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**A review on microbiological decontamination of fresh produce with nonthermal plasma**

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

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

3

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## 32 **Summary**

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

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

## 45 **Keywords**

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

## 48 **Microorganisms and foodborne outbreak**

49 Currently, the global burden of foodborne diseases due to the presence of contaminating and pathogenic  
50 microorganisms in food remains high although in the 1990s some foodborne illnesses declined due to an intensive and  
51 focused effort in checking some parts of the food chain (Purayidathil and Ibrahim, 2012; Braden and Tauxe, 2013;  
52 WHO, 2015). In 2015, the Foodborne Diseases Active Surveillance Network (FoodNet) identified 20,107 confirmed  
53 cases of infections, 4,531 hospitalisations and 77 deaths caused by nine pathogens transmitted through food at 10 sites,  
54 which encompassed 15% of the U.S. population. The incidence of confirmed cases per 100,000 were reported for  
55 *Salmonella* (1589), *Campylobacter* (1297), *Shigella* (553), *Cryptosporidium* (331), Shiga Toxin-Producing *E. coli*  
56 (STEC) non-O157 (164), STEC O157 (095), *Vibrio* (039), *Yersinia* (029), *Listeria* (024) and *Cyclospora* (013).  
57 Compared with the incidence rate in 2012–2014, the incidence of confirmed infections in 2015 was significantly higher  
58 for STEC non-O157 (40% increase) and *Cryptosporidium* (59% increase) (Huang *et al.*, 2016). The 2014 overall  
59 incidence for the nine pathogens was significantly lower compared with the 1996–1998 data (-29%) with further  
60 reductions in 2006–2008 (-3%) and 2011–2013 (-8%) (CDC 2014). The incidence of infections and the number of  
61 hospitalisations were the highest for *Salmonella*; the percent of hospitalisation and deaths were the highest for *Listeria*.

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

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

84 A broad spectrum of microorganisms and food vehicles are involved in fresh produce-associated outbreaks. Norovirus  
85 was the main pathogen responsible (59% of foodborne illnesses in the US and 53% of foodborne illnesses in the EU)  
86 followed by *Salmonella* (18% of foodborne illnesses in the US and 20% of foodborne illnesses in the EU). Specifically,  
87 in the US, Norovirus outbreaks were strongly correlated with the consumption of salads; in the EU, this pathogen was  
88 mainly linked to berries (raspberries). *Salmonella* was the most common bacterial pathogen responsible for fresh  
89 produce outbreaks, accounting for nearly half of the outbreaks due to bacteria (53% in the US and 50% in the EU).

90 *Salmonella* outbreaks was the microorganism involved in the majority of sprout-associated outbreaks (14 in the US and  
91 11 in the EU). Regarding other microorganisms, *E. coli* and *Campylobacter* outbreaks were more prevalent in the US  
92 than in the EU. Regarding food vehicle, *E. coli* was associated with the consumption of various fresh vegetables, fruits

93 and sprouts, whereas *Campylobacter jejuni* was involved in fresh produce outbreaks linked to the consumption of salad,  
94 lettuce, tomatoes and melons (Callejon *et al.* 2015).  
95 Surveys of fresh produce have revealed contamination with pathogenic bacteria in commodities such as tomatoes,  
96 lettuce, salad greens, sprouting seeds, unpasteurized fruit juice, cantaloupe and nuts (EFSA 2013). At the same time,  
97 evaluation of prevalence and trends of bacterial contamination in fresh fruits and vegetables initiated in Europe. In  
98 Sweden, a survey on prepackaged ready-to-eat (RTE) mixed ingredient salad showed that 9% of the 141 samples were  
99 contaminated with *Listeria monocytogenes*. The results of this study indicate that pathogenic bacteria can be present in  
100 RTE salads in Sweden (Soderqvist *et al.* 2016). This public health concern should be addressed by improving the  
101 hygiene of the raw ingredients, the production environment and the cold temperature from the manufacturer to the  
102 consumer. There is a great need to address possible decontamination treatments for fresh fruit and vegetables from  
103 production systems that would otherwise lack adequate safety. However, such treatments raise issues of acceptability by  
104 consumers and the high costs incurred. The final use of fruits and vegetables in the food distribution chain, e.g., eaten  
105 raw versus heat treated, could also be adapted with regard to the microbiological risks that they could pose.

106

### 107 **Plasma Chemistry and Reactive Species**

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

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

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

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

130

### 131 **Plasma-Surface Interaction**

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

140  $A + (s) \rightarrow A(s) \text{ (Kb)}$ .

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

142  $A(s) + B(s) \rightarrow AB(s) + (s) \text{ (Kc)}$ , which then desorbs

143  $AB(s) \rightarrow AB + (s) \text{ (Ke)}$ , or which may spread onto the same surface **(Kd)**.

144 Alternatively, the reactive atoms may desorb without undergoing any reaction

145  $A(s) \rightarrow A + (s) \text{ (Ki)}$

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

147  $A(s) + B(s) \rightarrow AB + (s) \text{ (Kj)}$ .

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

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

152

### 153 **How plasma acts on the microorganisms**

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

158 One kinetic measurement parameter is known as Decimal value (D). This parameter has been used widely by studying  
159 sterilization by plasma. The D value is the time required to reduce an original concentration of microorganisms by 90%.

160 The first complete analysis of literature on low pressure cold plasma (LPCP) sterilization was shown by Moisan *et al.*  
161 (2001) regarding the role of UV photons and reactive species on the survival curve of microorganisms. In the classical  
162 sterilization process, such plots show a single straight line, while plasma sterilization shows a survival diagram with two  
163 or three different linear segments. The analysis of the three single steps in the survival curve suggested many basic  
164 mechanisms: (i) direct destruction by UV irradiation of the genetic material of the microorganism; (ii) erosion of the  
165 microorganism, atom by atom, through intrinsic photodesorption by UV irradiation to form volatile compounds  
166 combining atoms intrinsic to the microorganisms; and (iii) erosion of the microorganism, atom by atom, through  
167 etching. The etching results from the adsorption of reactive species from the plasma (glow or afterglow) on the  
168 microorganism with which they subsequently undergo a chemical reaction to form volatile compounds. In certain cases,  
169 the etching mechanism is enhanced by UV photons acting synergistically with the reactive species (Laroussi *et al.*  
170 2005). Laroussi *et al.* (2002) studied the germicidal effect of atmospheric pressure cold plasma. Depending on the type  
171 of microorganism, the type of medium in which the microorganisms are seeded, the method of exposure (direct or  
172 remote exposure) and the experimental work on the germicidal effects of cold atmospheric pressure plasma have shown  
173 survivor curves with different shapes, revealing that bacteria inactivation by non-equilibrium high pressure plasmas is a  
174 composite process. If UV is present in a dominant manner, the survivor curves often exhibit a first rapid step (small D  
175 value) followed by a second slower step. When the presence of UV is not dominant, such as in the case of an air  
176 plasma, single-slope survivor curves were mostly observed. However, in many cases, multi-slope curves have also been  
177 reported.

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

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



185 *et al.* 2002, 2005). However, different types of bacteria show a drastic reduction in cell viability. The diffusion of  
186 plasma-generated reactive species through the cell membrane induces the promotion of some reactions with the inner  
187 biomaterials: these reactions lead to cell death or non-viable cells (Lackmann and Bandow 2014). Recently, Guo *et al.*  
188 2015 postulated an explanation to justify the role of UV radiation in different plasma conditions. When UV radiation  
189 played a major role in the inactivation process, the gases were Ar or a N<sub>2</sub>/O<sub>2</sub> mixture in combination with microwave-  
190 driven discharge. In this context, the ionization energy of Ar is higher than N<sub>2</sub> and O<sub>2</sub>, making N<sub>2</sub> and O<sub>2</sub> ions (i.e., N<sub>2</sub><sup>+</sup>,  
191 N<sup>+</sup>, O<sub>2</sub><sup>+</sup>, and O<sup>+</sup>). In these conditions, the amount of positive nitrogen ions and negative oxygen ions was similar, and  
192 NO was generated with more respect to the electric discharge directly in the air. A similar mechanism happens with the  
193 excited state of NO. UV radiation in this experimental condition plays a main role in bacterial inactivation because their  
194 doses in the 200–300 nm wavelength range is higher than other experimental conditions.

195

#### 196 **Chemical reactions of plasma on microbial cell**

197 As described, the effect of plasma treatment on microbial cells is mainly due to the plasma ions and cell interactions.  
198 Commonly used oxygen and nitrogen gas plasmas are excellent sources of reactive oxygen-based and nitrogen-based  
199 reactive species (O, O<sub>2</sub>, O<sub>3</sub>, OH, NO, and NO<sub>2</sub>) because they have a direct oxidative effect on the outer surface of  
200 microbial cells. Atomic oxygen can potentially be a very effective sterilizing agent, with a chemical rate constant for  
201 oxidation at room temperature of approximately 10<sup>6</sup> times that of molecular oxygen (Misra *et al.* 2011).  
202 The lipid bilayer of microbial cells is more susceptible to atomic oxygen as the reactivity of atomic oxygen is much  
203 higher than that of molecular oxygen, which can degrade lipids, proteins and DNA of cells. The damage of the double  
204 bonds in the lipid bilayer causes impaired transportation of molecules in and out of the cell. The bombardment of  
205 reactive oxygen species (ROS) on the surface of bacterial cells also disrupts the membrane lipids. During plasma use,  
206 microorganisms are exposed to an intense bombardment by the radicals, which most likely provoke surface lesions that  
207 the living cell cannot repair sufficiently, a process termed “etching”. Plasma etching is based on the interaction of  
208 relative energetic ions and activated species with the molecules of the substrate. The accumulation of charges imparts  
209 an electrostatic force at the outer surface of cell membranes and can cause cell wall rupture called  
210 electropermeabilization, which is the same principle occurring in pulsed electric fields. During plasma treatment, where  
211 plasma initiates, catalyses, or helps sustain a complex biological response, compromised membrane structures (e.g.,  
212 peroxidation) or changes in the membrane-bound proteins and/or enzymes leads to complex cell responses and may  
213 affect many cells, as the affected cell then signals others.  
214 The reactive species in plasma have been widely associated with direct oxidative effects on the outer surface of  
215 microbial cells. The presence of water increases the effect of plasma: the highest efficiency in sanitization was observed

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

227

#### 228 **Plasma obtained at atmospheric pressure**

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

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

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

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

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

272

### 273 **Nonthermal plasma application on fresh produce**

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

277 type of technology used to generate the plasma, the feed gas, the voltage, the treatment time, the direct or indirect  
278 exposure, the species and the concentration of the tested microorganisms and the structural characteristics of the  
279 produce (Li and Farid 2016). It is only in the last ten years that researchers began to apply nonthermal plasma to the  
280 surface of different foods inoculated with many bacterial species. Of the 47 studies we found, 40% used cold plasma on  
281 fresh fruits and vegetables, 21% on dry fruits, nuts and seeds, 19% on protein foods such as meat and cold cuts, 10% on  
282 spices, 6% on liquids and 4% on the eggshells. All of these experiments were performed exclusively in the laboratory  
283 and, to our knowledge, real industrial applications have not yet been made. Indeed, these studies have shown both the  
284 ability of plasma processes to break down the microbial load and some limitations in the efficacy on biofilms, the  
285 capacity of penetration and a lack of knowledge on the nutritional effects (Fernández and Thompson 2012; Niemira  
286 2012; Pinela and Ferreira 2015; Surowsky *et al.* 2015; Thirumdas *et al.* 2015). The 34 studies on fresh foods, as defined  
287 previously, are shown in Tables 1–7 since they were the most involved in the foodborne outbreaks that occurred in  
288 Europe and industrialized countries. The studies were divided into seven groups based on the type of microorganism  
289 subjected to the treatment. The authors applied cold plasma on fresh vegetables (56%), dry fruits, nuts and seeds (29%)  
290 and spices (15%). Different plasma processes were used including plasma jet (34%), DBD (20%), MW (14%), low  
291 pressure plasma (12%), CD (3%), and other plasma processes (17%). Furthermore, the gas most widely used was air  
292 (44%), followed by pure Ar (17%), mixtures of He/O<sub>2</sub> and Ar/O<sub>2</sub> (12%) and pure N<sub>2</sub> (9%).

293 The inoculated microorganisms included *Escherichia coli* spp., *Salmonella* spp., *Listeria* spp., mesophilic bacteria,  
294 fungi, yeast, spores and bacilli and even other microorganisms involved in the decay process. The microorganisms that  
295 were most studied were *E. coli* spp., fungi, mesophilic bacteria and *Salmonella* spp. Notably, *E. coli* spp. and especially  
296 toxin-producing species and *Salmonella* spp. are often responsible for foodborne outbreaks. As reported above, in  
297 Europe, the main causative agent of these events is *Campylobacter jejuni* and *Campylobacter coli*, but in the tables, no  
298 studies are reported that used this microorganism. *Campylobacter jejuni* was used in one study where it was inoculated  
299 at a concentration of approximately 4 Log on skinless chicken breast and chicken thighs with the skin then subjected to  
300 a direct treatment with air plasma at atmospheric pressure (DBD) for three minutes; this treatment was enough to break  
301 down the microorganism of 2.45 Log and 3.11 Log, respectively (Dirks *et al.* 2012). The lack of studies on the plasma  
302 treatment of foods inoculated with *Campylobacter* spp. could be due to it is a microaerophilic microorganism and the  
303 difficulty in cultivation. To our knowledge, studies assessing the effectiveness of nonthermal plasma on food  
304 contaminated with Norovirus have not been published since Norovirus, which is a primary cause of acute gastroenteritis  
305 in both Europe and the United States, cannot be cultivated. There is only one study in which human faeces  
306 contaminated with Norovirus GII.4 were treated with cold atmospheric pressure plasma for 15 minutes to reach a  
307 reduction of 1.60 Log genomic equivalents ml<sup>-1</sup> (Ahlfeld *et al.* 2015).

308 To verify whether the effectiveness of the plasma treatment depended on the type of microorganism inoculated, the type  
309 of technology, the type of gas, the type of food, and the exposure time, statistical analyses were performed on the data  
310 reported in tables 1–7. The statistical analysis was conducted with the statistical package IBM SPSS Statistics 22.0  
311 using Spearman’s test, ANOVA, Probit regression analysis, and t test. At first, statistical analyses were performed by  
312 considering the data as a whole, and then the data were divided by the type of microorganism treated, the type of plasma  
313 process used, the type of gas for the generation of plasma, the treatment time, and the type of food treated. As shown in  
314 tables 1–7, the data are not homogeneous, such as the data regard the treated microorganism groups and the type of  
315 plasmas that were used. In some cases, the lack of homogeneity in the data prevented statistical analysis or resulted in  
316 no significant differences due to groups containing a low number of data. In order to evaluate the microorganism  
317 abatement due to the different process parameters, Log reduction was used. The mean and standard deviation of the  
318 abatement and the treatment time related to different parameters are shown in table 8. On average, the plasma  
319 treatments are able to reduce the microorganism on fresh produce by  $2.73 \pm 1.44$  Log, and this highlights their  
320 potentiality in food decontamination. The mean abatement of the seven groups of microorganisms ranges from  $3.25 \pm$   
321  $1.56$  Log for *Listeria* spp. to  $1.51 \pm 1.04$  Log for bacilli and spores, but there are no statistically significant difference  
322 between the groups.

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

332 Furthermore, the mean abatement obtained in relation to the type of gas used to generate the plasma amounted between  
333  $4.10 \pm 0.85$  Log reached with SF<sub>6</sub> and  $1.80 \pm 1.22$  Log with Ar. Moreover, the differences between the groups were  
334 statistically significant ( $F = 4.290$ ,  $p < 0.001$ ) and in particular, from mixtures Ar/O<sub>2</sub> and N<sub>2</sub> ( $p < 0.05$ ) and between the  
335 mixtures Ar/O<sub>2</sub> and Ar ( $p < 0.05$ ). This mixture was then proven to be the most effective ( $3.86 \pm 0.94$ ), immediately  
336 followed by air, that is the only gas used for generating plasma applied to all seven groups of microorganisms, and it  
337 reached a mean Log reduction of  $3.03 \pm 1.58$ . This is an important result because air is the least expensive gas, and this  
338 characteristic could be critical for the application of plasma treatment on an industrial scale. No correlation was found

339 between the abatement and processing time used during the experiments except for bacilli and spores ( $Rho = 0.730$ ,  $p$   
 340  $< 0.05$ ), which are the most resistant microorganisms among those surveyed. The treatment times ranged between  $22.22$   
 341  $\pm 7.54$  min for bacilli and spores and  $3.45 \pm 3.77$  min for *E. coli* spp. The ANOVA showed a statistically significant  
 342 difference between the treatment times used on different groups of microorganisms ( $F = 4.565$ ,  $p < 0.05$ ). Using the post  
 343 hoc Tukey's test, the most significant differences were found between *E. coli* spp. and mesophilic bacteria ( $p < 0.05$ ), *E*  
 344 *coli* spp. and bacilli and spore ( $p < 0.01$ ), *Listeria* spp. and bacilli and spores ( $p < 0.05$ ) and, finally, between fungi and  
 345 bacilli/spores ( $p < 0.05$ ). Therefore, *E. coli* spp. appears as the microorganism that requires little time to treat, and this is  
 346 definitely an important finding given the problems related to the disease because of toxin producers such as *E. coli*  
 347 O157:H7. The treatment time is also significantly different relative to the type of plasma used ( $F = 5.068$ ,  $p < 0.001$ ) and  
 348 in particular, between the treatment time with the plasma jet and MW ( $p < 0.001$ ) and between MW and "other"  
 349 treatments ( $p < 0.05$ ). In fact, the latter have proved to be the most rapid followed by those at low pressure, while MW  
 350 reached the highest treatment time. Plasma jet was the quickest among the most used treatments in the time to  
 351 microorganisms inactivation ( $4.26 \pm 6.45$  min). The mean treatment time of each group of microorganisms changes in  
 352 relation to the plasma process that is applied, except for *Salmonella* spp. mesophilic bacteria and fungi. Plasma jet and  
 353 DBD were the quickest in breaking the other group considered.

354 We did not find statistically significant differences in the Log reduction of *E. coli* spp., *Listeria* spp., mesophilic  
 355 bacteria, and bacilli/spores on the bases of the plasma process, even if *E. coli* spp. reached the higher abatement by MW  
 356 and DBD, *Listeria* spp. and mesophilic bacteria by DBD and bacilli/spores by MW. DBD was the most efficient  
 357 treatment applied to *Salmonella* spp. ( $p < 0.01$ ), while low pressure plasma was the better for the treatment of fungi ( $p <$   
 358  $0.05$ ). The air resulted in the most efficient gas in the abatement of *Listeria* spp. ( $p < 0.05$ ) and bacilli/spores ( $p < 0.05$ )  
 359 while no other differences were found for the other groups of microorganisms.

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

366

### 367 **Conclusion**

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

370 The low pressure processes are very effective, especially against fungi and yeast, but they need a vacuum system, so  
371 they cannot be used on-line. Nonthermal atmospheric plasma proves itself to be a suitable technology for use on fresh  
372 produce to reduce the microbial load that is present and to avoid reaching the minimum infective dose of pathogens.  
373 Considering the treatment time, it may be compatible with an industrial application using the DBD or the plasma jet.  
374 Nonthermal plasma has many benefits including a lower operating temperature, lower water consumption, lower cost,  
375 timely production of the acting agents, and a lack of residues during production when compared to thermal and  
376 chemical treatments (Thirumdas *et al.* 2015; Ziuzina *et al.* 2015; Li and Farid 2016). Moreover, this technology could  
377 be used for the degradation of chemical contaminants such as pesticides and mycotoxins as reported by Heo *et al.*  
378 (2014) and Ouf *et al.* (2015), respectively.

379 The nonthermal plasma treatment could be a step in a multi-stage process in which microorganisms, pathogens and  
380 chemical contaminants need to overcome in order to survive in the food environment. The correct combination of  
381 hurdles can ensure microbial safety, stability and quality of foods (Pinela and Ferreira 2015). For example, nonthermal  
382 plasma can be generated by applying electric fields to the gas that is inside packages (Ziuzina *et al.* 2012). Misra *et al.*  
383 (2014) studied the effects of cold plasma on packaged strawberries, and they reached a 3 Log reduction of the total plate  
384 count. Additionally, Min *et al.* (2016) inhibited *E. coli* O157:H7, *Salmonella*, *L. monocytogenes*, and Tulane virus  
385 inoculated on Romaine lettuce treated in package by a DBD process. Therefore, this treatment received increasing  
386 attention from the food industry because it can prevent the recontamination of fresh produce during the packaging step,  
387 and it has the potential to scale up for commercial application (Li and Farid 2016).

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

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

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

401 detailed. Presuming that plasma technology costs are higher commercially compared to the lab, there is a certain gain in  
402 energy efficiency and other engineering advantages. Each cold plasma technology has specific fixed and recurrent costs,  
403 which are difficult to predict; while other costs (like consumables, energy consumption and feed gas) can be predicted.  
404 The electricity can be scaled from the lab to commercial equipment, reaching values comparable to other industrial  
405 apparatus (up to 90 kW). The feed gases and their purity represent crucial costs: the price range for helium is 7.10–9.39  
406 € m<sup>-3</sup>. For oxygen and nitrogen purchased in gas cylinders, the price is about 2.25–9.22€ m<sup>-3</sup>, but if generated directly in  
407 the plant from the surrounding air the costs were abated to 0.02–0.15 € m<sup>-3</sup>. In the past few years, advances were made  
408 in realizing the industrial atmospheric plasma equipment devoted to non-food applications. On the whole, the trend is to  
409 decrease both energy and process gas consumption, leading to situations of 35 kW of energy and 360 l min<sup>-1</sup> for plasma  
410 jet technology and 100 l min<sup>-1</sup> for DBD plasma technology, which is less than the consumption calculated in Niemira  
411 (2012) due to the drastically decreased costs when not using feed gases. Despite considerable improvements, it is  
412 possible to consider Niemira's (2012) conclusions still applicable, especially regarding the grade uncertainty of the  
413 optimal mixture composition for the biocidal activity. The antimicrobial contribution of very expensive gases, such as  
414 helium, has to be verified with accuracy in order to justify the high price and to find the exact applications.  
415 All these observations show that nonthermal plasma technologies could be applied at the industrial scale, especially for  
416 the reduction of food spoilage microorganisms, foodborne pathogens and chemical contaminants. These outcomes  
417 represent a main goal for improving public health and reducing the economic impact of health care associated with  
418 foodborne outbreaks and removing unsold goods after the expiration date.

419

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422

#### 423 **Conflict of Interest**

424 No conflict of interest declared.

425

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- 580

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

Microorganism	Substrate	Max. Log reduction	Plasma Source	Process gas	Direct	Max exp.	Reference
					Indirect	time	
<i>E. coli</i> NCTC 12900	Cherry tomatoes	3.1 Log CFU sample <sup>-1</sup>	DBD	Air	Indirect	60 s	Ziuzina <i>et al.</i> 2014
	Strawberries	3.5 Log CFU sample <sup>-1</sup>				300 s	
<i>E. coli</i> BL21 and XL10	Iceberg lettuce	3.3 Log CFU sample <sup>-1</sup>	DBD	Air	Indirect	300 s	Ziuzina <i>et al.</i> 2015
<i>E. coli</i> BL21 and XL10 (biofilm 24h, 4°C)	Iceberg lettuce	3.0 Log CFU sample <sup>-1</sup>				300 s	
<i>E. coli</i> BL21 and XL10 (biofilm 48h, 4°C)	Iceberg lettuce	4.0 Log CFU sample <sup>-1</sup>				300 s	
<i>E. coli</i> DSM1116	Apples	4.6 Log CFU g <sup>-1</sup>	MW	Air (20 l min <sup>-1</sup> )	Indirect	10 min	Baier <i>et al.</i> 2015a
	Carrots	6.5 Log CFU g <sup>-1</sup>				300 s	
<i>E. coli</i> O157:H7 ATCC 35150	Dried fig	1.3 Log CFU g <sup>-1</sup>	MW	N <sub>2</sub>	Direct	10 min	Lee <i>et al.</i> 2015
<i>E. coli</i> ATCC 11775	Lettuce	1.5 Log CFU g <sup>-1</sup>	Streamer CD	Ar	Indirect	10 min	Bermúdez-Aguirre <i>et al.</i> 2013
	Carrots	0.5 Log CFU g <sup>-1</sup>				10 min	
	Tomatoes	1.7 Log CFU g <sup>-1</sup>				10 min	
<i>E. coli</i> K12 (DSM 11250)	Corn salad	2.7 Log CFU cm <sup>-2</sup>	APPJ	Ar (20 l min <sup>-1</sup> )	Direct	120 s	Baier <i>et al.</i> 2013
<i>E. coli</i> DSM 1116	Corn salad	4.1 Log CFU cm <sup>-2</sup>	APPJ	Ar + 0.1%O <sub>2</sub>	Semi	60 s	Baier <i>et al.</i> 2014
	Cucumbers	4.7 Log CFU cm <sup>-2</sup>			Direct	60 s	
	Apples	4.7 Log CFU cm <sup>-2</sup>				60 s	
	Tomatoes	3.3 Log CFU cm <sup>-2</sup>				20 s	

<i>E. coli</i> O104:H4 ST678	Corn salad	3.3 Log CFU cm <sup>-2</sup>	APPJ	Ar + 0.1%O <sub>2</sub>	Semi	120 s	Baier <i>et al.</i> 2015b
	Corn salad	3.4 Log CFU cm <sup>-2</sup>			Direct	60 s	
<i>E. coli</i> O157:H7 (C9490)	Almonds	1.3 Log CFU ml <sup>-1</sup>	APPJ	Air	Direct	20 s	Niemira 2012
<i>E. coli</i> O157:H7 (ATCC 35150)	Almonds	1.1 Log CFU ml <sup>-1</sup>			Direct	10 s	
<i>E. coli</i> O157:H7 (ATCC 43894)	Almonds	1.1 Log CFU ml <sup>-1</sup>			Direct	10 s	
<i>E. coli</i> type 1 (W00871)	Mango pericarps	> 3.0 Log CFU cm <sup>-2</sup>	Double APPJ	He + 0.5%O <sub>2</sub>	Direct	5 s	Perni <i>et al.</i> 2008a
	Honeydew melon pericarps	> 3.0 Log CFU cm <sup>-2</sup>				5 s	
<i>E. coli</i> type 1 (W00871)	Mango cut fruit	2.5 Log CFU cm <sup>-2</sup>	Double APPJ	He + 0.5%O <sub>2</sub>	Direct	30 s	Perni <i>et al.</i> 2008b
	Cantaloupe melon cut fruit	1.5 Log CFU cm <sup>-2</sup>				40 s	
<i>E. coli</i> O157:H7 ATCC 43894	Golden delicious apples	3.6 Log CFU ml <sup>-1</sup>	Gliding arc plasma	Air (40 l min <sup>-1</sup> )	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	Max exp.	Reference
					Indirect	time	
<i>S. enterica</i> serovar Typhimurium ATCC 14028	Cherry tomatoes	6.3 Log CFU sample <sup>-1</sup>	DBD	Air	Indirect	10 s	Ziuzina <i>et al.</i> 2014
<i>S. enterica</i> serovar Typhimurium ATCC	Strawberries	3.8 Log CFU sample <sup>-1</sup>				300 s	

14028									
<i>S. enterica</i> serovar Typhimurium ATCC	Iceberg lettuce	2.4 Log CFU sample <sup>-1</sup>	DBD	Air	Indirect	300 s		Ziuzina <i>et al.</i> 2015	
14028									
<i>S. enterica</i> serovar Typhimurium ATCC	Iceberg lettuce	4.1 Log CFU sample <sup>-1</sup>				300 s			
14028 (biofilm 24h, 4°C)									
<i>S. enterica</i> serovar Typhimurium ATCC	Iceberg lettuce	5.1 Log CFU sample <sup>-1</sup>				300 s			
14028 (biofilm 48h, 4°C)									
<i>S. Typhimurium</i> DT 104	Cabbage	1.5 Log CFU g <sup>-1</sup>	MW	N <sub>2</sub>	Direct	10 min		Lee <i>et al.</i> 2015	
	Lettuce	1.5 Log CFU g <sup>-1</sup>				10 min			
<i>S. enterica</i> serovar Typhimurium 4/74	Lettuce	2.7 Log CFU sample <sup>-1</sup>	APPJ	N <sub>2</sub>	Direct	15 min		Fernández <i>et al.</i>	
	Strawberries	1.8 Log CFU sample <sup>-1</sup>				15 min		2013	
	Potatoes	0.9 Log CFU sample <sup>-1</sup>				15 min			
<i>S. enterica</i> DSM 17058	Black pepper seeds	2.7 Log CFU g <sup>-1</sup>	APPJ	Ar	Direct	15 min		Hertwig <i>et al.</i> 2015a	
	Black pepper seeds	4.1 Log CFU g <sup>-1</sup>	MW	Air	Indirect	30 min			
<i>S. Anatum</i> F4317	Almonds	1.2 Log CFU ml <sup>-1</sup>	APPJ	Air	Direct	20 s		Niemira 2012	
<i>S. Stanley</i> H0558	Almonds	1.1 Log CFU ml <sup>-1</sup>	APPJ	Air	Direct	20 s			
<i>S. Enteritidis</i> PT30	Almonds	1.1 Log CFU ml <sup>-1</sup>	APPJ	Air	Direct	20 s			
<i>S. Typhimurium</i> (mono culture biofilm)	Iceberg lettuce	3.74 Log CFU cm <sup>-2</sup>	Cold oxygen plasma	Air	Direct	300 s		Jahid <i>et al.</i> 2015	



<i>S. Typhimurium</i> (mix culture biofilm)	Iceberg lettuce	1.74 Log CFU cm <sup>-2</sup>	Cold oxygen plasma			300 s	
<i>S. Stanley</i> H0558	Golden Delicious apples	3.7 Log CFU ml <sup>-1</sup>	Gliding arc plasma	Air (40 l min <sup>-1</sup> )	Indirect	180 s	Niemira and Sites 2008
<i>S. enterica</i> serovars Enteritidis PT30	Black pepper seeds	5.0 Log CFU g <sup>-1</sup>	Arc discharge plasma	Air (20 l min <sup>-1</sup> ) + Ar (14 l min <sup>-1</sup> )	Indirect	80 s	Sun <i>et al.</i> 2014

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

Microorganism	Substrate	Max. Log reduction	Plasma Source	Process gas	Direct	Max exp.	Reference
					Indirect	time	
<i>L. monocytogenes</i> NCTC 11994	Cherry tomatoes	6.7 Log CFU sample <sup>-1</sup>	DBD	Air	Indirect	120 s	Ziuzina <i>et al.</i> 2014
	Strawberries	4.2 Log CFU sample <sup>-1</sup>				120 s	
<i>L. monocytogenes</i> NCTC 11994	Iceberg lettuce	2.3 Log CFU sample <sup>-1</sup>	DBD	Air	Indirect	300 s	Ziuzina <i>et al.</i> 2015
<i>L. monocytogenes</i> NCTC 11994 (biofilm 24h, 4°C)	Iceberg lettuce	3.8 Log CFU sample <sup>-1</sup>				300 s	
<i>L. monocytogenes</i> NCTC 11994 (biofilm 48h, 4°C)	Iceberg lettuce	4.5 Log CFU sample <sup>-1</sup>				300 s	
<i>L. monocytogenes</i> KCTC 3569	Cabbage	2.1 Log CFU g <sup>-1</sup>	MW	He:O <sub>2</sub> (99:8:0:2)	Direct	10 min	Lee <i>et al.</i> 2015
	Lettuce	1.9 Log CFU g <sup>-1</sup>				10 min	

	Dried fig	1.6 Log CFU g <sup>-1</sup>				10 min	
<i>L. innocua</i> DSM 20649	Tomatoes	4.2 Log CFU cm <sup>-2</sup>	APPJ	Ar + 0.1%O <sub>2</sub>	Semi	20 s	Baier <i>et al.</i> 2014
					Direct		
<i>L. monocytogenes</i> Scott A	Mango cut fruit	2.5 Log CFU cm <sup>-2</sup>	Double APPJ	He + 0.5%O <sub>2</sub>	Direct	30 s	Perni <i>et al.</i> 2008b
	Cantaloupe melon cut fruit	2.0 Log CFU cm <sup>-2</sup>				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 <sup>-1</sup>	DBD	Air	Indirect		300 s	Misra <i>et al.</i> 2014a
	3.7 Log CFU g <sup>-1</sup>		90%N <sub>2</sub> +10% O <sub>2</sub>	Indirect		300 s	
	3.1 Log CFU g <sup>-1</sup>		65%O <sub>2</sub> +16%N <sub>2</sub> +19%CO <sub>2</sub>	Indirect		300 s	
Melon (fresh cut)	3.4 Log CFU g <sup>-1</sup>	DBD	Air	Direct		30 min + 30 min	Tappi <i>et al.</i> 2016
Cherry tomatoes	5.0 Log CFU sample <sup>-1</sup>	DBD	Air	Indirect		300 s	Ziuzina <i>et al.</i> 2014
Strawberries	1.6 Log CFU sample <sup>-1</sup>					60 s	
Apples	3.4 Log CFU g <sup>-1</sup>	MW	Air (20 l min <sup>-1</sup> )	Indirect		300 s	Baier <i>et al.</i> 2015a
Cucumbers	1.5 Log CFU g <sup>-1</sup>					10 min	
Tomatoes	3.3 Log CFU g <sup>-1</sup>					300 s	
Carrots	5.2 Log CFU g <sup>-1</sup>					300 s	
Black pepper seeds	4.0 Log CFU g <sup>-1</sup>	MW	Air (18 l min <sup>-1</sup> )	Indirect		60 min	Hertwig <i>et al.</i> 2015c

Crushed oregano	1.6 Log CFU g <sup>-1</sup>					30 min	
Paprika powder	3.3 Log CFU g <sup>-1</sup>					60 min	
Red pepper powder	1.0 Log CFU g <sup>-1</sup>	MW	N <sub>2</sub> , N <sub>2</sub> :O <sub>2</sub> (99.3:0.7), He, He:O <sub>2</sub> (99.8:0.2)	Direct		20 min	Kim <i>et al.</i> 2014
Black pepper seeds	0.7 Log CFU g <sup>-1</sup>	APPJ	Ar	Direct		15 min	Hertwig <i>et al.</i> 2015a
Black pepper seeds	2.0 Log CFU g <sup>-1</sup>	MW	Air	Indirect		30 min	
Blueberries	0.9 Log CFU g <sup>-1</sup>	APPJ	Air	Indirect		90 s	Lacombe <i>et al.</i> 2015
Chickpeas	2.0 Log CFU ml <sup>-1</sup> cm <sup>-2</sup>	Micro-discharge plasma	Air	Direct		300 s	Mitra <i>et al.</i> 2014
Iceberg lettuce	4.1 Log CFU cm <sup>-2</sup>	Cold oxygen plasma	Air	Direct		300 s	Jahid <i>et al.</i> 2015
Iceberg lettuce	1.6 Log CFU cm <sup>-2</sup>					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	Max exp.	Reference
					Indirect	time	
Yeast/molds	Strawberries	3.30 Log CFU g <sup>-1</sup>	DBD	Air	Indirect	300 s	Misra <i>et al.</i> 2014a
Yeast/molds	Strawberries	3.30 Log CFU g <sup>-1</sup>	DBD	90%N <sub>2</sub> +10%O <sub>2</sub>	Indirect	300 s	Misra <i>et al.</i> 2014b
	Strawberries	3.40 Log CFU g <sup>-1</sup>	DBD	65%O <sub>2</sub> +16%N <sub>2</sub> +19%CO <sub>2</sub>	Indirect	300 s	
Yeast/molds	Cherry tomatoes	5.0 Log CFU sample <sup>-1</sup>	DBD	Air	Indirect	120 s	Ziuzina <i>et al.</i> 2014

	Strawberries	1.4 Log CFU sample <sup>-1</sup>				300 s	
Yeast/molds	Black pepper seeds	3.1 Log CFU g <sup>-1</sup>	MW	Air (18 l min <sup>-1</sup> )	Indirect	300 s	Hertwig <i>et al.</i> 2015c
	Crushed oregano	1.8 Log CFU g <sup>-1</sup>				90 min	
	Paprika powder	No reduction				90 min	
<i>A. flavus</i> ATCC 200026	Red pepper powder	2.5 Log CFU g <sup>-1</sup>	MW	N <sub>2</sub>	Direct	20 min	Kim <i>et al.</i> 2014
		2.0 Log CFU g <sup>-1</sup>		He	Direct	20 min	
		0.4 Log CFU g <sup>-1</sup>		N <sub>2</sub> :O <sub>2</sub> (99.3:0.7)	Direct	20 min	
		0.3 Log CFU g <sup>-1</sup>		He:O <sub>2</sub> (99.8:0.2)	Direct	20 min	
Yeast/molds	Blueberries	1.2 Log CFU g <sup>-1</sup>	APPJ	Air	Indirect	120 s	Lacombe <i>et al.</i> 2015
<i>A. niger</i>	Date palm fruit	3.0 Log CFU cm <sup>-2</sup>	Double APPJ	Ar	Direct	9 min	Ouf <i>et al.</i> 2015
<i>S. cerevisiae</i> (NCYC 2843)	Mango pericarps	> 3.0 Log CFU cm <sup>-2</sup>	Double APPJ	He + 0.5%O <sub>2</sub>	Direct	10 s	Perni <i>et al.</i> 2008a
	honeydew melon pericarps	> 3.0 Log CFU cm <sup>-2</sup>				30 s	
<i>S. cerevisiae</i> (NCYC 2843)	Mango cut fruit	2.5 Log CFU cm <sup>-2</sup>	Double APPJ	He + 0.5%O <sub>2</sub>	Direct	40 s	Perni <i>et al.</i> 2008b
	Cantaloupe melon cut fruit	1.0 Log CFU cm <sup>-2</sup>	Double APPJ	He + 0.5%O <sub>2</sub>	Direct	40 s	
<i>A. flavus</i> WU 0211	Brown rice cereal bars	4.2 Log CFU g <sup>-1</sup>	APPJ	Ar	Direct	20 min	Suhem <i>et al.</i> 2013
<i>A. parasiticus</i> 631	Hazelnuts	5.0 Log CFU g <sup>-1</sup>	LPCP	SF6	Direct	300 s	Barasan <i>et al.</i> 2008
	Peanuts	3.3 Log CFU g <sup>-1</sup>				300 s	
	Pistachios	4.0 Log CFU g <sup>-1</sup>				300 s	

Natural fungi	Pistachios	2.0 Log CFU g <sup>-1</sup>	LPCP	Ar + O <sub>2</sub> (10:1)	Direct	60 s	Pignata <i>et al.</i> 2014
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**Table 6** Studies regarding the treatment of bacilli and spores on fresh produce.

Microorganism	Substrate	Max. Log reduction	Plasma Source	Process gas	Direct	Max exp.	Reference
					Indirect	time	
<i>B. cereus</i> spores ATCC 10876, ATCC 13061, W-1	Red pepper powder	No reduction	MW	N <sub>2</sub> , N <sub>2</sub> :O <sub>2</sub> (99.3:0.7), He, He:O <sub>2</sub> (99.8:0.2)	Direct	20 min	Kim <i>et al.</i> 2014
<i>B. subtilis</i> spores PS 832	Black pepper seeds	0.8 Log CFU g <sup>-1</sup>	APPJ	Ar	Direct	15 min	Hertwig <i>et al.</i> 2015a
<i>B. atrophaeus</i> spores WIS 39 6/3		1.3 Log CFU g <sup>-1</sup>				15 min	
<i>B. subtilis</i> spores PS 832	Black pepper seeds	2.4 Log CFU g <sup>-1</sup>	MW	Air	Indirect	30 min	
<i>B. atrophaeus</i> spores WIS 39 6/3		2.8 Log CFU g <sup>-1</sup>				30 min	
Total bacterial spores		0.6 Log CFU g <sup>-1</sup>	APPJ	Ar	Direct	15 min	
Total bacterial spores		1.7 Log CFU g <sup>-1</sup>	MW	Air	Indirect	30 min	
<i>B. subtilis</i> spores PS 832	Black pepper seeds	1.0 Log CFU g <sup>-1</sup>	APPJ	Ar, Ar + 0.2%N <sub>2</sub> and/or 0.13%O <sub>2</sub>	Direct	15 min	Hertwig <i>et al.</i> 2015b
Total bacterial spores	Black pepper seeds	3.0 Log CFU g <sup>-1</sup>	MW	Air (18 l min <sup>-1</sup> )	Indirect	30 min	Hertwig <i>et al.</i> 2015c

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

Microorganism	Substrate	Max. Log reduction	Plasma Source	Process gas	Direct	Max exp.	Reference
					Indirect	time	
Anaerobic mesophilic lactobacilli	Melon (fresh cut)	2.0 Log CFU g <sup>-1</sup>	DBD	Air	Direct	30 min + 30 min	Tappi <i>et al.</i> 2016
Anaerobic mesophilic lactococci	Melon (fresh cut)	2.5 Log CFU g <sup>-1</sup>				30 min + 30 min	
<i>P. agglomerans</i> (VCM)	Mango pericarps	> 3.0 Log CFU cm <sup>-2</sup>	Double APPJ	He + 0.5%O <sub>2</sub>	Direct	2.5 s	Perni <i>et al.</i> 2008a
<i>G.r liquefaciens</i> (NCIMB 9136)		> 3.0 Log CFU cm <sup>-2</sup>				2.5 s	
<i>P. agglomerans</i> (VCM)	honeydew melon pericarps	> 3.0 Log CFU cm <sup>-2</sup>				2.5 s	
<i>G. liquefaciens</i> (NCIMB 9136)		> 3.0 Log CFU cm <sup>-2</sup>				2.5 s	
<i>G. liquefaciens</i> (NCIMB 9136)	Mango cut fruit	2.0 Log CFU cm <sup>-2</sup>	Double APPJ	He + 0.5%O <sub>2</sub>	Direct	10 s	Perni <i>et al.</i> 2008b
	Cantaloupe melon cut fruit	2.5 Log CFU cm <sup>-2</sup>				20 s	
<i>A. hydrophila</i> planktonic	Iceberg lettuce	7.0 Log CFU ml <sup>-1</sup>	Cold oxygen plasma	Air	Direct	15 s	Jahid <i>et al.</i> 2014
<i>A. hydrophila</i> biofilm	Iceberg lettuce	3.0 Log CFU cm <sup>-2</sup>				300 s	
Aerobic psychrotrophic bacteria	Melon (fresh cut)	1.0 Log CFU g <sup>-1</sup>	DBD	Air	Direct	30 min + 30 min	Tappi <i>et al.</i> 2016

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

Microorganisms Log reduction (mean ± SD)								
Process parameter	All microorganism	<i>E. coli</i> spp.	<i>Salmonella</i> spp.	<i>Listeria</i> 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 <sub>2</sub>	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 <sub>6</sub>	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 <sub>2</sub> 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 $\pm$ SD)							
All processes	9.80 $\pm$ 14.07	3.45 $\pm$ 3.77	7.66 $\pm$ 7.62	4.59 $\pm$ 3.90	16.87 $\pm$ 20.37	6.91 $\pm$ 7.37	22.22 $\pm$ 7.54	16.90 $\pm$ 27.72
DBD	11.83 $\pm$ 19.66	4.20 $\pm$ 1.79	4.03 $\pm$ 2.16	3.80 $\pm$ 1.64	13.50 $\pm$ 22.84	4.40 $\pm$ 1.34		60.00 $\pm$ 0.00
Corona discharge	10.00 $\pm$ 0.00	10.00 $\pm$ 0.00						
Plasma jet	4.26 $\pm$ 6.45	0.74 $\pm$ 0.64	8.71 $\pm$ 7.84	0.50 $\pm$ 0.16	8.25 $\pm$ 9.55	4.71 $\pm$ 7.42	15.00 $\pm$ 0.00	0.11 $\pm$ 0.12
MW	18.80 $\pm$ 14.91	8.33 $\pm$ 2.89	16.67 $\pm$ 11.55	10.00 $\pm$ 0.00	25.00 $\pm$ 22.22	12.57 $\pm$ 9.34	28.00 $\pm$ 4.47	
Low pressure	4.00 $\pm$ 2.00					4.00 $\pm$ 2.00		
Other plasma process	3.66 $\pm$ 1.85		3.58 $\pm$ 1.77		5.00 $\pm$ 0.00			2.62 $\pm$ 3.36

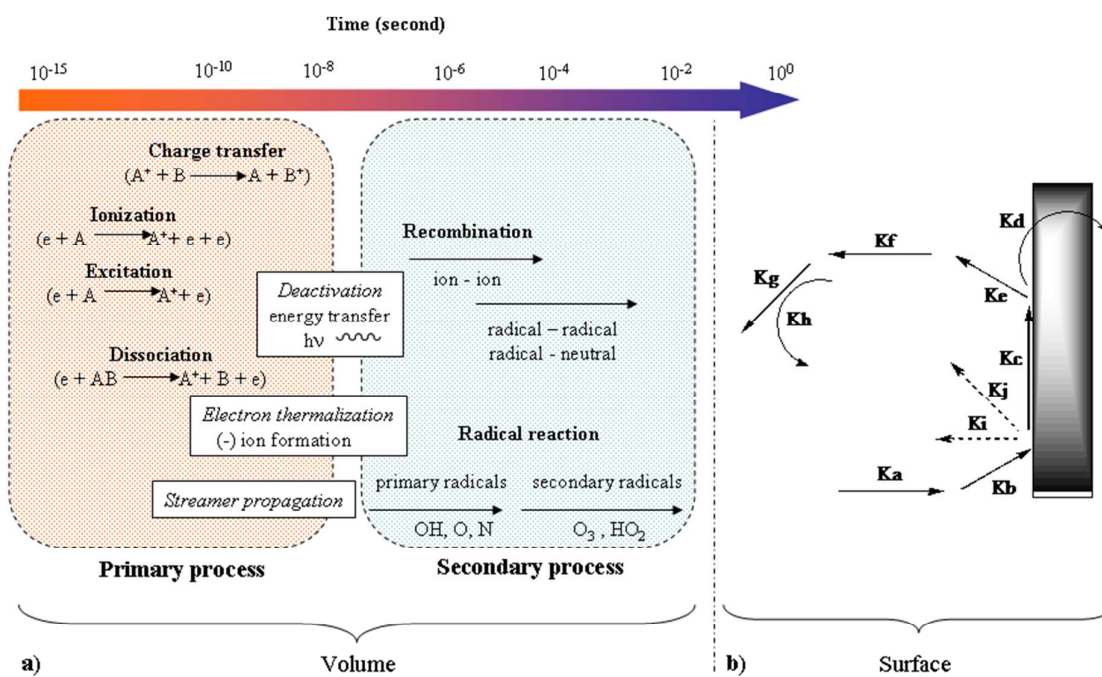
\*Data from only one study.



**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).

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