A Review on Non-thermal Atmospheric Plasma for Food Preservation: Mode of Action, Determinants of Effectiveness, and Applications

Mercedes López1,2*, Tamara Calvo1, Miguel Prieto1,2, Rodolfo Múgica-Vidal3, Ignacio Muro-Fraguas3, Fernando Alba-Elías3 and Avelino Alvarez-Ordóñez1,2

1 Department of Food Hygiene and Technology, Universidad de León, León, Spain, 2 Institute of Food Science and Technology, Universidad de León, León, Spain, 3 Department of Mechanical Engineering, Universidad de La Rioja, Logroño, Spain

Non-thermal Atmospheric Plasma (NTAP) is a cutting-edge technology which has gained much attention during the last decade in the food-processing sector as a promising technology for food preservation and maintenance of food safety, with minimal impact on the quality attributes of foods, thanks to its effectiveness in microbial inactivation, including of pathogens, spoilage fungi and bacterial spores, simple design, ease of use, cost-effective operation, short treatment times, lack of toxic effects, and significant reduction of water consumption. This review article provides a general overview of the principles of operation and applications of NTAP in the agri-food sector. In particular, the numerous studies carried out in the last decade aimed at deciphering the influence of different environmental factors and processing parameters on the microbial inactivation attained are discussed. In addition, this review also considers some important studies aimed at elucidating the complex mechanism of microbial inactivation by NTAP. Finally, other potential applications of NTAP in the agri-food sector, apart from food decontamination, are briefly described, and some limitations for the immediate industrial implementation of NTAP are discussed (e.g., impact on the nutritional and sensory quality of treated foods; knowledge on the plasma components and reactive species responsible for the antimicrobial activity; possible toxicity of some of the chemical species generated; scale-up by designing fit-for-purpose equipment).

Keywords: plasma, food safety, food quality, microbial inactivation, food processing

INTRODUCTION

At present, consumers demand safe foods which also meet other quality criteria that traditional food preservation methods do not meet, such as convenience, high nutritional and sensorial quality, long shelf life, freshness, additives-free status, environment-friendly processing and low production costs (Fernández et al., 2012). Heat treatments have been widely used for many years to extend shelf life and enhance food safety and are generally well-accepted by consumers. However, they present certain drawbacks, such as the loss of nutrients and the considerable reduction in the
organoleptic quality of some foods they cause (Jayasena et al., 2015). For this reason, a great deal of attention has been paid in recent years to the development of new preservation approaches, based on novel technologies, so-called "Emerging Food Preservation Technologies," whose objective is to inactivate microorganisms and enzymes without appreciably altering their nutritional, organoleptic and functional characteristics (Kim et al., 2014). These novel technologies include high hydrostatic pressures, pulsed electric fields, high-intensity ultrasounds, pulsed light, oscillating magnetic fields and, more recently, non-thermal atmospheric plasma (NTAP).

The term Plasma is used in Physics and Chemistry to designate the state of an ionized gas. Plasma is considered the fourth state of Matter. Although solid, liquid and gaseous states are more common on Earth due to their temperature and pressure conditions, they are, in global terms, exceptional, whereas plasma is the predominant state across the universe. It is estimated that up to 99% of Matter is found in this state, e.g., in auroras, the ionosphere, the solar wind, the sun and other stars (Lackmann and Bandow, 2014). In addition, it is also possible to artificially produce plasmas, and many of them are part of our daily lives (e.g., televisions or monitors with plasma screens, fluorescent tubes or neon lamps used in lighting and as indicators in equipment, etc.). They are also used for industrial purposes, e.g., to confer certain functional properties onto materials (paper, plastics, fabrics or electronic elements), to produce electrostatic precipitation of fine particles, to generate ozone, or as an active medium for chemical synthesis processes.

In order to produce plasma it is necessary to supply energy to a gas to cause its ionization. Plasmas can be classified according to their temperature into two large groups: thermal plasmas and cold plasmas (Lieberman and Lichtenberg, 2005). Thermal plasmas can reach temperatures of up to several thousand Celsius degrees and are used in applications where high temperatures are required, such as in casting processes in the metallurgical industry or in chemical synthesis processes (e.g., production of acetylene from natural gas). Cold plasmas, with temperatures close to ambient temperature, are on the contrary suitable for the treatment of heat sensitive materials.

These cold or non-thermal plasmas (NTAP) are generated by the application of an electric or electromagnetic field to a gas. The field energy causes the free electrons to accelerate and ionizes the gas atoms and molecules, which release more free electrons that in turn provoke new ionizations. In addition, excited electrons produce molecular dissociations, with the formation of new atoms and free radicals, also able to excite atoms and molecules to higher energy levels. Excited atoms and molecules, when returning to the more stable state, emit excess energy in the form of broad-spectrum electromagnetic radiation, including ultraviolet (UV) radiation. Consequently, the plasma is constituted basically by molecules and atoms in an excited state, positive and negative ions, free radicals, electrons, UV radiation and reactive oxygen and nitrogen species, such as ozone, superoxide, hydroxyl radicals, singlet oxygen, atomic oxygen, nitric oxide or nitrogen dioxide. Interestingly, all these agents show antimicrobial activity against a wide range of microorganisms, including bacteria, molds, yeasts, and even bacterial and fungal spores (Klämpf et al., 2012; Tseng et al., 2012; Takamatsu et al., 2015; Dasan et al., 2016).

### POTENTIAL OF NTAP AS A PRESERVATION TECHNOLOGY IN THE FOOD INDUSTRY

The possibility of using plasma as a surface decontamination technology was first pointed out in the late 60’s in an American patent (Menashi, 1968). However, NTAP was not implemented yet in the food industry at that point, since cold plasmas could only be obtained under vacuum and at small-scale conditions, which was expensive and not applicable in industrial settings (Lackmann and Bandow, 2014). Nonetheless, the technological advances experienced at the late 90’s in relation to the sources of plasma generation allowed the development of equipment capable of generating plasmas at atmospheric pressure, thus avoiding the need for vacuum cameras and pumps and facilitating continuous treatments with relatively simple and inexpensive equipment.

NTAP offers very important advantages for food industries, which makes it a very promising novel food preservation technology. Firstly, it allows short processing times. Indeed, it has been described that very short treatment times (between a few seconds and 2 min) can cause more than 5 log reductions for different microorganisms, including pathogens such as Salmonella Typhimurium, S. Enteritidis, Escherichia coli, Staphylococcus aureus, Listeria monocytogenes, Campylobacter jejuni, Campylobacter coli, Aeromonas hydrophila, and even sporulated microorganisms such as Bacillus cereus and Clostridium botulinum (Deng et al., 2007; Muranyi et al., 2007; Rowan et al., 2007; Song et al., 2009; Shi et al., 2011; Lee et al., 2012b; Jahid et al., 2014; Ziuzina et al., 2014). In addition, it is effective at room temperature, which makes it particularly interesting for heat-sensitive products, and can be used to treat pre-packaged foods (Fröhling et al., 2012a; Rød et al., 2012; Ziuzina et al., 2014; Jayasena et al., 2015), which prevents their subsequent recontamination. Finally, its non-toxic nature and the reduced consumption of water and chemical agents result in a significant reduction of effluents, which is beneficial not only from an economic but also from an environmental point of view.

This set of advantages has led in recent years to explore the use of NTAP for food preservation, and there are already numerous studies, focused on characterizing its antimicrobial effectiveness and on deciphering the inactivation mechanisms involved. Nevertheless, a great research effort is still necessary to accomplish its successful implementation at industrial level as a safe and effective alternative to traditional preservation methods, with the main challenges arising from the difficulty in interpreting the data obtained by different research groups which use very diverse equipment and operating conditions, resulting in very different plasmas in terms of properties and, consequently, with very different antimicrobial effectiveness. However, some general conclusions can be drawn on various aspects related to the mechanisms of microbial inactivation by NTAP and the
factors that determine its lethal efficacy, which will be discussed in the following sections of this review article.

MECHANISMS OF MICROBIAL INACTIVATION BY NTAP

Although several studies have tried to elucidate the mode of microbial inactivation by various plasmas obtained under atmospheric conditions, the specific mechanisms leading to microbial death are not precisely known yet.

It is well-known that UV radiation with wavelengths in the 220–280 nm range is capable of inhibiting microbial growth by inducing the formation of DNA thymine dimers. Indeed, UV light has been used for years for the decontamination of water, air and surfaces. However, the contribution of UV radiation to the antimicrobial effect of plasmas obtained at atmospheric pressure is controversial. Thus, although some researchers hypothesize that UV-C radiation present in plasma plays an important inactivating role (Boudam et al., 2006; Eto et al., 2008; Muranyi et al., 2010), most authors (Laroussi and Leipold, 2004; Deng et al., 2006; Lee et al., 2006; Dobrynin et al., 2009, 2011; Joshi et al., 2011; Miao and Yun, 2011; Reineke et al., 2015) believe that UV radiations are not generated at the most effective wavelengths or are absorbed by the gas molecules themselves (Reineke et al., 2015) and, therefore, are not involved in microbial inactivation (Patil et al., 2014; Surowsky et al., 2014). Indeed, Reineke et al. (2015) compared the effectiveness of different plasmas for the inactivation of Bacillus atrophaeus and Bacillus subtilis spores and found that, although plasmas containing oxygen and nitrogen emitted four times more UV radiation than pure argon plasmas, the greatest lethal effect was achieved when pure argon was used as the working gas. These authors suggested that the antimicrobial effect was determined by reactive species of oxygen and nitrogen generated in the pure gas, and especially by hydroxyl radicals. Other authors have also tested the contribution of UV light by exposing E. coli cells (Liu et al., 2008; Gweon et al., 2009) and Bacillus cereus (van Bokhorst-van de Veen et al., 2015) and B. subtilis spores (Hong et al., 2009) to the action of plasma by intercalating between the plasma generation point and the treatment medium a lithium fluoride filter or a fused silica quartz plate transparent to UV light, which allows cell/spore exposure to the radiation potentially emitted by the plasma source but avoids contact with chemical reactive species. These studies demonstrated that the contribution of UV light to microbial inactivation was negligible, compared to that observed for direct exposition to plasma. In addition, exposure of B. cereus vegetative cells to nitrogen plasma has been shown to result in a transcriptional response in which the expression of genes involved in UV damage repair (uvrA, uvrB) was unaffected, while various genes involved in the response to oxidative stress, such as those encoding nitric oxide dioxygenase, as well as membrane-associated enzymes that catalyze oxidation-reduction reactions, were overexpressed (Mols et al., 2013). Finally, it has also been shown that microorganisms exhibiting variability in resistance to UV light exhibit a similar tolerance against NTAP. For instance, although B. cereus spores were more sensitive to UV light than spores of Geobacillus stearothermophilus and B. atrophaeus, a given NTAP treatment produced a similar degree of inactivation for the three species (van Bokhorst-van de Veen et al., 2015), suggesting that the mechanism of inactivation through both technologies was different.

There exists on the contrary a general agreement in that reactive chemical species generated through gas ionization exert an antimicrobial effect through a direct and non-specific attack on various microbial structures and components, including cellular envelopes, DNA and proteins (Song et al., 2009; Colagar et al., 2010; Surowsky et al., 2014; Yost and Joshi, 2015).

Lesions at the Cellular Envelopes

Various authors (Dobrynin et al., 2009, 2010; Miao and Yun, 2011; Tseng et al., 2012; Tian et al., 2015) have identified the mechanical or oxidative damage caused to cellular envelopes as the main cause of death after NTAP treatments. The mechanical erosion of cellular envelopes could be due either to the impact of plasma energy particles, such as electrons and excited atoms (Butcher et al., 2016), or to the accumulation of charges in certain parts of the cell surface, which would lead to its permeabilization through the formation of pores, in a phenomenon known as electroporation (Laroussi et al., 2003). Moreover, damage at this level would facilitate the release of important intracellular components and the invasion of reactive species which would attack other cellular components, such as DNA and proteins, thus accelerating the inactivation process. In addition to this mechanical effect, neutral reactive species generated in plasma, such as atomic oxygen, hydroxyl and peroxyl radicals, metastable oxygen or ozone, are capable of causing oxidative damage to various cellular structures and macromolecules. If this damage is very intense and exceeds the physiological repair capacity, it will lead to microbial death (Leipold et al., 2010). In fact, the use of substances that chelate reactive species has been shown to reduce the antimicrobial efficacy of NTAP. Thus, several sequestering agents of various reactive oxygen species, such as catalase, superoxide dismutase, dimethyl sulfoxide, thiourea, sodium azide, and various antioxidants (glutathione, ascorbic acid, sodium pyruvate, mannitol, histidine, or tocopherol) have been reported to protect microorganisms, such as E. coli (Joshi et al., 2011; Yost and Joshi, 2015) or P. aeruginosa (Takamatsu et al., 2015), from the action of NTAP. Yost and Joshi (2015) showed that previous incubation of E. coli with catalase provided a greater protective effect than vitamin E and thiourea, concluding that peroxides such as H₂O₂ could be the major chemical species responsible for microbial inactivation by NTAP. On the other hand, Takamatsu et al. (2015) observed that the presence of dimethyl sulfoxide or sodium azide resulted in lower P. aeruginosa inactivation rates than those observed when catalase or superoxide dismutase were used, suggesting that hydroxyl radicals and singlet oxygen were the main agents involved in microbial inactivation.

Reactive species exert their oxidative effect especially on the polyunsaturated fatty acids of the cytoplasmic membrane (Laroussi and Leipold, 2004), which are very susceptible to lipid peroxidation phenomena. Lipid peroxidation can compromise
cell viability by modifying membrane properties, decreasing its permeability and even its integrity (Joshi et al., 2011; Hosseinizadeh Colagar et al., 2013). Lipid peroxidation is a chain reaction that begins with the attack of unsaturated fatty acids by reactive oxygen species (or any reactive species), which extract a hydrogen atom from a methylene group (-CH2), giving rise to the formation of a lipid radical (L•) which can react rapidly with an oxygen molecule to give a peroxyl radical (LOO•). These radicals can extract new hydrogen atoms from other lipids and become hydroperoxides (LOOH), which undergo chemical degradative phenomena to produce very toxic degradation compounds, such as alkoxy (LO•), peroxyl and hydroxyl radicals and reactive aldehydes, including malondialdehyde and 4-hydroxynonenal. In fact, malondialdehyde is commonly used as a biological marker of oxidative stress (Liu et al., 2008), and a linear correlation between the content of this compound in E. coli, the time of exposure to NTAP and microbial viability has been described (Joshi et al., 2011; Hosseinizadeh Colagar et al., 2013; Yost and Joshi, 2015). These intermediate reactive compounds also behave as secondary toxic messengers of the reactive species generated in the plasma, amplifying their lethal damage to the cell (Joshi et al., 2011). Furthermore, many have greater stability than the plasma reactive species and, consequently, a much longer cytotoxic action, and can disseminate from the membrane toward more distant molecules, like DNA (Yost and Joshi, 2015), where they exert their action by interacting with nucleotides, inducing important modifications and cross-links and making cell growth and DNA repair more difficult (del Rio et al., 2005). In S. Typhimurium, malondialdehyde has been shown to be capable of inducing base insertions, deletions and substitutions, thus potentially contributing to significant DNA alterations (Dobrynin et al., 2009). In addition, some of the aldehydes generated in the peroxidation process, including malondialdehyde, are capable of forming cross-links in the polypeptide chains of proteins, affecting the activity of enzymes and membrane-associated proteins. To sum up, NTAP causes an uncontrolled process of lipid peroxidation which results in (i) changes in the membrane chemical composition, ultrastructural organization and permeability, (ii) a decrease in membrane fluidity, and (iii) the inactivation of membrane-associated enzymes, thereby compromising cellular viability.

The existence of damages in the cellular envelopes upon exposure to NTAP has been repeatedly demonstrated by using direct and indirect methods. Micrographs obtained using scanning electron microscopy for both vegetative cells (Staphylococcus aureus, E. coli, B. subtilis, Campylobacter jejuni, Citrobacter freundii, Pseudomonas aeruginosa, Enterococcus faecalis, Saccharomyces cerevisiae, Candida albicans) and bacterial (B. cereus, B. subtilis and G. stearothermophilus) and fungal (Aspergillus parasiticus, Aspergillus flavus) spores have allowed the identification of morphological alterations, such as size reductions, changes of shape, surface structure modifications, presence of pores in membranes and envelope disruptions (Yu et al., 2006; Hong et al., 2009; Miao and Jierong, 2009; Joshi et al., 2011; Miao and Yun, 2011; Bermúdez-Aguirre et al., 2013; Ryu et al., 2013; Zhang et al., 2013; Kim et al., 2014; Surowsky et al., 2014; Ma et al., 2015; van Bokhorst-van de Veen et al., 2015; Butscher et al., 2016; Cui et al., 2016a; Danse et al., 2016; Gabriel et al., 2016b; Lai et al., 2016; Nishime et al., 2017). For bacterial spores, a loss of refringence, not accompanied by the onset of germination, has been observed for B. cereus by phase contrast microscopy (van Bokhorst-van de Veen et al., 2015). Damages in the cell envelopes have also been evidenced indirectly by determining the release of different intracellular components, including small ions such as K+ (Zhang et al., 2013), ATP (Cui et al., 2016a), nucleic acids (Zhang et al., 2013; Tian et al., 2015; Cui et al., 2016a), proteins (Liu et al., 2008; Miao and Jierong, 2009; Miao and Yun, 2011; Zhang et al., 2013; Tian et al., 2015; Cui et al., 2016a) or dipicolinic acid in the case of bacterial spores (Tseng et al., 2012), or by monitoring the entry into the cell of fluorescent dyes which cannot cross intact membranes, such as propidium iodide (Joshi et al., 2011; Surowsky et al., 2014; Ma et al., 2015; Tian et al., 2015), the decrease in membrane potential or its depolarization (Joshi et al., 2011; Surowsky et al., 2014; Tian et al., 2015; Yost and Joshi, 2015), as well as the inability of cells to retain the Gram staining (Muranyi et al., 2010; Joshi et al., 2011).

**Damages at Intracellular Level**

However, it appears that microbial cell envelopes are not the only damaged cell structure. Indeed, reactive species, such as singlet oxygen, hydrogen peroxide, nitric oxide and excited atoms and molecules, can rapidly and easily diffuse into the cells, even when the membrane is intact, and oxidize many macromolecules (Dobrynin et al., 2011; Joshi et al., 2011; Yost and Joshi, 2015). Recently, Ziuizina et al. (2015b) detected and quantified, in L. monocytogenes and E. coli, the intracellular pool of reactive oxygen species after exposure to NTAP, using fluorescence techniques. They observed an increase in their concentration after NTAP treatment, which was more pronounced in L. monocytogenes. Similarly, Joshi et al. (2011), using highly selective fluorescent compounds for singlet oxygen and hydrogen peroxide quantification, found that the singlet oxygen concentration inside E. coli cells increased steadily within a 60 second NTAP treatment, whereas hydrogen peroxide content reached a maximum value after 12s of treatment. In S. cerevisiae, an increase in the intracellular concentration of reactive species of oxygen and, especially, of nitric oxide has also been reported (Ryu et al., 2013). Once inside the cell, reactive substances provoke damage not only on lipids, but also on proteins, nucleic acids and carbohydrates. In order to neutralize the deleterious effects of such oxidant species or to repair the damage they cause, microorganisms have a number of mechanisms of elimination and transformation of these compounds, such as catalases, superoxide dismutases and glutathione and thioredoxin systems, among others. Nevertheless, when the accumulation of reactive oxygen species exceeds the cellular detoxifying capacity, and their concentration surpasses a critical level, the microbial cell suffers from oxidative stress, which induces the expression of certain genes and the activation of different defense responses (Pomposiello and Demple, 2002). For instance, in E. coli, the regulators oxyRS and soxRS are involved in the cellular response to oxidative stress induced by NTAP. Indeed, the exposure of E.
Sharma et al. showed that hydroxyl radicals (OH\(^*\)) and, according to some authors, superoxides (O\(_2^-*\)) are capable of interacting with various macromolecules (NO), are capable of interacting with various macromolecules and, according to some authors (Yost and Joshi, 2015), DNA and proteins are especially susceptible.

**DNA Damage**

Damage to DNA is caused through the oxidation of bases. For example, oxidation of guanine generates 8-hydroxy-2’-deoxyguanosine (OHdG), a compound which is used as a marker of oxidative DNA damage (Joshi et al., 2011; Yost and Joshi, 2015). The oxidation of DNA bases leads to a transversion (substitution of a pyrimidine for a pyrimidine or vice versa), which alters the bonds between DNA bases, causing changes in DNA conformation, and frequently leading to errors in the reading of the strand, mutagenesis and cell death. Some of the chemical species of plasma also react with deoxyribose carbons, breaking the N-glucosidic bond, thus generating apurinic or apyrimidine sites (abasic sites). All these changes may eventually lead to DNA strand ruptures (Hosseinzhadeh Colagar et al., 2013; Ryu et al., 2013; Yost and Joshi, 2015). Microorganisms can nevertheless respond to a NTAP treatment with DNA fragmentation as a marker of oxidative damage. Indeed, transcriptional studies using microarrays have demonstrated that up to 18 genes involved in modifications in the conformation and three-dimensional structure of proteins and enzymes (Surovsky et al., 2013). In addition, the inactivation of some enzymes, their activity could also be compromised by the oxidation of their cofactors (Colagar et al., 2010). Actually, the oxidation of just one amino acid in a protein can affect its function (Lackmann et al., 2013). These latter authors showed that the loss of 3-phosphate glyceraldehyde dehydrogenase activity after NTAP treatment was due to the oxidation of a cysteine residue (cys150) of its active site, which is known to be irreversible (Brandes et al., 2009). However, it is still unknown which enzymes and proteins are specific targets for the action of NTAP. In the particular case of vegetative cells, Hosseinzhadeh Colagar et al. (2013) analyzed E. coli proteins after exposure to plasma using sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) techniques and found that high molecular weight proteins, of between 50 and 90 KDa, were especially sensitive to NTAP, observing a decrease in their content as treatment time increased. In addition, these authors also observed that the concentration of free amino acids linearly increased with exposure time, which suggested that NTAP is not only able to break peptide bonds, but also to fragment proteins liberating free amino acids. Concerning bacterial spores, several authors (Klämpfl et al., 2012; Tseng et al., 2012) have pointed out that the effectiveness of NTAP against these resistant forms is due to the inactivation of various enzymes responsible for spore germination, such as GerA and GerB, as well as to the inactivation of channel proteins involved in the transport of ions and dipicolinic acid, which also results in the inability to initiate germination processes.

**Damage to Proteins**

According to some authors, proteins and cytoplasmic enzymes would also suffer damage during plasma treatments. Indeed, reactive species are able to (i) break peptide bonds, (ii) oxidize amino acid side chains, especially of sulfur amino acids, such as methionine and cysteine, and amino acids harboring aromatic rings (tryptophan, phenylalanine and tyrosine), (iii) produce crosslinks within proteins, and (iv) give rise to aggregation phenomena, which are favored by the formation of intra- and inter-molecular disulfide bonds. All these effects result in modifications in the conformation and three-dimensional structure of proteins and enzymes (Surovsky et al., 2013). In addition, all the chemical species of plasma also react with deoxyribose carbons, breaking the N-glucosidic bond, thus generating apurinic or apyrimidine sites (abasic sites). All these changes may eventually lead to DNA strand ruptures (Hosseinzhadeh Colagar et al., 2013; Ryu et al., 2013; Yost and Joshi, 2015). Microorganisms can nevertheless respond to a NTAP treatment with DNA fragmentation as a marker of oxidative damage. Indeed, transcriptional studies using microarrays have demonstrated that up to 18 genes involved in modifications in the conformation and three-dimensional structure of proteins and enzymes (Surovsky et al., 2013). In addition, the inactivation of some enzymes, their activity could also be compromised by the oxidation of their cofactors (Colagar et al., 2010). Actually, the oxidation of just one amino acid in a protein can affect its function (Lackmann et al., 2013). These latter authors showed that the loss of 3-phosphate glyceraldehyde dehydrogenase activity after NTAP treatment was due to the oxidation of a cysteine residue (cys150) of its active site, which is known to be irreversible (Brandes et al., 2009). However, it is still unknown which enzymes and proteins are specific targets for the action of NTAP. In the particular case of vegetative cells, Hosseinzhadeh Colagar et al. (2013) analyzed E. coli proteins after exposure to plasma using sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) techniques and found that high molecular weight proteins, of between 50 and 90 KDa, were especially sensitive to NTAP, observing a decrease in their content as treatment time increased. In addition, these authors also observed that the concentration of free amino acids linearly increased with exposure time, which suggested that NTAP is not only able to break peptide bonds, but also to fragment proteins liberating free amino acids. Concerning bacterial spores, several authors (Klämpfl et al., 2012; Tseng et al., 2012) have pointed out that the effectiveness of NTAP against these resistant forms is due to the inactivation of various enzymes responsible for spore germination, such as GerA and GerB, as well as to the inactivation of channel proteins involved in the transport of ions and dipicolinic acid, which also results in the inability to initiate germination processes.

**Indirect Mechanism of Action**

Finally, apart from the direct effects caused by reactive species present in plasmas, an indirect mechanism of action, through the formation of other cytotoxic compounds, has been also described. Thus, Yost and Joshi (2015) showed that hydroxyl radicals (OH\(^*\)) can be generated from H\(_2\)O\(_2\) and superoxide radicals (O\(_2^-\)\(_2\)) in the presence of transition metals such as iron or copper. Although OH\(^*\) radicals can be produced by different routes, the Haber-Weiss reaction is the most habitual (Lemire et al., 2012; Tseng et al., 2012; Perni et al., 2012; Klämpfl et al., 2012; Yost and Joshi, 2015). Microorganisms can nevertheless respond to a NTAP treatment with DNA fragmentation as a marker of oxidative damage. Indeed, transcriptional studies using microarrays have demonstrated that up to 18 genes involved in modifications in the conformation and three-dimensional structure of proteins and enzymes (Surovsky et al., 2013). In addition, the inactivation of some enzymes, their activity could also be compromised by the oxidation of their cofactors (Colagar et al., 2010). Actually, the oxidation of just one amino acid in a protein can affect its function (Lackmann et al., 2013). These latter authors showed that the loss of 3-phosphate glyceraldehyde dehydrogenase activity after NTAP treatment was due to the oxidation of a cysteine residue (cys150) of its active site, which is known to be irreversible (Brandes et al., 2009). However, it is still unknown which enzymes and proteins are specific targets for the action of NTAP. In the particular case of vegetative cells, Hosseinzhadeh Colagar et al. (2013) analyzed E. coli proteins after exposure to plasma using sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) techniques and found that high molecular weight proteins, of between 50 and 90 KDa, were especially sensitive to NTAP, observing a decrease in their content as treatment time increased. In addition, these authors also observed that the concentration of free amino acids linearly increased with exposure time, which suggested that NTAP is not only able to break peptide bonds, but also to fragment proteins liberating free amino acids. Concerning bacterial spores, several authors (Klämpfl et al., 2012; Tseng et al., 2012) have pointed out that the effectiveness of NTAP against these resistant forms is due to the inactivation of various enzymes responsible for spore germination, such as GerA and GerB, as well as to the inactivation of channel proteins involved in the transport of ions and dipicolinic acid, which also results in the inability to initiate germination processes.

**Indirect Mechanism of Action**

Finally, apart from the direct effects caused by reactive species present in plasmas, an indirect mechanism of action, through the formation of other cytotoxic compounds, has been also described. Thus, Yost and Joshi (2015) showed that hydroxyl radicals (OH\(^*\)) can be generated from H\(_2\)O\(_2\) and superoxide radicals (O\(_2^-\)\(_2\)) in the presence of transition metals such as iron or copper. Although OH\(^*\) radicals can be produced by different routes, the Haber-Weiss reaction is the most habitual (Lemire et al., 2012; Tseng et al., 2012; Perni et al., 2012; Klämpfl et al., 2012; Yost and Joshi, 2015). Microorganisms can nevertheless respond to a NTAP treatment with DNA fragmentation as a marker of oxidative damage. Indeed, transcriptional studies using microarrays have demonstrated that up to 18 genes involved in modifications in the conformation and three-dimensional structure of proteins and enzymes (Surovsky et al., 2013). In addition, the inactivation of some enzymes, their activity could also be compromised by the oxidation of their cofactors (Colagar et al., 2010). Actually, the oxidation of just one amino acid in a protein can affect its function (Lackmann et al., 2013). These latter authors showed that the loss of 3-phosphate glyceraldehyde dehydrogenase activity after NTAP treatment was due to the oxidation of a cysteine residue (cys150) of its active site, which is known to be irreversible (Brandes et al., 2009). However, it is still unknown which enzymes and proteins are specific targets for the action of NTAP. In the particular case of vegetative cells, Hosseinzhadeh Colagar et al. (2013) analyzed E. coli proteins after exposure to plasma using sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) techniques and found that high molecular weight proteins, of between 50 and 90 KDa, were especially sensitive to NTAP, observing a decrease in their content as treatment time increased. In addition, these authors also observed that the concentration of free amino acids linearly increased with exposure time, which suggested that NTAP is not only able to break peptide bonds, but also to fragment proteins liberating free amino acids. Concerning bacterial spores, several authors (Klämpfl et al., 2012; Tseng et al., 2012) have pointed out that the effectiveness of NTAP against these resistant forms is due to the inactivation of various enzymes responsible for spore germination, such as GerA and GerB, as well as to the inactivation of channel proteins involved in the transport of ions and dipicolinic acid, which also results in the inability to initiate germination processes.
This reaction consists in the transformation of the ferric cation to ferrous cation \((Fe^{3+} + O_2^{-} \rightarrow Fe^{2+} + O_2)\) by the superoxide radical. Afterwards, the ferrous cation reacts with \(H_2O_2\) to produce \(Fe^{3+}\), hydroxyl anions and hydroxyl radicals, which also exert and antimicrobial activity. Nitric oxide (NO) can also react with oxygen or superoxide radicals to form nitrogen dioxide (NO\(_2\)), peroxynitrates (ONOO\(^-\) or ONO\(_2\)) and nitrous anhydride (N\(_2\)O\(_3\)) (Han et al., 2013), which all have a broad spectrum of antimicrobial activity, although they differ in reactivity, stability and biological activity (Fang, 2004).

**FACTORS DETERMINING THE ANTIMICROBIAL EFFECTIVENESS OF NTAP**

There are several factors known to influence the microbial inactivation achieved through NTAP treatments. Some of these factors are related to the conditions used for plasma generation and application, while others are associated with the particular properties of the microorganism targeted or with the characteristics of the treatment medium or food. The following sections of this review article will compile and discuss the information available in the literature in relation to these aspects.

**Processing Parameters**

NTAP may be obtained through electrical discharges, which may be accomplished by using very diverse systems, e.g., corona discharge, micro hollow cathode discharge, atmospheric pressure plasma jet, gliding arc discharge, dielectric barrier discharge (DBD), radiofrequency (rf) and microwaves. In any case, electrical discharges are initiated and sustained through electron collision processes under the action of specific electric or electromagnetic fields. Among all the existing plasma sources, DBD and plasma jets are the most widely explored configurations in food research, due to their easiness of construction and adoption. Indeed, there are some commercially available DBD and plasma jet configurations. Nevertheless, regardless of the equipment used, the molecular composition of plasma will depend on the energy supplied, which in turn is determined by voltage, power and excitation frequency, and even on the composition and flow rate of the working gas, with these parameters being very variable among different NTAP units, which makes it very difficult to carry out direct comparisons among research studies executed employing different equipment and NTAP processing conditions. However, it is possible to draw some general conclusions on how some processing parameters affect the level of microbial inactivation achieved through NTAP treatments.

With processing times described in the literature ranging from a few seconds up to several minutes, it is well-known that microbial inactivation increases with processing time, although not in all cases exponentially. Thus, a wide variety of inactivation kinetics are observed in the published data, where it is frequent to find both linear, concave-upward (tailing) and concave-downward (shoulders) kinetics (Lee et al., 2011; Calvo et al., 2016, 2017).

Voltage (difference of potential between two points per unit of charge, expressed in Volts), power (electrical energy consumed per unit of time, expressed in Watts) and frequency (number of cycles per second, expressed in Hertz) have a great effect on the antimicrobial effectiveness of NTAP, as they are known to determine the input energy and, consequently, the chemical reactive species generated and their concentration. It has been repeatedly demonstrated that an increase in voltage increases microbial inactivation of both vegetative cells and bacterial spores (Table 1). In fact, Liu et al. (2013) have found, after using different voltages to generate helium plasmas, that the content of reactive chemical species and, in particular, of \(N_2^+\), OH, He and O, and the level of inactivation achieved for \(S.\) aureus, increased with the voltage applied. It has also been shown that, working under a constant voltage, an increase in excitation frequency or in power gives rise to greater lethal effects in a wide range of microbial species (Table 1). Taking these findings into account it would be advisable to use high voltages, power, and frequencies for the generation of plasmas. However, some technological constraints, including high costs, and potential negative impacts on food quality, due to an excessive temperature rise, represent a limit toward the use of extreme processing conditions in industrial practice (Kim et al., 2011; Alkawareek et al., 2012; Daeschlein et al., 2012; Butscher et al., 2016).

Another variable to take into account when considering the antimicrobial effectiveness of NTAP treatments is the working gas used. A wide variety of gases have been used to generate plasma, with the most frequent ones being nitrogen, oxygen, carbon dioxide, argon, helium, air, or mixtures of some of these gases (Table 1). All of them give rise to plasmas capable of achieving certain level of microbial inactivation, and there is no general agreement on which one is the most effective gas. Lee et al. (2011) compared the effectiveness against \(L.\) monocytogenes of plasmas obtained with helium or nitrogen and observed a higher inactivation level for nitrogen-based plasmas. Other studies have described that air-based plasmas are most effective in inactivating \(E.\) coli O157:H7, \(B.\) cereus, \(S.\) aureus, \(L.\) monocytogenes, \(L.\) innocua and various serovars of \(S.\) enteritica, such as \(S.\) Anatum, \(S.\) Stanley, \(S.\) Typhimurium and \(S.\) Enteritidis, than plasmas generated with nitrogen (Marsili et al., 2002; Niemira, 2012; Calvo et al., 2016, 2017). On the contrary, Takamatsu et al. (2015) obtained a greater lethal effect against \(S.\) aureus and \(P.\) aeruginosa with nitrogen and \(CO_2\) based plasmas than with plasmas generated with air, \(O_2\) or argon, and Rowan et al. (2007) concluded that oxygen is the gas of choice, over \(CO_2\) or nitrogen, when generating plasmas for the inactivation of \(E.\) coli, \(C.\) jejuni, \(C.\) coli, \(L.\) monocytogenes, \(S.\) Typhimurium, \(S.\) Enteritidis and \(B.\) cereus spores. On the other hand, it seems to exist an agreement, although with some exceptions (Reineke et al., 2015), in that the addition of small amounts of oxygen to noble gases, such as helium (Gweon et al., 2009; Kim et al., 2011, 2013; Lee et al., 2011, 2012a; Galvin et al., 2013) and argon (Surowsky et al., 2014), or nitrogen (Lee et al., 2011, 2012b) improves the antimicrobial effectiveness of NTAP against vegetative cells and spore-forming bacteria (Table 1). This effect is mainly attributed to a higher formation of reactive oxygen species, such as hydroxyl and hydroperoxy radicals, atomic
### TABLE 1 | Summary of major research articles assessing the influence of processing parameters on the performance of non-thermal atmospheric plasma as a food decontamination technique.

| References            | Treatment regime | Foods/medium               | Microorganism               | Main findings                                                                                     |
|-----------------------|------------------|----------------------------|-----------------------------|---------------------------------------------------------------------------------------------------|
| Marsili et al. (2002) | 23 kV; Pulse rate: 320 pps; N₂, CO₂, air (10 L/min); Up to 50 s; batch | 0.1% peptone solution       | E. coli NCTC 9001, S. aureus NCTC 4135, S. Enteritidis NCTC PT4, B. cereus NCTC KD4                  | Air gas facilitated a greater inactivation after a 50 s treatment. B. cereus was the most susceptible microorganism. |
| Deng et al. (2007)    | 16–25 kV; 1,000–2,500 Hz; Air; Up to 30 s | Almonds                    | E. coli 12965S (Collection of the Dept of Food Science and Nutrition, University of Minnesota) | Effectiveness increased with the applied voltage and the frequency. Cells at log growth phase were more sensitive than those at stationary phase. |
| Rowan et al. (2007)   | 23.5 kV; Pulse rate: 124 pps; N₂, O₂, CO₂, air (10 L/min); Up to 30 s; 4°C; batch | Distilled water             | B. cereus NCTC 11145 spores                       | The order of effectiveness was: O₂ > CO₂ > air > N₂                                             |
| Muranyi et al. (2008) | 170 W; Air (0–80% RH); 20°C; For up to 7 s | PET foil                   | Bacillus subtilis DSM 4181 spores, Aspergillus niger DSM 1957 spores | For A. niger, increased effectiveness at relative gas humidity of 70%; in contrast, B. subtilis spores showed slightly poorer inactivation at high gas humidity. |
| Miao and Jierong (2009) | 13.56 MHz; 20–120 W; O₂ (20–100 cm³/min); Up to 5 s; batch | Polyvinyl chloride          | E. coli*                                       | The optimum conditions (highest efficiency) are 100 W and O₂ 60 cm³/min.                          |
| Song et al. (2009)    | 13.56 MHz; 75–150 W; Helium (10 L/min); Up to 120 s | Sliced cheese and ham       | 3-strain cocktail of L. monocytogenes (ATCC 19114, 19115, and 19111) | Increased effectiveness at high input power. Higher D values were obtained in sliced ham. |
| Jung et al. (2010)    | 13.56 MHz; 75 and 100 W; Helium and argon (6 L/min); Up to 120 s | Slide glass                 | Bacillus subtilis Spores*                      | Helium plasma was much less efficient than argon plasma; increased effectiveness at high input power; increased effectiveness at high discharge power. |
| Leipold et al. (2010) | 10.4 W; 21.7 kHz; 1.8 W–0.36 kW; Air; Up to 340 s | Knife                      | Listeria innocua (DMR10011)                  | Increased effectiveness at high air moisture content; S. Enteritidis resulted to be more plasma sensitive; increased effectiveness at high input power. |
| Ragni et al. (2010)   | 12.7 kHz; 15 W; Air (RH 35 and 65%); 25°C; Up to 90 min | Shell eggs                  | S. Enteritidis MB2509, S. Typhimurium T5       | Increased effectiveness at high air moisture content; S. Enteritidis resulted to be more plasma sensitive. |
| Yun et al. (2010)     | 13.56 MHz; 75–150 W; Helium (4 L/min); Up to 120 s | Disposable plastic trays, aluminum foil, and paper cups | 3-strain cocktail of L. monocytogenes (ATCC 19114, 19115, and 19111) | Increased effectiveness at high input power. The lowest D values were obtained on disposable plastic trays. |
| Kim et al. (2011)     | 13.56 MHz; 75–125 W; Helium (10 L/min) and He+O₂ (10 sccm); Up to 90 s | Sliced bacon                | L. monocytogenes (KCTC 3596), E. coli (KCTC 1682), S. Typhimurium (KCTC 1925) | Increased effectiveness at high input power and with the addition of oxygen to the working gas. |
| Lee et al. (2011)     | 2 kW; 50 kHz; He, N₂ (7 L/min), He+O₂, N₂+O₂ (0.07 L/min); Up to 2 min | Agar plates, slices of cooked chicken breast and ham | L. monocytogenes KCTC 3596                     | N₂ was more effective than He. The addition of O₂ to both gases improved their effectiveness, being N₂+O₂ the most effective mixture. The highest and the lowest levels of inactivation were obtained on agar plates and sliced chicken breast, respectively. |
| Alkawareek et al. (2012) | 6 kW; 20–40 kHz; 0.5% O₂+ 99.5% helium (2 L/min); Up to 240 s | Agar plates                 | Pseudomonas aeruginosa PA01 (ATCC BAA-47)     | Increased effectiveness at high frequencies.                                                      |
| Fröhling et al. (2012b) | 27.12 MHz; 10–40 W; Argon (20 L/min); Up to 4 min | Gelrite®-a polysaccharide gel | L. innocua DSM 20649; E. coli DSM 1116         | Increased effectiveness at high discharge power.                                                   |
| Niemira (2012)        | 47 kHz; 5.49 W; Air and N₂; Distance from the plasma jet: 2–6 cm; Up to 20 s | Almonds                     | S. Anatui F4317, S. Stanley H0558, S. Enteritidis PT30, E. coli O157:H7 (C9490, ATCC 35150, ATCC 43894) | Air was more effective. The greatest reductions were observed for E. coli O157:H7 C9490 at a 6cm distance. |
| Bermúdez-Aguirre et al. (2013) | 3.95–12.83 kHz; 60 Hz; Argon (455.33 sccm); Up to 10 min | Lettuce, baby carrots, tomatoes | E. coli ATCC 11 775                           | Effectiveness increased with voltage and decreased with increasing contamination level. Tomatoes, followed by lettuce, were easier to decontaminate than carrots. |

(Continued)
| References                  | Treatment regime | Foods/medium                  | Microorganism                        | Main findings                                                                                                                                 |
|-----------------------------|------------------|-------------------------------|--------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------|
| Han et al. (2013)           | 56 and 70 kV; 50 Hz; Air, 90% N₂ + 10% O₂, 65% O₂ + 30% CO₂ + 5% N₂; Direct and indirect exposure; Up to 120 s; batch | Phosphate Buffered Saline (PBS)       | *E. coli* ATCC 25922, *E. coli* NCTC 12900, *L. monocytogenes* NCTC 11994                                                                   | Greater reduction of viability using higher voltage and working gas mixtures with higher oxygen content. Indirect mode of exposure more effective than direct exposure. *L. monocytogenes* resulted to be more sensitive to plasma treatment. |
| Zuzina et al. (2013)        | 40 kV; Air; Direct and indirect exposure; Up to 300 s; batch | Maximum recovery diluent (M RD) or PBS | *E. coli* ATCC 25922                                                                                                                           | Direct exposure resulted to be more effective than indirect exposure, especially at lower plasma treatment times. The gas mixture 65% O₂ + 30% CO₂ + 5% N₂ was the most effective; increased effectiveness at high air moisture content and after direct exposure to plasma. |
| Patti et al. (2014)         | 50 Hz; 70 kV; Air (3–70% RH); 90% N₂ + 10% O₂, 65% O₂ + 30% CO₂ + 5% N₂; 20°C; Direct and indirect exposure; Up to 120 s; batch | *B. atrophaeus* standard spore strips (Sportrol® Namsa®) |                                                                                                                                            |                                                                                                                        |
| Edelblute et al. (2015)     | 24 kV, 500 Hz; Air (5, 10 L/min); Up to 3 min | Brain Heart Infusion (BHI) agar plates | *E. coli* ATCC 25922 and *S. epidermidis* ATCC 12225                                                                                       | Effectiveness decreased with the flow rate                                                                                                     |
| Takamatsu et al. (2015)     | 16 kHz; 9 kV; 10 W; Ar, O₂, N₂, CO₂, air (1 L/min); 20°C; Up to 120 s; batch | PBS                                                                                     | *S. aureus* ATCC 25923, *P. aeruginosa* ATCC 27853                                                                                       | The greatest antimicrobial effect was obtained with N₂ and CO₂.                                                                                     |
| Butscher et al. (2016)      | 6–10 kHz; 5–15 kHz; Argon; Up to 60 min | Wheat grains; Polycarbonate membrane filters | *Geobacillus stearothermophilus* (ATCC 7953) spores                                                                                          | Higher efficiency by applying faster pulse frequency or higher pulse voltage. Less decontamination effect on wheat grains than on polystyrene. |
| Calvo et al. (2016)         | 1 kHz; 1 W; O₂, N₂ (5–15 L/min); Up to 4 min | Polycarbonate membrane filters       | *L. monocytogenes* CECT 4301, *L. innocua* CECT 910                                                                                       | A higher sensitivity to plasma was observed when the treatment was performed using air; increases in flow rate from 5 to 10 L/min caused an acceleration of bacterial inactivation when air was used; gas flow rate hardly affected NTAP efficiency when nitrogen was used. |
| Cui et al. (2016a)          | 300-600 W; N₂; Up to 3 min | Lettuce; Stainless steel coupons    | Biofilms of *Escherichia coli* O157:H7 (CICC 21530)                                                                                       | Significant lower inactivation was observed at 300 W; The combination of plasma and clove oil exhibited a remarkable synergistic effect. |
| Cui et al. (2016b)          | 300-600 W; N₂ (100 sccm); Up to 3 min | Eggshell                             | *Salmonella Enteritidis* (OCC 21482); *Salmonella Typhimurium* (OCC 22956)                                                                 | Significant lower inactivation was observed at 300 W; The combination of plasma and thyme oil exhibited a remarkable synergistic effect. |
| Gabriel et al. (2016a)      | 2.45 GHz; 450 and 650 W; Air (5 L/min); Up to 25 min; batch | Young coconut liquid endosperm       | Multi-strain cocktails of *E. coli* O157:H7 (7 strains), *S. enterica* (5 strains), *L. monocytogenes* (2 strains) and spoilage bacteria (Klebsiella spp., *Staphylococcus* spp., and *Kluyvera* spp.) | Significant lower inactivation was observed at 450 W; *S. enterica* exhibited the greatest plasma resistance, while *L. monocytogenes* and *Staphylococcus* spp. exhibited the least. |
| Lai et al. (2016)            | 4 W; Air (2–7 m/s); 52–90% RH | Sterilized distilled water          | *Micrococcus luteus* ATCC 4698, *Staphylococcus epidermidis* ATCC 12228, *E. coli* ATCC 10536, *Serratia marcescens* ATCC 6911, *Pseudomonas abalgenes* ATCC 14909. | The inactivation efficacy increased with flow rate and decreased with relative humidity. The inactivation efficacy at 90% R.H. dropped to 10% of the value measured at 55% R.H. |
| Calvo et al. (2017)          | 1 kHz; 1 W; O₂, N₂ (5–15 L/min); Up to 12 min | Polycarbonate membrane filters      | *S. Typhimurium* CECT 443, *S. Enteritidis* CECT 4300                                                                                        | Microbial inactivation was reduced when air was used and with increasing flow rates. |

1Non-specified strain.

RH: relative humidity; pps: pulses per second; sccm: standard cubic centimeters per minute.
oxygen, hydrogen peroxide, singlet oxygen and ozone, all of them with a high antibacterial activity.

Another processing parameter determining the antimicrobial effectiveness of NTAP is the gas moisture content. In fact, it has been occasionally observed (Dobrynin et al., 2011) that the use of completely dry gases is ineffective for E. coli inactivation, and there are several studies which show that an increase in the water content of the gas improves its effectiveness (Table 1). Thus, Ragni et al. (2010) reported that an increase in the air relative humidity from 35 to 65% increased the inactivation of S. Enteritidis and S. Typhimurium from 2.5 to 4.5 log cycles, and attributed this effect to a higher concentration of hydroxyl radicals in the plasma. Similar results were found by Patil et al. (2014) for B. atrophaeus spores. These authors used plasmas with different moisture content (3, 10, 30, 50, and 70%), and obtained a 5 to 6 log reduction at humidities of 3 and 10%, while a complete inactivation was observed at higher humidities. These authors related this higher antimicrobial activity to the increased generation of numerous reactive species, such as N2O3, H2O2, HNO3, or hydroxyl radicals, and, especially, to the decomposition of ozone in the presence of water, with the consequent formation of highly oxidizing species, such as hydroxyl and hydroperoxy radicals, superoxide anion and H2O2. On the contrary, a recent study by Lai et al. (2016) has shown that when the relative air humidity increased from 52 to 81% or from 62 to 81%, the inactivation efficiency of NTAP against E. coli and Staphylococcus epidermidis decreased by 87 and 58%, respectively, which was linked to a decrease in the concentration of negative ions in plasma. These apparently contradictory results could be explained by the existence of an optimum moisture value for achieving a maximum antimicrobial activity. For instance, in the particular case of Aspergillus niger, a progressive increase in microbial inactivation was observed up to a moisture content of 70%, which was the optimum moisture content for NTAP generation (Muranyi et al., 2008).

Gas flow rate also seems to influence the effectiveness of NTAP treatments (Table 1). Lai et al. (2016) reported a steady increase in E. coli, S. epidermidis and P. alcaligenes inactivation when air flow rates increased from 2 to 7 meters/s, linked to a linear rise in the concentration of negative ions in the generated plasma. Results from our research group have demonstrated that the effect of gas flow rate on L. monocytogenes and L. innocua inactivation through NTAP depended on the type of gas used to generate plasma. Indeed, increases in flow rate from 5 to 10 L/min caused an acceleration of bacterial inactivation when air was used, while an additional increase of gas flow from 10 to 15 L/min had a minor impact on microbial inactivation. On the other hand, gas flow rate hardly affected NTAP treatment efficiency when nitrogen was used to generate plasma (Calvo et al., 2016). A similar behavior was also observed for S. Enteritidis and S. Typhimurium (Calvo et al., 2017). In contrast, Edelblute et al. (2015) found that an increase in air flow rate from 5 to 10 liters per minute was accompanied by a loss of efficacy of NTAP for the inactivation of E. coli and S. epidermidis, and attributed this effect to a reduction in ozone and NO2 concentration. Moreover, Miao and Jierong (2009), using oxygen as working gas, suggested the existence of an optimal flow rate for the inactivation of E. coli, reporting that, once this optimal flow rate is surpassed, the lethal effect achieved is reduced. According to these authors, at low flow rates, the number of reactive species, mainly constituted by oxygen radicals, is lower than at high flow rates, but they have a higher average energy and, therefore, the likelihood of each one colliding with microbial cells increases and, consequently, also their antimicrobial effectiveness. However, at higher flow rates the number of reactive species will be higher, but they will have a lower average energy, and their antimicrobial action would be comparatively lower.

NTAP effectiveness can also depend on whether treatments are carried out directly or indirectly. In direct treatments, the product is physically located in the field where plasma is generated, and, therefore, it is in intimate contact with all the photons and chemical species produced. In contrast, in indirect treatments plasma is produced at some distance from the product, and is usually displaced toward it, using a rapid flow of the feed gas. In principle, one would expect that indirect treatments would be less effective in microbial inactivation. Patil et al. (2014) demonstrated that the direct exposure of B. atrophaeus spores to plasmas generated with gases of different composition (air; a mixture of nitrogen [90%] and oxygen [10%]; a mixture of oxygen [65%], CO2 [30%], and nitrogen [5%]) caused the inactivation of at least 6 log cycles, whereas for indirect exposures inactivation rates ranged from 2.1 to 6.3 log units, depending on the type of gas used. Nevertheless, other authors have obtained conflicting results. Thus, Han et al. (2013) found that indirect treatments were more effective against E. coli and L. monocytogenes than direct treatments, and suggested that this fact could be due to the recombination of reactive radicals with a short life before reaching the microorganisms in indirect treatments, giving rise to new chemical species with strong bactericidal effects. However, there are also some authors (Ziuza et al., 2015a) who did not detect differences in effectiveness between direct and indirect treatments when treating L. monocytogenes, E. coli and S. aureus biofilms.

Finally, it should be noted that, in the case of indirect treatments, another parameter that seems to determine NTAP lethality is the distance between the point of plasma generation and the sample. Thus, the results obtained by several authors show that, in general, the antimicrobial effectiveness decreases as that distance increases (Gabriel et al., 2016b; Nishime et al., 2017).

**Microbial-Related Factors**

One of the key advantages of NTAP in comparison to other non-thermal food preservation technologies is its ability to inactivate not only bacteria, molds and yeasts, but also mold ascospores and bacterial spores (Muranyi et al., 2007; Rowan et al., 2007; Shi et al., 2011; Klämpfl et al., 2012; Dasan et al., 2016).

In general, molds and yeasts are more tolerant against the action of plasma than bacteria, and vegetative cells are more sensitive than bacterial spores (Lee et al., 2006; Muranyi et al., 2007; Rowan et al., 2007; Hong et al., 2009; Shi et al., 2011; Klämpfl et al., 2012; Tseng et al., 2012; Takamatsu et al., 2015). However, results obtained by numerous authors studying the effectiveness of NTAP under the same experimental conditions for a wide range of microbial groups have demonstrated that
differences in NTAP resistance among these microbial groups are not as marked as those observed for thermal and other non-thermal processing technologies.

Some studies, in particular, provide interesting insights into the inter-kingdom and inter-species variability in NTAP resistance. Klämpfl et al. (2012) evaluated the effectiveness of an air plasma for the inactivation of vegetative cells of 15 bacterial species, including *E. coli, P. aeruginosa, S. aureus, E. faecalis, B. cereus, B. pumilus, and C. difficile*, one yeast species (*Candida albicans*), and spores of four bacterial species (*B. subtilis, B. pumilus, B. atrophaeus* and *G. stearothermophilus*). They observed that after 30 s of NTAP treatment, between 4 and 6 log reductions were achieved for vegetative cells, while after a 1 min exposure under the same working conditions, from 1 (*G. stearothermophilus*) to 4 (*B. subtilis*) log reductions were attained for bacterial spores. Lee et al. (2006), using a plasma obtained from a mixture of helium and oxygen, also reported a higher sensitivity to NTAP for vegetative cells than for yeasts and bacterial spores, with D values of 0.3 min for *E. coli* and *S. aureus*, 2 min for *S. cerevisiae*, and 14 min for *B. subtilis* spores. Similarly, Takamatsu et al. (2015) found that treatments of around 1 min with a nitrogen-based plasma were required in order to achieve 6 log cycles of inactivation for *E. coli, P. aeruginosa, E. faecalis* and *S. aureus*, while 5 and 15 min were required to achieve the same inactivation levels for *Aspergillus niger* and *B. cereus* spores, respectively. Moreover, Tseng et al. (2012) described D values of 0.50 min for vegetative cells of *E. coli* and *B. subtilis*, while D values obtained for bacterial spores of species belonging to the genus *Bacillus, Geobacillus* and *Clostridium* (*B. subtilis, G. stearothermophilus, C. sporogenes, C. perfringens, C. difficile, and C. botulinum Type A and type B*) ranged between 2.66 (C. *perfringens*) and 8.04 (C. *botulinum type A*) min. Interestingly, spores of *G. stearothermophilus*, generally used as biological indicators of heat sterilization treatments given their extreme thermoresistance, showed a similar NTAP resistance to that of spores of *B. subtilis*, a mesophilic microorganism, suggesting that mechanisms of inactivation by heat and plasma differ, at least to a certain extent. Similar observations were also made by van Bokhorst-van de Veen et al. (2015), who hardly found differences in NTAP resistance among spores of *B. cereus, G. stearothermophilus* and *B. atrophaeus*, obtaining between 3.7 and 4.9 log reductions after a 20 min treatment with a nitrogen-based plasma, while identified large inter-species variations in spore resistance to heat, UV light and chemical oxidants (hydrogen peroxide and sodium hypochlorite).

The higher NTAP resistance exhibited by bacterial spores may be due to the low water content and the high concentration of dipicolinic acid in the spore core, the low permeability of the spore inner membrane, the very robust spore coat, which represents a physical barrier to external agents, the special conformation of the spore DNA, which is saturated by a group of soluble acid proteins (SASP), the presence of detoxifying enzymes in the spore cortex, or the absence of metabolic activity within the spore, which limits the activity of plasma-generated components (Henriques and Moran, 2007). In a recent study by Reineke et al. (2015) analyzing two *B. subtilis* mutant strains, unable to synthesize dipicolinic acid during sporulation and two of the major SASPs, respectively, it was reported that only this latter strain was more sensitive to NTAP than the wild type strain, what suggested that DNA stabilization through SASPs may be responsible, at least in part, for the increased resistance of bacterial spores against NTAP.

The higher NTAP resistance exhibited by yeasts and molds in comparison to vegetative bacterial cells could be due to differences between prokaryotic and eukaryotic cells in cellular structure and molecular composition. On the one hand, the fungal genome is protected by the nuclear membrane, which constitutes an additional diffusion barrier that would contribute to increase the cell resistance to DNA damaging agents. On the other hand, the fungal cell wall is very thick and consists of rigid layers of polysaccharides, which would provide further protection to the cell (Klämpfl et al., 2012).

In relation to bacterial vegetative cells, Gram-positive bacteria are generally considered to be more resistant to NTAP than Gram-negative bacteria due to the fact that they have a thicker layer of peptidoglycan in their cell wall, which increases the rigidity of cellular envelopes, making them more resistant to mechanical damage and hindering the diffusion of reactive species through the envelopes (Lee et al., 2006; Ziuzina et al., 2014, 2015a; Edelblute et al., 2015; Jayasena et al., 2015; Yong et al., 2015a; Puligundla et al., 2017). However, other studies have found similar resistance to NTAP for particular species of both bacterial groups (Lee et al., 2006; Klämpfl et al., 2012; Tseng et al., 2012; Takamatsu et al., 2015), and, even, some authors have described strains of *L. innocua* (Baier et al., 2014), *L. monocytogenes* (Gabriel et al., 2016a), *B. cereus* (Marsili et al., 2002), *S. aureus* and *E. faecalis* (Nishime et al., 2017) as being more sensitive to NTAP than strains of *E. coli*, *S. Typhimurium*, *S. Enteritidis*, *Vibrio para-haemolyticus* and *Pseudomonas aeruginosa*, respectively. These results seem to indicate that it is not possible to draw general conclusions on whether Gram-positive and Gram-negative bacteria have differential sensitiveness against NTAP.

Intraspecific variability in NTAP resistance may also exist. Indeed, Galvin et al. (2013) and Burts et al. (2009) have reported differences of around 1 log cycle of inactivation among *S. aureus* strains exposed either to helium-based or air-based plasmas. Similar results have been described by Niemira (2012) for a group of *E. coli* O157:H7 strains exposed to air plasmas.

It is well-known that the cellular physiological state markedly determines microbial resistance to different inactivation treatments. In fact, bacterial cells harvested during exponential growth phase are known to be more sensitive against heat, pulsed electric fields and high hydrostatic pressures than cells obtained in the stationary phase of growth (Álvarez et al., 2000; Martínez et al., 2003; Mañas and Mackey, 2004). However, based on the results available in the literature, the microbial physiological state exerts little or no influence at all on microbial resistance against NTAP. Thus, no significant differences in D-values were found among *E. coli* (Yu et al., 2006; Deng et al., 2007) and *S. Typhimurium* (Fernández et al., 2013) cultures obtained in logarithmic, early and late stationary phases of growth.

It is worth noting that NTAP treatments have been shown to be capable of inactivating different bacteria (e.g., *S. aureus, E.*
**Characteristics of the Treatment Medium or Food**

The most important factors related to the treatment medium properties determining microbial resistance to NTAP are the medium composition, in the case of liquids and liquid foods, and the topography and certain physical properties, in the case of solid foods.

For liquids, only those plasma-generated reactive species with a relatively long life, such as ozone, atomic oxygen, nitric oxide or hydrogen peroxide, would have the capacity to diffuse through the medium and interact with microbial cells (Ziuzina et al., 2013). However, NTAP has been shown to be very effective in liquid media (Montenegro et al., 2002; Shi et al., 2011; Ziuzina et al., 2013; Surowsky et al., 2014), which has been attributed to the generation of new secondary reactive species through the interaction of the chemical species of plasma with each other or with water or other molecules present in the liquid or gaseous phase of the medium. For example, the coexistence of ozone and hydrogen peroxide in water results in highly...
reactive species such as hydroperoxides and hydroxyl radicals. In addition, atomic oxygen, upon reaction with water, can generate hydrogen peroxide and singlet oxygen (Surowsky et al., 2014; Takamatsu et al., 2015), and the interaction of nitric oxide and superoxide produces peroxynitrite, a highly reactive compound that can readily diffuse through cell membranes (Ercan et al., 2016).

Several authors have reported that NTAP treatment of water and other aqueous solutions causes an increase in both their electrical conductivity and oxidation-reduction potential (Tian et al., 2015; Xu et al., 2016). In addition, several studies have detected through various analytical techniques the presence of reactive oxygen species and reactive nitrogen species in plasma treated water (Ryu et al., 2013; Surowsky et al., 2014; Ercan et al., 2016). However, apart from the presence of these highly reactive species, the rapid acidification that NTAP treatment causes in non-buffered solutions, due to the dissociation of water and the formation of nitric acid and nitrous acid if nitrogen is available, is also believed to be responsible for the inactivation capacity of NTAP in liquid media. Several authors (Rowan et al., 2007; Chen et al., 2009; Naitali et al., 2010; Ryu et al., 2013; Ma et al., 2015) have shown that the pH of plasma treated water decreases gradually with treatment time up to values close to pH 3.0. This same behavior has been observed in non-buffered saline solutions (Oehmigen et al., 2010; Ryu et al., 2013; Ziuizina et al., 2013), which would justify the greater antimicrobial effectiveness of NTAP in these media as compared to that observed in media containing substances with buffering capacity (Chen et al., 2009; Naitali et al., 2010; Ryu et al., 2013; Ziuizina et al., 2013). However, several authors have observed that the level of microbial inactivation attained in NTAP treated media (reaching ∼pH 3.0) is much higher than that observed for media acidified at pH 3.0 with different acidulants (Chen et al., 2009; Liu et al., 2010; Naitali et al., 2010; Ercan et al., 2016). In order to explain this behavior, several authors have speculated about the possibility that low pH values could contribute to the stabilization of some of the reactive species generated in plasmas (Yost and Joshi, 2015) or to the formation of new compounds with antimicrobial potential (Naitali et al., 2010). A possible synergistic effect of low pH and NTAP-derived reactive species on microbial inactivation has been also proposed (Oehmigen et al., 2010; Sun et al., 2012).

The influence of treatment medium composition on plasma effectiveness in liquid media has been scarcely studied, but some reports have shown that the presence of some salts, such as carbonates, phosphates and sodium chloride (Chen et al., 2009; Ryu et al., 2013), reduce the efficacy of NTAP treatments, through reacting with the chemically active species or due to their buffering capacity. The presence of organic matter can also influence the effectiveness of NTAP. Indeed, Rowan et al. (2007) observed a greater inactivation of various enteropathogenic microorganisms in poultry washing water than in distilled water, which was attributed to the formation of nitric acid and carbonic acid from fat and proteins present in the medium. On the other hand, the inactivation of molds and yeasts in complex media has been shown to be much less effective than in a saline solution or water (Ryu et al., 2013).

Regarding microbial inactivation on solid media, NTAP is a very efficient technology for surface decontamination of abiotic surfaces, such as stainless steels, various packaging materials, paper, glass and plastics of diverse nature (Muranyi et al., 2007; Leipold et al., 2010; Miao and Yun, 2011; Klämpfl et al., 2012; Patil et al., 2014; Butscher et al., 2016; Gabriel et al., 2016b). In addition, it is also highly effective for surface decontamination of a wide variety of foods, such as fruits, vegetables, spices, nuts, cereal grains, seeds, seaweed, meat and meat products, eggs, egg products, cheese slices, and dried squid shreds (Table 2). However, microbial survival on different solids is conditioned by surface characteristics, which will determine its possible heating during NTAP treatment, the degree of microbial adhesion, as well as the level of formation and/or adsorption of active species. In particular, surface roughness, porosity and topography are of special relevance (Song et al., 2009; Noriega et al., 2011). A large number of studies have demonstrated a great variability in NTAP effectiveness against both vegetative cells and bacterial spores when identical treatments were applied onto different surfaces (Yun et al., 2010; Noriega et al., 2011; Yong et al., 2015a; Butscher et al., 2016). For example, Miao and Yun (2011) studied the inactivation of E. coli on PET (polyethylene terephthalate), PVC (polytetrafluoroethylene) and PTFE (polytetrafluoroethylene- teflon), and obtained the greatest lethal effect on PET, while the lowest inactivation was found on PTFE. These authors attributed the observed differences to differences in surface wettability of the materials, which was higher for PET, followed by PVC and, finally, PTFE. Fernández et al. (2013) obtained 2.7 log reductions in S. Typhimurium viability on polycarbonate filters after a 2 min NTAP treatment, while it took 15 min to reach 2.7; 1.8 and 0.9 log cycles of inactivation on lettuce, strawberries and cut potato, respectively. Similarly, Lee et al. (2011) and Butscher et al. (2016), comparing the effectiveness of NTAP for the inactivation of L. monocytogenes and G. stearothermophilus on abiotic surfaces and foods, observed that the highest antimicrobial activities were obtained on abiotic surfaces, such as agar plates or polypropylene grains. In addition, it has also been demonstrated that the effectiveness of NTAP as a food decontamination technique varies among different foods. Thus, inactivation of E. coli was more effective on tomato than on lettuce (Bermúdez-Aguirre et al., 2013) or strawberries (Ziuizina et al., 2014), also being faster on carrots than on apples (Baier et al., 2015). For L. monocytogenes a greater lethal effect was also observed on cheese slices than on ham (Song et al., 2009), on sliced ham than on chicken breast filets (Lee et al., 2011), or on tomato than on strawberries (Ziuizina et al., 2014). These results show that food topography should be taken into account when designing effective NTAP treatments. Additionally, differences can be also attributed to the fact that bacteria could have been in the form of planktonic cultures on the surface, biofilms or internalized in the food tissues, and it is well-known that this can show a very important influence on the inactivation effectiveness of NTAP (Ziuizina et al., 2015b; Berardinelli et al., 2016).

Overall, there is a unanimous agreement in that on smooth and polished surfaces the anti-microbial efficiency of NTAP is very high (Noriega et al., 2011; Bermúdez-Aguirre et al., 2013; Fernández et al., 2013; Kim et al., 2014; Butscher et al., 2016; Gabriel et al., 2016b).
| Product | Treatment regime | Maximal log reduction | Impact on quality attributes | References |
|---------|------------------|-----------------------|------------------------------|------------|
| **MEAT AND MEAT PRODUCTS** | | | | |
| Pork loin | He or mixtures He+O₂: 10 slpm; 3 kV; 50 kHz; 3 mm distance | Listeria monocytogenes ~0.59 log | Loss of lightness. Significant reductions in sensory quality parameters (appearance, color, odor, acceptability). | Kim et al., 2013 |
| Pork | Mixtures of N₂ and O₂: 15 kHz; 2 W | Listeria monocytogenes ~2.0 log Escherichia coli O157:H7 ~2.5 log Salmonella Typhimurium ~2.7 log, in 10 min | Minor changes in color and taste. No effects in texture. | Jayasena et al., 2015 |
| Frozen and unfrozen pork | Air; 2.5 m/s; 20 kV; 58 kHz; 25 mm distance | Listeria monocytogenes ~1.0 log Escherichia coli O157:H7 ~1.5 log, in 2 min | Only significant impact on the sensory characteristics (color and appearance) of unfrozen pork | Choi et al., 2016 |
| Beef | N₂+O₂: 15 kHz; 2 W | Listeria monocytogenes ~1.9 log Escherichia coli O157:H7 ~2.8 log Salmonella Typhimurium ~2.6 log, in 10 min | Minor changes in color and taste. No effects in texture. | Jayasena et al., 2015 |
| Chicken meat and skin | He (5 L/min)+O₂ (100 mL/min); 16 kV; 30 kHz; 1 cm distance | Listeria innocua ~1.0 log on skin, in 8 min >3.0 log on meat, in 4 min | Not assessed | Noriega et al., 2011 |
| Chicken breast and thigh | Air | Salmonella enterica ~2.5 log Campylobacter jejuni ~2.4 log, in 3 min | Not assessed | Dirks et al., 2012 |
| Beef jerky | Ar; 20,000 sccm; 200 W; 1 cm distance | Staphylococcus aureus ~1.8 log, in 8 min | No changes in texture and color | Kim et al., 2014 |
| Ar; 15 kHz | Listeria monocytogenes ~2.4 log Escherichia coli O157:H7 ~2.7 log Salmonella Typhimurium ~3.0 log Aspergillus flavus ~3.2 log, in 10 min | No significant changes in metmyoglobin content, shear force, and myofibrillar fragmentation index; changes in peroxides content and color parameters; negative effects on flavor, off-odor, and overall acceptability | Yong et al., 2017 |
| Cooked chicken breast | N₂ (7 L/min)+O₂ (0.07 L/min); 2 kV; 50 kHz; 4 cm distance | Listeria monocytogenes ~4.7 log, in 2 min | Not assessed | Lee et al., 2011 |
| Cooked ham | N₂ (7 L/min)+O₂ (0.07 L/min); 2 kV; 50 kHz; 4 cm distance | Listeria monocytogenes ~6.5 log, in 2 min | Not assessed | Lee et al., 2011 |
| Ham | He; 13.56 MHz; 150 W; 0.6 mm distance | Listeria monocytogenes ~1.7 log, in 2 min | Not assessed | Song et al., 2009 |
| Bacon | He (10 L/min)+O₂ (10 sccm); 13.56 MHz; 125 W; 3 mm distance | Listeria monocytogenes ~2.6 log Escherichia coli ~3.0 log Salmonella Typhimurium ~1.0 log, Total aerobic bacteria ~4.6 log, in 1.5 min | No physical damage on surface tissues. Higher lightness. Lipid oxidation was not observed | Kim et al., 2011 |
| Bresaola | Ar (70%) + O₂ (30%); 27.8 kV; 15.5 W | Listeria innocua >1.5 log, in 10 s | Loss of redness. Induced lipid oxidation. | Red et al., 2012 |
| **DAIRY PRODUCTS** | | | | |
| Cheese | He; 13.56 MHz; 150 W; 0.6 mm distance | Listeria monocytogenes ~8.5 log, in 2 min Escherichia coli ~2.7 log, in 1 min Salmonella Typhimurium ~3.1 log, in 45 s | Not assessed | Song et al., 2009 |
| Air; 15 kHz; 250 W | | | Not assessed | Yong et al., 2015a |
| Cheddar cheese | Ar; 15 kHz; 2 W | Listeria monocytogenes ~5.8 log Escherichia coli O157:H7 ~3.6 log Salmonella Typhimurium ~2.1 log, in 10 min | No changes in color and sensory appearance. Significant reductions in flavor and overall acceptability. Increased off-odor | Yong et al., 2015b |
| **EGGS** | | | | |
| Cooked egg white | N₂ (7 L/min)+O₂ (0.07 L/min); 2 kV; 50 kHz; 4 cm distance | Listeria monocytogenes ~6.7 log Total aerobic bacteria ~2.9 log, in 2 min | Loss of lightness. No impact on sensory attributes (color, flavor, texture, taste, acceptability). | Lee et al., 2012b |
| Cooked egg yolk | N₂ (7 L/min)+O₂ (0.07 L/min); 2 kV; 50 kHz; 4 cm distance | Listeria monocytogenes ~7.1 log Total aerobic bacteria ~2.3 log, in 2 min | Loss of lightness. Significant reductions in flavor, taste and overall acceptability | Lee et al., 2012b |
TABLE 2 | Continued

| Product                  | Treatment regime | Maximal log reduction                                                                 | Impact on quality attributes                                                                 | References |
|-------------------------|------------------|----------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------|------------|
| **FISH AND SEAFOOD**    |                  |                                                                                       |                                                                                                |            |
| Dried squid shreds      | Air; 4 cfm; 4 KHz; 58 kHz; 25 mm distance | Staphylococcus aureus ~0.9 log<br>Marine bacteria ~1.6 log<br>Aerobic bacteria ~2.0 log<br>Yeast and molds ~0.9 log, in 3 min | No significant changes in color characteristics and volatile basic nitrogen content; moisture and thiobarbituric acid reactive substances levels were altered; no significant impact on sensory characteristics (color, flavor, taste, texture, acceptability, overall acceptance) | Choi et al., 2017 |
| **FRUIT, CEREALS AND VEGETABLES** |                  |                                                                                       |                                                                                                |            |
| Blueberries             | Air; 4 cfm; 47 KHz; 549 W; 7.5 cm distance | Total aerobic count ~0.8 log<br>Yeast and molds ~1.2 log, in 2 min | Significant reductions in firmness and anthocyanins; surface color was significantly impacted | Lacombe et al., 2015 |
| Strawberries            | Air; 70 kV; 50 Hz; 140–160 mm distance | Listeria monocytogenes ~4.2 log<br>Escherichia coli O157:H7 ~3.5 log<br>Salmonella Typhimurium ~3.8 log, in 5 min | Not assessed                                                                                   | Ziuza et al., 2014 |
| Pears                   | Air; 5 slm; 500 V | Salmonella spp. ~0.2 log, in 0.5 s | Changes in physicochemical properties were within an acceptable range | Wang et al., 2012 |
| Apples                  | Ar (5 L/min)+O₂ (0.1%); 10 kV; 8 W; 17 mm distance | Escherichia coli ~4.7 log, in 1 min | Not assessed                                                                                  | Baier et al., 2014 |
| Cherry tomatoes         | Air; 70 kV; 50 Hz; 140–160 mm distance | Listeria monocytogenes ~6.7 log, in 2 min<br>Escherichia coli ~3.1 log, in 1 min<br>Salmonella Typhimurium ~6.3 log, in 10 s<br>Aerobic mesophilic bacteria ~4.0 log, in 2 min<br>Yeast and molds ~6.0 log, in 2 min | Not assessed                                                                                  | Ziuza et al., 2014 |
| Tomato                  | Ar (5 L/min)+O₂ (0.1%); 10 kV; 8 W; 17 mm distance | Escherichia coli ~3.3 log, in 1 min | Not assessed                                                                                  | Baier et al., 2014 |
| Corn salad              | Ar (5 L/min)+O₂ (0.1%); 10 kV; 8 W; 17 mm distance | Escherichia coli ~4.1 log, in 1 min | No changes in color were observed                                                              | Baier et al., 2014 |
| Cucumber                | Air; 5 slm; 500 V | Salmonella spp. ~0.4 log, in 0.5 s | Changes in physicochemical properties were within an acceptable range | Wang et al., 2012 |
| Carrots                 | Ar; 5 slm; 500 V | Salmonella spp. ~1.0 log, in 0.5 s | Changes in physicochemical properties were within an acceptable range | Wang et al., 2012 |
| Tomato                  | Ar (5 L/min)+O₂ (0.1%); 10 kV; 8 W; 17 mm distance | Salmonella spp. ~0.4 log, in 0.5 s | Changes in physicochemical properties were within an acceptable range | Wang et al., 2012 |
| Lettuce                 | Air; 4.5 com distance | Aeromonas hydrophila ~5 log, in 15 s | Not assessed                                                                                  | Jahid et al., 2014 |
| Rapeseed seeds          | Air; 2 L/min; 20 kV; 58 kHz; 25 mm distance | Escherichia coli ~2.0 log<br>Bacillus cereus ~1.2 log<br>Salmonella spp. ~1.8 log<br>Total aerobic bacteria ~2.2 log<br>Yeast and molds ~2.0 log, in 3 min | Physicochemical (weight, length, moisture content) and sensory characteristics (appearance, color, flavor, taste, texture) were unaffected. | Puligundla et al., 2017 |
| Orange juice            | Air; 20 kV; 60 kHz; 1.14 W/cm²; batch | Escherichia coli ~5.0 log, in 8 s<br>Staphylococcus aureus ~5.0 log, in 12 s<br>Candida albicans ~5.0 log, in 25 s | Almost no effect on vitamin C content, pH, turbidity or °Brix | Shi et al., 2011 |
| Black peppercorns       | Air; 20 L/min +Ar (14 L/min); 4 cm distance | Salmonella ~5.0 log, in 80 sec | Minimal changes in color                                                                        | Sun et al., 2014 |
| Almonds                 | Air; 30 kV; 2 kHz | Escherichia coli ~5 log, in 30 sec | Not assessed                                                                                  | Deng et al., 2007 |
| Wheat grains            | Air (28 L/min); 8 kV; 10 kHz | Geobacillus stearothermophilus spores ~3.0 log, in 60 min | Functional wheat grain properties (falling number, gluten content) were not negatively affected. | Butscher et al., 2016 |

slpm, standard liters per minute; sccm, standard cubic centimeters per minute; cfm, cubit feet meter.
2016; Cui et al., 2016a), whereas rough, porous and irregular surfaces, such as those of some foods, offer numerous places for microorganisms to fix and hide, thus avoiding the action of plasma (Fernández et al., 2012; Bermúdez-Aguirre et al., 2013; Butscher et al., 2016; Cui et al., 2016a). In fact, some studies using electron microscopy have shown that inoculated microorganisms are capable of finding shelter within various food irregularities, such as cracks, grooves or gaps (Fernández et al., 2013; Jahid et al., 2014; Ziuzina et al., 2014; Cui et al., 2016a).

APPLICATIONS OF NTAP IN THE FOOD INDUSTRY

Once the mechanisms of microbial inactivation by NTAP and the factors that determine its lethal efficacy have been described, the following section of the review article will discuss the potential of this novel technology for specific applications within food processing industries.

NTAP as a Decontamination Technique

NTAP can be used as a decontamination technique for foods, packaging materials, equipment, and even the processing environment itself.

In relation to surface decontamination of foods, several studies have shown the potential of NTAP to improve the microbiological quality of a wide range of solid foods, including strawberries, tomatoes, chicken breast filets, ham, cheese slices, carrots, melon, or lettuce, and liquid foods, such as milk, apple and orange juices, and coconut liquid endosperm (Table 2). Although microbial inactivation rates obtained widely vary among studies, surfaces and microbial species tested, promising results have been reported. For example, with 2 min treatments, 4 to 8 logarithmic reductions were obtained for L. monocytogenes in tomatoes, cooked chicken breast filets and ham, cooked egg white and egg yolk, and cheese slices (Song et al., 2009; Lee et al., 2011, 2012b; Ziuzina et al., 2014), and 4.5 log units for E. coli in carrots (Baier et al., 2015a). Even treatments as short as 10 and 15 s have been able to reduce the population of A. hydrophila in lettuce in 5 log cycles (Jahid et al., 2014) and that of S. Typhimurium in tomatoes in 6 log units (Ziuzina et al., 2014). However, most studies have not evaluated the impact of NTAP treatments on the nutritional and sensory properties of such foods, despite the fact that plasma-generated reactive species could lead to organoleptic changes in the end product. Moreover, information available in the literature is very variable, with some authors identifying minor changes in color, texture, appearance, taste or aroma of foods, while others have reported important modifications in sensory attributes after NTAP treatment (Table 2). The observed discrepancies are probably due to the variability in equipment, plasma generation conditions or type of food used in validation studies. Indeed, several authors have shown how these factors influence the maintenance of food sensory attributes. For example, the application of similar NTAP treatments significantly modified the color of carrots (Baier et al., 2015) and the taste of cooked egg yolk (Lee et al., 2012b), but did not affect these quality attributes in apples and cooked egg white, respectively. Moreover, NTAP treatments using helium as working gas caused changes in pork loin color, whereas this quality attribute was not modified in treatments using a mixture of helium and oxygen (Kim et al., 2013). Baier et al. (2014) also reported changes in leaf lettuce color after a NTAP treatment, which only occurred when the distance between food and plasma generation source was low (5 mm). Furthermore, Rod et al. (2012) described that an increase in potency, treatment time and storage time was accompanied by an increased lipid oxidation and rancidity of bresaola. Similarly, a NTAP treatment of 5–10 min has been shown to induce lipid oxidation in cheese samples (Yong et al., 2015b). However, no significant changes in the fatty acid composition of beef jerky were detected after exposure to plasma for 5 min (Kim et al., 2014).

Although the vast majority of studies have focused on evaluating the potential of NTAP for the decontamination of solid foods, some authors have addressed its efficacy in liquid foods, obtaining also in some occasions promising results. Thus, up to 5 log reductions have been obtained for E. coli in orange and apple juice after a treatment of 8 and 40 s, respectively, and treatments of 12 and 25 s had a similar lethality effect for S. aureus and Candida albicans, respectively (Shi et al., 2011; Liao et al., 2018).

NTAP can be also used for the decontamination of food packaging materials. Currently, the most widely used methods with this aim are based on the employment of dry or wet heat or chemical agents, such as peracetic acid or hydrogen peroxide, which can be applied at relatively high temperatures, ranging from 65 to 80°C, to increase their effectiveness. However, most polymers used as packaging materials do not withstand high temperatures. In addition, some chemical agents also present other drawbacks, such as their difficult handling, maximum limits allowed and the possible generation of toxic waste substances. NTAP is an attractive alternative to chemical decontamination agents, due to its effectiveness as a decontamination technique which successfully inactivates microorganisms, including bacterial and fungal spores, on abiotic surfaces (Muranyi et al., 2007, 2008, 2010; Klämpfl et al., 2012). For example, Muranyi et al. (2007), assessing the efficacy of NTAP for the inactivation of vegetative cells (E. coli, S. aureus, S. Mons, and D. radiodurans), bacterial spores (B. atrophaeus, B. pumilus, C. botulinum, C. sporogenes) and fungal spores (A. niger) on PET, achieved, with treatment times as short as 1 second, between 5.6 and 6.9 log reductions for vegetative cells, between 5.1 and 6.1 log reductions for bacterial spores and 3 log reductions for A. niger conidiospores.

NTAP can even be used for the decontamination of food once packaged, using dielectric barrier discharge equipment (Fröhling et al., 2012a; Rod et al., 2012; Ziuzina et al., 2014; Jayasena et al., 2015). The reactive species generated in this case would simultaneously decontaminate both the packaging material and the food itself. Moreover, once the treatment concludes, the plasma species recombine, the atmosphere returns to its initial composition, and recontamination of the end product after NTAP treatment is thus avoided. The use of NTAP for the decontamination of food once packaged, has been successfully
studied in cherry tomatoes, strawberries, bresaola and pork and beef meat by several research groups (Fröhling et al., 2012a; Rod et al., 2012; Ziuzina et al., 2014; Jayasena et al., 2015).

Another potential application of NTAP in the food industry is the decontamination of food processing surfaces and equipment. In fact, a novel plasma system has been designed to decontaminate slicers, in which the cutting blade of the equipment constitutes one of the electrodes of the dielectric discharge system (Leipold et al., 2010). These authors obtained an effective inactivation of L. innocua inoculated on the cutting blade, representing a novel approach for decontaminating food processing equipment which could be applied to other surfaces, such as conveyors.

Recently, several authors have described the fact that water treated by NTAP, so-called plasma-activated water (PAW), has relevant antimicrobial activity, which persist over a long period of time (Kamgang-Youbi et al., 2009; Ercan et al., 2013; Zhang et al., 2013). This offers new possibilities for decontamination of both surfaces and food, through the treatment of water by NTAP, which will be then used for decontamination purposes. This approach may be a promising alternative to the chemical agents currently used for the sanitation of surfaces, equipment and minimally processed vegetables. In fact, the potential of plasma-activated water has recently been revealed for the decontamination of strawberries (Ma et al., 2015) and mushrooms (Xu et al., 2016). Indeed, a bactericidal effect similar to that obtained using sodium hypochlorite solutions (Issa-Zacharia et al., 2010), with no changes in color or firmness, was observed for strawberries treated with plasma-activated water, with log reductions for S. aureus ranging from 1.7 to 2.3 log depending on the exposure time (Ma et al., 2015). Log reductions achieved following a similar approach for treated mushrooms were between 1.5 and 0.7 log after a week of storage, and mushrooms maintained their initial quality attributes (Xu et al., 2016).

**Other Applications of NTAP in Food Processing**

Apart from being used as a decontamination technique, NTAP may also represent an alternative food processing method which can be used, for example, to obtain the characteristic color of cured meat products without adding nitrates, extend the shelf-life of oils used for coating cookies, reduce the cooking time of cereals, improve the extraction of essential oils or modify the functional properties of flours.

As already mentioned, the interaction of plasma with water results in the generation of reactive oxygen and nitrogen species, including nitrates and nitrites (Oehmigen et al., 2010), reaching concentrations of up to 1,050 ppm (Hao et al., 2014). The generation of nitrates during plasma treatment may raise a flag since use of nitrates in food is restricted, with maximum allowed concentrations, due to their potential toxicological effects. However, Jung et al. (2015) manufactured frankfurters by replacing the nitrates of the curing salts by plasma-treated water and did not detect significant differences in their microbiological or organoleptic quality during 28 days of storage at refrigeration. Interestingly, the residual nitrite content was 30% lower in sausages processed with plasma-treated water. Similar results have been also obtained by directly treating the meat butter with NTAP at different stages of the sausage making process, and a patent for NTAP as a system to eliminate nitrates in meat products has been recently filed (Lim et al., 2015).

NTAP can be used to modify the surface properties of various materials, such as paper, polymers or electronic equipment, to confer them certain functional properties of interest. The idea of using this technology to achieve surface modifications in foods is innovative. NTAP has been used to modulate hydrophobicity or hydrophilicity of oil coatings on cookies, in order to improve palatability and appearance (Misra et al., 2014). These authors demonstrated that NTAP increased surface hydrophobicity, resulting in a greater extension of the incorporated oil and in a more accurate infiltration of oil, without affecting the color, odor and appearance of cookies. Thus, it allowed the preservation of the functionality of the sprayed oil, also decreasing the amount of oil required, which is interesting from a nutritional and economic point of view. A modification of surface properties after NTAP treatments has also been observed for basmati rice (Thirumdas et al., 2015). In this case, NTAP increased the hydrophilicity and water absorption rate of the cereal, and decreased the cooking time, from 20 to 13 min. Electron microscopy images of the treated rice grains revealed the presence of cracks and depressions on their surface, which probably provide routes for faster absorption of water. Although this experiment was carried out under vacuum conditions, which does not allow a direct extrapolation of the results, similar results are expected at atmospheric pressure conditions. Similarly, NTAP is currently being also proposed as a promising technology for the physico-chemical modification of processing surfaces, such as those of food or samples.

**TABLE 3 | Suggested information to be provided in research studies related to non-thermal atmospheric plasma.**

| Category                  | Variable and minimum description to be included |
|---------------------------|-------------------------------------------------|
| Process equipment         | Type of equipment, type of discharge, and electrode configuration |
|                           | Equipment model and vendor name                 |
|                           | Distance between the point of plasma generation and the sample |
| Processing conditions     | Type of gas                                      |
|                           | Gas flow rate and gas moisture content           |
|                           | Energy supplied: voltage, power, and frequency   |
|                           | Processing time                                  |
|                           | Composition of the generated plasma             |
| Food or sample            | Type of food or sample: detailed description     |
|                           | Water activity, pH and composition of the sample |
|                           | Time—temperature history                         |
| Microbial factors         | Genus, species, and strain(s) used               |
|                           | Initial microbial load                           |
|                           | Pre-history of the microbial inoculum            |
|                           | Description of the procedure followed for preparing the inoculum and enumerating microorganisms |
food-contact materials, in a way to prevent microbial adhesion and formation of biofilms, therefore limiting episodes of food cross-contamination with persistent microorganisms colonizing food processing environments. These surface modification techniques are sometimes focused at changing electronegativity, hydrophobicity or morphology/topography of surfaces (Bazaka et al., 2015).

Another possible application of NTAP is its use to improve those processes that involve mass transfer. Indeed, exposure of lemon skins to plasma has been shown to increase the yield of extracted essential oils (Kodama et al., 2014). However, these results should be taken with caution, since essential oils can be oxidized to a certain extent, depending on the gas used for NTAP generation.

Finally, NTAP has been also evaluated as a methodology capable of inducing modifications in the functional properties of proteins. Misra et al. (2015) showed that the secondary structure of gluten became more stable when wheat flour had been treated with an air plasma, and observed significant changes in the rheological properties of the doughs obtained. These changes, both in viscosity and elasticity, depended on the treatment conditions, applied voltage and exposure time. Thus, NTAP can be used as an innovative strategy in order to modulate the functionality of wheat flour during processing of bread, pasta, noodles, cookies, and others.

CONCLUSIONS AND FUTURE PROSPECTS

NTAP is a promising food decontamination technology capable of inactivating bacteria, yeasts, molds, fungal and bacterial spores both on abiotic surfaces (e.g., packaging materials, food processing environments and equipment) and on foods. In addition, NTAP has also other different innovative applications of great interest for food quality and safety improvement.

Although the exact mechanism of microbial inactivation by NTAP is not completely known yet, the cellular envelopes, DNA and proteins are recognized as potential targets, and damages produced to them are the result of the simultaneous action of the different components and reactive species occurring in plasma when the microbial detoxifying capacity is overcome, resulting in the accumulation of dysfunctional macromolecules that compromise cellular viability. The multi-target nature of NTAP could justify the great antimicrobial effectiveness of NTAP in comparison with other technologies that affect a single component or cellular structure. The relative contribution of each individual damage to the total lethal effect of NTAP is unknown yet, since, on the one hand, the composition of plasmas may be very different depending on the conditions of production and, on the other hand, the magnitude of the damage could be in turn dependent on the type of microorganism and the time of treatment (Muranyi et al., 2010; Fröhling et al., 2012b; Tseng et al., 2012; Han et al., 2013, 2016).

Inadequate reporting of experimental methodologies may hinder independent verification and validation of results among different laboratories and equipment units. It is therefore advisable to establish a set of recommended guidelines for conducting and reporting NTAP experiments which would help improve the reliability of future experimental data, thus facilitating process optimization for industrial implementation. Some suggestions on this regard are provided in Table 3.

However, the industrial implementation of NTAP with these aims still requires substantial research efforts, which should be especially focused at (i) assessing the impact that NTAP shows on the nutritional and sensory quality of treated foods in order to facilitate the design of preservation regimes capable of inhibiting pathogenic and spoilage microorganisms while maintaining food quality attributes; (ii) identifying the plasma components and reactive species responsible for the antimicrobial activity of NTAP, which will allow a better selection of processing conditions; (iii) confirming the lack of toxicity of the chemical species generated during NTAP treatments; (iv) developing combined preservation treatments, within a hurdles technology concept, where other inactivation approaches showing a synergic effect when applied together with NTAP will be used; (v) and designing fit-for-purpose equipment susceptible to be easily adopted in a processing line, being compact, energetically efficient, and cost-effective.

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

ACKNOWLEDGMENTS

The authors would like to acknowledge the financial support from Junta de Castilla y León (LE077G18) and Ministerio de Economía y Competitividad (AGL2017-82779-C2).

REFERENCES

Alkawareek, M. Y., Algwari, Q. T., Laverty, G., Gorman, S. P., Graham, W. G., O’Connell, D., et al. (2012). Eradication of Pseudomonas aeruginosa biofilms by atmospheric pressure non-thermal plasma. PLoS ONE 7, e44289. doi: 10.1371/journal.pone.0044289

Alvarez, I., Raso, J., Palop, A., and Sala, F.J. (2000). Influence of different factors on the inactivation of Salmonella senftenberg by pulsed electric fields. Int. J. Food Microbiol. 55, 143–146. doi: 10.1016/S0168-1605(00)00173-2

Álvarez-Ordóñez, A., Fernández, A., Bernardo, A., and López, M. (2009). Comparison of acids on the induction of an acid tolerance response in Salmonella Typhimurium, consequences for food safety. Meat Sci. 81, 65–70. doi: 10.1016/j.meatsci.2008.06.019

Álvarez-Ordóñez, A., Fernández, A., Bernardo, A., and López, M. (2010). Acid tolerance in Salmonella Typhimurium induced by culturing in the presence of organic acids at different growth temperatures. Food Microbiol. 27, 44–49. doi: 10.1016/j.fm.2009.07.015

Álvarez-Ordóñez, A., Fernández, A., López, M., Arenas, R., and Bernardo, A. (2008). Modifications in membrane fatty acid composition of...
Salmonella Typhimurium in response to growth conditions and their effect on heat resistance. *Int. J. Food Microbiol.* 123, 212–219. doi: 10.1016/j.ifm.2008.01.015

Baier, M., Ehlbeck, J., Knorr, D., Herpich, W. B., and Schlüter, O. (2015). Impact of plasma processed air (PPA) on quality parameters of fresh produce. *Postharvest Biol. Tech.* 100, 120–126. doi: 10.1016/j.postharvbio.2014.09.005

Baier, M., Görgen, M., Ehlbeck, J., Knorr, D., Herpich, W. B., and Schlüte, O. (2014). Non-thermal atmospheric pressure plasma: screening for gentle process conditions and antibacterial efficiency on perishable fresh produce. *Innov. Food Sci. Emerg. Technol.* 22, 147–157. doi: 10.1016/j.ifset.2014.01.011

Bazaka, K., Jacob, M. V., Chrzankowski, W., and Ostrikov, K. (2015). Anti-bacterial surfaces: Natural agents, mechanisms of action, and plasma surface modification. *RSC Adv.* 5, 48739–48759. doi: 10.1039/C4RA17244B

Berardinelli, A., Pasquali, F., Cevoli, C., Trevisani, M., Ragni, L., Mancusi, L., Berardinelli, A., Pasquali, F., Cevoli, C., Trevisani, M., Ragni, L., Mancusi, L., Burts, M. L., Alexeff, I., Meek, E. T., and McCullers, J. A. (2009). Use of trichloroacetic acid for decontamination of Bacillus subtilis spores using cold atmospheric plasmas. *IEEE Trans. Plasma Sci.* 37, 631–636. doi: 10.1109/TPS.2006.877739

Dirks, B. P., Dobrynin, D., Fridman, G., Mukhin, Y., Fridman, A., and 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, 22–28. doi: 10.4315/0362-028X-JFP-11-153

Dobrynin, D., Fridman, G., Friedman, A., and Fridman, A. (2009). Physical and biological mechanisms of direct plasma interaction with living tissue. *New J. Phys.* 11, 115020. doi: 10.1088/1367-2630/11/11/115020

Edelblute, C. M., Malik, M. A., and Heller, L. C. (2015). Surface-dependent inactivation of model microorganisms with shielded sliding plasma discharge and applied air flow. *Bioelectrochemistry* 103, 22–27. doi: 10.1016/j.bioelechem.2014.08.013

Ercan, U. K., Smith, J., Ji, H. F., Brooks, A. D., and Joshi, S. G. (2016). Chemical changes in nonthermal plasma-treated N-acetylcysteine (NAC) solution and their contribution to bacterial inactivation. *Sci. Rep.* 6, 20365. doi: 10.1038/srep20365

Ercan, U. K., Wang, H., Ji, H. F., Fridman, G., Brooks, A. D., and Joshi, S. G. (2013). Nonequilibrium plasma-activated antimicrobial solutions are broad-spectrum and retain their efficacies for extended period of time. *Plasma Process. Polym.* 10, 544–555. doi: 10.1002/ppap.201200104

Eto, H., Ono, Y., Ogino, A., and Nagatsu, M. (2008). Low-temperature sterilization of wrapped materials using flexible sheet-type dielectric barrier discharge. *Appl. Phys. Lett.* 93, 221502. doi: 10.1063/1.3039808

Fang, F. C. (2004). Antimicrobial reactive oxygen and nitrogen species: concepts and controversies. *Nat. Rev. Microbiol.* 2, 820–832. doi: 10.1038/nrmicro1004

Fernández, A., Noriega, E., and Thompson, A. (2013). Inactivation of Salmonella enterica serovar Typhimurium on fresh produce by cold atmospheric gas plasma technology. *Food Microbiol.* 33, 24–29. doi: 10.1016/j.fm.2012.08.007

Fernández, A., Shearer, N., Wilson, D. R., and Thompson, A. (2012). Effect of microbial loading on the efficiency of cold atmospheric gas plasma inactivation of Salmonella enterica serovar Typhimurium. *Int. J. Food Microbiol.* 152, 175–180. doi: 10.1016/j.ifm.2011.02.038

Flynn, P. B., Higginbotham, S., Alshraiedeh, N. H., Gorman, S. W., Graham, W. G., and Gilmore, B. F. (2015). Bactericidal efficacy of atmospheric pressure non-thermal plasma (APTP) against the ESKEAPE pathogens. *Int. J. Antimicrob. Agents* 46, 101–107. doi: 10.1016/j.ijantimicag.2015.02.026

Frohling, A., Baier, M. J., Ehlbeck, J., Knorr, D., and Quinlan, O. (2012b). Atmospheric pressure plasma treatment of Listeria innocua and Escherichia coli at polysaccharide surfaces: inactivation kinetics and flow cytometric characterization. *Innov. Food Sci. Emerg. Technol.* 13, 142–150. doi: 10.1016/j.ifset.2011.11.002

Frohling, A., Durek, J., Schnabel, U., Ehlbeck, J., Bolling, J., and Schlüter, O. (2012a). Indirect plasma treatment of fresh pork: decontamination efficiency and effects on quality attributes. *Innov. Food Sci. Emerg. Technol.* 16, 381–390. doi: 10.1016/j.ifset.2012.09.001

Gabriel, A. A., Aba, R. P. M., Tayamora, D. J. L., Colombo, J. C. R., Siringan, M. A. T., Rosario, L. M. D., et al. (2014). Reference organism selection for microwave atmospheric pressure plasma jet treatment of young coconut liquid endosperm. *Food Control* 56, 76–82. doi: 10.1016/j.foodcont.2014.03.043

Gabriel, A. A., Clarisse, M. C., Ugay, F., Siringan, M. A. T., Rosario, L. M. D., Tumlos, R. B., et al. (2016b). Atmospheric pressure plasma jet inactivation of...
Lim, Y. B., Park, S., Kim, H. R., Yong, H. I., Kim, S. H., and Lee, H. J. (2015). **Plasma Treatment Process for Processed Meat and Plasma Treatment Apparatus for Processed Meat.** Korea Patent Submission Number 10-2015-0029641. Submission date at March 3, 2015.

Liu, F., Sun, P., Bai, N., Tian, Y., Zhou, H., Wei, S., et al. (2010). Inactivation of bacteria in an aqueous environment by a direct-current, cold-atmospheric-pressure air plasma microjet. *Plasma Process. Polym.* 7, 231–236. doi: 10.1002/ppap.200900070

Liu, H., Chen, J., Yang, L., and Zhou, Y. (2008). Long-distance oxygen plasma sterilization: effects and mechanisms. *Appl. Surf. Sci.* 254, 1815–1821. doi: 10.1016/j.apsusc.2007.07.152

Liu, X. H., Hong, F., Guo, Y., Zhang, J., and Fang, J. (2015). Non-thermal plasma-activated water inactivation of food-borne pathogen on fresh produce. *J. Hazard. Mater.* 300, 643–651. doi: 10.1016/j.jhazmat.2015.07.061

Mañas, P., and Mackey, B. M. (2004). Morphological and physiological changes of *Staphylococcus aureus* by an atmospheric non-thermal plasma jet. *Plasma Sci. Technol.* 15, 339–342. doi: 10.1088/1009-0630/15/5/009

Ma, R., Wang, G., Tian, Y., Wang, K., Zhang, J., and Fang, J. (2015). Non-thermal plasma-activated water inactivation of food-borne pathogen. *J. Hazard. Mater.* 300, 643–651. doi: 10.1016/j.jhazmat.2015.07.061

Miao, H., and Yun, G. (2011). The sterilization of *Listeria innocua*. *Food Microbiol.* 28, 1293–1300. doi: 10.1016/j.fm.2009.02.010

Nishime, T. M. C., Borges, A. C., Koga-Ito, C. Y., Machida, M., Hein, L. R. O., and Kostov, K. G. (2017). Non-thermal atmospheric pressure plasma jet applied to inactivation of different microorganisms. *Surf. Coat. Technol.* 312, 19–24. doi: 10.1016/j.surfcoat.2016.07.076

Noriega, E., Shama, G., Laca, A., Díaz, M., and Kong, M. G. (2011). Cold atmospheric gas plasma disinfection of meat and chicken meat contaminated with *Listeria innocua*. *Food Microbiol.* 28, 1293–1300. doi: 10.1016/j.fm.2011.05.007

Oehmigen, K., Hahnel, M., Brandenburg, R., Wilke, C., Weltmann, K., and von Woedtke, T. (2010). The role of acidification for antimicrobial activity of atmospheric pressure plasma in liquids. *Plasma Process. Polym.* 7, 250–257. doi: 10.1002/ppap.200900770

Pan, Y., Breidt, F., and Katheriour, S. (2006). Resistance of *Listeria monocytogenes* biofilm to sanitizing agents in a simulated food processing environment. *Appl. Environ. Microbiol.* 72, 7711–7717. doi: 10.1128/AEM.01056-06

Patil, S., Moiseev, T., Misra, N. N., Cullen, P. J., Moisner, J. P., Keener, K. M., et al. (2014). Influence of high voltage atmospheric cold plasma process parameters and role of relative humidity on inactivation of *Bacillus atropaeus* spores inside a sealed package. *J. Hosp. Infect.* 88, 162–169. doi: 10.1016/j.jhin.2014.08.009

Peri, S., Shama, G., Hobman, J. L., Lund, P. A., Kershaw, C. J., Hidalgo-Arroyo, G. A., et al. (2007). Probing bacterial mechanisms induced by cold atmospheric plasmas with *Escherichia coli* mutants. *Appl. Phys. Lett.* 90: 73902. doi: 10.1063/1.2458162

Pomposiello, P. J., and Demple, B. (2002). Global adjustment of microbial physiology during free radical stress. *Adv. Microb. Physiol.* 46, 319–341. doi: 10.1016/S0065-994X(02)00670-9

Puligundla, P., Kim, J. W., and Mok, C. (2017). Effect of corona discharge plasma jet treatment on decontamination and spouting of raspessed (*Brassica napus L.* seeds. *Food Control* 71, 376–382. doi: 10.1016/j.foodcont.2016.07.021

Purevdorj, D., Igruna, N., Shimoda, M., Ariyoda, O., and Hayakawa, I. (2001). Kinetics of inactivation of *Bacillus* spores using low temperature argon plasma at different microwave power densities. *Acta Biotechnol.* 21, 333–342. doi: 10.1002/1521-3846(20011121)21:4<333::AID-ABIO333>3.0.CO;2-6

Ragni, L., Berardinelli, A., Vannini, L., Montanari, C., Sirri, F., Guerzoni, M. E., et al. (2010). Non-thermal atmospheric gas plasma device for surface decontamination of shell eggs. *J. Food Eng.* 100, 125–132. doi: 10.1016/j.jfoodeng.2010.03.036

Reineke, K., Langer, K., Hertwig, C., Ehbeck, J., and Schlüter, O. (2015). The impact of different process gas compositions on the inactivation effect of an atmospheric pressure plasma jet on *Bacillus* spores. *Innov. Food Sci. Emerg. Technol.* 20, 112–118. doi: 10.1016/j.ifset.2015.03.019

Red, S. K., Hansen, F., Leipold, F., and Knochel, S. (2012). Cold atmospheric pressure plasma treatment of ready-to-eat meat: inactivation of *Listeria innocua* and changes in product quality. *Food Microbiol.* 30, 233–238. doi: 10.1016/j.fm.2011.12.018

Rowan, N. J., Espie, S., Harrower, J., Anderson, J. G., Marsili, L., and MacGregor, S. J. (2007). Pulsed-plasma gas discharge inactivation of microbial pathogens in chilled poultry wash water. *J. Food Prot.* 70, 2805–2810. doi: 10.4315/0362-028X-70.12.2805

Ryu, Y. H., Kim, Y. H., Lee, J. Y., Shim, G. B., Uhm, H. S., Park, G., et al. (2013). Effects of background fluid on the efficiency of inactivating yeast with non-thermal atmospheric pressure plasma. *PLoS ONE* 8, e66231. doi: 10.1371/journal.pone.0066231

Sharma, A., Collins, G., and Pruden, A. (2009). Differential gene expression in *Escherichia coli* following exposure to nonthermal atmospheric pressure plasma. *J. Appl. Microbiol.* 107, 1440–1449. doi: 10.1111/j.1365-2672.2009.04323.x

Shi, X., and Zhu, X. (2009). Biofilm formation and food safety in food industries. *Trends Food Sci Technol.* 20, 407–413. doi: 10.1016/j.tifs.2009.01.054

Shi, X. M., Zhang, G. J., Wu, X. L., Li, Y. X., Ma, Y., and Shao, X. J. (2011). Effect of low-temperature plasma on microorganism inactivation and quality of freshly squeezed orange juice. *IEEE T. Plasma Sci.* 39, 1591–1597. doi: 10.1109/TPS.2011.2142017

Song, H. P., Kim, B., Choe, J. M., Jung, S., Moon, S. Y., Choe, W., et al. (2009). Evaluation of atmospheric pressure plasma to improve the safety of slice cheese and ham inoculated by 3-strain cocktail *Listeria monocytogenes*. *Food Microbiol.* 26, 432–436. doi: 10.1016/j.fm.2009.02.010
Sun, P., Wu, H. Y., Bai, N., Zhou, H. X., Wang, R. X., Feng, H. Q., et al. (2012). Inactivation of *Bacillus subtilis* spores in water by a direct-current, cold atmospheric-pressure air plasma microjet. *Plasma Process Polym.* 9, 157–164. doi: 10.1002/ppap.201100041

Sun, S., Anderson, N. M., and Keller, S. (2014). Atmospheric pressure plasma treatment of black peppercorns inoculated with *Salmonella* and held under controlled storage. *J. Food Sci.* 79, 2441–2446. doi: 10.1111/1750-38 41.12696

Surowsky, B., Fischer, A., Schlüter, O., and Knorr, D. (2013). Cold plasma effects on enzyme activity in a model food system. *Innov. Food Sci. Emerg. Technol.* 19, 146–152. doi: 10.1016/j.ifset.2013.04.002

Surowsky, B., Frohling, A., Gottschalk, N., Schlüter, O., and Knorr, D. (2014). Impact of cold plasma on *Citrobacter freundii* in apple juice: inactivation kinetics and mechanisms. *Int. J. Food Microbiol.* 174, 65–71. doi: 10.1016/j.ijfoodmicro.2013.12.031

Takamatsu, T., Uehara, K., Sasaki, Y., Hidaka, M., Matsumura, Y., Iwasawa, A., et al. (2015). Microbial inactivation in the liquid phase induced by multigas plasma jet. *PLoS ONE* 10, e0132381. doi: 10.1371/journal.pone.0132381

Thirumadas, R., Deshmukh, R. R., and Annapure, U. S. (2015). Effect of low temperature plasma processing on physicochemical properties and cooking quality of basmati rice. *Innov. Food Sci. Emerg. Technol.* 31, 83–90. doi: 10.1016/j.ifset.2015.08.003

Tian, Y., Ma, R., Zhang, Q., Feng, H., Liang, Y., Zhang, J., et al. (2015). Assessment of the physicochemical properties and biological effects of water activated by non-thermal plasma above and beneath the water surface. *Plasma Process. Polym.* 12, 439–449. doi: 10.1002/ppap.201400082

Tseng, S., Abramzon, N., Jackson, J. O., and Lin, W.-J. (2012). Gas discharge plasmas are effective in inactivating *Bacillus* and *Clostridium* spores. *Appl. Microbiol. Biotechnol.* 93, 2563–2570. doi: 10.1007/s00253-011-3661-0

van Bokhorst-van de Veen, H., Xie, H., Esveld, E., Abee, T., Mastwijk, H., and Nierop Groot, M. (2015). Inactivation of chemical and heat-resistant spores of *Bacillus* and *Geobacillus* by nitrogen cold atmospheric plasma evokes distinct changes in morphology and integrity of spores. *Food Microbiol.* 45, 26–33. doi: 10.1016/j.fm.2014.03.018

Vluguels, M., Shama, G., Deng, X. T., Greenacre, E. J., Brocklehurst, T. F., and Kong, M. G. (2005). Atmospheric plasma inactivation of biofilm-forming bacteria for food safety control. *IEEE T. Plasma Sci.* 33, 824–828. doi: 10.1109/TPS.2005.844524

Wang, R. X., Nian, W. F., Wu, H. Y., Feng, H. Q., Zhang, K., Zhang, J., et al. (2012). Atmospheric-pressure cold plasma treatment of contaminated fresh fruit and vegetable slices: inactivation and physiochemical properties evaluation. *Eur. Phys. J. D.* 66, 276. doi: 10.1140/epjd/e2012-30053-1

Xu, Y., Tian, Y., Ma, R., Liu, Q., and Zhang, J. (2016). Effect of plasma activated water on the postharvest quality of button mushrooms, *Agaricus bisporus*. *Food Chem.* 197, 436–444. doi: 10.1016/j.foodchem.2015.10.144

Yong, H. I., Kim, H. J., Park, S., Kim, K., Choe, W., Yoo, S. J., et al. (2015b). Pathogen inactivation and quality changes in sliced cheddar cheese treated using flexible thin-layer dielectric barrier discharge plasma. *Food Res. Int.* 69, 57–63. doi: 10.1016/j.foodres.2014.12.008

Yong, H. I., Lee, H., Park, S., Park, J., Choe, W., Jung, S., et al. (2017). Flexible thin-layer plasma inactivation of bacteria and mold survival in beef jerky packaging and its effects on the meat's physicochemical properties. *Meat Sci.* 123, 151–156. doi: 10.1016/j.meatsci.2016.09.016

Yost, A. D., and Joshi, S. G. (2015). Atmospheric nonthermal plasma-treated PBS inactivates *Escherichia coli* by oxidative DNA damage. *PLoS ONE* 10(10): e0139903. doi: 10.1371/journal.pone.0139903

Yu, H., Perni, S., Shi, J. J., Wang, D. Z., Kong, M. G., and 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, 1532–1539. doi: 10.1111/j.1365-2672.2006.03033.x

Yun, H., Kim, B., Jung, S., Kruk, Z. A., Kim, D. B., Choe, W., et al. (2010). Inactivation of *Listeria monocytogenes* inoculated on disposable plastic tray, aluminum foil, and paper cup by atmospheric pressure plasma. *Food Control* 21, 1182–1186. doi: 10.1016/j.foodcont.2010.02.002

Zhang, Q., Liang, Y., Feng, H., Ma, R., Tian, Y., Zhang, J., et al. (2013). A study of oxidative stress induced by non-thermal plasma-activated water for bacterial damage. *Appl. Phys. Lett.* 102, 203701. doi: 10.1063/1.4807133

Ziuzina, D., Boehm, D., Patil, S., Cullen, P. J., and Bourke, P. (2015a). Cold plasma inactivation of bacterial biofilms and reduction of quorum sensing regulated virulence factors. *PLoS ONE* 10(9), e0138209. doi: 10.1371/journal.pone.0138209

Ziuzina, D., Han, L., Cullen, P. J., and Bourke, P. (2015b). Cold plasma inactivation of internalised bacteria and biofilms for *Salmonella enterica* serovar *Typhimurium*, *Listeria monocytogenes* and *Escherichia coli*. *International J. Food Microbiol.* 210, 53–61. doi: 10.1016/j.ijfoodmicro.2015.05.019

Ziuzina, D., Patil, S., Bourke, P., Keener, K., and Cullen, P. J. (2013). Atmospheric cold plasma inactivation of *Escherichia coli* in liquid media inside a sealed package. *J. Appl. Microbiol.* 114, 778–787. doi: 10.1111/jam.12087

Ziuzina, D., Patil, S., Cullen, P. J., Keener, K. M., and Bourke, P. (2014). Atmospheric cold plasma inactivation of *Escherichia coli* serovar *Typhimurium* and *Listeria monocytogenes* inoculated on fresh produce. *Food Microbiol.* 42, 109–116. doi: 10.1016/j.fm.2014.02.007

**Conflict of Interest Statement:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

*Copyright © 2019 López, Calvo, Prieto, Múgica-Vidal, Muro-Fraguas, Alba-Elías and Alvarez-Ordóñez. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.*