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1 Sanitisation of fresh-cut celery and radicchio by gas plasma treatments in water 2 medium

3
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6
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12 13 **Abstract**

14
15 The antimicrobial efficacy of dielectric barrier discharge atmospheric gas plasma (DBD) was tested
16 against *Listeria monocytogenes* and shigatoxin-producing *Escherichia coli* serogroups O157 and
17 O26. Challenge tests were carried out with samples of cut celery and radicchio leaves inoculated
18 with a mix of five strains of *L. monocytogenes* or the two strains of *E. coli* immersed in deionised
19 water. The treatment efficacy was also assessed considering only the contaminated deionised water.
20 For deionised inoculated water alone, a treatment time-dependent strong effect was observed and a
21 pathogens reduction higher than 6 Log CFU/mL was obtained after 40 min of treatment. With the
22 vegetables presence in the liquid medium, the efficacy appeared reduced and related to the
23 treatment time, microorganism, substrate and storage duration (reduction up to 2.5 and 3.7 Log
24 CFU/cm² for *L. monocytogenes* and *E. coli*, respectively). No significant changes were observed on
25 celery visual attributes, soluble solids content and textural parameters. A significant decrease of the
26 Chroma colour parameter during storage was noted in treated radicchio samples respect to control
27 ones.

28
29 **Keywords:** gas plasma, bacterial decontamination, fresh cut vegetables, quality, storage.

34 **1. Introduction**

35

36 Chlorine or other sanitizers are currently used in the washing steps of fresh-cut products to reduce
37 the number of pathogens and spoilage bacteria on their surface. However, the surviving bacteria can
38 grow during storage, reducing the efficacy of the sanitation steps. Despite the general idea that
39 sanitizers are used to reduce the microbial population of produce, their main effect is to maintain
40 the microbial quality of the water by avoiding cross-contamination between clean and contaminated
41 products. Strong concern has recently arisen for the presence of chlorine toxic residues in fresh-cut
42 produce when the appropriate level of chlorine in washing water is exceeded. A ban of chlorine for
43 fresh-cut produce sanitation was introduced in the food legislation in Germany and Switzerland (Gil
44 et al., 2009) and might be introduced in future in the European legislation, as well.

45 The ionized gas, named gas plasma, whose antimicrobial efficacy has been proven in the last
46 decade towards different types of microorganism, is one of the new techniques that are being
47 explored in the food panorama (Niemira, 2012). The gas plasma attractiveness lies mainly in the
48 possibility to conduct the treatment at atmospheric conditions without altering the temperature of
49 the product.

50 The efficacy of the gas plasma treatment, based on the action of oxidizing species and reactive
51 molecules, is affected by the type of microorganism and substrate characteristics (Berardinelli et al.,
52 2012; Guo et al., 2015), and in the electrical conditions (applied energy level and gas mixture) used
53 to generate the discharge Atmospheric gas plasma can be obtained by using power sources ranging
54 from direct current (DC) and low frequency, to radio frequency and microwave power supplies
55 involving a different energy transfer to gas particles (Moreau et al., 2008).

56 When the air is used as working gas, reactive oxygen and nitrogen species can be generated at
57 atmospheric conditions (Ragni et al., 2010). These molecules are metastable excited oxygen, ozone,
58 hydroxyl and nitric oxide radicals. Excited nitrogen molecules characterize also non thermal
59 electrical discharge (Laroussi and Leipold, 2004).

60 If the atmospheric discharge is generated close to a water based liquid surface, the reactive species
61 can diffuse into the aqueous environment and can induce complex chemical reactions responsible
62 for the microorganism inactivation. This interaction involves the generation of nitric/nitrous acids
63 and a consequent acidification of the liquid media. The acidification conditions seemed to have a
64 significant role in the decontamination mechanism; moreover, peroxides, such as hydrogen
65 peroxide H_2O_2 and superoxide anions ($O_2^{\bullet-}$), generated by the interaction with the gaseous phase,
66 correlated with the liquid antimicrobial potential (Ikawa et al., 2010; Shainsky et al., 2012).

67 At present, several investigations have attempted to clarify different aspects related to a possible
68 application of this technique on solid substrates. In particular on food products, different
69 applications were described according to the microorganisms and the physical and chemical
70 properties of the specific food matrix, (Misra et al., 2011). In contrast, the decontamination
71 mechanisms derived from a plasma-liquid interaction are not yet clear. Studies conducted on plasma
72 generated in contact with liquids regard mainly the water sanitation in terms of reduction of organic
73 pollutants and microorganisms (Malik et al., 2001).

74 Complex reactions can occur in the liquid phase inducing the formation of biologically active
75 species. However, the presence of organic compounds could influence the reaction channels and
76 consequently the oxidation effects. The possible inhibition role of the substrate immersed in the
77 liquid has not been fully investigated.

78 In order to understand the role of the plasma generated species produced in liquid phase, research
79 was conducted utilizing surface dielectric barrier discharge plasma and atmospheric air as a
80 working gas for treatment of deionised water (Oehmigen et al., 2010; von Woedtke et al., 2011).
81 The main results suggested that NO radical oxidation products such as the peroxyxynitrate (ONOO-)
82 and the peroxyxynitrous acid (ONOOH) could mainly affect the decontamination process. These
83 strong oxidant products are characterised by an extremely low stability and their detection on the
84 liquid media appeared difficult.

85 The technique could preserve the sensorial characteristics of the product and the microbial quality
86 of the liquid medium, but for common practical use some important aspects related to the influence
87 of the different food matrixes should be clarified.

88 The present work assessed the decontamination efficacy of the atmospheric gas plasma generated
89 by a DBD device with parallel plates placed on a batch of deionised water and explored, for the first
90 time in the literature panorama, the role of vegetable substrates in the treatment performances. The
91 effect was assessed towards a mix of *Listeria monocytogenes* strains and a mix of *Escherichia coli*
92 O157 and O26 shigatoxin-producing strains experimentally inoculated in deionised water or on the
93 surface of cut celery (*Apium graveolens*) and radicchio (red chicory, *Cichorium intybus* L.) leaves
94 samples that were subsequently immersed in water. An in-depth analysis of the interaction between
95 the gas phase and the liquid medium was also carried out together with the evaluation of the
96 possible product side effects.

97

98 **2. Materials and Methods**

99

100 *2.1 Gas plasma generator*

101 A DBD generator was used for the experiments. A temperature of $33 \pm 1^\circ\text{C}$ and a saturation
102 condition were observed inside the hermetic chamber after 30 min of the tested treatment
103 conditions.

104 The discharge was driven between a couple of parallel plate electrodes. One of the two electrodes
105 was made of brass and covered by a glass sheet (5 mm width) while the other was made of stainless
106 still. The voltage at the electrodes was generated by an high voltage transformer and power
107 switching transistors supplied by a stabilized DC power supply (Elektro-Automatik GmbH &
108 Co.KG, EA-PS 2042-06B).

109 The electrode was confined in a plastic hermetic chamber ($135 \text{ mm} \times 220 \text{ mm} \times 178 \text{ mm}$) housing
110 the product to be processed. A fan placed over the electrodes (at about 15 mm) increases the speed
111 of the plasma species against the target and to stir the liquid fluid.

112 All the assessments (chemical characterisation, microbiological and qualitative assessments) were
113 conducted at atmospheric conditions (at $26 \pm 1^\circ\text{C}$ and 53% R.H.) by using air as working gas with a
114 supply voltage of 19.15 V ($3.15 \pm 0.5 \text{ A}$). A schematic of the electrodes configuration is shown in
115 Figure 1.

116

117 *2.2 Chemical characterisation of the discharge*

118 The chemical characterisation of the emission was evaluated by acquiring the spectrum irradiance
119 ($\mu\text{W cm}^{-2}$) from 200 to 450 nm by using an optic fibre probe (Avantes, FCUV400- 2) placed at
120 about 10 mm from the discharge and connected to a spectrometer (Avantes, AvaSpec-2048,
121 resolution of 2.4 nm). The irradiance values were acquired after 3 min from the ignition of the
122 generator in order to achieve stable conditions verified through preliminary tests.

123

124 *2.3 Microbiological assessments*

125 A layout of the microbiological assessments performed on deionized water, celery pieces and
126 radicchio leaves immediately after the treatments (30 and 60 min) and after further 5 d of storage at
127 4°C and 80% R.H. is shown in Figure 2. Treatment times were selected after preliminary tests
128 aiming at identifying the longest time corresponding to acceptable quality parameters of fresh cut
129 products assessed immediately after the treatments. Selected times are not compatible with the
130 current practice of commercial fresh-cut line, but this procedure could be used for sanitation steps in
131 tanks.

132

133 *2.3.1 Growth of bacteria and preparation of inoculum*

134 Five strains of *Listeria monocytogenes* (LM LR 102 0227-359, vi 51028, 0113-131, vi51010) and
135 two strains of *Escherichia coli* (O157:H7 VTx, Oxoid NTCT12900; O26:nt VT+, isolated from
136 milk) were used in this study. Cultures were grown at 37°C using brain heart infusion (BHI, Oxoid,
137 Basingstoke, United Kingdom) and tryptic soy broth (TSB, Oxoid) for *L. monocytogenes* and *E.*
138 *coli*, respectively.

139 Broth cultures were stored at -80°C with the addition of 25% glycerol. Tryptic soy agar (TSA)
140 plates were streaked from these stocks and stored at 4°C. Cultures for inactivation experiments were
141 inoculated from single colonies on these agar plates and grown at 37°C for 21 h. Single colonies of
142 each strain of *L. monocytogenes* and *E. coli* were harvested and suspended in Phosphate-Buffered
143 Saline pH 7.0 (PBS) at a cell density of approximately 10⁸ CFU/mL (0.08-0.1 Absorbance at 625
144 nm). The number of bacteria was determined by plating the appropriate decimal dilutions on TSA
145 and incubating at 37°C for 24 h.

146

147 2.3.2 Deionised water

148 A mix of *L. monocytogenes* strains and a mix of *E. coli* strains were inoculated in 150 mL of
149 deionised water samples at a cell density of approx.10⁶ CFU/mL. After 10, 20, 40 and 60 min of
150 treatment three aliquots of 1 mL each were harvested and the number of inoculated bacteria was
151 determined as described above.

152

153 2.3.3 Vegetables (celery and radicchio) in deionised water

154 Ten grams of celery pieces with a thickness of 3 mm and portions of radicchio leaves
155 (approximately 16 cm²) were layered on the bottom of plastic boxes. To contaminate the vegetables,
156 one hundred microliter of the suspensions of bacteria (*L. monocytogenes* or *E. coli*) in PBS (at a cell
157 density of approximately 10⁸ CFU/mL) were spotted on the surface of celery or the radicchio
158 leaves. Leaves were incubated at room temperature under laminar flow in a biohazard cabinet until
159 the inoculum was completely dried (approximately 60 min). Inoculated leaves were submerged with
160 deionised water (150 mL) and treated for 30 or 60 min, whereas the control samples were held in
161 the biohazard cabinet for the same period of time. After the treatment, the vegetables were pull out
162 from the water.

163

164 2.3.4. Determination of bacterial survival

165 Immediately after treatments, treated and control vegetables and water samples were analysed
166 separately to assess the viability of *L. monocytogenes* and *E. coli* cells. Vegetables were
167 homogenized in Buffered Peptone Water (BPW, Oxoid) with a Stomacher® (Seward, UK) for 2

168 min at normal speed and allowed to stay for 1 h at room temperature in order to increase the
169 recovery of the stressed cells. On water samples, in order to increase the sensitivity of the test, 10
170 ml of each sample were filtered through Microcheck II beverage monitor (Pall Italia, Buccinasco,
171 MI, Italy). In order to recover the microbial cells injured by the gas plasma treatment, serial 1:10
172 decimal dilution of BPW after vegetable homogenization and filters of water samples were plated
173 on TAL (Thin Agar Layer) plates (Wu and Fung, 2001). These plates are characterised by a layer of
174 selective/differential isolation agar overlaid by non selective Tryptic Soy Agar (TSA, Oxoid). *L.*
175 *monocytogenes* and *E. coli* were enumerated by plating the appropriate decimal dilutions of the
176 samples on Agar Listeria according to Ottaviani and Agosti (ALOA, Biolife) and Sorbitol
177 MacConkey Agar for (SMAC, Oxoid), which were overlaid with 14 mL of TSA (Wu and Fung,
178 2001). The isolated colonies grown on the TSA-SMAC plates that have the characteristics of *E. coli*
179 were differentiated on the basis of their colour. Five isolated colonies for each phenotype (sorbitol
180 fermenting or not-fermenting) were tested with *E. coli* O26 and *E. coli* O157 latex agglutination
181 test, respectively, to confirm the identification and thus exclude from the count the generic *E. coli*
182 that can potential contaminate the vegetables.

183 Colonies were enumerated on TAL plates after incubation at 37°C for 24 h. Upon the observation of
184 no colonies, the BPW homogenates were tested with the methods ISO 11290 and ISO 16654 to
185 detect the presence of *L. monocytogenes* and *E. coli*, respectively. The challenge test was repeated
186 three times to evaluate the reproducibility of results.

187 Viability of *L. monocytogenes* and *E. coli* cells in treated and control samples was assessed also in
188 vegetables after 5 d of storage at 4°C (80% of R.H.) in plastic boxes wrapped within a perforated
189 low density polyethylene film in order to preserve the product hydration. Three replicates per each
190 pathogen/treatment and time/storage day combinations were tested.

191 The survival of bacteria in the plasma-treated samples was determined measuring the reduction of
192 viable cells that was expressed as the difference between the logarithms of the colony counts of the
193 untreated and treated samples ($\text{Log } N_0 - \text{Log } N$).

194

195 2.4 Qualitative assessments

196 A layout of the qualitative assessments conducted on deionized water, celery pieces and radicchio
197 leaves before and immediately after the treatments (30 and 60 min) and after further 1 and 5 d of
198 storage at 4°C and 80% R.H. is shown in Figure 3.

199

200 2.4.1 Deionised water

201 Concentration (mg L^{-1}) of nitrite and nitrate (ion chromatography method, APAT CNR IRSA 4020
202 Man 29 2003) (spectrophotometric method, APAT CNR IRSA 4050 Man 29 2003) generated in
203 150 ml of deionised water after 5, 20, 40 and 60 min of treatment, were evaluated. The analyses
204 were conducted after about 2-3 h from the end of the treatment. pH values were recorded using pH
205 meter (GLP 22, CRISON) immediately after the treatment.

206

207 2.4.2 Vegetables (celery and radicchio) in deionised water

208 All qualitative parameters of vegetables were assessed before, immediately after the treatment (30
209 and 60 min) and after further storage of 1 and 5 d (at 4°C and 80% R.H.). The same sample
210 preparation conditions used for the microbiological tests were considered. Control samples
211 consisted of selected vegetable submerged in 150 mL of water for the same treatment time (30 and
212 60 min) at room temperature.

213 For the celery samples, the results of the image analysis, mechanical parameters obtained by a
214 compression test and soluble solid content (SSC) were evaluated.

215 For image analysis, a digital camera mod. D7000 (Nikon, Shinjuku, Japan) equipped with a 60 mm
216 lens mod. AF-S micro, Nikkor (Nikon, Shinjuku, Japan) was used to acquire digitalized images of
217 celery pieces (exposition time $\frac{1}{2}$ sec; F-stop $f/16$) placed inside a black box under controlled
218 lighting condition. The digitalized images were analysed with Image Pro-Plus v. 6.2, (Media
219 Cybernetics, USA). On the basis of the chromatic characteristics, two different pixel ranges were
220 defined corresponding to “green” and “not green” areas. All pixels were then evaluated by the
221 model in terms of percentage of each area on the total.

222 Compression test (speed of 0.5 mm s^{-1} and a maximum deformation of 90%) was conducted by
223 means of a Texture Analyser mod. TA-HDi500 (Stable Micro Systems, Surrey, UK) equipped with
224 a 50 N load cell and a 6 mm diameter stainless steel cylinder. Firmness (N) (F, the first peak force
225 value representing the limit of the flesh elasticity), work required to rupture the flesh ($\text{N}\cdot\text{s}$) (area
226 under the curve from 0 s to F) and gradient (G, between 0 s and F) were extracted from the force
227 versus time curves.

228 SSC was determined at 20°C by measuring the refractive index with a digital refractometer mod.
229 PR1 (Atago Co. Ltd, Tokyo, Japan) calibrated with distilled water. For each sample, SSC was
230 determined in triplicate on the juice obtained from 5 pieces of celery.

231 For the radicchio samples, colour parameters were evaluated by means of a reflectance colorimeter
232 (Minolta Chroma Meter CR-400, Minolta Italia S.p.A). For each sample, an average value of three
233 measurements was calculated. The CIELab system L^* , a^* and b^* , was considered (CIE, 1976).

234 Chroma values were also calculated ($C^* = \sqrt{a^{*2} + b^{*2}}$).

235 2.5 Data analysis

236

237 Significant differences (P -level < 0.05) between control and treated samples at the same storage and
238 treatment time were found by using analysis of variance (ANOVA with LSD post-hoc test) and the
239 Kruskal-Wallis, in case of significance of the Levene test (Statistica 7.0, StatSoft Inc., Tulsa, Okla.,
240 U.S.A.). Significant differences were also explored during the storage within the same sample
241 (control or treated) and the same treatment time, for vegetables qualitative assessments only.

242

243 3. Results and discussion

244

245 3.1 Chemical characterisation of the discharge

246

247 The emission spectra of the tested DBD plasma generator is shown in Figure 4. The emission peaks
248 of OH ($\lambda=280$ nm) and NO ($\lambda= 226$ - 248 nm) radicals can be detected together to the dominant
249 peaks related to the neutral nitrogen molecules N_2 named second positive system ($\lambda= 290$ - 440 nm)
250 and to the positive ion N_2^+ ($\lambda=391$ nm).

251

252 3.2 Deionised water

253

254 *L. monocytogenes* and *E. coli* survival in deionised water submitted to gas plasma treatment is
255 summarized in Figure 5. During the first 10 min, cell numbers remained constant for *L.*
256 *monocytogenes* whereas a slight reduction was observed for *E. coli* (0.4 Log CFU/ml reduction).
257 After 20 min of treatment the reduction of both *L. monocitogenes* and *E.coli* were statistically
258 significant (0.8 and 1.7 Log CFU/mL reduction). The numbers of viable target bacteria detected in
259 the water samples treated for 40 minutes were < 1 Log CFU/ mL, corresponding to a reduction
260 higher than 6 Log CFU / mL.

261 Nitrite and nitrate concentration (mg L^{-1}) and pH values measured on treated deionised water are
262 reported in Table 1. The nitrate content increases linearly with the treatment time up to 111.31 mg
263 L^{-1} , while the nitrite content increases exponentially up to 6.96 mg L^{-1} . As expected, the pH
264 decreases from 6.45 (initial value) to an equilibrium value of 2.51 (60 min of treatment).

265 By comparing the microbiological results with the chemical composition of the deionised water, it
266 appears that highest decontamination results can be observed for both microorganisms after
267 treatments of 40 and 60 min. After these times, the pH of the solution reaches values lower than 3

268 and probably positively affects the generation of peroxynitrates (ONOO-) and the peroxynitrous
269 acids (ONOOH) (Von Woedtke et al., 2011).

270

271 3.3 Vegetables (*celery and radicchio*) in deionised water

272

273 The results of gas plasma treatments on *L. monocytogenes* and *E. coli* inoculated on the surface of
274 celery are shown in Figure 6 and 7, respectively. Any or only slight significant differences between
275 control and treated samples were observed after 30 min and 60 min of treatment (-0.35 Log CFU/g
276 and -0.57 Log CFU/g reduction for *L. monocytogenes* respectively and no reduction for *E. coli*).
277 After storage, for both tested pathogens, no significant differences were recorded.

278 The gas plasma treatment appears to have a bactericidal effect on *L. monocytogenes* and *E. coli* on
279 radicchio leaves (Figures 8 and 9). Immediately after 30 min and 60 min of treatment, the number
280 of viable *L. monocytogenes* was significantly lower in comparison to the control. However, the
281 detected differences were not high: 1 and 1.3 Log CFU/cm², respectively. After storage at 4°C for 5
282 d, the differences between control and treated samples were -1.7 and -2.5 Log after 30 and 60 min
283 of treatment, respectively. For *E. coli*, no significant differences between controls and treated
284 samples were observed after 30 minutes, whereas a significant difference was measured after 60
285 min (-2.2 Log CFU/cm²). After storage no decontamination effects were observed after 30 min, but
286 a significant lower number of viable *E. coli* (-3.7 Log CFU/cm²) in comparison with controls was
287 detected after 60 min.

288 Gas plasma is a surface active preservation technology that is efficient in decontaminating the
289 surface of specified matrices, but could be not efficient in decontaminating the core of the matrix.
290 The higher decontamination efficacy of gas plasma on radicchio in comparison to celery might be
291 linked to the different structure of the surface of the two vegetables. Regarding the porous structure
292 of celery, pathogens cells might have migrated inside the celery cut avoiding the exposure to the
293 treatment. Another aspect that might have influenced the different efficacies might be linked to the
294 different chemical composition of vegetables. In particular, antimicrobial properties of radicchio
295 were described (Verma et al., 2013). These antimicrobial properties are confirmed by the lower pH
296 of radicchio washing water (pH 2.7) in comparison to celery washing water (pH 3.8) after the
297 treatment of 60 min. Similarly, a different efficacy linked to the different fresh-cut produces was
298 registered on washing water treated by a titanium dioxide (TiO₂) photocatalytic system (Selma et
299 al., 2008). Finally, a third aspect deserve further investigations. Different efficacies might be due to
300 two different counts or compositions of the microbial population naturally colonising the surface of
301 the two tested vegetables. Higher counts of naturally colonising bacteria might suggest a higher

302 competition rate and lower survival of inoculated pathogen bacteria. However results of previous
303 studies, does not fully support this hypothesis since significantly higher total bacteria counts were
304 described in minimally processed celery in comparison to minimally processed radicchio (6-7 log₁₀
305 CFU/g vs 4 log₁₀ CFU/g) (Lavelli et al., 2009; Lopez et al., 2005). Further studies on the
306 composition of naturally colonizing microbial population of the two vegetables need to be
307 performed.

308 The gas plasma treatment was efficient in reducing the number of *L. monocytogenes* and *E. coli*
309 cells naturally migrating from experimentally inoculated vegetables to deionised water used to
310 submerge the vegetables. For celery test, significant lower numbers of viable *L. monocytogenes* (-
311 1.8 Log CFU/mL) and *E. coli* (-1.3 Log CFU/mL) were observed in deionised water after a
312 treatment of 30 min; after 60 min significant differences were highlighted only for *L.*
313 *monocytogenes* (-2.2 Log CFU/mL).

314 The gas plasma treatment was efficient in the inactivation of *L. monocytogenes* and *E. coli* cells that
315 migrated from radicchio leaves to deionised water. In particular, 30 min treatments achieved a
316 reduction of 2.5 Log for *L. monocytogenes* and above 3.8 Log (undetectable level) for *E. coli*. After
317 60 min of treatment, a reduction above 5 Log (undetectable level) was observed for *L.*
318 *monocytogenes*. Whereas the reduction of *E. coli* was 3.5 Log. The apparent discrepancy between
319 the results observed after 30 and 60 min of treatment for *E. coli* can be related to microbial cells that
320 are progressively released from the radicchio leaves to water. Mean values of water pH values of
321 4.14 (± 0.07) and 3.06 (± 0.03) were observed after 30 min whereas pH values of 3.68 (± 0.03) and
322 2.74 (± 0.07) were registered after 60 min, for water with celery and radicchio, respectively.

323 The differences between celery and radicchio water decontamination, might be due to a different
324 influence of the two food matrices on the pH and the diffusion of gas plasma reactive species into
325 water.

326 In the normal full-scale washing process leafy vegetables are usually washed at 333 ± 50 kg h⁻¹
327 according to Van Haute et al. (2015). For a possible industrial application, the performance of
328 different configurations or more powerful generators that can be reduce the treatment time should
329 be analysed also in relation to the presence of organic matter in the liquid medium.

330 As concerning the quality parameters of celery, results of the image analysis, in terms of percentage
331 of green area, are reported in Table 2. No significant differences were observed between control and
332 treated samples, at the same storage time, while significant differences were detected after 5 d of
333 storage within the same control and treated sample and within the same treatment time.

334 Results of mechanical parameters are summarized in Table 3. In general, for both treatments and
335 during the storage, significant differences were not observed between treated and control celery
336 pieces. No significant modification in mechanical properties were detected during storage.

337 The results of soluble solid content measurements are shown in Table 4. No significant differences
338 were observed between control and treated samples, at the same storage time. As expected,
339 significant differences were observed after 5 d of storage within the same control and treated
340 sample and within the same treatment time. Gas plasma treatments did not induce changes in the
341 soluble solid content.

342 Results of colour measurement, in term of L*, a*, b* and C*, conducted on the control and treated
343 radicchio samples, are summarized in Table 5. For the treated samples (both 30 and 60 min), the
344 Chroma parameter (C*) decreases significantly during storage from about 31 to 20 after 5 d of
345 storage, while for the control sample, this parameter decreases slightly (from about 31 to 26 after 60
346 min and from 30 to 23 after 30 min of treatment). The brightness (L*) decreases during the storage
347 both in treated and control samples. During the storage, b* parameter increases and a* decreases
348 both in treated and control samples, as a consequence of the browning.

349

350 **4. Conclusions**

351

352 The decontamination potential of oxidative species generated by the diffusion of the ionised gas in
353 deionised water was tested towards a mix of *Listeria monocytogenes* strains and a mix of
354 *Escherichia coli*. A bacteria survival lower than 1 Log CFU/mL was detected after 40 min of
355 treatment involving a pH values lower than 3. The presence of vegetable samples in the water
356 seemed to affect the reaction pathways and to induce a lower acidification of liquid medium
357 according to the type of substrate.

358 In relation to the vegetable decontamination, the efficacy is related to the specific microorganism,
359 kind of vegetable, treatment time and duration of storage. A more pronounced bactericidal effect
360 was observed in radicchio leaves samples (up to -3.7 Log CFU/cm²) in contrast with to cut celery
361 pieces (up to -0.57 Log CFU/g). However, the treatment induced a more rapid loss of the radicchio
362 leaves during the storage.

363 For a possible application of this technique a balance between sanitisation benefits and side effects,
364 in terms of a visual point of view but also in reference to the nutritional and toxicological aspects,
365 should be considered. More powerful equipment could be considered in order to strongly reduce the
366 treatment time. The high level of soil particles or organic matter that characterise the wash water
367 can represent possible limitations. Even if other studies should be conducted in order to evaluate the

368 role of the inorganic and organic matter in the decontamination efficacy, in the light of the observed
369 results the technique provides a first overview to investigate the possibility to sanitize wash water in
370 a discontinuous (batch) system process.

371

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375

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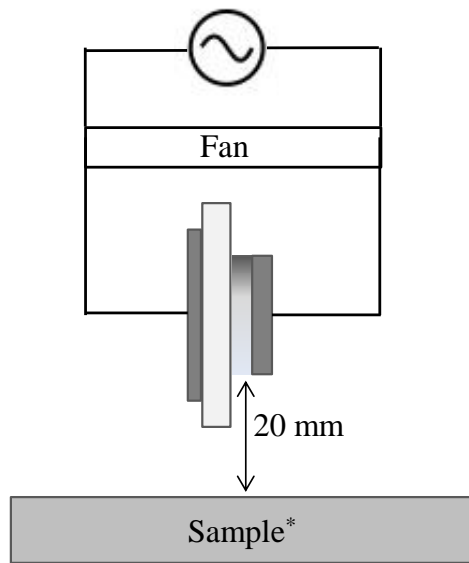
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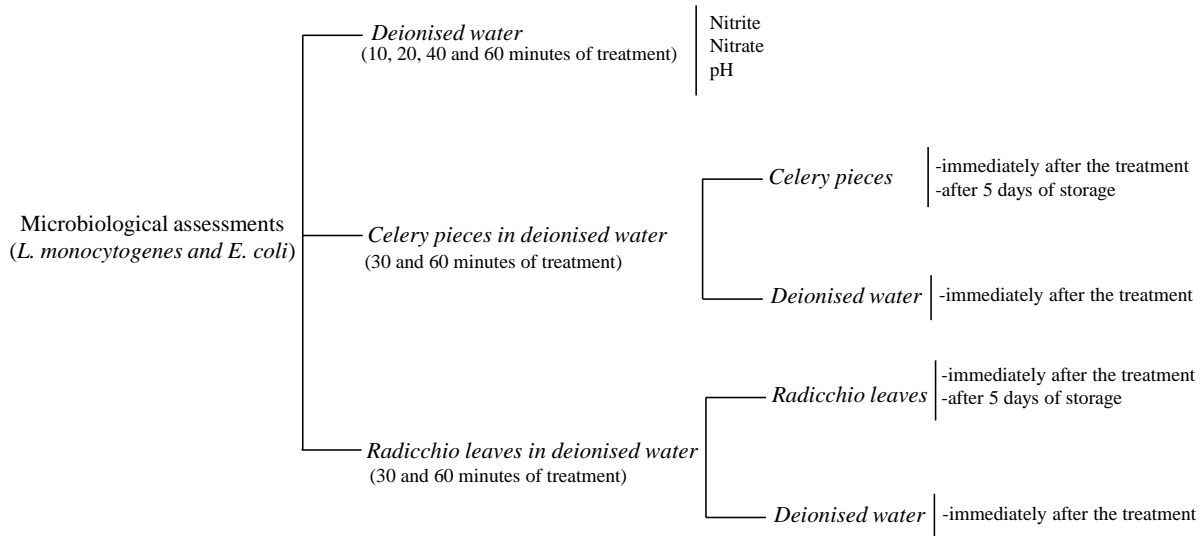


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451 **Figure 1.** Schematic of the electrodes configuration. *Deionised water or vegetables in deionised
452 water.

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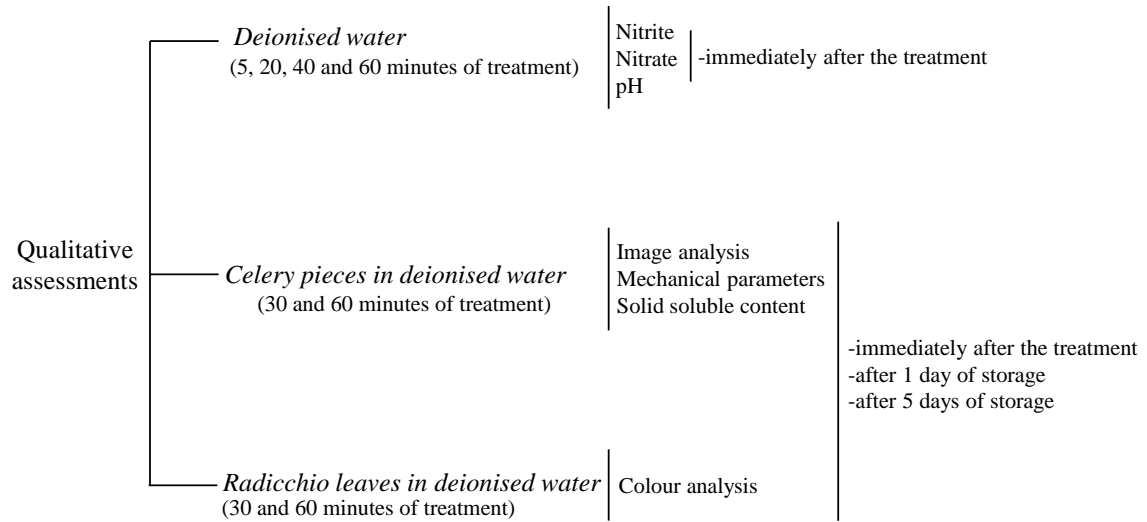


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456 **Figure 2.** Layout of the microbiological assessments.

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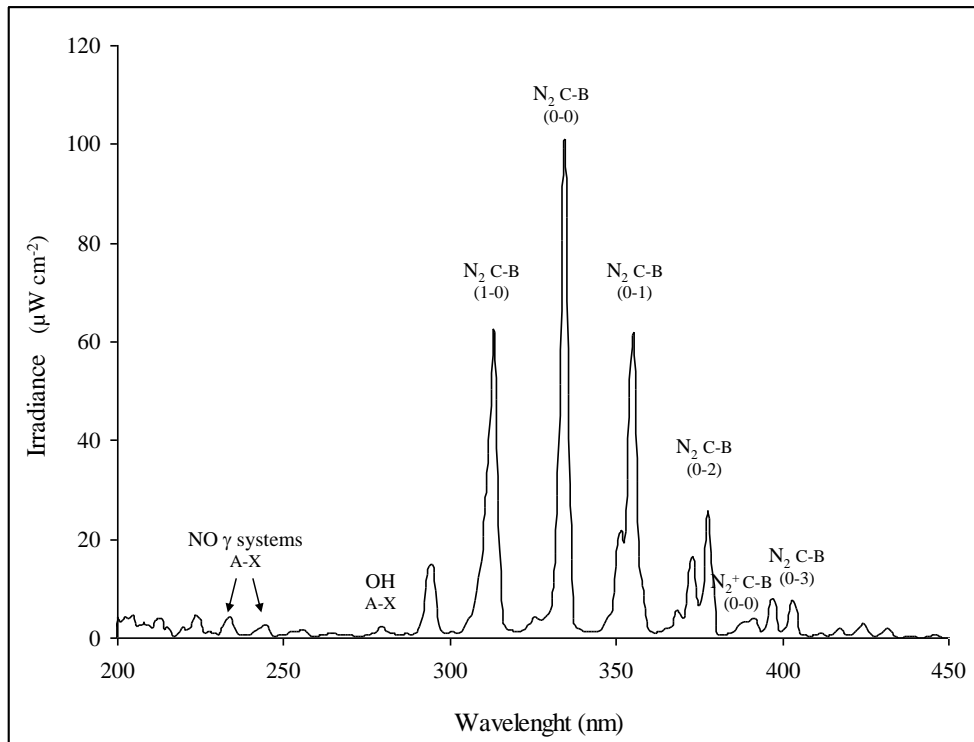


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460 **Figure 3.** Layout of the qualitative assessments.

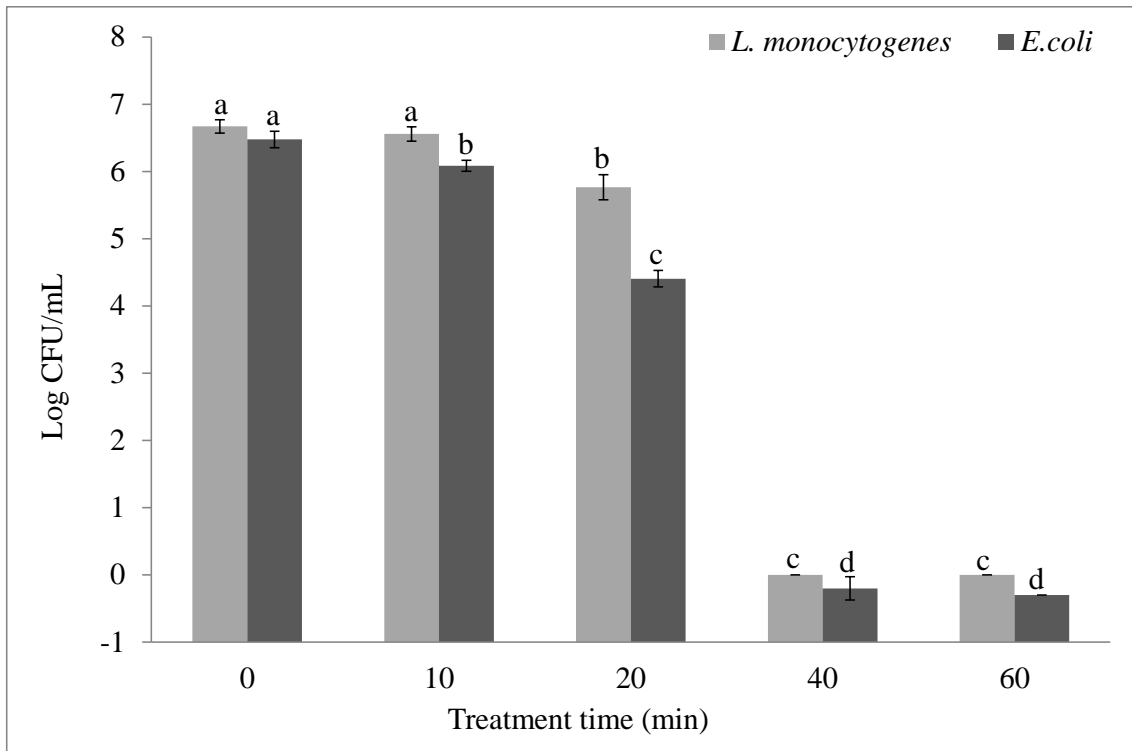
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463 **Figure 4.** Irradiance ($\mu\text{W cm}^{-2}$) of the Dielectric Barrier Discharge (input voltage of 19.15 V, at 26
 464 $\pm 1^\circ\text{C}$ and 53% R.H.).

465



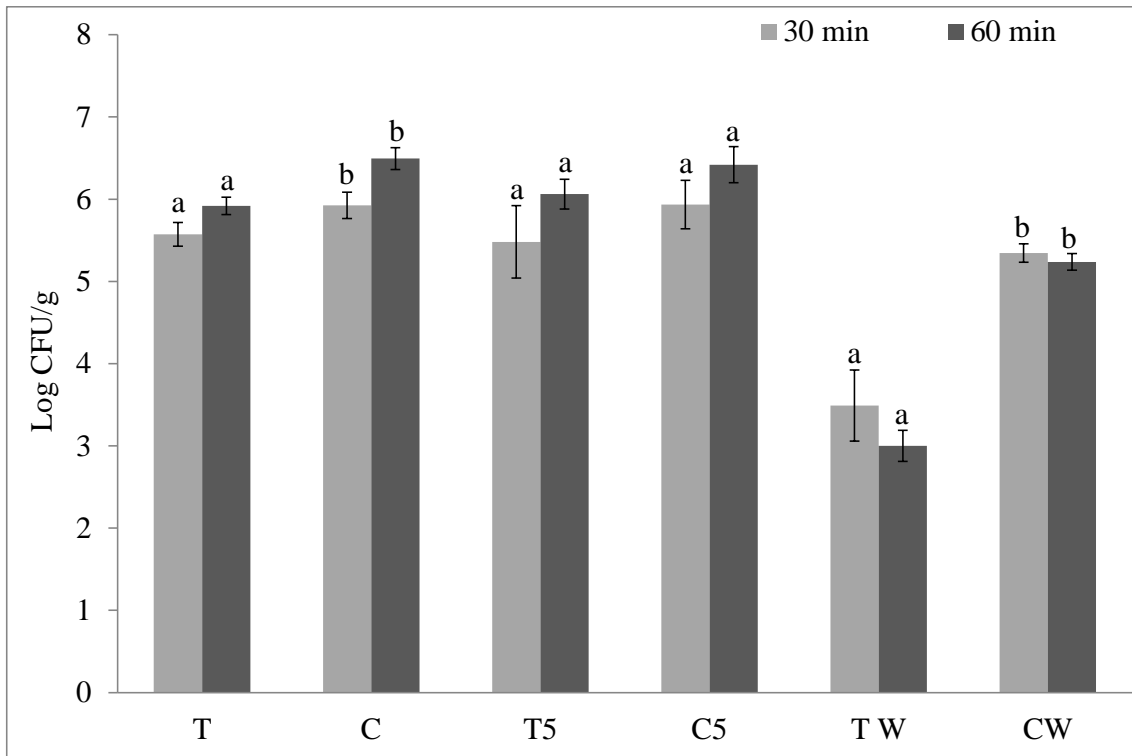
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467 Note: the same lowercase letters show not significant differences between the means at different treatment time (n=3, p-
 468 level<0.05).

469

470 **Figure 5.** *L. monocytogenes* and *E. coli* survival in deionised water (error bars indicate standard
 471 deviation).

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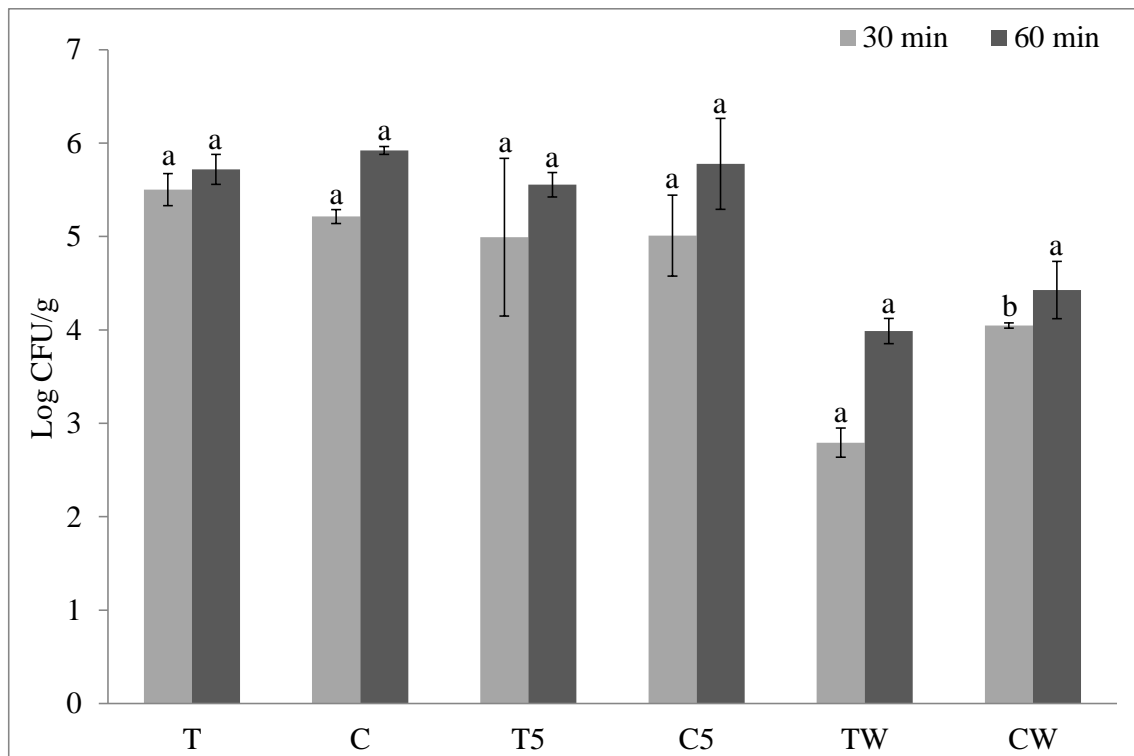
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474 *Note: The same lowercase letters show not significant differences for the same storage and treatment time, between
 475 control and treated samples (n=3, p-level<0.05).

476

477 **Figure 6.** *L. monocytogenes* survival on cut celery (T: treated samples immediately after the
 478 treatment, C: control samples immediately after the treatment time, T5: treated samples after
 479 storage, C: control samples after storage, TW: treated water samples and CW: control water sample,
 480 error bars indicate standard deviation).

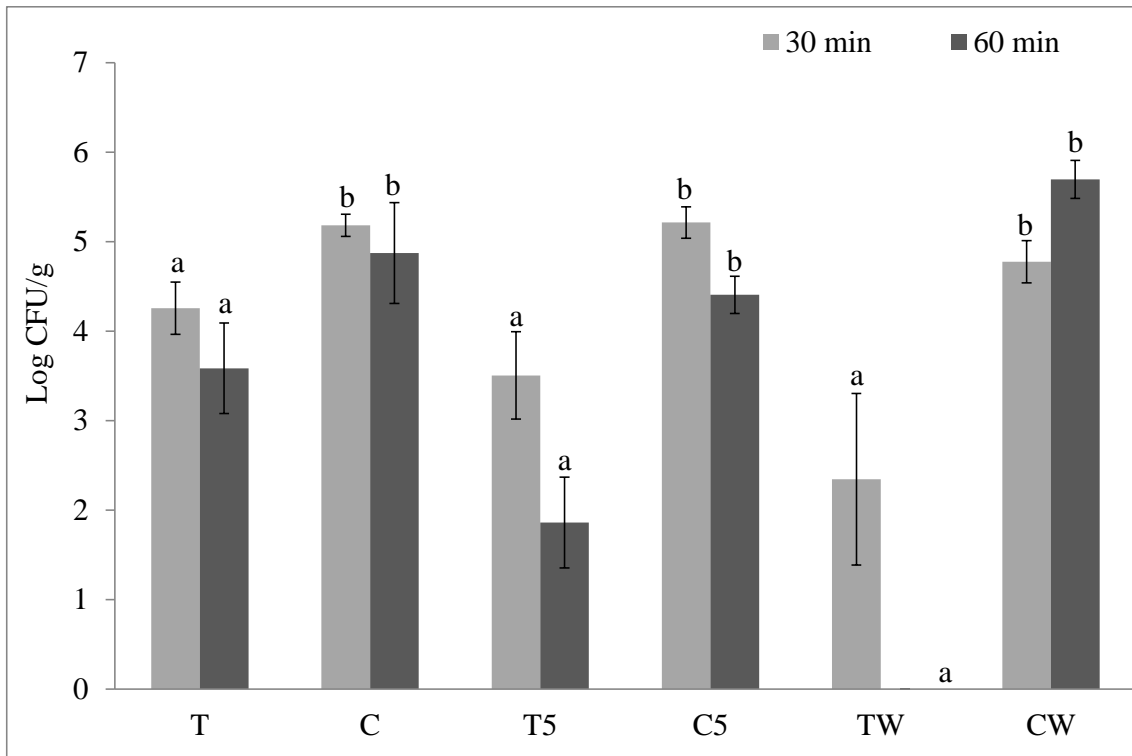
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483 **Figure 7.** *E.coli* survival on cut celery (T: treated samples immediately after the treatment, C:
 484 control samples immediately after the treatment time, T5: treated samples after storage, C: control
 485 samples after storage, TW: treated water samples and CW: control water sample, error bars indicate
 486 standard deviation). *See figure 6 note.

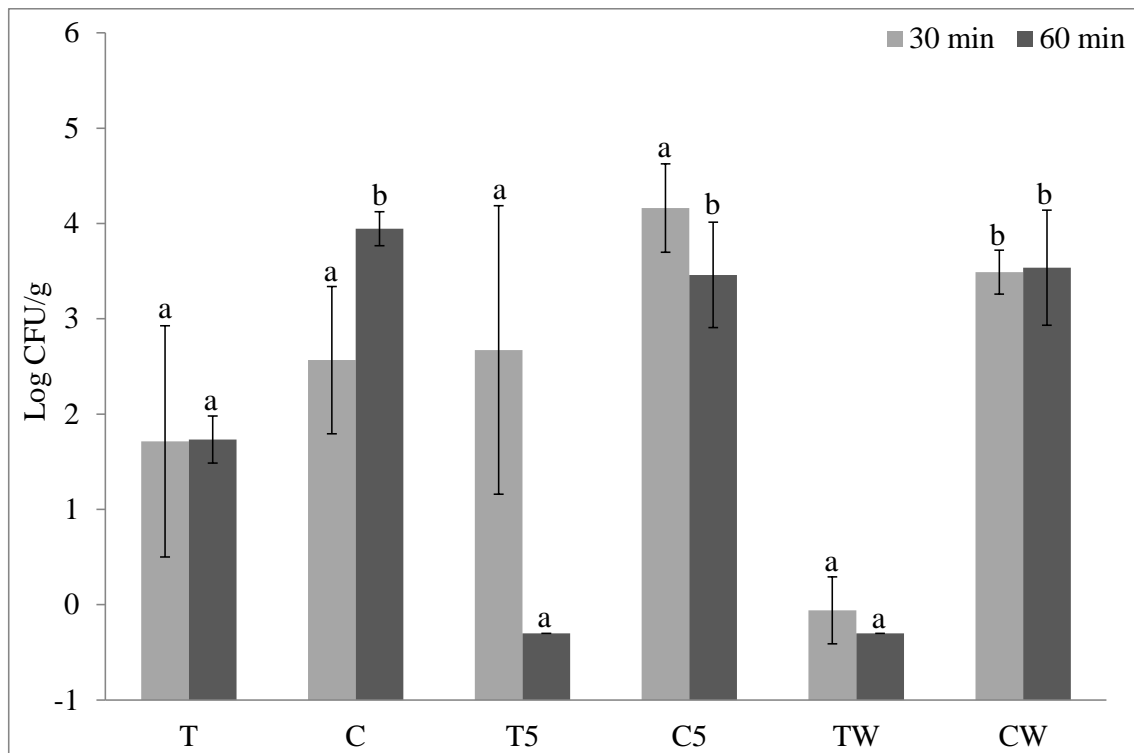
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489 **Figure 8.** *L. monocytogenes* survival on radicchio leaves (T: treated samples immediately after the
 490 treatment, C: control samples immediately after the treatment time, T5: treated samples after
 491 storage, C: control samples after storage, TW: treated water samples and CW: control water sample,
 492 error bars indicate standard deviation). *See figure 6 note.

493



494

495 **Figure 9.** *E. coli* survival on radicchio leaves (T: treated samples immediately after the treatment,
 496 C: control samples immediately after the treatment time, T5: treated samples after storage, C:
 497 control samples after storage, TW: treated water samples and CW: control water sample, error bars
 498 indicate standard deviation). *See figure 6 note.

499 **Table 1.** Chemical characterisation of deionised water.

Treatment time (minutes)	Nitrite (mg L ⁻¹)*	Nitrate (mg L ⁻¹)*	pH
0	0	0	6.45(0.05)
5	0.67	1.84	3.72(0.18)
20	5.54	43.84	3.11(0.14)
40	5.85	65.28	2.74(0.12)
60	6.96	111.31	2.51(0.17)

500 Note: standard deviations in brackets; *expanded uncertainty of measurement of 10%.

501 **Table 2.** Mean values of the percentage of celery green area.

Treatment time	Sample	Immediately after the treatment	After 1 day	After 5 days
30 min	T	97.5 (8.1) ^{a,A}	98.9 (7.9) ^{a,A}	66.4 (7.5) ^{a,B}
	C	93.9 (5.1) ^{a,A}	93.8 (5.3) ^{a,A}	71.5 (8.9) ^{a,B}
60 min	T	97.5 (6.3) ^{a,A}	94.2 (9.2) ^{a,A}	72.7 (10.2) ^{a,B}
	C	94.6 (5.9) ^{a,A}	92.1 (8.4) ^{a,A}	68.4 (8.9) ^{a,B}

502 Note: T: treated, C: control. The same lowercase letters show not significant differences for the same storage and
 503 treatment time, between control and treated samples while the same uppercase letters show not significant differences
 504 during the storage, within the same sample, control or treated and the same treatment time (p-level < 0.05). Standard
 505 deviations in brackets.
 506

507 **Table 3.** Mean values of the celery mechanical parameters.

Treatment time	Storage	Sample	F (N)	Area (N s)	Gradient (N s ⁻¹)
30 min	Immediately after the treatment	T	26.4 (14.8) ^{a,A}	37.4 (22.6) ^{a,A}	6.6 (3.7) ^{a,A}
		C	29.2 (18.1) ^{a,A}	44.6 (25.1) ^{a,A}	7.7 (5.1) ^{a,A}
	1 day	T	26.0 (8.9) ^{a,A}	40.0 (17.2) ^{a,A}	8.5 (2.5) ^{a,A}
		C	25.1 (7.9) ^{a,A}	56.1 (29.1) ^{a,A}	7.0 (2.6) ^{a,A}
	5 days	T	28.3 (8.2) ^{a,A}	46.1 (19.3) ^{a,A}	7.6 (2.6) ^{a,A}
		C	32.2 (7.6) ^{a,A}	46.6 (16.5) ^{a,A}	8.1 (2.3) ^{a,A}
60 min	Immediately after the treatment	T	32.6 (6.1) ^{a,A}	53.9 (23.7) ^{a,A}	9.4 (2.9) ^{a,A}
		C	29.8 (4.4) ^{a,A}	62.8 (18.9) ^{a,A}	8.2 (2.4) ^{a,A}
	1 day	T	29.4 (6.6) ^{a,A}	65.7 (20.5) ^{a,A}	6.2 (2.6) ^{a,A}
		C	26.7 (5.3) ^{a,A}	60.0 (21.9) ^{a,A}	6.9 (2.1) ^{a,A}
	5 days	T	26.7 (11.6) ^{a,A}	41.0 (21.6) ^{a,A}	6.4 (3.7) ^{a,A}
		C	28.7 (7.9) ^{a,A}	42.3 (14.1) ^{a,A}	6.5 (2.6) ^{a,A}

508 Note: T: treated, C: control. The same lowercase letters show not significant differences for the same storage and
 509 treatment time, between control and treated samples while the same uppercase letters show not significant differences
 510 during the storage, within the same sample, control or treated and the same treatment time (p-level < 0.05). Standard
 511 deviations in brackets.
 512

513 **Table 4.** Mean values of the celery soluble solids content (°Brix).

Treatment time	Sample	Immediately after the treatment	After 1 day	After 5 days
30 min	T	2.30 (0.16) ^{a,A}	2.39 (0.18) ^{a,A}	3.11 (0.36) ^{a,B}
	C	2.12 (0.32) ^{a,A}	2.49 (0.12) ^{a,A}	2.98 (0.18) ^{a,B}
60 min	T	1.86 (0.34) ^{a,A}	2.23 (0.23) ^{a,A}	3.37 (0.42) ^{a,B}
	C	2.09 (0.16) ^{a,A}	2.22 (0.14) ^{a,A}	3.23 (0.64) ^{a,B}

514 Note: T: treated, C: control. The same lowercase letters show not significant differences for the same storage and
 515 treatment time, between control and treated samples while the same uppercase letters show not significant differences
 516 during the storage, within the same sample, control or treated and the same treatment time (p-level < 0.05). Standard
 517 deviation in brackets

518

519 **Table 5.** Results of colour analysis of radicchio leaves.

Treatment time	Storage time	Sample	Colour parameters				
			L*	a*	b*	C*	
60 min	Before the treatment	T	36.9 (2.1) ^{a,A}	31 (1.8) ^{a,A}	2 (1.4) ^{a,A}	31.1 (1.8) ^{a,A}	
		C	36.5 (2.4) ^{a,A}	30.8 (1.7) ^{a,A}	2.2 (0.9) ^{a,A}	30.9 (1.7) ^{a,A}	
	Immediately after the treatment	T	33.6 (4.7) ^{a,A,B}	27.2 (2.2) ^{a,B}	6.9 (1.1) ^{a,B}	28.1 (2.2) ^{a,A}	
		C	34.4 (4.3) ^{a,A,B}	29 (4.5) ^{a,A,B}	7.4 (1.6) ^{a,B}	27.2 (3.7) ^{a,A,B}	
	1 day	T	32.5 (3.2) ^{a,B}	22.4 (3.7) ^{a,C}	7.5 (2.0) ^{a,B}	24 (2.2) ^{a,B}	
		C	32.2 (1.9) ^{a,B}	26.6 (4.9) ^{a,B,C}	7 (0.6) ^{a,B}	27.5 (3.9) ^{a,A,B}	
	5 days	T	30.4 (3.6) ^{a,B}	18.6 (5.2) ^{a,C}	7.1 (3.7) ^{a,B}	20.6 (1.1) ^{a,C}	
		C	30 (3.9) ^{a,B}	24.5 (1.8) ^{b,C}	7.1 (0.7) ^{a,B}	26.2 (1.8) ^{b,B}	
	30 min	Before the treatment	T	37.9 (3.3) ^{a,A}	32.1 (2.1) ^{a,A}	2.8 (0.7) ^{a,A}	32.2 (2.9) ^{a,A}
			C	36.7 (1.7) ^{a,A}	30.9 (2.8) ^{a,A}	1.8 (0.7) ^{b,A}	29.9 (1.8) ^{a,A}
Immediately after the treatment		T	34.1 (3.9) ^{a,A}	30.1 (2.4) ^{a,A}	6 (0.6) ^{a,B}	31.1 (2.4) ^{a,A}	
		C	34.6 (2.4) ^{a,A}	30 (1.7) ^{a,A}	6 (0.6) ^{a,B}	30.6 (1.7) ^{a,A}	
1 day		T	28.8 (6.4) ^{a,B}	24.5 (2.8) ^{a,B}	6.2 (1.5) ^{a,B}	25.3 (2.6) ^{a,B}	
		C	28.6 (4.8) ^{a,B}	24.4 (5.8) ^{a,B}	5.2 (1.4) ^{a,B}	24.9 (5.9) ^{a,B}	
5 days		T	25.5 (3.6) ^{a,B}	19.2 (2.4) ^{a,C}	6.3 (1.0) ^{a,B}	20.4 (2.5) ^{a,C}	
		C	25.5 (8.5) ^{a,B}	22.5 (3.8) ^{a,B}	5.3 (1.5) ^{a,B}	23.2 (3.6) ^{a,B}	

520

521 Note: T: treated, C: control. The same lowercase letters show not significant differences for the same storage and
522 treatment time, between control and treated samples while the same uppercase letters show not significant differences
523 during the storage, within the same sample, control or treated and the same treatment time (p-level < 0.05). Standard
524 deviations in brackets.

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