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

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Abstract

The antimicrobial efficacy of dielectric barrier discharge atmospheric gas plasma (DBD) was tested against *Listeria monocytogenes* and shigatoxin-producing *Escherichia coli* serogroups O157 and O26. Challenge tests were carried out with samples of cut celery and radicchio leaves inoculated with a mix of five strains of *L. monocytogenes* or the two strains of *E. coli* immersed in deionised water. The treatment efficacy was also assessed considering only the contaminated deionised water. For deionised inoculated water alone, a treatment time-dependent strong effect was observed and a pathogens reduction higher than 6 Log CFU/mL was obtained after 40 min of treatment. With the vegetables presence in the liquid medium, the efficacy appeared reduced and related to the treatment time, microorganism, substrate and storage duration (reduction up to 2.5 and 3.7 Log CFU/cm^2 for *L. monocytogenes* and *E. coli*, respectively). No significant changes were observed on celery visual attributes, soluble solids content and textural parameters. A significant decrease of the Chroma colour parameter during storage was noted in treated radicchio samples respect to control ones.

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

1. Introduction

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

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

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

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

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

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

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

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

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

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

2. Materials and Methods

2.1 Gas plasma generator

A DBD generator was used for the experiments. A temperature of 33 ± 1°C and a saturation condition were observed inside the hermetic chamber after 30 min of the tested treatment conditions.

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

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

All the assessments (chemical characterisation, microbiological and qualitative assessments) were conducted at atmospheric conditions (at 26 ± 1°C and 53% R.H.) by using air as working gas with a supply voltage of 19.15 V (3.15 ± 0.5 A). A schematic of the electrodes configuration is shown in Figure 1.

2.2 Chemical characterisation of the discharge

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

2.3 Microbiological assessments

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

2.3.1 Growth of bacteria and preparation of inoculum

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

Broth cultures were stored at −80°C with the addition of 25% glycerol. Tryptic soy agar (TSA) plates were streaked from these stocks and stored at 4°C. Cultures for inactivation experiments were inoculated from single colonies on these agar plates and grown at 37°C for 21 h. Single colonies of each strain of *L. monocytogenes* and *E. coli* were harvested and suspended in Phosphate-Buffered Saline pH 7.0 (PBS) at a cell density of approximately 10^8 CFU/mL (0.08-0.1 Absorbance at 625 nm). The number of bacteria was determined by plating the appropriate decimal dilutions on TSA and incubating at 37°C for 24 h.

2.3.2 Deionised water

A mix of *L. monocytogenes* strains and a mix of *E. coli* strains were inoculated in 150 mL of deionised water samples at a cell density of approx.10^6 CFU/mL. After 10, 20, 40 and 60 min of treatment three aliquots of 1 mL each were harvested and the number of inoculated bacteria was determined as described above.

2.3.3 Vegetables (celery and radicchio) in deionised water

Ten grams of celery pieces with a thickness of 3 mm and portions of radicchio leaves (approximately 16 cm^2) were layered on the bottom of plastic boxes. To contaminate the vegetables, one hundred microliter of the suspensions of bacteria (*L. monocytogenes* or *E. coli*) in PBS (at a cell density of approximately 10^8 CFU/mL) were spotted on the surface of celery or the radicchio leaves. Leaves were incubated at room temperature under laminar flow in a biohazard cabinet until the inoculum was completely dried (approximately 60 min). Inoculated leaves were submerged with deionised water (150 mL) and treated for 30 or 60 min, whereas the control samples were held in the biohazard cabinet for the same period of time. After the treatment, the vegetables were pull out from the water.

2.3.4. Determination of bacterial survival

Immediately after treatments, treated and control vegetables and water samples were analysed separately to assess the viability of *L. monocytogenes* and *E. coli* cells. Vegetables were 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 ½ 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 (N·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 ($C^* = \sqrt{a^{*2} + b^{*2}}$).

2.5 Data analysis

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

3. Results and discussion

3.1 Chemical characterisation of the discharge

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

3.2 Deionised water

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

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

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

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

3.3 Vegetables (celery and radicchio) in deionised water

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

The gas plasma treatment appears to have a bactericidal effect on *L. monocytogenes* and *E. coli* on radicchio leaves (Figures 8 and 9). Immediately after 30 min and 60 min of treatment, the number of viable *L. monocytogenes* was significantly lower in comparison to the control. However, the detected differences were not high: 1 and 1.3 Log CFU/cm^2, respectively. After storage at 4°C for 5 d, the differences between control and treated samples were -1.7 and -2.5 Log after 30 and 60 min of treatment, respectively. For *E. coli*, no significant differences between controls and treated samples were observed after 30 minutes, whereas a significant difference was measured after 60 min (-2.2 Log CFU/cm^2). After storage no decontamination effects were observed after 30 min, but a significant lower number of viable *E. coli* (-3.7 Log CFU/cm^2) in comparison with controls was detected after 60 min.

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

competition rate and lower survival of inoculated pathogen bacteria. However results of previous studies, does not fully support this hypothesis since significantly higher total bacteria counts were described in minimally processed celery in comparison to minimally processed radicchio (6-7 $\log_{10}$ CFU/g *vs* 4 $\log_{10}$ CFU/g) (Lavelli et al., 2009; Lopez et al., 2005). Further studies on the composition of naturally colonizing microbial population of the two vegetables need to be performed.

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

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

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

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

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

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

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

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

4. Conclusions

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

In relation to the vegetable decontamination, the efficacy is related to the specific microorganism, kind of vegetable, treatment time and duration of storage. A more pronounced bactericidal effect was observed in radicchio leaves samples (up to -3.7 Log CFU/cm^2) in contrast with to cut celery pieces (up to -0.57 Log CFU/g). However, the treatment induced a more rapid loss of the radicchio leaves during the storage.

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

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

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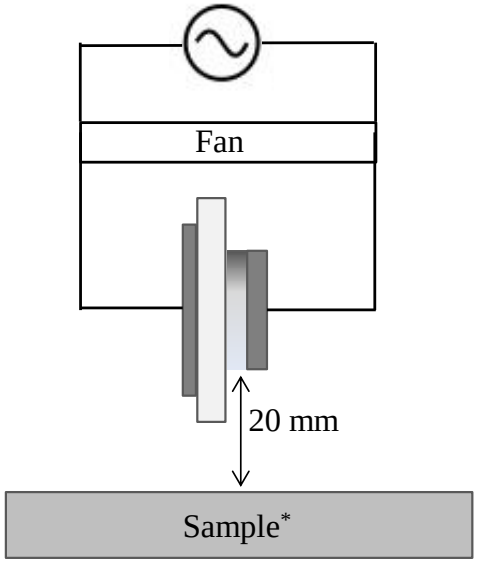

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450

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

452 water.

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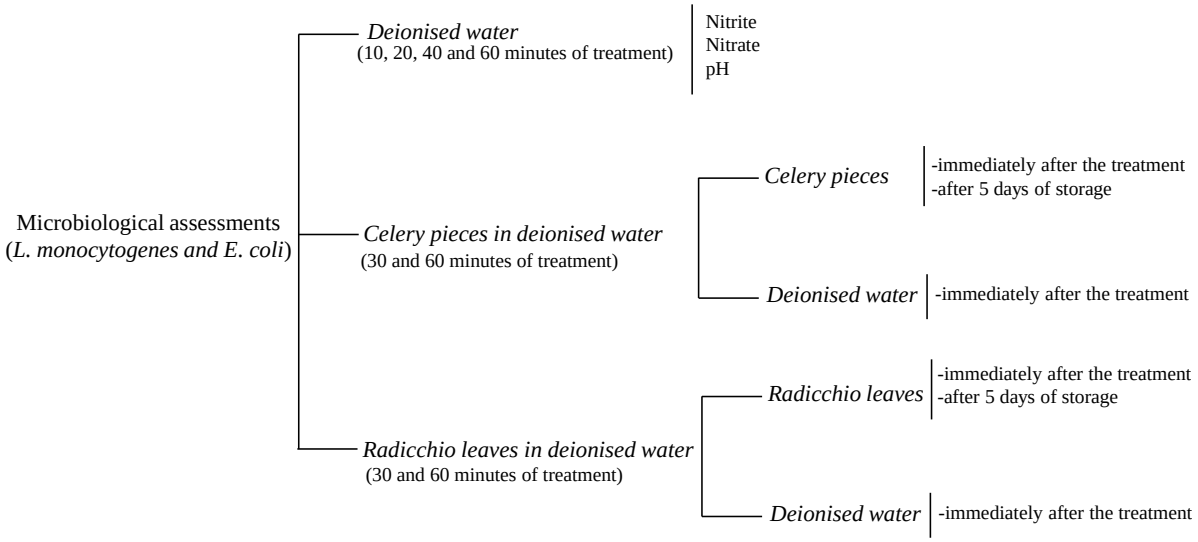
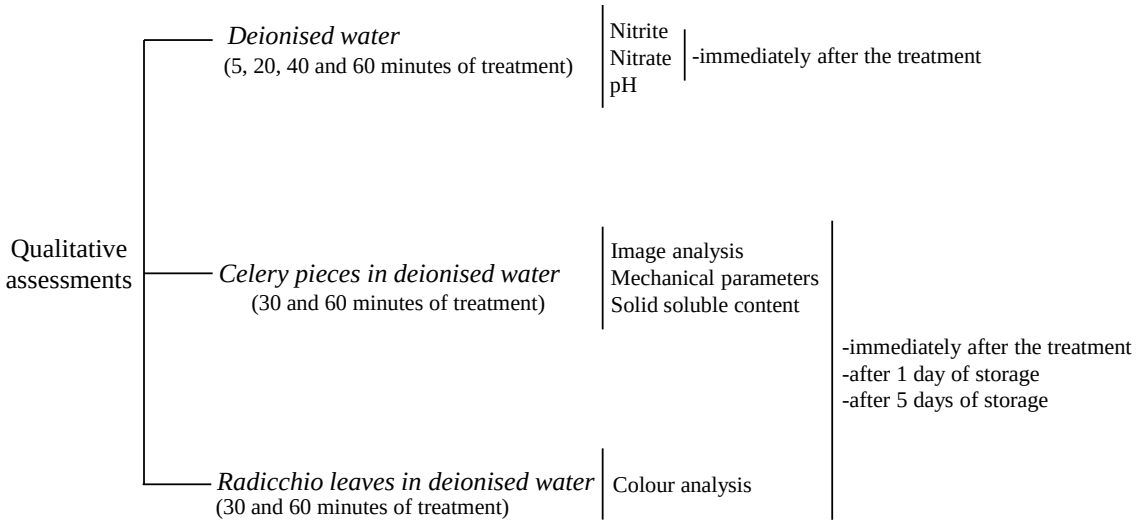


454

455

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

457

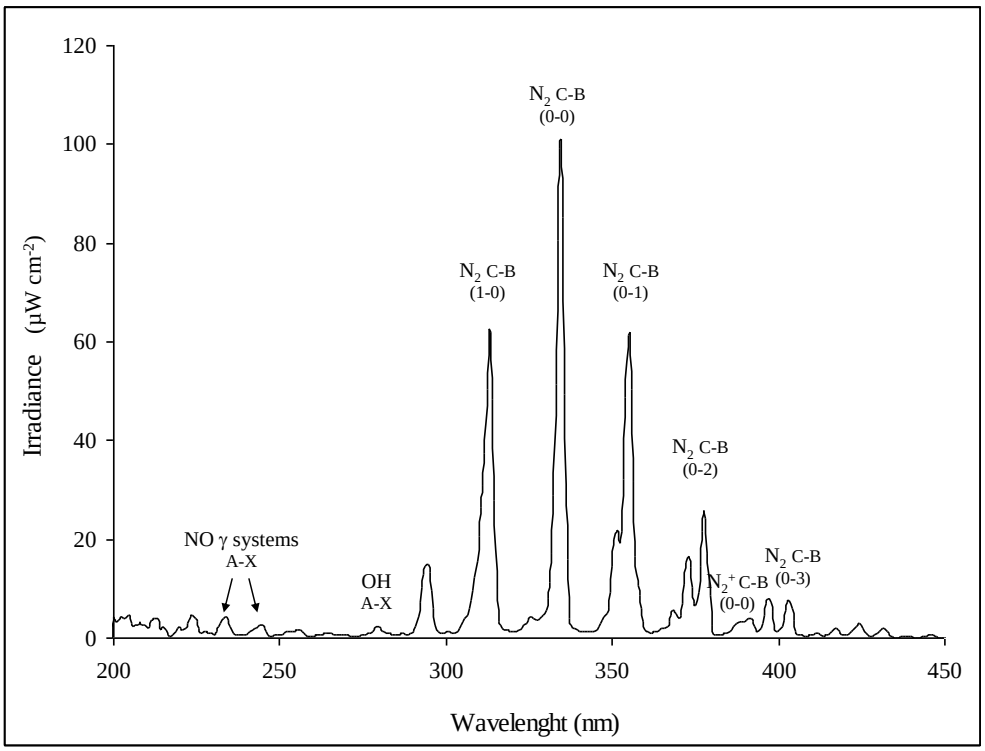


458

459

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

461

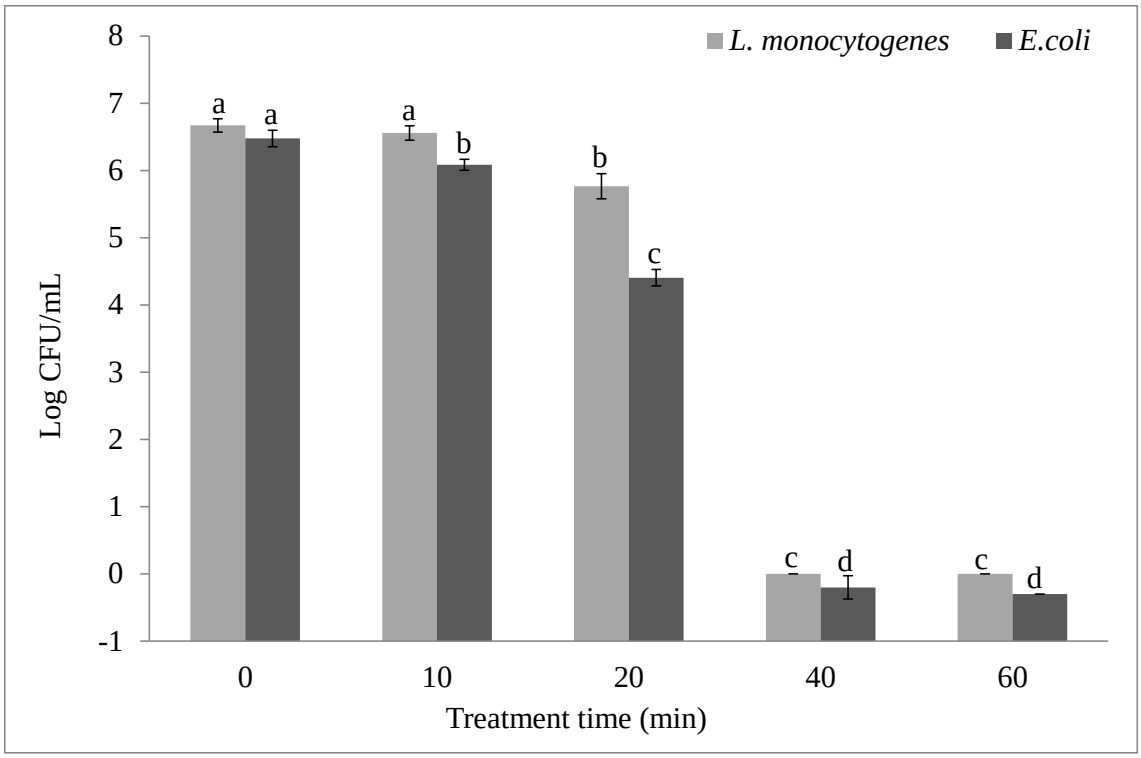


462

463 **Figure 4.** Irradiance (μW cm^{-2}) of the Dielectric Barrier Discharge (input voltage of 19.15 V, at 26

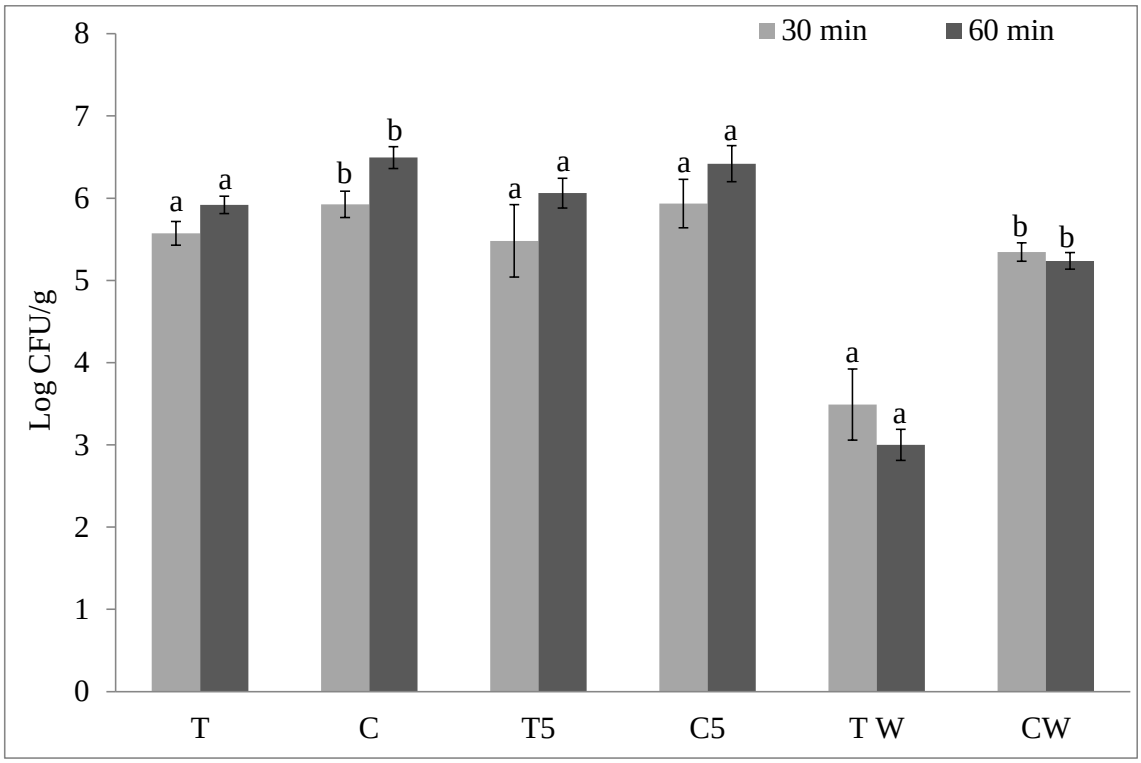
464 ± 1°C and 53% R.H.).

465



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

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



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

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

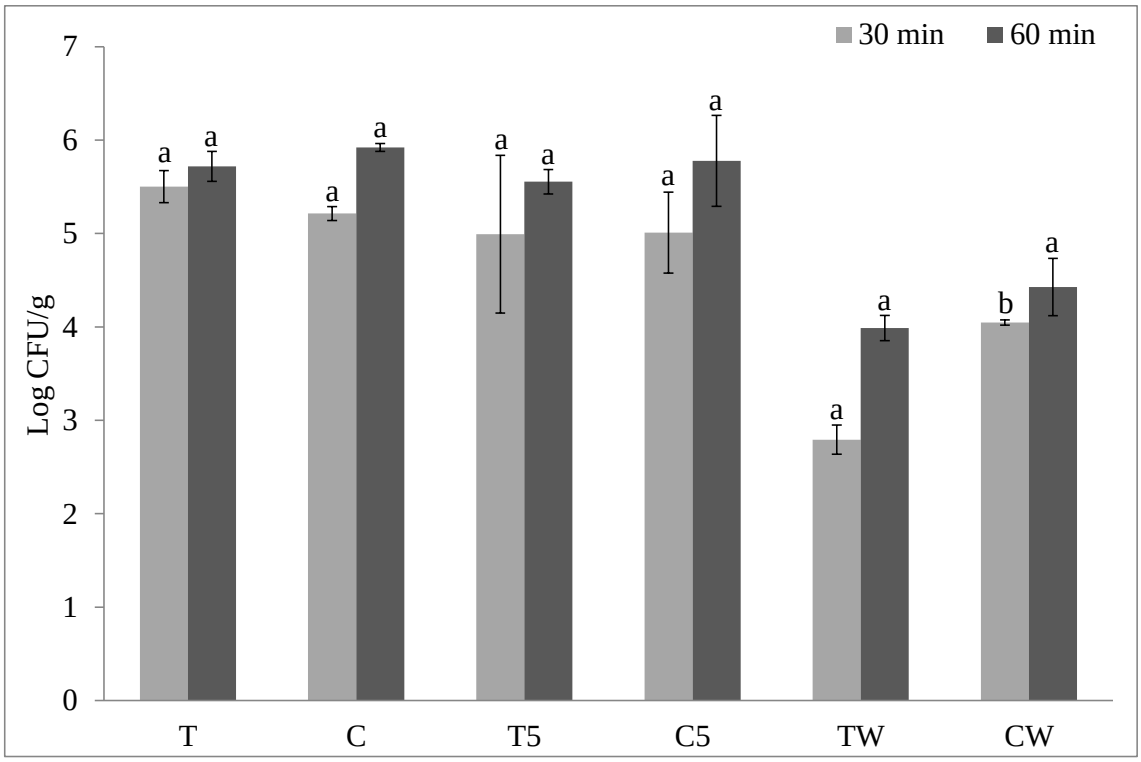


Figure 7. *E.coli* survival on cut celery (T: treated samples immediately after the treatment, C: control samples immediately after the treatment time, T5: treated samples after storage, C: control samples after storage, TW: treated water samples and CW: control water sample, error bars indicate standard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

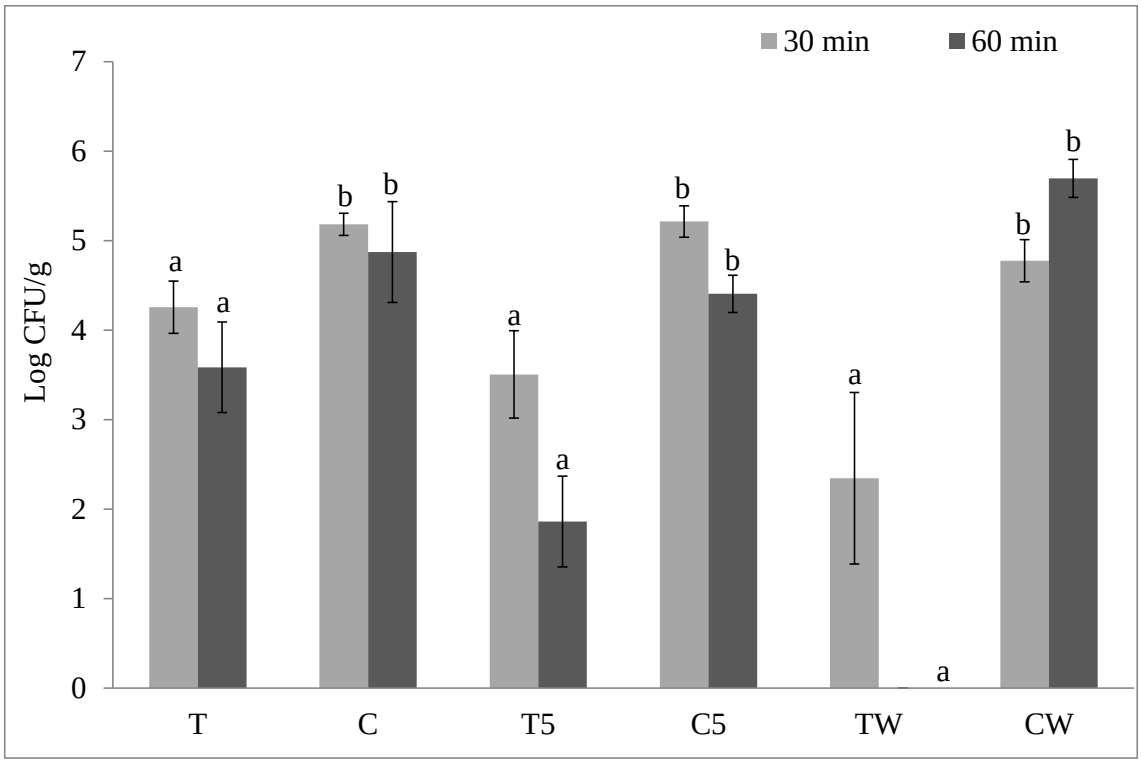


Figure 8. *L. monocytogenes* survival on radicchio leaves (T: treated samples immediately after the treatment, C: control samples immediately after the treatment time, T5: treated samples after storage, C: control samples after storage, TW: treated water samples and CW: control water sample, error bars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

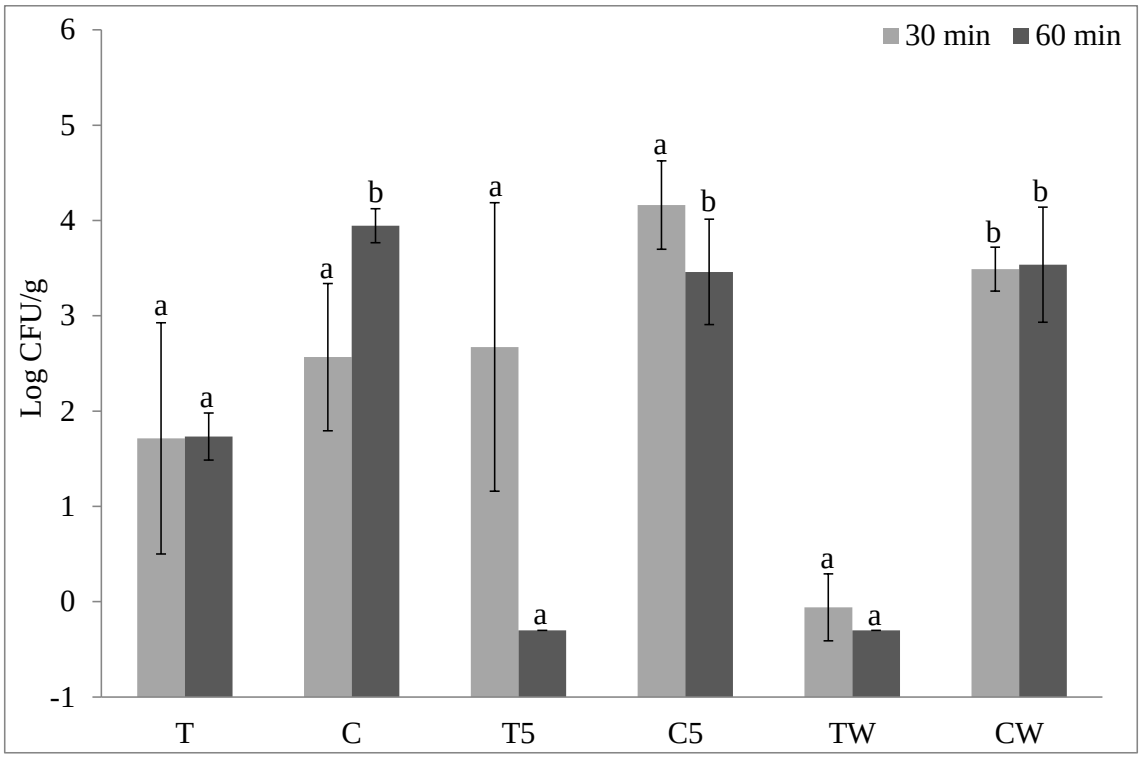


Figure 9. *E. coli* survival on radicchio leaves (T: treated samples immediately after the treatment, C: control samples immediately after the treatment time, T5: treated samples after storage, C: control samples after storage, TW: treated water samples and CW: control water sample, error bars indicate 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.
525
526