A review on microbiological decontamination of fresh produce with nonthermal plasma

NONTHERMAL PLASMA IN FRESH PRODUCE DECONTAMINATION: A REVIEW ON MICROORGANISMS INVOLVED IN FOODBORNE OUTBREAKS.

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NONTHERMAL PLASMA IN FRESH PRODUCE DECONTAMINATION: A REVIEW ON MICROORGANISMS INVOLVED IN FOODBORNE OUTBREAKS.

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Summary

Food safety is a critical public health issue for consumers and the food industry because microbiological contamination of food causes considerable social and economic burdens on health care. Most foodborne illness comes from animal production, but as of the mid-1990s in the United States and more recently in the European Union, the contribution of fresh produce to foodborne illness has rapidly increased.

Recent studies have suggested that sterilisation with nonthermal plasma could be a viable alternative to the traditional methods for the decontamination of heat-sensitive materials or food because this technique proves capable of eliminating microorganisms on surfaces without altering the substrate. In the last ten years, researchers have used nonthermal plasma in a variety of food inoculated with many bacterial species. All of these experiments were conducted exclusively in a laboratory and, to our knowledge, this technique has not been used in an industrial setting. Thus, the purpose of this review is to understand whether this technology could be used at the industrial level. The latest researches using nonthermal plasma on fresh produce were analysed. These evaluations have focused on the Log reduction of microorganisms and the treatment time.

Keywords

Decontamination, foodborne outbreak, fresh produce, pathogenic microorganism, nonthermal plasma

Microorganisms and foodborne outbreak

Currently, the global burden of foodborne diseases due to the presence of contaminating and pathogenic microorganisms in food remains high although in the 1990s some foodborne illnesses declined due to an intensive and focused effort in checking some parts of the food chain (Purayidathil and Ibrahim, 2012; Braden and Tauxe, 2013; WHO, 2015). In 2015, the Foodborne Diseases Active Surveillance Network (FoodNet) identified 20,107 confirmed cases of infections, 4,531 hospitalisations and 77 deaths caused by nine pathogens transmitted through food at 10 sites, which encompassed 15% of the U.S. population. The incidence of confirmed cases per 100,000 were reported for *Salmonella* (15·89), C*ampylobacter* (12·97), *Shigella* (5·53), *Cryptosporidium* (3·31), Shiga Toxin-Producing *E. coli* (STEC) non-O157 (1·64), STEC O157 (0·95), *Vibrio* (0·39), *Yersinia* (0·29), *Listeria* (0·24) and *Cyclospora* (0·13). Compared with the incidence rate in 2012–2014, the incidence of confirmed infections in 2015 was significantly higher for STEC non-O157 (40% increase) and *Cryptosporidium* (59% increase) (Huang *et al*., 2016). The 2014 overall incidence for the nine pathogens was significantly lower compared with the 1996–1998 data (-29%) with further reductions in 2006–2008 (-3%) and 2011–2013 (-8%) (CDC 2014). The incidence of infections and the number of hospitalisations were the highest for *Salmonella*; the percent of hospitalisation and deaths were the highest for *Listeria*.

The range of infections and the food sources that transmits them have changed as new pathogens have emerged or are better detected, the high-risk population has increased, the previous syndromes of unknown cause have been linked to foodborne infection, and the nature and sources of the food we eat has changed (Braden and Tauxe, 2013). Most foodborne illnesses come from animal production, but as of the mid-1990s in the US and more recently in the EU, the contribution of fresh produce to foodborne illness has rapidly increased (Nguyen-the *et al.* 2016). Many studies are reporting foodborne outbreaks due to fresh produce in the United State, the European Union, and in Australia over the last twenty years (Callejon *et al.* 2015; Nuesch-Inderbinen and Stephan 2016; Yeni *et al.* 2016). Food of non-animal origin comprise a wide range of fruit, vegetables, salads, seeds, nuts, cereals, herbs, spices, fungi, and algae. Food of non-animal origin are commonly consumed in a variety of forms: (i) ready-to-eat (RTE) foods in which the constituents are raw or minimally processed (e.g., fresh-cut and prepackaged) and (ii) foods that are processed with heat or other inactivation treatments. Food of non-animal origin are a major component of almost all meals. Mixed-ingredient RTE salads are considered healthy and convenient and are popular with consumers.

From 2004 to 2012, the United States and European Union have reported a total of 377 and 198 fresh produce-associated outbreaks, respectively. This high number of outbreaks linked to fresh produce may be due to improved surveillance, but it might also be related to changes in consumer food preferences, food production and distribution practices, as well as the emergence of new foodborne pathogens (Harris *et al*. 2003; Sivapalasingam *et al*. 2004, Callejon *et al.* 2015). In the United States, the absolute number of outbreaks due to fresh produce ranges from 23 to 60 per year and does not show a clear trend during this period. In fact, there were substantial increases in 2006 (57 outbreaks), 2008 (51 outbreaks), and 2011 (60 outbreaks) (CDC 2014). In the European Union, the number of outbreaks fluctuate between 10 and 42, underlining increases in 2006 (29 outbreaks), 2009 (34 outbreaks) and 2010 (44 outbreaks). In the EU, sprouted fenugreek seeds (a fresh produce) were involved in the major food outbreak in 2011, which resulted in 3000 cases of bloody diarrhoea, 852 cases of haemolytic-uremic syndrome (HUS) and 53 deaths.

A broad spectrum of microorganisms and food vehicles are involved in fresh produce-associated outbreaks. Norovirus was the main pathogen responsible (59% of foodborne illnesses in the US and 53% of foodborne illnesses in the EU) followed by *Salmonella* (18% of foodborne illnesses in the US and 20% of foodborne illnesses in the EU). Specifically, in the US, Norovirus outbreaks were strongly correlated with the consumption of salads; in the EU, this pathogen was mainly linked to berries (raspberries). *Salmonella* was the most common bacterial pathogen responsible for fresh produce outbreaks, accounting for nearly half of the outbreaks due to bacteria (53% in the US and 50% in the EU). *Salmonella* outbreaks was the microorganism involved in the majority of sprout-associated outbreaks (14 in the US and 11 in the EU). Regarding other microorganisms, *E. coli* and *Campylobacter* outbreaks were more prevalent in the US than in the EU. Regarding food vehicle, *E. coli* was associated with the consumption of various fresh vegetables, fruits

and sprouts, whereas *Campylobacter jejuni* was involved in fresh produce outbreaks linked to the consumption of salad, lettuce, tomatoes and melons (Callejon *et al.* 2015).

Surveys of fresh produce have revealed contamination with pathogenic bacteria in commodities such as tomatoes, lettuce, salad greens, sprouting seeds, unpasteurized fruit juice, cantaloupe and nuts (EFSA 2013). At the same time, evaluation of prevalence and trends of bacterial contamination in fresh fruits and vegetables initiated in Europe. In Sweden, a survey on prepackaged ready-to-eat (RTE) mixed ingredient salad showed that 9% of the 141 samples were contaminated with *Listeria monocytogenes*. The results of this study indicate that pathogenic bacteria can be present in RTE salads in Sweden (Soderqvist *et al.* 2016). This public health concern should be addressed by improving the hygiene of the raw ingredients, the production environment and the cold temperature from the manufacturer to the consumer. There is a great need to address possible decontamination treatments for fresh fruit and vegetables from production systems that would otherwise lack adequate safety. However, such treatments raise issues of acceptability by consumers and the high costs incurred. The final use of fruits and vegetables in the food distribution chain, e.g., eaten raw versus heat treated, could also be adapted with regard to the microbiological risks that they could pose.

Plasma Chemistry and Reactive Species

The term ‘‘plasma’’ refers to a partially or completely ionized gas consisting of photons, ions, free electrons and atoms in their fundamental or excited states having a net neutral charge. The free electric charges (electrons and ions) make plasma electrically conductive, internally interactive, and strongly responsive to electromagnetic fields.

Electrons and photons are usually designated as ‘‘light’’ species in contrast to the rest of the constituents designated as ‘‘heavy’’ species. The chemical effects occurring in an electrical discharge are the consequence of energy injection into a gas stream by way of electron-impact processes under the influence of an electric field. Collisions of energetic electrons with neutral species produce ionizations, fragmentations of molecules, and electronic, vibrational, and rotational excitations of the neutral gas. Plasma chemistry can be divided into two parts: 1) a volume chemistry, which addresses the formation and loss reactions of species in the discharge volume, and 2) a surface chemistry, implying adsorption and desorption of molecules at the substrate surface or etching (Fridman 2008).

The elementary processes in nonthermal plasma (NTP) volume can be broadly divided into a primary process and a secondary process based on the timescale of streamer propagation. Figure 1a summarizes the typical timescale of the elementary processes in NTP. The primary process (typical timescale of approximately 10^{-8} s) includes ionization, excitation, dissociation, light emission, and charge transfer. The efficiency of the primary process highly depends on the energization methods and their parameters, such as the pulse, direct current (DC) + pulse, alternating current (AC), AC + pulse or DC, voltage rise-time, and frequency.The products of primary processes (electrons, radicals, ions, and

excited molecules) go to subsequent chemical reactions in the secondary process. Some additional radical species and reactive molecules are also formed by radical-neutral recombination in the secondary process. The timescale of the secondary process is very fast (approximately 10^{-3} s), so the residence time of the gas in the NTP reactor has modest or no influence on the overall results. The total efficiency η_T of the NTP process will be the product of the efficiencies of the primary process ($\eta_{Primary}$) and of the chemical reactions in the secondary process ($\eta_{Secondary}$) (i.e.),$\eta_T = \eta_{Primary} \cdot \eta_{Secondary}$ (Kim 2004).

Plasma-Surface Interaction

Many fundamental processes take place at the plasma-substrate interface. The plasma can deliver kinetic energy through ions accelerated in the sheaths and by vibrationally-excited molecules, potential energy through charged ions and metastable states, chemical energy through plasma-produced reactive atoms, radicals and electromagnetic energy from the decay of electronically excited species. The interactions with the surface are complex and regulated by specific rate constants (K). The relation between the kinetics of surface processes and the kinetics of processes taking place within the plasma near the surface can be seen in Figure 1b. The atom and molecule entering the plasma are converted into activated species with kinetic rate constant **Ka**, following a specific reaction channel. The activated species arrive at the surface and may be adsorb there

A + (s) → A(s) (**Kb**).

After adsorption, they may make a chemical reaction with the surface

A(s) + B(s) → AB(s) + (s) (**Kc**), which then desorbs

AB(s) → AB + (s) (**Ke**), or which may spread onto the same surface (**Kd**).

Alternatively, the reactive atoms may desorb without undergoing any reaction

A(s) → A + (s) (**Ki**)

or may undergo associative desorption with a reactive atom already on the surface

A(s) + B(s) → AB + (s) (**Kj**).

Reactive species may couple in the plasma to form larger nuclei of materials and dust particles (**Kg**).

Finally, the product resulting from the recombination between desorbed species in the plasma may return to the surface (**Kh**) (D'Angelo 2010).. Note that e represents an electron, (s) an open surface site, A(s) a specie A bound to the surface, B(s) a specie B bound on the surface.

How plasma acts on the microorganisms

The cytoplasmic membrane is the barrier between the inner compartment of the cell and the external environment, and it is the main, and a very crucial target for most of the techniques of decontamination (chemical and/or physical), targeting the membrane structure or specific functions. Comprehension of the kinetics of cell inactivation by experimental investigation is the most important step to obtain a consistent temporal measure of microbial destruction.

One kinetic measurement parameter is known as Decimal value (D). This parameter has been used widely by studying sterilization by plasma. The D value is the time required to reduce an original concentration of microorganisms by 90%. The first complete analysis of literature on low pressure cold plasma (LPCP) sterilization was shown by Moisan *et al.* (2001) regarding the role of UV photons and reactive species on the survival curve of microorganisms. In the classical sterilization process, such plots show a single straight line, while plasma sterilization shows a survival diagram with two or three different linear segments. The analysis of the three single steps in the survival curve suggested many basic mechanisms: (i) direct destruction by UV irradiation of the genetic material of the microorganism; (ii) erosion of the microorganism, atom by atom, through intrinsic photodesorption by UV irradiation to form volatile compounds combining atoms intrinsic to the microorganisms; and (iii) erosion of the microorganism, atom by atom, through etching. The etching results from the adsorption of reactive species from the plasma (glow or afterglow) on the microorganism with which they subsequently undergo a chemical reaction to form volatile compounds. In certain cases, the etching mechanism is enhanced by UV photons acting synergistically with the reactive species (Laroussi *et al*. 2005). Laroussi *et al.* (2002) studied the germicidal effect of atmospheric pressure cold plasma. Depending on the type of microorganism, the type of medium in which the microorganisms are seeded, the method of exposure (direct or remote exposure) and the experimental work on the germicidal effects of cold atmospheric pressure plasma have shown survivor curves with different shapes, revealing that bacteria inactivation by non-equilibrium high pressure plasmas is a composite process. If UV is present in a dominant manner, the survivor curves often exhibit a first rapid step (small D value) followed by a second slower step. When the presence of UV is not dominant, such as in the case of an air plasma, single-slope survivor curves were mostly observed. However, in many cases, multi-slope curves have also been reported.

Montie *et al*. (2000) proposed three mechanisms of cell destruction in the case of high-pressure cold plasmas assuming the presence of oxygen and moisture in the gas mixture: 1) the susceptibility of unsaturated fatty acids to attacks by hydroxyl radicals caused lipid peroxidation as confirmed by Dolezova and Lukes (2015); 2) the susceptibility of amino acids to oxidation caused protein; and 3) the formation of base adducts, which are generated through reactions with oxygen radicals that caused DNA oxidation.

Mendis *et al*. (2000) suggested that the membrane rupture of gram-negative bacteria is caused by charge accumulation on the outer surface of the membrane; gram-positive bacteria do not undergo visible morphological changes (Laroussi

et al. 2002, 2005). However, different types of bacteria show a drastic reduction in cell viability. The diffusion of plasma-generated reactive species through the cell membrane induces the promotion of some reactions with the inner biomaterials: these reactions lead to cell death or non-viable cells (Lackmann and Bandow 2014). Recently, Guo *et al*. 2015 postulated an explanation to justify the role of UV radiation in different plasma conditions. When UV radiation played a major role in the inactivation process, the gases were Ar or a N_2/O_2 mixture in combination with microwave-driven discharge. In this context, the ionization energy of Ar is higher than N_2 and O_2, making N_2 and O_2 ions (i.e., N_2^+, N^+, O_2^+, and O^+). In these conditions, the amount of positive nitrogen ions and negative oxygen ions was similar, and NO was generated with more respect to the electric discharge directly in the air. A similar mechanism happens with the excited state of NO. UV radiation in this experimental condition plays a main role in bacterial inactivation because their doses in the 200–300 nm wavelength range is higher than other experimental conditions.

Chemical reactions of plasma on microbial cell

As described, the effect of plasma treatment on microbial cells is mainly due to the plasma ions and cell interactions. Commonly used oxygen and nitrogen gas plasmas are excellent sources of reactive oxygen-based and nitrogen-based reactive species (O, O_2, O_3, OH, NO, and NO_2) because they have a direct oxidative effect on the outer surface of microbial cells. Atomic oxygen can potentially be a very effective sterilizing agent, with a chemical rate constant for oxidation at room temperature of approximately 10^6 times that of molecular oxygen (Misra *et al.* 2011).

The lipid bilayer of microbial cells is more susceptible to atomic oxygen as the reactivity of atomic oxygen is much higher than that of molecular oxygen, which can degrade lipids, proteins and DNA of cells. The damage of the double bonds in the lipid bilayer causes impaired transportation of molecules in and out of the cell. The bombardment of reactive oxygen species (ROS) on the surface of bacterial cells also disrupts the membrane lipids. During plasma use, microorganisms are exposed to an intense bombardment by the radicals, which most likely provoke surface lesions that the living cell cannot repair sufficiently, a process termed “etching”. Plasma etching is based on the interaction of relative energetic ions and activated species with the molecules of the substrate. The accumulation of charges imparts an electrostatic force at the outer surface of cell membranes and can cause cell wall rupture called electropermeabilization, which is the same principle occurring in pulsed electric fields. During plasma treatment, where plasma initiates, catalyses, or helps sustain a complex biological response, compromised membrane structures (e.g., peroxidation) or changes in the membrane-bound proteins and/or enzymes leads to complex cell responses and may affect many cells, as the affected cell then signals others.

The reactive species in plasma have been widely associated with direct oxidative effects on the outer surface of microbial cells. The presence of water increases the effect of plasma: the highest efficiency in sanitization was observed

in moist organisms in comparison to dry organisms. One potential application of plasma in decontamination is based on the damage the deoxyribonucleic acid (DNA) in the chromosomes by plasma reactive species. Radiobiology studies conducted by Wiseman and Halliwell (1996) showed that the formation of ROS (hydroxyl radicals, hydrogen peroxide, and superoxide anion) near DNA stimulates a strong biocidal effect. The use of plasma results in malondialdehyde (MDA) formation in microbial cells, which is responsible for DNA adduct formation, leading to cell damage. In particular, reactive species interact with water, leading to the formation of OH^- ions, which are most reactive and harmful to the cells. These radicals that formed in the hydration layer around DNA are responsible for 90% of DNA damage. Hydroxyl radicals can then react with organics in its proximity leading to subsequent oxidation and consequently, to DNA destruction as well as destruction of cellular membranes and other cell components. Several active species can react with cells, but reactive oxygen species such as oxygen radicals (especially single state oxygen) can produce significant effects on cells by reacting with various macromolecules (Thirumdas *et al.* 2015).

Plasma obtained at atmospheric pressure

Non-equilibrium atmospheric pressure discharges can operate in a wide range of temperatures and pressures and are often called partial discharges (PD). PDs are gas discharges that are restricted electrical discharges. These discharges can occur in the presence of a solid or liquid dielectric and frequently show a non-stationary character (a transition between different plasma modes). The plasma is described by generative technology with the following main PDs: dielectric-barrier discharges (DBD), corona discharges (CD), microwave discharges (MW) and atmospheric pressure plasma jet (APPJ).

Siemens invented the DBD for the generation of ozone in 1857. Thereafter, it was established that the discharge takes place in a number of individual filamentary breakdown channels in a plane-parallel gap with insulated electrodes. It was shown that the plasma parameters of the micro-discharges (breakdown channels) can be controlled and modulated, and therefore, the DBD process can be optimised for applications of interest. DBD installations have various electrode configurations and are characterized by the presence of one or more solid dielectric layers (glass, quartz, and ceramic) placed between the metal electrodes. The gap between the electrodes with the dielectric can range from 100 mm to many centimetres. In atmospheric pressure environments, under 10 kV AC conditions, a few mm distance between the electrodes is common. Multiple set-ups of the electrode systems are also common, and joint and non-joint electrode configurations are possible (Denes and Manolache 2004). The dielectric layer plays an important role for (i) limiting the discharge current and avoiding the arc transition (it enables to work with a continuous/pulsed mode) and (ii) distributing random streamers on the electrode surface and ensuring a homogeneous treatment (Tendero et al 2006).

The second type of PDs is CD. Corona discharges are often called negative, positive, bipolar, AC, DC, or high frequency (HF), depending on the polarity of the stressed electrodes, whether one or both positive and negative ions are implicated in the current conduction, and on the nature of the driving field. Corona discharges are exclusive in comparison to other plasmas due to the presence of a large low field drift region positioned between the ionization zone and the passive (low field) electrode. Ions and electrons penetrating the above mentioned drift space will undergo neutralization, excitation and recombination reactions including both electrons and neutral and charged molecular and atomic species. Nevertheless, because of the multiple inelastic collision processes in the atmospheric pressure environment, the charged active species running off from the ionization zone (electrons and ions) will have energies lower than the ionization energies, and as a consequence, neutral chemistry (free radical chemistry) will typify the drift region (Denes and Manolache 2004).

Microwave discharges (MW) are produced by electromagnetic waves with frequencies above hundreds of MHz. The discharge usually burns in a box, where the waves are in resonance. Because of the necessity for a microwave-generating apparatus and the need for protection, this type of plasma, in general, seems to be of minor interest in biotechnology, but it was often used in the basic research of NTP interactions with biomaterials. However, MW belongs to one of the few NTP plasma sources already certified for medical use (Scholtz *et al*. 2015).

The last type of PDs is the atmospheric pressure plasma jet (APPJ) that can operate with radio frequency (RF) power or microwave power. The ionized gas from the plasma jet flows out through a nozzle and is directed on a substrate situated a few millimetres to a few centimetres downstream. This APPJ source configuration has been used for many applications such as the surface treatment of different materials and biomedical applications and for example, the induction of apoptosis in cancer cells. An apparatus with characteristics similar to APPJ is the APP torch system, but the plasma is generated between the tip of the centre of the electrode and the ground electrode near the exit of the torch. A relatively low electron and gas temperature characterizes the APPJs because gas molecules are dissociated between the electrodes in a glow micro-discharge. In the case of plasma torches, a very high voltage of 10 to 50 kV is generally applied, and the reactive gas is dissociated in an arc discharge. Consequently, a typical atmospheric pressure plasma torch is predisposed to have a significantly greater gas temperature and plasma density than that found in APPJs (Kim et al 2016).

Nonthermal plasma application on fresh produce

The potential of the nonthermal plasma technology in food decontamination has emerged since the mid-90s when many studies that evaluated the effectiveness of plasma on pure cultures of many microorganisms started being published (Surowsky *et al.* 2015). These studies found that the plasma inactivation capacity depended on many factors such as the

type of technology used to generate the plasma, the feed gas, the voltage, the treatment time, the direct or indirect exposure, the species and the concentration of the tested microorganisms and the structural characteristics of the produce (Li and Farid 2016). It is only in the last ten years that researchers began to apply nonthermal plasma to the surface of different foods inoculated with many bacterial species. Of the 47 studies we found, 40% used cold plasma on fresh fruits and vegetables, 21% on dry fruits, nuts and seeds, 19% on protein foods such as meat and cold cuts, 10% on spices, 6% on liquids and 4% on the eggshells. All of these experiments were performed exclusively in the laboratory and, to our knowledge, real industrial applications have not yet been made. Indeed, these studies have shown both the ability of plasma processes to break down the microbial load and some limitations in the efficacy on biofilms, the capacity of penetration and a lack of knowledge on the nutritional effects (Fernández and Thompson 2012; Niemira 2012; Pinela and Ferreira 2015; Surowsky *et al.* 2015; Thirumdas *et al.* 2015). The 34 studies on fresh foods, as defined previously, are shown in Tables 1–7 since they were the most involved in the foodborne outbreaks that occurred in Europe and industrialized countries. The studies were divided into seven groups based on the type of microorganism subjected to the treatment. The authors applied cold plasma on fresh vegetables (56%), dry fruits, nuts and seeds (29%) and spices (15%). Different plasma processes were used including plasma jet (34%), DBD (20%), MW (14%), low pressure plasma (12%), CD (3%), and other plasma processes (17%). Furthermore, the gas most widely used was air (44%), followed by pure Ar (17%), mixtures of He/O_2 and Ar/O_2 (12%) and pure N_2 (9%).

The inoculated microorganisms included *Escherichia coli* spp., *Salmonella* spp., *Listeria* spp., mesophilic bacteria, fungi, yeast, spores and bacilli and even other microorganisms involved in the decay process. The microorganisms that were most studied were *E. coli* spp., fungi, mesophilic bacteria and *Salmonella* spp. Notably, *E. coli* spp. and especially toxin-producing species and *Salmonella* spp. are often responsible for foodborne outbreaks. As reported above, in Europe, the main causative agent of these events is *Campylobacter jejuni* and *Campylobacter coli*, but in the tables, no studies are reported that used this microorganism. *Campylobacter jejuni* was used in one study where it was inoculated at a concentration of approximately 4 Log on skinless chicken breast and chicken thighs with the skin then subjected to a direct treatment with air plasma at atmospheric pressure (DBD) for three minutes; this treatment was enough to break down the microorganism of 2·45 Log and 3·11 Log, respectively (Dirks *et al.* 2012). The lack of studies on the plasma treatment of foods inoculated with *Campylobacter* spp. could be due to it is a microaerophilic microorganism and the difficulty in cultivation. To our knowledge, studies assessing the effectiveness of nonthermal plasma on food contaminated with Norovirus have not been published since Norovirus, which is a primary cause of acute gastroenteritis in both Europe and the United States, cannot be cultivated. There is only one study in which human faeces contaminated with Norovirus GII.4 were treated with cold atmospheric pressure plasma for 15 minutes to reach a reduction of 1·60 Log genomic equivalents ml^{-1} (Ahlfeld *et al.* 2015).

To verify whether the effectiveness of the plasma treatment depended on the type of microorganism inoculated, the type of technology, the type of gas, the type of food, and the exposure time, statistical analyses were performed on the data reported in tables 1–7. The statistical analysis was conducted with the statistical package IBM SPSS Statistics 22.0 using Spearman's test, ANOVA, Probit regression analysis, and t test. At first, statistical analyses were performed by considering the data as a whole, and then the data were divided by the type of microorganism treated, the type of plasma process used, the type of gas for the generation of plasma, the treatment time, and the type of food treated. As shown in tables 1–7, the data are not homogeneous, such as the data regard the treated microorganism groups and the type of plasmas that were used. In some cases, the lack of homogeneity in the data prevented statistical analysis or resulted in no significant differences due to groups containing a low number of data. In order to evaluate the microorganism abatement due to the different process parameters, Log reduction was used. The mean and standard deviation of the abatement and the treatment time related to different parameters are shown in table 8. On average, the plasma treatments are able to reduce the microorganism on fresh produce by 2.73 ± 1.44 Log, and this highlights their potentiality in food decontamination. The mean abatement of the seven groups of microorganisms ranges from 3.25 ± 1.56 Log for *Listeria* spp. to 1.51 ± 1.04 Log for bacilli and spores, but there are no statistically significant difference between the groups.

Regarding the plasma processes that were studied, plasma jet was applied on all microorganisms that were considered followed by DBD and MW, which were tested on all microorganisms except bacilli/spores and the so called "other" respectively. The abatements obtained with the different plasma processes range between 3.55 ± 1.63 Log for those identified as other treatment and 1.23 ± 0.64 Log for corona discharge plasma. The ANOVA revealed a statistically significant difference comparing the mean abatements with the type of plasma used ($F = 4.996$, $p < 0.001$), especially between the DBD and the plasma jet ($p < 0.005$) and between the DBD and the MW ($p < 0.05$). This highlights the efficacy of the DBD more than the other two most used treatments, regardless of the type of microorganism or treated food. Additionally, low pressure plasma and the so called "other processes" reached good microorganism reduction, but they were not studied on all groups of microorganisms.

Furthermore, the mean abatement obtained in relation to the type of gas used to generate the plasma amounted between 4.10 ± 0.85 Log reached with SF6 and 1.80 ± 1.22 Log with Ar. Moreover, the differences between the groups were statistically significant ($F = 4.290$, $p < 0.001$) and in particular, from mixtures Ar/O_2 and N_2 ($p < 0.05$) and between the mixtures Ar/O_2 and Ar ($p < 0.05$). This mixture was then proven to be the most effective (3.86 ± 0.94), immediately followed by air, that is the only gas used for generating plasma applied to all seven groups of microorganisms, and it reached a mean Log reduction of 3.03 ± 1.58. This is an important result because air is the least expensive gas, and this characteristic could be critical for the application of plasma treatment on an industrial scale. No correlation was found

between the abatement and processing time used during the experiments except for bacilli and spores (Rho = 0.730, p <0.05), which are the most resistant microorganisms among those surveyed. The treatment times ranged between 22.22 ± 7.54 min for bacilli and spores and 3.45 ± 3.77 min for *E. coli* spp. The ANOVA showed a statistically significant difference between the treatment times used on different groups of microorganisms (F = 4.565, p <0.05). Using the post hoc Tukey's test, the most significant differences were found between *E. coli* spp. and mesophilic bacteria (p <0.05), *E coli* spp. and bacilli and spore (p <0.01), *Listeria* spp. and bacilli and spores (p <0.05) and, finally, between fungi and bacilli/spores (p <0.05). Therefore, *E. coli* spp. appears as the microorganism that requires little time to treat, and this is definitely an important finding given the problems related to the disease because of toxin producers such as *E. coli* O157:H7. The treatment time is also significantly different relative to the type of plasma used (F = 5.068, p <0.001) and in particular, between the treatment time with the plasma jet and MW (p <0.001) and between MW and "other" treatments (p <0.05). In fact, the latter have proved to be the most rapid followed by those at low pressure, while MW reached the highest treatment time. Plasma jet was the quickest among the most used treatments in the time to microorganisms inactivation (4.26 ± 6.45 min). The mean treatment time of each group of microorganisms changes in relation to the plasma process that is applied, except for *Salmonella* spp. mesophilic bacteria and fungi. Plasma jet and DBD were the quickest in breaking the other group considered.

We did not find statistically significant differences in the Log reduction of *E. coli* spp., *Listeria* spp., mesophilic bacteria, and bacilli/spores on the bases of the plasma process, even if *E. coli* spp. reached the higher abatement by MW and DBD, *Listeria* spp. and mesophilic bacteria by DBD and bacilli/spores by MW. DBD was the most efficient treatment applied to *Salmonella* spp. (p < 0.01), while low pressure plasma was the better for the treatment of fungi (p < 0.05). The air resulted in the most efficient gas in the abatement of *Listeria* spp. (p < 0.05) and bacilli/spores (p < 0.05) while no other differences were found for the other groups of microorganisms.

Plasma treatments achieved a higher mean reduction in microorganisms on fresh vegetables followed by dry fruits, nuts, and seeds and spices. This higher mean Log reduction on fresh fruit and vegetables could be due to the higher activity water since the humidity or amount of water plays a fundamental role in the production of reactive species to achieve fast inactivation of microorganisms (Guo *et al.* 2015). Moreover, as reported by Surowsky *et al.* (2015), food structures can create physical barriers to the plasma penetration, and the surface of spices (e.g., peppercorn) with cracks, grooves, and pits might cause shadow effects for the emitted UV photons and other reactive species (Hertwig *et al.* 2015a).

Conclusion

The application of plasma technology on an industrial scale is possible only if it reaches sufficient levels of effectiveness, efficiency and economic and environmental sustainability.

The low pressure processes are very effective, especially against fungi and yeast, but they need a vacuum system, so they cannot be used on-line. Nonthermal atmospheric plasma proves itself to be a suitable technology for use on fresh produce to reduce the microbial load that is present and to avoid reaching the minimum infective dose of pathogens. Considering the treatment time, it may be compatible with an industrial application using the DBD or the plasma jet. Nonthermal plasma has many benefits including a lower operating temperature, lower water consumption, lower cost, timely production of the acting agents, and a lack of residues during production when compared to thermal and chemical treatments (Thirumdas *et al.* 2015; Ziuzina *et al.* 2015; Li and Farid 2016). Moreover, this technology could be used for the degradation of chemical contaminants such as pesticides and mycotoxins as reported by Heo *et al.* (2014) and Ouf *et al.* (2015), respectively.

The nonthermal plasma treatment could be a step in a multi-stage process in which microorganisms, pathogens and chemical contaminants need to overcome in order to survive in the food environment. The correct combination of hurdles can ensure microbial safety, stability and quality of foods (Pinela and Ferreira 2015). For example, nonthermal plasma can be generated by applying electric fields to the gas that is inside packages (Ziuzina *et al.* 2012). Misra *et al.* (2014) studied the effects of cold plasma on packaged strawberries, and they reached a 3 Log reduction of the total plate count. Additionally, Min *et al.* (2016) inhibited *E. coli* O157:H7, *Salmonella*, *L. monocytogenes*, and Tulane virus inoculated on Romaine lettuce treated in package by a DBD process. Therefore, this treatment received increasing attention from the food industry because it can prevent the recontamination of fresh produce during the packaging step, and it has the potential to scale up for commercial application (Li and Farid 2016).

The nutritional and organoleptic characteristics of fresh produce treated by plasma technology should be taken into account because it does not negatively affect the consumer's buying decision. Some authors studied the impact of plasma treatment on colour, pH, vitamins, fat and enzymes, but the results were not always uniform. Sometimes the researchers encountered some colour changes that adversely affected the appearance of the product, while the inactivation of enzymes such as polyphenol oxidase and peroxidase would be useful since they catalyse the browning reaction at cut surfaces. Furthermore, there is a lack of evidence regarding organoleptic evaluations of fresh produce treated by nonthermal plasma (Surowsky *et al.* 2015).

The aim of food industry is to produce healthy food with high nutritional and organoleptic quality, reducing the environmental impact, while raising their economic standards with a net profit. This matter must be taken into account for the evaluation of the economic and environmental sustainability of cold plasma treatments.

Niemira (2012) proposed some interesting considerations about the economic aspects of plasma in food safety and food processing. These calculations derive from the transposition and the elaboration of the costs of plasma in non-food commercial applications because the economic data presented in most food processing publications are not well

detailed. Presuming that plasma technology costs are higher commercially compared to the lab, there is a certain gain in energy efficiency and other engineering advantages. Each cold plasma technology has specific fixed and recurrent costs, which are difficult to predict; while other costs (like consumables, energy consumption and feed gas) can be predicted. The electricity can be scaled from the lab to commercial equipment, reaching values comparable to other industrial apparatus (up to 90 kW). The feed gases and their purity represent crucial costs: the price range for helium is 7·10–9·39 € m^{-3}. For oxygen and nitrogen purchased in gas cylinders, the price is about 2·25–9·22€ m^{-3}, but if generated directly in the plant from the surrounding air the costs were abated to 0·02–0·15 ·€ m^{-3}. In the past few years, advances were made in realizing the industrial atmospheric plasma equipment devoted to non-food applications. On the whole, the trend is to decrease both energy and process gas consumption, leading to situations of 35 kW of energy and 360 l min^{-1} for plasma jet technology and 100 l min^{-1} for DBD plasma technology, which is less than the consumption calculated in Niemira (2012) due to the drastically decreased costs when not using feed gases. Despite considerable improvements, it is possible to consider Niemira's (2012) conclusions still applicable, especially regarding the grade uncertainty of the optimal mixture composition for the biocidal activity. The antimicrobial contribution of very expensive gases, such as helium, has to be verified with accuracy in order to justify the high price and to find the exact applications.

All these observations show that nonthermal plasma technologies could be applied at the industrial scale, especially for the reduction of food spoilage microorganisms, foodborne pathogens and chemical contaminants. These outcomes represent a main goal for improving public health and reducing the economic impact of health care associated with foodborne outbreaks and removing unsold goods after the expiration date.

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Conflict of Interest

No conflict of interest declared.

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Table 1 Studies regarding the treatment of *Escherichia coli* spp. on fresh produce.

Microorganism	Substrate	Max. Log reduction	Plasma Source	Process gas	Direct Indirect	Max exp. time	Reference
E. coli NCTC 12900	Cherry tomatoes	3·1 Log CFU sample^{-1}	DBD	Air	Indirect	60 s	Ziuzina *et al*. 2014
	Strawberries	3·5 Log CFU sample^{-1}				300 s	
E. coli BL21 and XL10	Iceberg lettuce	3·3 Log CFU sample^{-1}	DBD	Air	Indirect	300 s	Ziuzina *et al.* 2015
E. coli BL21 and XL10 (biofilm 24h, 4°C)	Iceberg lettuce	3·0 Log CFU sample^{-1}				300 s	
E. coli BL21 and XL10 (biofilm 48h, 4°C)	Iceberg lettuce	4·0 Log CFU sample^{-1}				300 s	
E. coli DSM1116	Apples	4·6 Log CFU g^{-1}	MW	Air (20 l min^{-1})	Indirect	10 min	Baier *et al.* 2015a
	Carrots	6·5 Log CFU g^{-1}				300 s	
E. coli O157:H7 ATCC 35150	Dried fig	1·3 Log CFU g^{-1}	MW	N_2	Direct	10 min	Lee *et al.* 2015
E. coli ATCC 11775	Lettuce	1·5 Log CFU g^{-1}	Streamer CD	Ar	Indirect	10 min	Bermúdez-Aguirre *et*
	Carrots	0·5 Log CFU g^{-1}				10 min	*al.* 2013
	Tomatoes	1·7 Log CFU g^{-1}				10 min	
E. coli K12 (DSM 11250)	Corn salad	2·7 Log CFU cm^{-2}	APPJ	Ar (20 l min^{-1})	Direct	120 s	Baier *et al.* 2013
E. coli DSM 1116	Corn salad	4·1 Log CFU cm^{-2}	APPJ	Ar + 0·1%O_2	Semi	60 s	Baier *et al.* 2014
	Cucumbers	4·7 Log CFU cm^{-2}			Direct	60 s	
	Apples	4·7 Log CFU cm^{-2}				60 s	
	Tomatoes	3·3 Log CFU cm^{-2}				20 s	

E. coli O104:H4 ST678	Corn salad	3.3 Log CFU cm^{-2}	APPJ	Ar + 0.1%O_2	Semi	120 s	Baier *et al.* 2015b
	Corn salad	3.4 Log CFU cm^{-2}			Direct	60 s	
E. coli O157:H7 (C9490)	Almonds	1.3 Log CFU ml^{-1}	APPJ	Air	Direct	20 s	Niemira 2012
E. coli O157:H7 (ATCC 35150)	Almonds	1.1 Log CFU ml^{-1}			Direct	10 s	
E. coli O157:H7 (ATCC 43894)	Almonds	1.1 Log CFU ml^{-1}			Direct	10 s	
E. coli type 1 (W00871)	Mango pericarps	> 3.0 Log CFU cm^{-2}	Double APPJ	He + 0.5%O_2	Direct	5 s	Perni *et al.* 2008a
	Honeydew melon pericarps	> 3.0 Log CFU cm^{-2}				5 s	
E. coli type 1 (W00871)	Mango cut fruit	2.5 Log CFU cm^{-2}	Double APPJ	He + 0.5%O_2	Direct	30 s	Perni *et al.* 2008b
	Cantaloupe melon cut fruit	1.5 Log CFU cm^{-2}				40 s	
E. coli O157:H7 ATCC 43894	Golden delicious apples	3.6 Log CFU ml^{-1}	Gliding arc plasma	Air (40 l min^{-1})	Indirect	120 s	Niemira and Sites 2008

Table 2 Studies regarding the treatment of *Salmonella* spp. on fresh produce.

Microorganism	**Substrate**	**Max. Log reduction**	**Plasma Source**	**Process gas**	**Direct Indirect**	**Max exp. time**	**Reference**
S. enterica serovar Typhimurium ATCC 14028	Cherry tomatoes	6.3 Log CFU $sample^{-1}$	DBD	Air	Indirect	10 s	Ziuzina *et al.* 2014
S. enterica serovar Typhimurium ATCC	Strawberries	3.8 Log CFU $sample^{-1}$				300 s	

14028							
S. enterica serovar Typhimurium ATCC 14028	Iceberg lettuce	2·4 Log CFU sample^{-1}	DBD	Air	Indirect	300 s	Ziuzina *et al.* 2015
S. enterica serovar Typhimurium ATCC 14028 (biofilm 24h, 4°C)	Iceberg lettuce	4·1 Log CFU sample^{-1}				300 s	
S. enterica serovar Typhimurium ATCC 14028 (biofilm 48h, 4°C)	Iceberg lettuce	5·1 Log CFU sample^{-1}				300 s	
S. Typhimurium DT 104	Cabbage	1·5 Log CFU g^{-1}	MW	N_2	Direct	10 min	Lee *et al.* 2015
	Lettuce	1·5 Log CFU g^{-1}				10 min	
S. enterica serovar Typhimurium 4/74	Lettuce	2·7 Log CFU sample^{-1}	APPJ	N_2	Direct	15 min	Fernández *et al.* 2013
	Strawberries	1·8 Log CFU sample^{-1}				15 min	
	Potatoes	0·9 Log CFU sample^{-1}				15 min	
S. enterica DSM 17058	Black pepper seeds	2·7 Log CFU g^{-1}	APPJ	Ar	Direct	15 min	Hertwig *et al.* 2015a
	Black pepper seeds	4·1 Log CFU g^{-1}	MW	Air	Indirect	30 min	
S. Anatum F4317	Almonds	1·2 Log CFU ml^{-1}	APPJ	Air	Direct	20 s	Niemira 2012
S. Stanley H0558	Almonds	1·1 Log CFU ml^{-1}	APPJ	Air	Direct	20 s	
S. Enteritidis PT30	Almonds	1·1 Log CFU ml^{-1}	APPJ	Air	Direct	20 s	
S. Typhimurium (mono colture biofilm)	Iceberg lettuce	3·74 Log CFU cm^{-2}	Cold oxygen plasma	Air	Direct	300 s	Jahid *et al.* 2015

S. Typhimurium (mix colture biofilm)	Iceberg lettuce	1·74 Log CFU cm^{-2}	Cold oxygen plasma			300 s	
S. Stanley H0558	Golden Delicious apples	3·7 Log CFU ml^{-1}	Gliding arc plasma	Air (40 l min^{-1})	Indirect	180 s	Niemira and Sites 2008
S. enterica serovars Enteritidis PT30	Black pepper seeds	5·0 Log CFU g^{-1}	Arc discharge plasma	Air (20 l min^{-1}) + Ar (14 l min^{-1})	Indirect	80 s	Sun *et al.* 2014

Table 3 Studies regarding the treatment of *Listeria* spp. on fresh produce.

Microorganism	**Substrate**	**Max. Log reduction**	**Plasma Source**	**Process gas**	**Direct Indirect**	**Max exp. time**	**Reference**
L. monocytogenes NCTC 11994	Cherry tomatoes	6·7 Log CFU $sample^{-1}$	DBD	Air	Indirect	120 s	Ziuzina *et al.* 2014
	Strawberries	4·2 Log CFU $sample^{-1}$				120 s	
L. monocytogenes NCTC 11994	Iceberg lettuce	2·3 Log CFU $sample^{-1}$	DBD	Air	Indirect	300 s	Ziuzina *et al.* 2015
L. monocytogenes NCTC 11994 (biofilm 24h, 4°C)	Iceberg lettuce	3·8 Log CFU $sample^{-1}$				300 s	
L. monocytogenes NCTC 11994 (biofilm 48h, 4°C)	Iceberg lettuce	4·5 Log CFU $sample^{-1}$				300 s	
L. monocytogenes KCTC 3569	Cabbage	2·1 Log CFU g^{-1}	MW	He:O_2 (99·8:0·2)	Direct	10 min	Lee *et al.* 2015
	Lettuce	1·9 Log CFU g^{-1}				10 min	

	Dried fig	1.6 Log CFU g^{-1}				10 min	
L. innocua DSM 20649	Tomatoes	4.2 Log CFU cm^{-2}	APPJ	Ar + 0.1%O_2	Semi Direct	20 s	Baier *et al.* 2014
L. monocytogenes Scott A	Mango cut fruit	2.5 Log CFU cm^{-2}	Double APPJ	He + 0.5%O_2	Direct	30 s	Perni *et al.* 2008b
	Cantaloupe melon cut fruit	2.0 Log CFU cm^{-2}				40 s	

Table 4 Studies regarding the treatment of aerobic mesophilic bacteria on fresh produce.

Substrate	Max. Log reduction	Plasma Source	Process gas	Direct Indirect	Max exp. time	Reference
Strawberries	2.4 Log CFU g^{-1}	DBD	Air	Indirect	300 s	Misra *et al.* 2014a
	3.7 Log CFU g^{-1}		90%N_2+10% O_2	Indirect	300 s	
	3.1 Log CFU g^{-1}		65%O_2+16%N_2+19%CO_2	Indirect	300 s	
Melon (fresh cut)	3.4 Log CFU g^{-1}	DBD	Air	Direct	30 min + 30 min	Tappi *et al.* 2016
Cherry tomatoes	5.0 Log CFU $sample^{-1}$	DBD	Air	Indirect	300 s	Ziuzina *et al.* 2014
Strawberries	1.6 Log CFU $sample^{-1}$				60 s	
Apples	3.4 Log CFU g^{-1}	MW	Air (20 l min^{-1})	Indirect	300 s	Baier *et al.* 2015a
Cucumbers	1.5 Log CFU g^{-1}				10 min	
Tomatoes	3.3 Log CFU g^{-1}				300 s	
Carrots	5.2 Log CFU g^{-1}				300 s	
Black pepper seeds	4.0 Log CFU g^{-1}	MW	Air (18 l min^{-1})	Indirect	60 min	Hertwig *et al.* 2015c

Crushed oregano	1·6 Log CFU g^{-1}				30 min	
Paprika powder	3·3 Log CFU g^{-1}				60 min	
Red pepper powder	1·0 Log CFU g^{-1}	MW	N_2, N_2:O_2 (99·3:0·7), He, He:O_2 (99·8:0·2)	Direct	20 min	Kim *et al.* 2014
Black pepper seeds	0·7 Log CFU g^{-1}	APPJ	Ar	Direct	15 min	Hertwig *et al.* 2015a
Black pepper seeds	2·0 Log CFU g^{-1}	MW	Air	Indirect	30 min	
Blueberries	0·9 Log CFU g^{-1}	APPJ	Air	Indirect	90 s	Lacombe *et al.* 2015
Chickpeas	2·0 Log CFU ml^{-1} cm^{-2}	Micro-discharge plasma	Air	Direct	300 s	Mitra *et al.* 2014
Iceberg lettuce	4·1 Log CFU cm^{-2}	Cold oxygen plasma	Air	Direct	300 s	Jahid *et al.* 2015
Iceberg lettuce	1·6 Log CFU cm^{-2}				300 s	

Table 5 Studies regarding the treatment of fungi and yeast on fresh produce.

Microorganism	**Substrate**	**Max. Log reduction**	**Plasma Source**	**Process gas**	**Direct Indirect**	**Max exp. time**	**Reference**
Yeast/molds	Strawberries	3·30 Log CFU g-^{1}	DBD	Air	Indirect	300 s	Misra *et al.* 2014a
Yeast/molds	Strawberries	3·30 Log CFU g^{-1}	DBD	90%N_2+10%O_2	Indirect	300 s	Misra *et al.* 2014b
	Strawberries	3·40 Log CFU g^{-1}	DBD	65%O_2+16%N_2+19 %CO_2	Indirect	300 s	
Yeast/molds	Cherry tomatoes	5·0 Log CFU $sample^{-1}$	DBD	Air	Indirect	120 s	Ziuzina *et al.* 2014

	Strawberries	1.4 Log CFU sample^{-1}				300 s	
Yeast/molds	Black pepper seeds	3.1 Log CFU g^{-1}	MW	Air (18 l min-1)	Indirect	300 s	Hertwig *et al.* 2015c
	Crushed oregano	1.8 Log CFU g^{-1}				90 min	
	Paprika powder	No reduction				90 min	
A. flavus ATCC 200026	Red pepper powder	2.5 Log CFU g^{-1}	MW	N_2	Direct	20 min	Kim *et al.* 2014
		2.0 Log CFU g^{-1}		He	Direct	20 min	
		0.4 Log CFU g^{-1}		N_2:O_2 (99.3:0.7)	Direct	20 min	
		0.3 Log CFU g^{-1}		He:O_2 (99.8:0.2)	Direct	20 min	
Yeast/molds	Blueberries	1.2 Log CFU g^{-1}	APPJ	Air	Indirect	120 s	Lacombe *et al.* 2015
A. niger	Date palm fruit	3.0 Log CFU cm-^{2}	Double APPJ	Ar	Direct	9 min	Ouf *et al.* 2015
S. cerevisae (NCYC 2843)	Mango pericarps	> 3.0 Log CFU cm^{-2}	Double APPJ	He + 0,5%O_2	Direct	10 s	Perni *et al.* 2008a
	honeydew melon pericarps	> 3.0 Log CFU cm^{-2}				30 s	
S. cerevisae (NCYC 2843)	Mango cut fruit	2.5 Log CFU cm-^{2}	Double APPJ	He + 0,5%O_2	Direct	40 s	Perni *et al.* 2008b
	Cantaloupe melon cut fruit	1.0 Log CFU cm^{-2}	Double APPJ	He + 0,5%O_2	Direct	40 s	
A. flavus WU 0211	Brown rice cereal bars	4.2 Log CFU g^{-1}	APPJ	Ar	Direct	20 min	Suhem *et al.* 2013
A. parasiticus 631	Hazelnuts	5.0 Log CFU g^{-1}	LPCP	SF6	Direct	300 s	Barasan *et al.* 2008
	Peanuts	3.3 Log CFU g^{-1}				300 s	
	Pistachios	4.0 Log CFU g^{-1}				300 s	

Natural fungi	Pistachios	2·0 Log CFU g^{-1}	LPCP	Ar + O_2 (10:1)	Direct	60 s	Pignata *et al.* 2014

Table 6 Studies regarding the treatment of bacilli and spores on fresh produce.

Microorganism	**Substrate**	**Max. Log reduction**	**Plasma Source**	**Process gas**	**Direct Indirect**	**Max exp. time**	**Reference**
B.cereus spores ATCC 10876, ATCC 13061, W-1	Red pepper powder	No reduction	MW	N_2, N_2:O_2 (99·3:0·7), He, He:O_2 (99·8:0·2)	Direct	20 min	Kim *et al.* 2014
B. subtilis spores PS 832	Black pepper seeds	0·8 Log CFU g^{-1}	APPJ	Ar	Direct	15 min	Hertwig *et al.* 2015a
B. atrophaeus spores WIS 39 6/3		1·3 Log CFU g^{-1}				15 min	
B. subtilis spores PS 832		2·4 Log CFU g^{-1}	MW	Air	Indirect	30 min	
B. atrophaeus spores WIS 39 6/3		2,8 Log CFU g^{-1}				30 min	
Total bacterial spores		0·6 Log CFU g^{-1}	APPJ	Ar	Direct	15 min	
Total bacterial spores		1·7 Log CFU g^{-1}	MW	Air	Indirect	30 min	
B. subtilis spores PS 832	Black pepper seeds	1·0 Log CFU g^{-1}	APPJ	Ar, A r+ 0·2%N_2 and/or 0·13%O_2	Direct	15 min	Hertwig *et al.* 2015b
Total bacterial spores	Black pepper seeds	3·0 Log CFU g^{-1}	MW	Air (18 l min^{-1})	Indirect	30 min	Hertwig *et al.* 2015c

Table 7 Studies regarding the treatment of other bacteria on fresh produce.

Microorganism	Substrate	Max. Log reduction	Plasma Source	Process gas	Direct Indirect	Max exp. time	Reference
Anaerobic mesophilic lactobacilli	Melon (fresh cut)	2·0 Log CFU g^{-1}	DBD	Air	Direct	30 min + 30 min	Tappi *et al.* 2016
Anaerobic mesophilic lactococci	Melon (fresh cut)	2·5 Log CFU g^{-1}				30 min + 30 min	
P. agglomerans (VCM)	Mango pericarps	> 3·0 Log CFU cm^{-2}	Double APPJ	He + 0·5%O_2	Direct	2·5 s	Perni *et al.* 2008a
G.r liquefaciens (NCIMB 9136)		> 3·0 Log CFU cm^{-2}				2·5 s	
P. agglomerans (VCM)	honeydew melon pericarps	> 3·0 Log CFU cm^{-2}				2·5 s	
G. liquefaciens (NCIMB 9136)		> 3·0 Log CFU cm^{-2}				2,5 s	
G. liquefaciens (NCIMB 9136)	Mango cut fruit	2·0 Log CFU cm^{-2}	Double APPJ	He + 0·5%O_2	Direct	10 s	Perni *et al.* 2008b
	Cantaloupe melon cut fruit	2·5 Log CFU cm^{-2}				20 s	
A. hydrophila planktonic	Iceberg lettuce	7·0 Log CFU ml^{-1}	Cold oxygen plasma	Air	Direct	15 s	Jahid *et al.* 2014
A. hydrophila biofilm	Iceberg lettuce	3·0 Log CFU cm^{-2}				300 s	
Aerobic psychrotrophic bacteria	Melon (fresh cut)	1·0 Log CFU g^{-1}	DBD	Air	Direct	30 min + 30 min	Tappi *et al.* 2016

Table 8 Mean Log reduction and treatment time for different processing parameters.

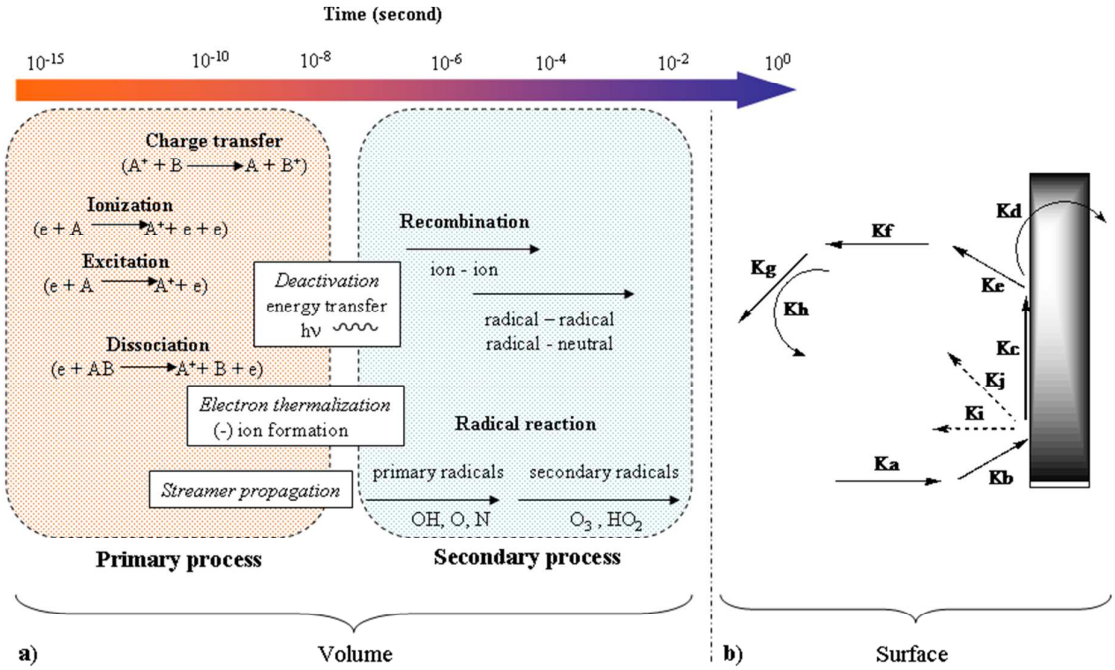
	Microorganisms Log reduction (mean ± SD)							
Process parameter	**All microorganism**	***E. coli* spp.**	***Salmonella* spp.**	***Listeria* spp.**	**Mesophilic bacteria**	**Fungi/yeast**	**Bacilli/spores**	**Other**
All processes	2·73 ± 1·44	2·94 ± 1·41	2·87 ± 1·60	3·25 ± 1·56	2·69 ± 1·34	2·55 ± 1·40	1·51 ± 1·04	2·91 ± 1·50
DBD	3·49 ± 1·33	3·38 ± 0·40	4·34 ± 1·46	4·30 ± 1·59	3·20 ± 1·16	3·28 ± 1·27		1·83 ± 0·76
Corona discharge	1·23 ± 0·64	1·23 ± 0·64						
Plasma jet	2·32 ± 1·16	2·84 ± 1·23	1·65 ± 0·77	2·90 ± 1·15	0·82 ± 0·18	2·56 ± 1·14	0·92 ± 0·30	2·75 ± 0·42
MW	2·35 ± 1·52	4·13 ± 2·63	2·37 ± 1·50	1·87 ± 0·25	2·81 ± 1·37	1·44 ± 1·21	1·98 ± 1·21	
Low pressure	3·58 ± 1·25					3·58 ± 1·25		
Other plasma process	3·55 ± 1·63		3·54 ± 1·35		2·58 ± 1·34			5·00 ± 2·83
Air	3·03 ± 1·58	3·19 ± 1·62	3·20 ± 1·69	4·30 ± 1·59	2·84 ± 1·31	2·25 ± 1·66	2·47 ± 0·57	3·10 ± 2·30
N_2	1·74 ± 0·64		1·68 ± 0·65			2·50 ± 0·00*		
He	1·25 ± 0·83					2·00 ± 0·00*		
Ar	1·80 ± 1·22	1·61 ± 0·92			0·70 ± 0·00*	3·62 ± 0·88	0·90 ± 0·36	
SF_6	4·10 ± 0·85					4·10 ± 0·85		
He mixture	2·32 ± 0·77	2·50 ± 0·71		2·02 ± 0·33		1·96 ± 1·24		2·75 ± 0·42
N_2 mixture	1·99 ± 1·53				2·60 ± 1·42	2·37 ± 1·70	0·50 ± 0·71	
Ar mixture	3·86 ± 0·94	3·92 ± 0·68				2·03 ± 0·00*		
Fresh vegetables	3·08 ± 1·37	3·25 ± 1·29	3·02 ± 1·59	3·42 ± 1·54	3·02 ± 1·34	2·73 ± 1·17		2·91 ± 1·50
Dry fruits, nuts, seeds	2·18 ± 1·42	1·20 ± 0·14	1·14 ± 0·02			3·72 ± 1·12		
Spices	1·92 ± 1·35		3·93 ± 1·16		2·10 ± 1·30	1·44 ± 1·21	1·51 ± 1·04	

	Treatment time (minute) (mean ± SD)							
All processes	9.80 ± 14.07	3.45 ± 3.77	7.66 ± 7.62	4.59 ± 3.90	16.87 ± 20.37	6.91 ± 7.37	22.22 ± 7.54	16.90 ±27.72
DBD	11.83 ± 19.66	4.20 ± 1.79	4.03 ± 2.16	3.80 ± 1.64	13.50 ± 22.84	4.40 ± 1.34		60.00 ± 0.00
Corona discharge	10.00 ± 0.00	10.00 ± 0.00						
Plasma jet	4.26 ± 6.45	0.74 ± 0.64	8.71 ± 7.84	0.50 ± 0.16	8.25 ± 9.55	4.71 ± 7.42	15.00 ± 0.00	0.11 ± 0.12
MW	18.80 ± 14.91	8.33 ± 2.89	16.67 ± 11.55	10.00 ± 0.00	25.00 ± 22.22	12.57 ± 9.34	28.00 ± 4.47	
Low pressure	4.00 ± 2.00					4.00 ± 2.00		
Other plasma process	3.66 ± 1.85		3.58 ± 1.77		5.00 ± 0.00			2.62 ± 3.36

*Data from only one study.

Figure captions

Figure 1 Time evolution of the elementary processes in NTP volume (a) and schematic surface processes (b) (modified from Kim *et al*. 2004 and D'Angelo 2010).


Time (second)
10-15
10-10
10-8
10-6
10-4
10-2
100
Charge transfer
(A+ + B → A + B+)
Ionization
(e + A → A+ + e + e)
Excitation
(e + A → A+ + e)
Dissociation
(e + AB → A+ + B + e)
Deactivation
energy transfer
hν
Electron thermalization
(-) ion formation
Streamer propagation
Recombination
ion - ion
radical – radical
radical - neutral
Radical reaction
primary radicals
secondary radicals
OH, O, N
O3 , HO2
Primary process
Secondary process
a)
Volume
b)
Surface
Kd
Kf
Kg
Ke
Kh
Kc
Kj
Ki
Ka
Kb