequency–Driven Cold Plasma

RF-driven cold plasma systems can take different forms. For example, the system can be arranged to place the treated food between the electrodes, forming an electrode contact system. One example is a study by Deng et al. (2007). Almonds inoculated with *E. coli* were treated with cold plasma from a dielectric barrier discharge apparatus operating at frequencies of 1.0–2.5 kHz (Deng et al. 2007). The plasma feed gas was air, and the almonds were positioned in the space between the dielectric material and the ground electrode. A treatment for 30 s at 25 kV, 2.0 kHz yielded reductions of 4 log, and treatment at a higher frequency, 2.5 kHz, resulted in a 5 log reduction.

Alternatively, the RF-driven electrodes may be held above the food product, with the cold plasma blown onto the surface to be treated. An example of this type of cold plasma system is the plasma jet. As with the systems previously described, the composition of the feed gas can be varied so as to modify the efficacy of the plasma. A study with almonds examined the effect of air- or nitrogen-based cold plasma from a plasma jet (Niemira 2011). The system operated at 47 kHz, 524 W. **Figure 4** shows this system in operation. Exposure time and distance were varied along with the feed gas. Isolates of *Salmonella* and *E. coli* O157:H7 were inoculated onto whole almonds. Short treatment with cold plasma significantly reduced both pathogens. The greatest reduction observed was 1.34 log of *E. coli* O157:H7 after a 20 s treatment at 6 cm spacing. Nitrogen was generally less effective compared with dry air (Niemira 2011). This finding agrees with other research that suggests a critical role for oxygen radicals. The systems described above operate at power levels of up to 1 kW. In contrast, Gweon et al. (2009) treated *E. coli* with an RF-glow discharge system in which input power was kept relatively low (less than 70 W) so as to ensure plasma temperature was below 50°C. This was done to eliminate thermal effects. The feed gas was either helium or helium + oxygen. Augmenting the feed gas with up to 0.15% oxygen enhanced sterilization by up to 40%. To isolate the effects of UV from the direct chemical interactions of

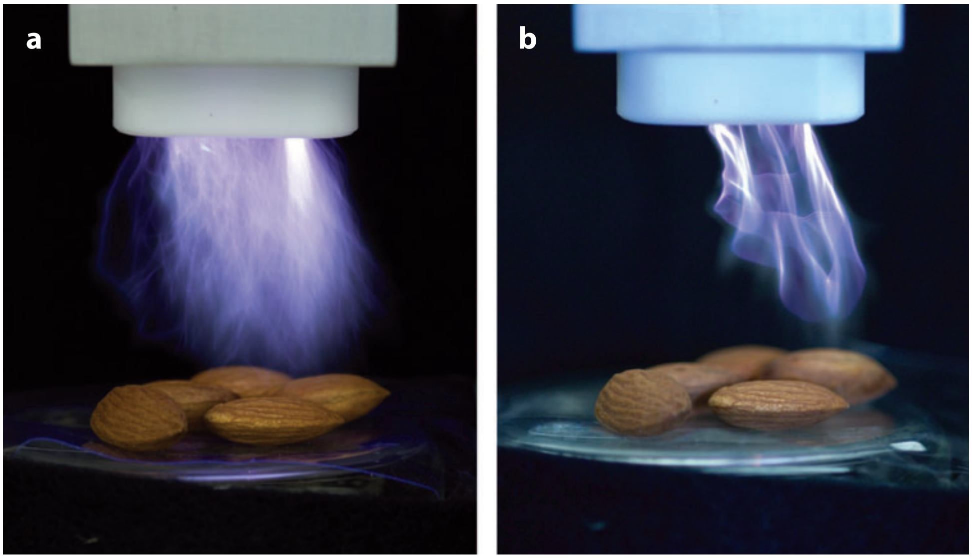

Figure 4

Cold plasma treatment of almonds. Plasma jet, 47 kHz at 524 W, feed gas is air. (*a*) Real-time image shows normal distribution of plasma. (*b*) High-speed photography shows individual plasma arcs within the overall plasma plume.

the oxygen and helium radicals, a UV transparent fused silica plate was placed between the plasma and the treatment sample. Resultant kill of the *E. coli*, due solely to UV, was negligible. Therefore, in contrast to previous research (Sureshkumar et al. 2010) that ascribed a significant role to UV, Gweon et al. (2009) concluded that the contribution of the UV irradiation from the cold plasma was negligible and that the inactivation process was dominantly controlled by oxygen radicals, rather than heat or UV photons.

Further complicating the interpretation of data is a recent study by Fernandez et al. (2011). Using a nitrogen-based cold plasma jet (~1 W, 1 kHz), this work suggested that excessive microbial loading in test procedures may lead to shielding of subsurface cells by the inactivated membranes of cells on the surface (Fernandez et al. 2011). This finding, derived from experiments with *S*. Enterica, has implications for future research and expansion of cold plasma beyond surface treatments.

Direct Current and Standard-Frequency Alternating Current

Direct current (DC) was widely used in early forms of glow discharges and continues to be used in a variety of nonfood applications. Systems in which the electricity is at a standard 60 Hz frequency are referred to as alternating current (AC) cold plasma systems. Although they are therefore a subset of RF plasmas, their integration with conventional electric power supplies justifies regarding them as a separate type of system.

In order to test this type of discharge, an atmospheric pressure plasma jet device at peak current of 300 mA was used to investigate the role of the charged particles in microbial inactivation (Lu et al. 2009). The cold plasma plume produced charged particles. These play a minor role when 97%:3% He:N_2 is used as working gas, but are more significant when 97%:3% He:O_2 is used. The authors concluded that negative ions O_2^- may be key species in the antimicrobial mode of action, along with O and reaction products such as O_3 and metastable state O_2^*. Reaction products from other gases, such as excited He^*, N_2, and N_2^+ were thought to have no significant direct effect on the inactivation of bacteria. The authors also concluded that heat and UV play little or no role in the inactivation process (Lu et al. 2009).

Both DC and AC systems have the advantage of relying on standard commercial electrical current. Although transformers must be used to step up voltages to the levels required to ionize gases, no frequency modulation electronics are required. One study evaluated the efficacy of a cold plasma generated in an AC gliding arc, 15 kV at 60 Hz. This cold plasma discharge was applied to outbreak strains of *E. coli* O157:H7 and *Salmonella* Stanley on agar plates and inoculated onto the surfaces of Golden Delicious apples (Niemira & Sites 2008). The feed gas was air. Higher flow rates (up to 40 l min^{-1}) and longer treatment times (up to 3 minutes) were most effective. Reductions obtained for both pathogens ranged from 2.9 to 3.7 log. The authors reported that the maximum temperature reached was 50.8°C (28°C above ambient).

As with the microwave- and RF-pumped systems described above, AC cold plasma systems can be used with a variety of gases and gas mixtures. Although changing the gas does not change the pressure, altering the gas composition can lower the voltage necessary to achieve ionization, thereby increasing the plasma density. Chicken muscle and chicken skin were treated with either 100% helium or 98%:2% He:O_2 plasma from an annular AC cold plasma system (Noreiga et al. 2011). The presence of oxygen and higher voltages enhanced kills, resulting in up to 3 log reductions of *Listeria innocua* after a 10 second treatment. The authors noted that surface topology of the food greatly affected the efficacy of the process.

As can be seen with the previous studies, gases that are easier to ionize (e.g., helium) may not be as effective at killing pathogens as gases that are harder to ionize (e.g., oxygen). This suggests research that deliberately introduces variant components into the plasma mixture. Using inoculated plates and a needle electrode, *S.* Typhimurium was treated with DC discharges at up to 12 kV in atmospheric pressure air (Machala et al. 2010). This system used electro-spraying of treated water through a needle electrode. The authors found that indirect exposure of contaminated surfaces to neutral active species was almost as efficient as the direct exposure to the plasma, whereas applying only UV radiation from the plasma had no antimicrobial effects. Radicals and reactive oxygen species were identified as dominant biocidal agents.

Chemical augmentation: the process of injecting a compound into the feed gas to enhance performance of the resulting cold plasma

The question remains, therefore, as to the relative significance of the antimicrobial effects contributed by UV or heat versus that accomplished by direct chemical interaction. The studies that cite UV as a key factor (Lassen et al. 2005, Tran et al. 2008, Sureshkumar et al. 2010) used low pressure cold plasma systems, whereas those that conclude little or no effect from UV (Gweon et al. 2009, Machala et al. 2010) used systems operating at ambient pressure. This fundamental difference in equipment design may be a key factor in the determination of mode of action and is therefore a key area for future research.

CHEMICAL AUGMENTATION OF COLD PLASMA TO ENHANCE EFFICACY

The reaction products of a cold plasma ionization stream derive from the input feed gases. For example, a pure argon stream can only result in neutral, active, or ionized argon species. Where dry air is used, intermediate products are various species of oxygen and nitrogen, with an extremely minor contribution from trace gases. Plasma products include O, O_3, O_2^*, and NO_X. However, the composition of the cold plasma may also be determined through the use of chemical augmentation. For example, by injection of water to the carrier gas, the addition of hydrogen can yield new reaction products, including reactive species, such as H and H_2O_2. Augmentation of the feed gas with organic compounds introduces carbon chemistry to the cold plasma mixture. Thus, injection of even simple compounds can alter the performance of cold plasma systems.

Water

The ionization potential of gases can be reduced significantly under conditions of high ambient humidity (Becker et al. 2005). Introduction of water vapor can lead to the production of atomic species that enhance the antimicrobial efficacy of plasma via UV production and direct chemical interactions (Moison et al. 2001). In tests with low-pressure inductively coupled plasma, von Keudell et al. (2010) confirmed the role of UV in the 200–250 nm range for the inactivation of microorganisms. Secondary modes of action include synergism between ion bombardment of a surface with the simultaneous reaction of active species such as O, O_2^*, or H, as is derived from water injection (von Keudell et al. 2010). Burlica et al. (2010) examined this phenomenon using pure water exposed to a pulsed plasma–gliding arc reactor equipped with a spray nozzle. Both H_2 and H_2O_2 formation rates were highest with an argon carrier gas, and production was suppressed with carrier gases (air and nitrogen) where significant amounts of nitrates are formed.

Essential Oils

In addition to nonreactive compounds, some research has examined the use of the injection into the cold plasma stream of compounds with independent antimicrobial activity. A range of potential compounds may be suitable for such applications, including volatile oils and other relatively high molecular weight compounds. Gaunt & Hughes (2004) examined the antimicrobial efficacy of a cold plasma discharge augmented with several different injected compounds. In tests with *E. coli* and *S. aureus*, kill efficiency of a corona discharge was enhanced by the introduction of ethyl alcohol or cinnamon oil, but reduced by introduction of volatilized tea tree oil. Using a thermal plasma discharge, *E. coli* and *S. aureus* were treated with volatilized β-pinene and orange oil (Gaunt et al. 2005). Both compounds effectively reduced the viability of the bacteria but only after extended exposures of 1 h and 3 h for *S. aureus* and *E. coli*, respectively. The authors showed that ionized products, rather than electrically neutral products, were shown to be the most effective component of the plasma discharge. Further research is necessary to evaluate this approach.

It is likely that controlled chemical augmentation of the plasma stream allows for optimization of antimicrobial effects, modulation of sensory impacts, and other, as yet undefined, applications and effects of cold plasma. Chemical analysis of the reaction products is an essential part of this process, to avoid the production of unwanted compounds that might negatively impact the safety and/or quality of the treated foods.

ECONOMICS OF COLD PLASMA

Consideration of the economics of cold plasma is facilitated somewhat by the fact that plasma is not an entirely new technology and has a variety of nonfood commercial applications. For delicate medical devices, low-temperature plasma sterilization was determined to be more cost effective than chemical sterilization (Adler et al. 1998). Although these implementations can be useful in estimating the economics for food safety and food processing applications, key elements remain unresolved. Foremost is the need to lower temperatures of plasma from those suitable for glass, plastics, textiles, etc. to those suitable for food products. The engineering research necessary for this has been detailed in the examples cited above. Without these technical data, it is difficult to meaningfully extrapolate capital costs and operational expenses.

As might be expected, most studies at the current state of investigation are concerned with establishing and optimizing efficacy and antimicrobial efficiency. Where economics are discussed, it is usually in a general sense, e.g., low-cost and reliable (Lee et al. 2005), but without specific economic details. Deng et al. (2007) describe cold plasma processing as having low equipment and operational cost but similarly without further detail. As the technology is developed, it is expected to become increasingly effective from an antimicrobial standpoint, but also in terms of cost efficacy. One study that addressed this (Niemira & Sites 2008) described a lab-scale gliding arc cold plasma system, which obtained reductions in pathogen populations orders of magnitude greater than an earlier version of the apparatus, using an order of magnitude less feed gas in shorter times. The authors describe the more advanced form of the system as consuming 0.017–0.051 kWh, compared with 0.038–0.10 kWh for an earlier study (Niemira et al. 2005), a ∼55% reduction in the electricity used.

As the technology moves from the lab scale to commercial scale, absolute capital costs will naturally be higher but may be offset by improvements in energy efficiency and overall engineering scale efficiencies. Precisely how costs will scale for application to industrial food processing will be influenced by the nature of the cold plasma technology used and the intended application. For very large cold plasma emitter arrays, regardless of design, certain key elements will represent recognizable cost inputs. Fixed costs will be power supplies, treatment chambers, control electronics, etc. Recurrent costs will result from wear and tear of seals, conveyors, electrodes, monitoring probes, thermocouples, etc. These types of costs will be specific to the technology used and cannot readily be predicted in a generic manner.

Ongoing costs based on consumables, however, can be predicted with some degree of confidence. This is because all cold plasma systems, of whatever design, must use electricity and a feed gas of some kind. Additional costs for injected compounds (see above) may range from trivial (water) to significant (essential oils, chemical antimicrobials). Electricity costs will scale upward from the smaller, lab-scale equipment. Power consumption of these lab-scale experimental systems varies according to the size of the emitter, ranging from 15 W (Laroussi & Lu 2005) to 900 W (Niemira & Sites 2008). For the sake of comparison, note that a regular home toaster uses approximately 1,500 W. Assuming a scale factor of two orders of magnitude in going from lab scale to commercial scale, power consumption of plasma may be as much as 90 kW, comparable to other types of large scale industrial equipment. Assuming \$0.05 per kWh, electricity cost per 1,000 hours of operation would be approximately \$4,500.

Operational costs become critical with respect to the feed gas used. Helium is more readily ionized than many other gases, which reduces the required ionization voltage and in turn, the amount of electricity consumed and the cost of the power supply. For this reason, a number of experimental cold plasma systems use feed gases that are predominantly or entirely composed of helium. Depending on purity, helium ranges in price from \$2.12–\$3.79 per m^3, excluding surcharges (USGS 2008). Pure nitrogen and pure oxygen range in price from \$3–\$15 per m^3 when purchased in gas cylinders. Nitrogen and oxygen cost much less (\$0.03–\$0.05 and \$0.06–\$0.10 per m^3, respectively) if generated in-situ from the surrounding air (PEM 2010). Filtered air is essentially zero-cost, excluding the costs of tubing, nozzles, etc., which would be fixed costs for any gas type. Gas consumption in lab-scale cold plasma systems can range from 5 l min^{-1} to 40 l min^{-1}. Using the same scaling factor as with electricity consumption, i.e., two orders of magnitude, a commercial cold plasma system may use as much as 500–4,000 l min^{-1}, 5–40 m^3 min^{-1}, or 300–2,400 m^3 h^{-1}.

Therefore, estimated feed gas costs for 1,000 hours of operation are: helium, \$636,000–\$9,096,000; nitrogen, \$9,000–\$72,000; oxygen, \$18,000–\$144,000; air, ∼\$0. Defined mixtures of pure gases would have intermediate costs, depending on proportionality.

CONCLUSIONS AND RECOMMENDATIONS FOR KEY FUTURE RESEARCH AREAS

Cold plasma is a flexible antimicrobial process with the potential for application to a wide variety of foods. Research has shown that cold plasma effectively reduces human pathogens, such as *E. coli* O157:H7, *Salmonella*, *L. monocytogenes*, *S. aureus*, and *Shigella* spp. A variety of types of cold plasma systems are the subject of active research and development worldwide. These technologies build on a wide base of knowledge from nonfood applications and progress in technology development is expected to continue to be rapid.

There remains a need to establish the antimicrobial mode of action of cold plasma treatment systems. It is likely that contributions are made by UV-mediated effects and the more direct chemical effects resulting from interactions with radicals, reactive atomic species, charged particles (such as heavy ions or electrons), or reactive plasma chemistry products (such as O_3, NO_x, etc). The specific balance of these contributions depends on the cold plasma system in question, the feed gas used, the applied voltages, the time of flight between cold plasma generation and interaction with the target, etc. Understanding the mode of action is a key step toward optimization of the technology for specific applications in the food processing industry.

Much of the research with cold plasma has been in surface sanitization. A key area of future research will be targeting food products that have a complex surface, e.g., skin-on chicken pieces, porous nuts, and stem scar or blossom ends of fruits. The extent to which cold plasma is limited to surface treatment versus application analogous to gas-phase antimicrobial treatment will determine how it may best be commercially used.

Although research into the antimicrobial efficacy of cold plasma is the first priority, additional research must follow to determine the impact of efficacious treatments on sensory parameters, such as taste, flavor, and aroma. Also, research must be conducted to determine chemical residue effects, potential toxicological profile changes, and other data that will be precursor requirements for determining regulatory status of the technology. At present, the U.S. Food and Drug Administration has not issued regulations governing the use of cold plasma as an antimicrobial process for foods. An understanding of the primary mode of action of cold plasma will be a key part of this process. Regulations governing processes or chemical additives are distinct from those governing radiation-based treatments (U.S. FDA 2008). The relative significance of reactive chemical species versus UV in the antimicrobial effects of cold plasma will have to be defined. As the data presented above shows, these modes of action may depend to a large extent on the design of the cold plasma equipment.

Inputs to cold plasma systems are electricity and a feed gas of some type, whether air, a pure gas, or a defined mixture. Also, as the technology matures, it should be available for use at different levels of throughput. Although this means that, in the strictest sense, cold plasma is a chemical input-free process that could be used in smaller-scale processing, the extent to which it may be applied to organic foods or locally grown foods is not clear. From both a conceptual standpoint and a regulatory framework, more information is required to clarify how this class of technologies could be used in these fast-growing market segments.

Cold plasma systems that use helium will be significantly more expensive to operate at a commercial scale than cold plasma systems that use air or other mixtures of nitrogen and oxygen. Even used as a minor component of an overall gas mix, the disparity in price between these gases is a significant factor to be considered in scale-up to commercial processing. The specific antimicrobial contribution, if any, of high-cost gases, such as helium, argon, etc., should be carefully established to identify niche applications for which the price premium might be justified.

SUMMARY POINTS

1. Cold plasma is an effective, flexible antimicrobial process with potential applications to a wide variety of foods.
2. The technologies used to produce cold plasma are many and varied, with strengths and limitations that are relevant to specific applications.
3. The antimicrobial mode of action for cold plasma has not yet been fully elucidated but derives primarily from UV and reactive products of the gas ionization process.
4. Antimicrobial efficacy of cold plasma tends to be enhanced when oxygen is used in the feed gas.
5. More information is needed on the sensory impact of foods treated with cold plasma.
6. Cold plasma systems that use helium are significantly more expensive to operate at a commercial scale than systems that use nitrogen, oxygen, or air.

FUTURE ISSUES

1. Contribution of injected water or volatile antimicrobial compounds within feed gas.
2. Design requirements for using cold plasma in refrigerated and/or high-moisture environments.

3. Optimization of feed gas composition to balance UV and reactive species-mediated modes of action.
4. Economic modeling: scale-up factors, capital costs, operational costs, consumables, commercial-scale throughput, etc.

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