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Effects of sanitizing treatments with atmospheric cold plasma, SDS and lactic acid on verotoxin-producing *Escherichia coli* and *Listeria monocytogenes* in red chicory (radicchio)

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\*Manuscript

**1 Effects of Sanitizing Treatments with Atmospheric Cold Plasma, SDS and Lactic Acid on 2**  
**verotoxin-producing *Escherichia coli* and *Listeria monocytogenes* in Red Chicory (radicchio)**

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9

## 9     **Abstract**

10     The main objective of this study was to evaluate the synergistic effect of atmospheric cold plasma  
 11     (ACP), Sodium Dodecyl Sulphate (SDS) and lactic acid (LA) on *L. monocytogenes* and  
 12     verotoxinproducing *E. coli* in red chicory. Experimentally inoculated samples were pre-treated with  
 13     either SDS, or SDS + LA for 5, 10 or 15 min. Pre-treated samples were then submerged in deionised  
 14     water and either exposed to ACP generated by dielectric barrier discharge device (DBD: fixed  
 15     parameters: 19.15 V and 3.15 A) for 15 min or left untreated. All combinations of treatments were  
 16     evaluated for sensory effects. Viable counts of verotoxin-producing *E. coli* on red chicory decreased  
 17     by more than 4 logs (4.78 Log CFU/cm<sup>2</sup> vs control) after a treatment with LA+SDS for 5 min and  
 18     ACP for 15 min and often dropped below the limit of quantification. *L. monocytogenes* showed a  
 19     higher tolerance to this sanitizing treatment and the level of inactivation was higher than 3 logs  
 20     (3.77 Log CFU/cm<sup>2</sup> vs control) only by increasing the duration of the washing step in LA+SDS to 15  
 21     min. The different treatments had no detrimental effects on colour, freshness and texture of red  
 22     chicory, but odour and overall acceptability of the samples treated by ACP slightly decreased during  
 23     storage. Further optimization of treatment parameters for maintaining fresh characteristics are needed,  
 24     but the effectiveness of combinations of sanitizers and ACP on other pathogens and fresh produce  
 25     worth to be investigated.

## 26    **Highlights**

- 27    • Red chicory leaves were treated with Lactic Acid, SDS and ACP to control VTEC and Listeria
- 28    • LA and SDS had synergistic effect with ACP in inactivation of VTEC and Listeria
- 29    • Freshness, colour and texture of the leaves were slightly affected by the analysed treatments

30    **Keywords:** Sanitizing Treatments; Atmospheric Cold Plasma; Sodium Dodecyl Sulphate; Lactic

31 Acid; verotoxin-producing *Escherichia coli*; *Listeria monocytogenes*

## 32 **1. Introduction**

33 The supply of safe food remains one of the major concerns of food industry. Among food, leafy greens  
34 are relatively vulnerable to pathogen contamination such as *Escherichia coli*, *Listeria* and  
35 *Salmonella*. Salad vegetables have been frequently associated with foodborne outbreaks (Denis,  
36 Zhang, Leroux, Trudel, & Bietlot, 2016; Friesema et al., 2008; Lynch, Tauxe, & Hedberg, 2009;  
37 Söderström et al., 2008).

38 During processing, suitable disinfectants such as chlorine dioxide, ozone, and peracetic acid, are used  
39 with the aim to reduce the number of microorganisms naturally contaminating fresh produces and  
40 prevent cross-contamination (Banach, Sampers, Haute, & van der Fels-Klerx, 2015). Relatively  
41 recently, chlorine was discovered to react with organic compounds with the formation of carcinogenic  
42 chlorinated compounds in water (chloramines and trihalomethanes) (Gil, Selma, López-Gálvez, &  
43 Allende, 2009). Since then, there have been many attempts to find alternative washing treatments.

44 A novel processing technology, Atmospheric Cold Plasma (ACP), has shown promising results  
45 (Berardinelli et al., 2016; Mir, Shah, & Mir, 2016; Pasquali et al., 2016). The antimicrobial  
46 mechanism is yet to be elucidated, but it is known that ACP is a source of multiple chemically reactive  
47 species with a high bactericidal activity, including reactive oxygen (ROS) and reactive nitrogen  
48 species (RNS) (Liao et al., 2016). Among the ROS, ozone, atomic oxygen, singlet oxygen,  
49 superoxide, peroxide, and hydroxyl radicals, independently or in synergy, are expected to play a role  
50 in the bacterial inactivation process (Joshi et al., 2011; Ziuzina, Boehm, Patil, Cullen, & Bourke,  
51 2015).

52 Our previous study demonstrated that ACP in water medium was efficient in the inactivation of *L.*  
53 *monocytogenes* and *E. coli* cells inoculated on radicchio leaves, but a significant reduction was

observed only in the planktonic bacteria (i.e. the microbial cells that migrated to the washing water) (Berardinelli et al., 2016). In this respect, the use of a surfactant might increase the sanitation efficacy of ACP by improving the washing effect. Some studies have reported that combinations of surfactants, such as Sodium Dodecyl Sulphate (SDS), with organic acids have synergistic effect (Beuchat, Mann, & Alali, 2012; Zhao, Zhao, & Doyle, 2009). Besides the antimicrobial property by lowering pH, Lactic Acid (LA) is a permeabilizer of the Gram-negative bacterial outer membrane and showed a strong sensitizing effect to SDS in *E. coli* and *Salmonella* Typhimurium (Alakomi, Skyttä, Saarela, & Helander, 2005). Unfortunately, LA and SDS also caused detrimental effects on visual quality and texture of lettuce in modified atmosphere packages during storage at 4 °C, although these effects can depend on experimental condition such as slice the lettuce into pieces before the sanitation step (Guan, Huang, & Fan, 2010).

According to recent studies, the presence of plasma-induced acidic pH, the reactive oxygen (ROS) and nitrogen (RNS) species and their interactions, give further rise to reactive products, which get stabilized and exhibit strong antimicrobial properties (Ercan, Smith, Ji, Brooks, & Joshi, 2016; Oehmigen et al., 2010).

This study aimed at evaluating the synergistic effect of ACP, SDS and LA on *L. monocytogenes* and verotoxin-producing *E. coli* in red chicory. This perennial plant is often used as a colourful salad garnish due to the leaves' bitter flavour. Red chicory was chosen as a model of leafy vegetables because of its sensitivity to discoloration and sogginess.

## 2. Material and Methods

### 2.1. Samples

Radicchio, also known as red chicory (*Cichorium intybus* L.) was purchased at a local supermarket and immediately transported to laboratory in a cool bag. The outer 3 or 4 leaves and core were removed from the red chicory head and discarded. The remaining leaves were washed under tap water

for 10 s and drained carefully, then left under laminar flow in a biohazard cabinet to dry (approximately 30 min) and exposed to germicidal UV-light (254 nm) for 30 min (both sides).

## 2.2. *Growth of bacteria and preparation of inoculum*

Five strains of verotoxin-producing *E. coli* (VTEC) (O157:H7 ATCC 35150, O157:Hnt VT+ isolated from cattle carcass, O26:H11 VT+, isolated from milk, O26:H11 VT+, isolated from milk filter, O26:H11 FV4028 received from *E. coli* reference laboratory Lugo (Spain)) and five strains of *L. monocytogenes* (LR 102 0227-359, vi 51028, 0113-131, vi51010) were used. Cultures were grown at 37°C using Tryptic Soy Broth (TSB, Oxoid) and Brain Heart Infusion (BHI, Oxoid, Basingstoke, United Kingdom) for VTEC and *L. monocytogenes*, 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 VTEC and *L. monocytogenes* 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. *Inoculation of samples*

One hundred microliter of the suspensions of bacteria (VTEC or *L. monocytogenes*) in PBS (at a cell density of approximately  $10^8$  CFU/mL) were spotted on the red chicory leaves. Bacteria would be expected to adhere in higher numbers on soil contaminated spots than would those on the clean vegetables, therefore 100 µl of horticultural soil water solution (0.1% w/v), which was freshly boiled for 60 min, was spotted on 10 points of each red chicory leave (external part). The inoculum with VTEC or *L. monocytogenes* was made on the soil contaminated spots, then leaves were airdried on a mesh in a laminar flow biosafety hood at room temperature for 1 hour. The inocula were spotted in a

102 well-identified area of approximately 2 cm by 5 cm (10 cm<sup>2</sup>) at each side of the central leave vein.  
 103 The inoculated red chicory leaves were then put in plastic box and hold at 8-10°C overnight.

#### 104 2.4. *Preliminary washing steps*

105 After the overnight storage the radicchio leaves were cut and the inoculated portions (10 cm<sup>2</sup>) were  
 106 submerged in water solutions containing SDS (0.05% w/v), LA (2% w/v) + SDS (0.05% w/v) or NaCl  
 107 (0.85% w/v). All reagents were purchased from Sigma-Aldrich (Milano, Italy). Treatments were  
 108 performed at room temperature (20-25°C) for 5, 10 or 15 min. During this period, the washing effect  
 109 was increased by gently tilting the containers for 5 s every 2 min. After this step samples were rinsed  
 110 with 18 ml of sterile deionized water.

#### 111 2.5. *Plasma settings and treatments*

112 ACP treatment of red chicory was carried out as previously described (Berardinelli et al., 2016). In  
 113 brief, radicchio samples were subjected to the effect of plasma in a plastic hermetic chamber housing  
 114 a Dielectric Barrier Discharge (DBD) plasma source; a fan mounted above the electrode was used to  
 115 direct the plasma species against the sample. Samples were placed below the plasma source in plastic  
 116 boxes filled with deionised water to a height of 0.6 cm. The distance between the fluid and the  
 117 electrodes was 2 cm. Voltage at the electrodes was produced by high voltage (HV) transformers and  
 118 power switching transistors supplied by a stabilized DC power supply (ElektroAutomatik GmbH &  
 119 Co.KG, EA-PS 2042-06B). Treatment parameters were fixed at 19.15 V and  $3.15 \pm 0.5$  A for 15 min.

120 Challenge tests with *L. monocytogenes* and VTEC were performed independently. To ensure result  
 121 reproducibility, each experiment (preliminary washing and ACP) was repeated three times with three  
 122 replicate samples each.



## 123 2.6. Microbiological assessments

124 The viability of VTEC and *L. monocytogenes* cells was assessed after each challenge test. The samples  
125 were homogenised in 50 ml of buffered peptone water (BPW, Oxoid, Basingstoke, United Kingdom)  
126 with a Stomacher® (Seward, UK) at normal speed for 2 min. Serial ten-fold dilutions were performed  
127 and plated onto Thin Agar Layer (TAL) plates for colony counting after storage at room temperature  
128 for one hour. The TAL method involves overlaying 14 mL of nonselective medium (Tryptic Soy Agar  
129 Oxoid, TSA, Basingstoke, United Kingdom) onto a pre-poured, pathogen-specific selective medium,  
130 in order to allow the recovery of sub-lethally injured cells (Wu and Fung, 2001). Agar Listeria  
131 according to Ottaviani and Agosti (ALOA, Biolife, Milan, Italy) was used as selective and differential  
132 medium for *L. monocytogenes*. Oxoid™ MacConkey Agar (MAC, Oxoid, Basingstoke, United  
133 Kingdom) was used for the detection and isolation of *E. coli*. Ten lactose fermenting colonies were  
134 picked from the countable plates and tested by latex agglutination kits for *E. coli* O26 and *E. coli*  
135 O157 (Oxoid™ Dryspot™ *E. coli* O157 and *E. coli* O26 test kit, Basingstoke, United Kingdom) and  
136 the numbers of serogroup specific colonies were calculated. Colonies were enumerated on TAL plates  
137 after incubation at 37°C for 24 h. Upon the observation of no colonies, BPW homogenates were tested  
138 with the methods ISO 16654 and ISO 11290 to detect the presence of VTEC and *L. monocytogenes*,  
139 respectively.

140 Lethality was calculated as the difference between the logarithms of colony counts of untreated and  
141 treated samples ( $\text{Log } N_0 - \text{Log } N$ ).

## 142 2.7. Qualitative assessments

143 Non inoculated red chicory leave fragments (approximately 2 cm by 5 cm) were submerged in water  
144 solution of SDS and LA+SDS (at concentrations reported above) for 5, 10 or 15 min. After this step  
145 the samples were washed with deionized water and immersed in 90 ml of deionized water.

146 The plasma treatment was carried out for 15 min as described in the microbiological section. Each  
 147 treatment was repeated three times. Colour of red chicory was assessed with a reflectance colorimeter  
 148 (Minolta Chroma Meter CR-400, Konica Minolta Sensing Europe, Cinisello Balsamo, Italy). Three  
 149 measurements were performed for each sample before and after each treatment. The analysis was  
 150 repeated after one day of storage (at 4°C and 80% of R.H). Colour differences were identified using  
 151 the CIE L\*a\*b\* coordinates and the parameter C\* (Chroma) was calculated from the a\* and b\*  
 152 coordinates (C.I.E. 2007).

153 Sensory test was conducted with 12 untrained assessors, considering 5 attributes: freshness, colour,  
 154 odour, texture and overall acceptability, as described by Pasquali et al. (2016).

155 pH values of water solutions before and immediately after the plasma treatment were also recorded  
 156 (pH meter, GLP 22, CRISON).

## 157 2.8. *Statistical analysis*

158 Unless elsewhere noted, data were reported as the arithmetic mean  $\pm$  Standard Deviation (SD). The  
 159 R Stats Package for Windows (CRAN, R-project) was used for statistical analyses. Data were  
 160 analysed for normality and homoscedasticity (Shapiro-Wilk and Levene test, respectively). For  
 161 calculating the statistical significance of the results one-way ANOVA and All Pairwise Multiple  
 162 Comparison Procedures, were used. The Kruskal–Wallis was used in case of significance of Shapiro-  
 163 Wilk and Levene test.

164

## 165 **3. Results and Discussion**

### 166 3.1. *Effect of Lactic Acid, SDS and ACP on VTEC*

167 Cold plasma demonstrates high efficacy in terms of reduction of VTEC cell number when associated  
 168 with a preliminary washing step in lactic acid (Figure 1. Panel A). The majority of the samples (6 out

169 of 9) inoculated with VTEC ( $6.72 \pm 0.27$  Log CFU/cm<sup>2</sup>) and treated with LA+SDS for 5 min and  
 170 ACP for 15 min had counts below the level of quantification. However, the presence of VTEC was  
 171 detected in these samples after 24 h of enrichment, meaning that the numbers of viable VTEC cells  
 172 were in a range between the limit of detection (LOD, 1 CFU in 10 cm<sup>2</sup> = -1 Log CFU/cm<sup>2</sup>) and the  
 173 theoretical limit of quantification (LOQ, 1 CFU in 1 ml of the sample homogenate approximately  
 174 equal 0.7 Log CFU/cm<sup>2</sup>). Also one out of 9 samples, not treated with ACP but washed with LA+SDS,  
 175 was below LOQ. In order to calculate the differences between the microbial counts before and after  
 176 treatments, the data points below the LOQ were arbitrarily assigned a value equal to 0.65 Log  
 177 CFU/cm<sup>2</sup> for statistical analyses, which biases estimates upward.

178 Data distributions that do not follow the normal distribution can be represented graphically in a box  
 179 plot (also known as a box-and-whisker plot) reporting median values, quartiles (25% and 75%)  
 180 minimum and maximum values (Figure 1). Reduction of VTEC counts was significantly higher in  
 181 samples treated with ACP, SDS and LA ( $4.78 \pm 0.66$ ) in comparison to samples treated with only two  
 182 of the three sanitizing methods ( $2.53 \pm 1.30$  mean Log CFU/cm<sup>2</sup> (LA + SDS) and  $2.69 \pm 0.38$  mean Log  
 183 CFU/cm<sup>2</sup> (SDS + ACP) and (Figure 1A).

184 Berardinelli et al. (2016) did not observe significant differences of VTEC counts on red chicory after  
 185 treatment with ACP for 30 min using the same plasma apparatus, whereas 60 min treatments gave a  
 186 significant reduction ( $-2.2$  mean Log CFU/cm<sup>2</sup> vs control). In this study, the effect on VTEC counts  
 187 was similar when the ACP treatment was applied for 15 min after washing the radicchio leaves with  
 188 SDS ( $-2.89$  mean Log CFU/cm<sup>2</sup> vs control) and the efficiency of the ACP treatment was much higher  
 189 after washing with LA+SDS ( $-4.78$  mean Log CFU/cm<sup>2</sup> vs control).

190 Several studies have highlighted that the large variability of results observed in challenge test with *E.*  
 191 *coli* on lettuce and other fresh produce aiming at assessing the sensitivity to ACP can depend on  
 192 experimental conditions (Bermúdez-Aguirre, Wemlinger, Pedrow, Barbosa-Cánovas, & GarciaPerez,

2013; Min et al., 2016; Song et al., 2015; Ziuzina et al., 2015). Because the attachment to surface and internal structures can be a relevant factor, in this study the experimental inoculation of red chicory leaves was performed prior to cutting. Moreover, the conditions used to facilitate attachment included the inoculation on soil contaminated spots and the incubation at room temperature for one hour, followed by overnight storage at 8-10 °C. The number of attached *E. coli* in the control samples was 5.58 mean Log CFU/cm<sup>2</sup>, with a recovery of 10.75% ( $\pm 9.25\%$ ) versus the inoculum. This difference between the number of *E. coli* in the inoculum and on control samples (approx. 1 Log CFU) can be due to the effect of washing with physiologic solution (NaCl 0.85% w/v).

The effects of lactic acid on *E. coli* O157:H7 was previously investigated by Velázquez, Barbini, Escudero, Estrada, & Guzmán (2009) who reported a 1.71 Log CFU reduction on lettuce after washing with 1% LA in bags with continuous agitation for 1 min. Similar results were reported by Akbas & Ölmez (2007). A significant reduction of *E. coli* (1.9 Log CFU/g) was observed after dipping lettuce in 0.5% LA for 2 min. The authors pointed out that any further reduction was observed by increasing the LA concentration from 0.5 to 1% or the treatment time from 2 to 5 min.

The synergistic effect of LA (0.5%) in combination with SDS (0.05%) was previously tested by Guan and colleagues (2010). The authors reported a reduction of  $0.41 \pm 0.12$  (Log CFU/g tissue) after washing for 5 min and observed detrimental effects on visual quality and texture of lettuce after storage in modified atmosphere package at 4°C for 7 and 14 days. However, these detrimental effects might be linked to the specific experimental conditions, because lettuce leaves were cut into pieces before the sanitation and the LA might more easily penetrate inside the lettuce leaves.

### 3.2. Effect of Lactic Acid, SDS and ACP on *L. monocytogenes*

The combination of LA (2%) +SDS (0.05%) treatment was very effective on *L. monocytogenes* (Figure 1B). Mean counts were often below LOQ, especially in the groups treated for 15 min, with

216 7 and 6 out of 9 samples below LOQ in the groups treated and not treated with ACP, respectively.  
 217 The *L. monocytogenes* counts in the control samples washed with NaCl 0.85% for 10 and 15 min  
 218 were  $5.17 \pm 0.13$  (mean Log CFU/cm<sup>2</sup>) and  $4.43 \pm 0.50$  (mean Log CFU/cm<sup>2</sup>), respectively (average  
 219 percent recovery  $2.50 \pm 0.75$  and  $2.40 \pm 0.44$ ). Washing with LA+SDS, with or without a subsequent  
 220 treatment with ACP, showed higher efficacy against *L. monocytogenes* in comparison to treatments  
 221 combinations excluding LA. Within LA + SDS treated samples, the higher the time of the SDS  
 222 treatment the higher the *L. monocytogenes* reduction values. In particular reduction values of -3.12  
 223 mean Log CFU/cm<sup>2</sup> vs control (LA + SDS 10 min) and -4.50 mean Log CFU/cm<sup>2</sup> vs control (LA +  
 224 SDS 15 min) were registered. However, the impact of the data below quantification limit should be  
 225 considered.

226 In the present study, SDS + ACP showed a limited efficacy against *L. monocytogenes* in  
 227 experimentally inoculated red chicory. A limited, although statistical significant, reduction of ACP  
 228 alone has been already described on red chicory after 30 and 60 min of treatment applied using the  
 229 same DBD system (1 and 1.30 Log CFU/cm<sup>2</sup> respectively) (Berardinelli et al., 2016).

230 Akbas & Ölmez (2007) reported that the populations of *L. monocytogenes* on iceberg lettuce samples  
 231 were significantly reduced ( $1.5 \text{ Log CFU g}^{-1}$ ) after dipping the inoculated samples in 0.5% LA for 2  
 232 min and that the effect was not significantly increased with treatment time of 5 min or LA  
 233 concentration 1%. The effectiveness of treatment with LA (or Levulinic Acid) plus SDS was  
 234 positively tested by several authors against Gram negative bacteria such as *Salmonella* spp. and *E.*  
 235 *coli* (Beuchat et al., 2012; Zhao et al., 2009). Inhibition of micro-organisms by organic acids depends  
 236 upon several factors including pH reduction, the ratio of undissociated species of the acid, chain  
 237 length, cell physiology and metabolism. SDS can denature protein surfaces and damage cell  
 238 membranes, and its bactericidal effect can be increased at low pH (Tejaswi Naidu & Prakash Prabhu,  
 239 2011; Zhao et al., 2009). Synergism between LA and SDS may be explained by the ability of LA to  
 240 increase the permeability of the bacterial cell membrane (Wang, Chang, Yang, & Cui, 2015).

241 These results underline the synergistic effect of the three sanitizing methods. A preliminar LA + SDS  
 242 treatment before the ACP treatment may act by improving the overall bactericidal effect of the  
 243 combined sanitizing treatment as well as the washing effect. By increasing the number of planktonic  
 244 cells detached from the leaf and released into the water, the number of cells damaged by ACP increase  
 245 suggesting a combination of LA + SDS + ACP as an alternative and efficient decontamination  
 246 treatment of red chicory to chlorine.

### 247 3.3. *Qualitative assessments*

248 Results of colour measurement, in term of  $L^*$ ,  $a^*$ ,  $b^*$  and  $C^*$ , are summarized in Table 1. The different  
 249 treatments had no significant effect on colour of red chicory and no differences were observed after  
 250 storage. The descriptive statistics shown in Table 2 indicate that the scores for freshness, colour and  
 251 texture were not affected by the treatments, but odour and overall acceptability of the samples treated  
 252 by ACP slightly decreased during storage. This is in agreement with the results reported by Pasquali  
 253 et al. (2016) for red chicory leaves treated by cold plasma and stored for 3 days. About the odour,  
 254 some assessors detected the typical ozone odour, as shown in the work conducted by Ragni et al.  
 255 (2016) by using the same device. An  $O_3$  concentration of about 7 ppm can be measured in a  $0.19\text{ m}^3$   
 256 volume chamber after 2.5 min of discharge.

257 After the treatments with (LA+SDS) 15 min + ACP 15 min and SDS 10 min + ACP 15 min, the pH  
 258 values of water solutions used to immerge the leaves were  $3.23\pm0.12$  and  $3.32\pm0.17$ , respectively.  
 259 Before the treatment with ACP the values of water solution pH were  $3.41\pm0.07$  and  $7.10\pm0.12$  for LA  
 260 + SDS and SDS, respectively. The low initial pH values of LA + SDS water solutions were probably  
 261 due to LA residues absorbed by the red chicory samples. As observed, ACP treatments were able to  
 262 reduce these values of about 5% and 53% for LA + SDS and SDS, respectively.

263

## 264 4. Conclusions

265 The results of this work clearly demonstrated that Lactic Acid (LA), SDS and atmospheric cold  
 266 plasma (ACP) had a synergistic effect on verotoxin-producing *E. coli* and *L. monocytogenes*. The

267 increased antimicrobial activity of ACP can be related to the perturbation of the bacterial cell  
 268 membranes produced by the preliminary treatment with a short chain weak acid (LA) and a surfactant  
 269 (SDS). The longer duration of the washing step needed to achieve a similar significant reductions for  
 270 the Gram positive species (*L. monocytogenes*) also suggests that the inactivation efficacy and the  
 271 magnitude of microbial cell damages depend on the microbial cell wall and membrane structure.  
 272 Further optimization of treatment parameters for maintaining fresh characteristics are needed. The  
 273 effectiveness of combinations of sanitizers and ACP on other pathogens and fresh produce worth to  
 274 be investigated.

275

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**\*Highlights (for review)**

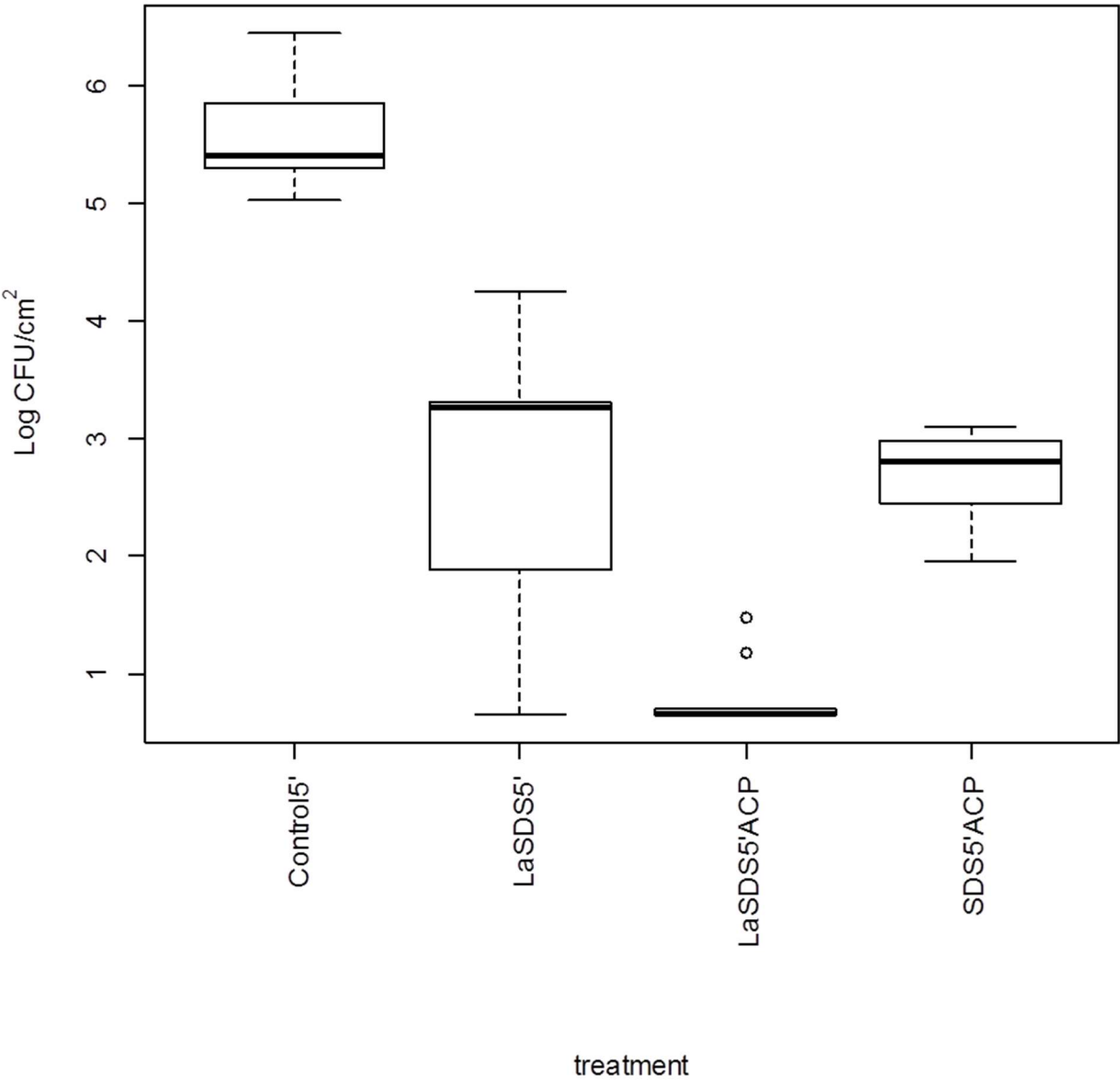
**Highlights**

- Red chicory leaves were treated with Lactic Acid, SDS and ACP to control VTEC and Listeria
- LA and SDS had synergistic effect with ACP in inactivation of VTEC and Listeria
- Freshness, colour and texture of the leaves were slightly affected by the analysed treatments

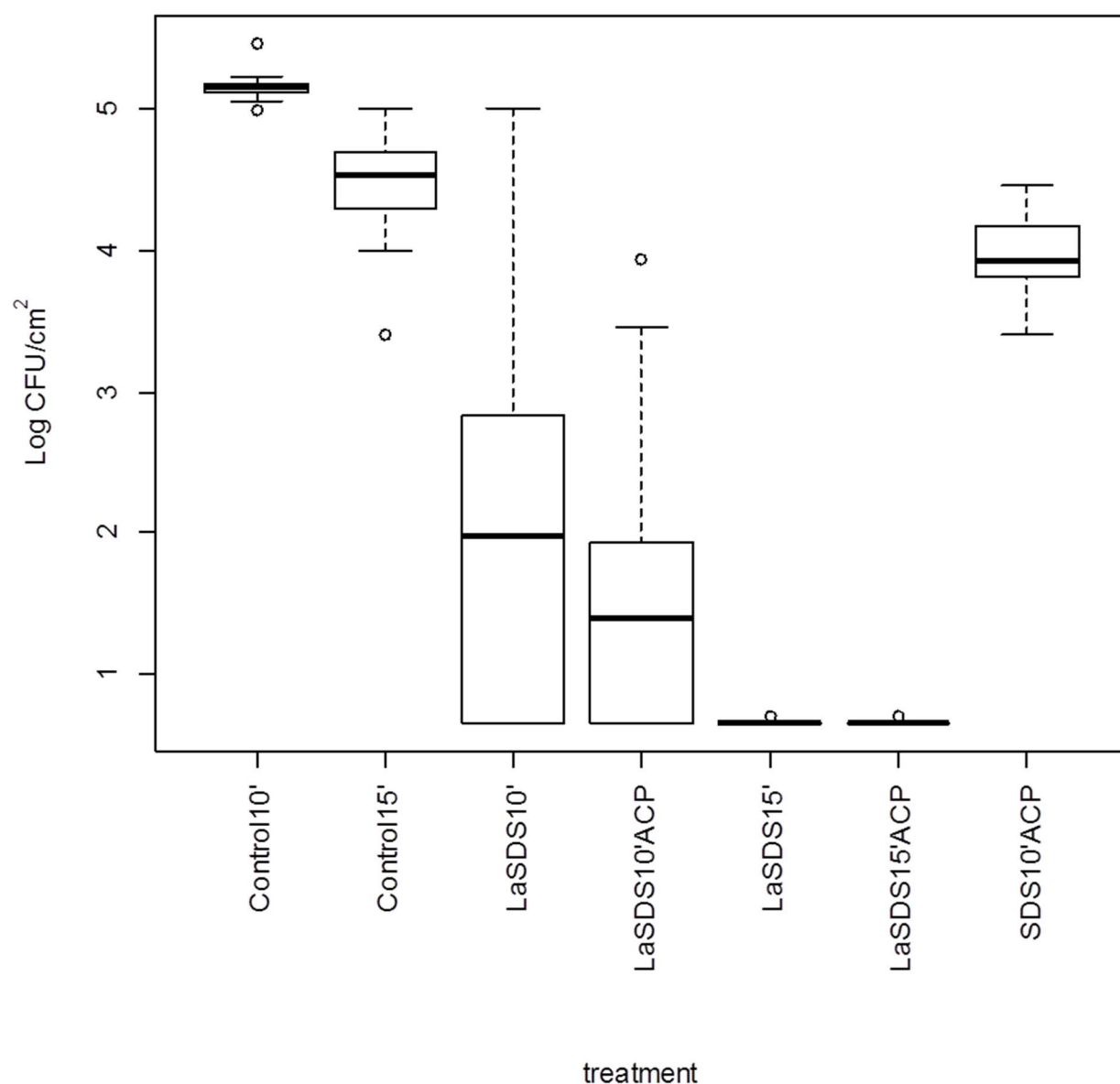
Figure

Figure 1

A



B



Figure

### Figure captions

**Figure 1** – Boxplot of microbial counts (Log CFU/cm<sup>2</sup>) in treated and control samples (verotoxin-producing *E. coli* (A); *L. monocytogenes* (B)).

The summary statistics used to create a box and whisker plot are the median of the data, the lower and upper quartiles (25% and 75%) and the minimum and maximum values (empty dots). Upper whisker =  $\min(\max(x), Q_3 + 1.5 * IQR)$ ; lower whisker =  $\max(\min(x), Q_1 - 1.5 * IQR)$ ; where  $IQR = Q_3 - Q_1$ , the box length. CFU numbers <1 were assigned a value equal to 0.9.

**Table 1.** Results of colour analysis of red chicory leaves stored for 1 day.

Treatment	Storage time	lour para			
		L*	a*	b*	C*
(LA+SDS) 5'	B A	32.2 ± 1.4 <sup>a</sup>	22.7 ± 1.2 <sup>a</sup>	7.4 ± 0.5 <sup>a</sup>	23.9 ± 1.3 <sup>a</sup>
		31.3 ± 2.3 <sup>a</sup>	24.0 ± 1.8 <sup>a</sup>	7.4 ± 0.5 <sup>a</sup>	25.2 ± 1.9 <sup>a</sup>
	1 day	32.4 ± 1.1 <sup>a</sup>	23.1 ± 1.6 <sup>a</sup>	7.2 ± 0.6 <sup>a</sup>	24.1 ± 1.6 <sup>a</sup>
(LA+SDS) 5'+ACP 15'	B A	34.5 ± 0.8 <sup>a</sup>	23.7 ± 1.5 <sup>a</sup>	7.0 ± 1.3 <sup>a</sup>	24.7 ± 1.5 <sup>a</sup>
		33.2 ± 1.7 <sup>a</sup>	24.8 ± 2.2 <sup>a</sup>	7.5 ± 0.6 <sup>a</sup>	25.9 ± 2.3 <sup>a</sup>
	1 day	34.4 ± 2.0 <sup>a</sup>	22.7 ± 1.3 <sup>a</sup>	7.9 ± 0.7 <sup>a</sup>	23.7 ± 1.2 <sup>a</sup>
(LA+SDS) 10'	B A	35.8 ± 1.1 <sup>a</sup>	25.5 ± 1.1 <sup>a</sup>	7.9 ± 0.5 <sup>a</sup>	26.7 ± 1.2 <sup>a</sup>
		34.1 ± 2.5 <sup>a</sup>	25.5 ± 2.5 <sup>a</sup>	8.0 ± 0.9 <sup>a</sup>	26.7 ± 1.0 <sup>a</sup>
	1 day	35.1 ± 2.4 <sup>a</sup>	24.2 ± 2.3 <sup>a</sup>	8.9 ± 0.6 <sup>a</sup>	24.2 ± 2.5 <sup>a</sup>
(LA+SDS) 10'+ACP 15'	B A	35.8 ± 1.4 <sup>a</sup>	27.3 ± 1.2 <sup>a</sup>	8.9 ± 0.6 <sup>a</sup>	28.7 ± 1.1 <sup>a</sup>
		34.7 ± 1.3 <sup>a</sup>	27.7 ± 1.0 <sup>a</sup>	8.2 ± 0.5 <sup>a</sup>	28.9 ± 1.3 <sup>a</sup>
	1 day	34.8 ± 1.4 <sup>a</sup>	26.3 ± 1.8 <sup>a</sup>	8.8 ± 0.8 <sup>a</sup>	27.2 ± 1.8 <sup>a</sup>
(LA+SDS) 15'	B A	32.8 ± 2.4 <sup>a</sup>	24.6 ± 1.1 <sup>a</sup>	8.6 ± 1.0 <sup>a</sup>	26.1 ± 1.1 <sup>a</sup>
		32.5 ± 2.5 <sup>a</sup>	25.1 ± 1.0 <sup>a</sup>	9.0 ± 0.6 <sup>a</sup>	26.7 ± 1.6 <sup>a</sup>
	1 day	31.3 ± 1.4 <sup>a</sup>	24.0 ± 1.8 <sup>a</sup>	8.9 ± 0.6 <sup>a</sup>	25.0 ± 0.7 <sup>a</sup>
(LA+SDS) 15'+ACP 15'	B A	33.9 ± 0.9 <sup>a</sup>	24.3 ± 1.2 <sup>a</sup>	7.9 ± 0.7 <sup>a</sup>	25.6 ± 1.2 <sup>a</sup>
		33.0 ± 2.5 <sup>a</sup>	25.5 ± 2.5 <sup>a</sup>	8.1 ± 0.8 <sup>a</sup>	26.8 ± 2.0 <sup>a</sup>
	1 day	34.4 ± 1.9 <sup>a</sup>	23.3 ± 2.3 <sup>a</sup>	8.8 ± 0.8 <sup>a</sup>	25.3 ± 2.3 <sup>a</sup>
SDS 10'	B A	31.0 ± 2.4 <sup>a</sup>	24.4 ± 2.4 <sup>a</sup>	7.4 ± 1.0 <sup>a</sup>	25.5 ± 1.3 <sup>a</sup>
		31.1 ± 1.9 <sup>a</sup>	23.1 ± 1.9 <sup>a</sup>	7.2 ± 0.7 <sup>a</sup>	24.2 ± 1.9 <sup>a</sup>
	1 day	32.2 ± 1.6 <sup>a</sup>	22.9 ± 1.7 <sup>a</sup>	7.5 ± 0.3 <sup>a</sup>	23.9 ± 1.7 <sup>a</sup>
SDS 10'+ACP 15'	B A	31.6 ± 2.6 <sup>a</sup>	23.9 ± 2.5 <sup>a</sup>	7.6 ± 0.5 <sup>a</sup>	25.0 ± 1.5 <sup>a</sup>
		32.0 ± 3.2 <sup>a</sup>	22.8 ± 3.2 <sup>a</sup>	7.4 ± 0.9 <sup>a</sup>	24.0 ± 2.1 <sup>a</sup>
	1 day	31.4 ± 1.4 <sup>a</sup>	24.4 ± 1.3 <sup>a</sup>	7.5 ± 0.5 <sup>a</sup>	25.2 ± 1.3 <sup>a</sup>

Note: B: before the treatment, A: immediately after the treatment. The same lowercase letters denote no significant differences during storage, within the same sample and the same treatment time (Tukey test, P < 0.05).

**Table 2.** Results of sensory test of red chicory leaves stored for 1 days.

Treatment	Storage time	Sensory attributes				
		Freshness	Colour	Odour	Texture	Overall acceptability
(LA+SDS) 5'	B A	5.0 ± 0 <sup>a</sup>	5.0 ± 0 <sup>a</sup>	5.0 ± 0 <sup>a</sup>	5.0 ± 0 <sup>a</sup>	9.0 ± 0 <sup>a</sup>
		5.0 ± 0 <sup>a</sup>	5.0 ± 0 <sup>a</sup>	5.0 ± 0 <sup>a</sup>	5.0 ± 0 <sup>a</sup>	9.0 ± 0 <sup>a</sup>
	1 day	5.0 ± 0 <sup>a</sup>	5.0 ± 0 <sup>a</sup>	5.0 ± 0 <sup>a</sup>	5.0 ± 0 <sup>a</sup>	9.0 ± 0 <sup>a</sup>
(LA+SDS) 5'+ACP 15'	B A	5.0 ± 0 <sup>a</sup>	5.0 ± 0 <sup>a</sup>	5.0 ± 0 <sup>a</sup>	5.0 ± 0 <sup>a</sup>	9.0 ± 0 <sup>a</sup>
		5.0 ± 0 <sup>a</sup>	5.0 ± 0 <sup>a</sup>	4.8 ± 0.4 <sup>b</sup>	5.0 ± 0 <sup>a</sup>	8.8 ± 0.4 <sup>b</sup>
	1 day	5.0 ± 0 <sup>a</sup>	5.0 ± 0 <sup>a</sup>	4.8 ± 0.4 <sup>b</sup>	5.0 ± 0 <sup>a</sup>	8.0 ± 0 <sup>c</sup>
(LA+SDS) 10'	B A	5.0 ± 0 <sup>a</sup>	5.0 ± 0 <sup>a</sup>	5.0 ± 0 <sup>a</sup>	5.0 ± 0 <sup>a</sup>	9.0 ± 0 <sup>a</sup>
		5.0 ± 0 <sup>a</sup>	5.0 ± 0 <sup>a</sup>	5.0 ± 0 <sup>a</sup>	5.0 ± 0 <sup>a</sup>	9.0 ± 0 <sup>a</sup>
	1 day	5.0 ± 0 <sup>a</sup>	5.0 ± 0 <sup>a</sup>	5.0 ± 0 <sup>a</sup>	5.0 ± 0 <sup>a</sup>	9.0 ± 0 <sup>a</sup>
(LA+SDS) 10' +ACP 15'	B A	5.0 ± 0 <sup>a</sup>	5.0 ± 0 <sup>a</sup>	5.0 ± 0 <sup>a</sup>	5.0 ± 0 <sup>a</sup>	9.0 ± 0 <sup>a</sup>
		5.0 ± 0 <sup>a</sup>	5.0 ± 0 <sup>a</sup>	5.0 ± 0 <sup>a</sup>	5.0 ± 0 <sup>a</sup>	9.0 ± 0 <sup>a</sup>
	1 day	5.0 ± 0 <sup>a</sup>	5.0 ± 0 <sup>a</sup>	4.8 ± 0.4 <sup>b</sup>	5.0 ± 0 <sup>a</sup>	8.8 ± 0.4 <sup>b</sup>
(LA+SDS) 15'	B A	5.0 ± 0 <sup>a</sup>	5.0 ± 0 <sup>a</sup>	5.0 ± 0 <sup>a</sup>	5.0 ± 0 <sup>a</sup>	9.0 ± 0 <sup>a</sup>
		5.0 ± 0 <sup>a</sup>	5.0 ± 0 <sup>a</sup>	5.0 ± 0 <sup>a</sup>	5.0 ± 0 <sup>a</sup>	9.0 ± 0 <sup>a</sup>
	1 day	5.0 ± 0 <sup>a</sup>	5.0 ± 0 <sup>a</sup>	5.0 ± 0 <sup>a</sup>	5.0 ± 0 <sup>a</sup>	9.0 ± 0 <sup>a</sup>
(LA+SDS) 15' + ACP 15'	B A	5.0 ± 0 <sup>a</sup>	5.0 ± 0 <sup>a</sup>	5.0 ± 0 <sup>a</sup>	5.0 ± 0 <sup>a</sup>	9.0 ± 0 <sup>a</sup>
		5.0 ± 0 <sup>a</sup>	5.0 ± 0 <sup>a</sup>	4.8 ± 0 <sup>b</sup>	5.0 ± 0 <sup>a</sup>	9.0 ± 0 <sup>a</sup>
	1 day	5.0 ± 0 <sup>a</sup>	5.0 ± 0 <sup>a</sup>	4.8 ± 0 <sup>b</sup>	5.0 ± 0 <sup>a</sup>	8.8 ± 0.4 <sup>b</sup>
SDS 10'	B A	5.0 ± 0 <sup>a</sup>	5.0 ± 0 <sup>a</sup>	5.0 ± 0 <sup>a</sup>	5.0 ± 0 <sup>a</sup>	9.0 ± 0 <sup>a</sup>
		5.0 ± 0 <sup>a</sup>	5.0 ± 0 <sup>a</sup>	5.0 ± 0 <sup>a</sup>	5.0 ± 0 <sup>a</sup>	9.0 ± 0 <sup>a</sup>
	1 day	5.0 ± 0 <sup>a</sup>	5.0 ± 0 <sup>a</sup>	5.0 ± 0 <sup>a</sup>	5.0 ± 0 <sup>a</sup>	9.0 ± 0 <sup>a</sup>
SDS 10' + ACP 15'	B A	5.0 ± 0 <sup>a</sup>	5.0 ± 0 <sup>a</sup>	5.0 ± 0 <sup>a</sup>	5.0 ± 0 <sup>a</sup>	9.0 ± 0 <sup>a</sup>
		5.0 ± 0 <sup>a</sup>	5.0 ± 0 <sup>a</sup>	5.0 ± 0 <sup>a</sup>	5.0 ± 0 <sup>a</sup>	9.0 ± 0 <sup>a</sup>
	1 day	5.0 ± 0 <sup>a</sup>	5.0 ± 0 <sup>a</sup>	4.8 ± 0.4 <sup>b</sup>	5.0 ± 0 <sup>a</sup>	8.8 ± 0.4 <sup>b</sup>



Note: B: before the treatment, A:immediately after the treatment. The same lowercase letters denote no significant differences during storage, within the same sample and the same treatment time (Tukey test,  $P < 0.05$ ).