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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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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Abstract

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

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

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

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

1. Introduction

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

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

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

Our previous study demonstrated that ACP in water medium was efficient in the inactivation of *L. 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

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

2.4. Preliminary washing steps

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

2.5. Plasma settings and treatments

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

Challenge tests with *L. monocytogenes* and VTEC were performed independently. To ensure result reproducibility, each experiment (preliminary washing and ACP) was repeated three times with three 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^{TM} 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^{TM} Dryspot^{TM} *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 ± 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 ± 0.27 Log CFU/cm^2) 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^2 = -1 Log CFU/cm^2) 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^2). 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^2 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±0.66) in comparison to samples treated with only two
182 of the three sanitizing methods (2.53±1.30 mean Log CFU/cm^2 (LA + SDS) and 2.69±0.38 mean Log
183 CFU/cm^2 (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^2 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^2 vs control) and the efficiency of the ACP treatment was much higher
189 after washing with LA+SDS (-4.78 mean Log CFU/cm^2 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,

193 2013; Min et al., 2016; Song et al., 2015; Ziuzina et al., 2015). Because the attachment to surface and
194 internal structures can be a relevant factor, in this study the experimental inoculation of red chicory
195 leaves was performed prior to cutting. Moreover, the conditions used to facilitate attachment included
196 the inoculation on soil contaminated spots and the incubation at room temperature for one hour,
197 followed by overnight storage at 8-10 °C. The number of attached *E. coli* in the control samples was
198 5.58 mean Log CFU/cm^2, with a recovery of 10.75% (±9.25%) versus the inoculum. This difference
199 between the number of *E. coli* in the inoculum and on control samples (approx. 1 Log CFU) can be
200 due to the effect of washing with physiologic solution (NaCl 0.85% w/v).

201 The effects of lactic acid on *E. coli* O157:H7 was previously investigated by Velázquez, Barbini,
202 Escudero, Estrada, & Guzmán (2009) who reported a 1.71 Log CFU reduction on lettuce after
203 washing with 1% LA in bags with continuous agitation for 1 min. Similar results were reported by
204 Akbas & Ölmez (2007). A significant reduction of *E. coli* (1.9 Log CFU/g) was observed after dipping
205 lettuce in 0.5% LA for 2 min. The authors pointed out that any further reduction was observed by
206 increasing the LA concentration from 0.5 to 1% or the treatment time from 2 to 5 min.
207 The synergistic effect of LA (0.5%) in combination with SDS (0.05%) was previously tested by Guan
208 and colleagues (2010). The authors reported a reduction of 0.41±0.12 (Log CFU/g tissue) after
209 washing for 5 min and observed detrimental effects on visual quality and texture of lettuce after
210 storage in modified atmosphere package at 4°C for 7 and 14 days. However, these detrimental effects
211 might be linked to the specific experimental conditions, because lettuce leaves were cut into pieces
212 before the sanitation and the LA might more easily penetrate inside the lettuce leaves.

213 *3.2. Effect of Lactic Acid, SDS and ACP on L. monocytogenes*

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

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

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

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

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

3.3. Qualitative assessments

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

After the treatments with (LA+SDS) 15 min + ACP 15 min and SDS 10 min + ACP 15 min, the pH values of water solutions used to immerge the leaves were 3.23±0.12 and 3.32±0.17, respectively. Before the treatment with ACP the values of water solution pH were 3.41±0.07 and 7.10±0.12 for LA + SDS and SDS, respectively. The low initial pH values of LA + SDS water solutions were probably due to LA residues absorbed by the red chicory samples. As observed, ACP treatments were able to reduce these values of about 5% and 53% for LA + SDS and SDS, respectively.

4. Conclusions

The results of this work clearly demonstrated that Lactic Acid (LA), SDS and atmospheric cold 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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380

*Highlights (for review)

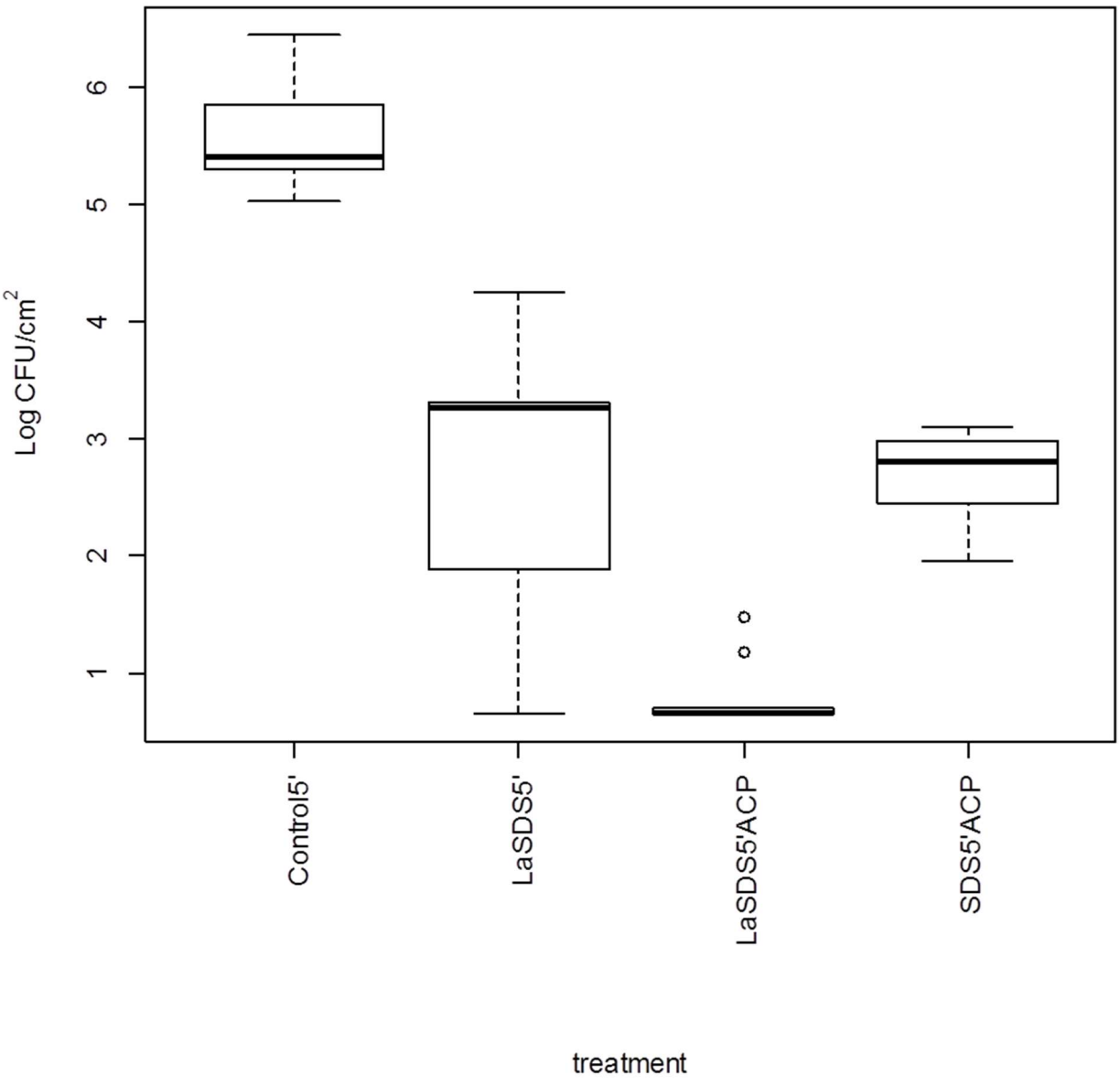
Highlights

- 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

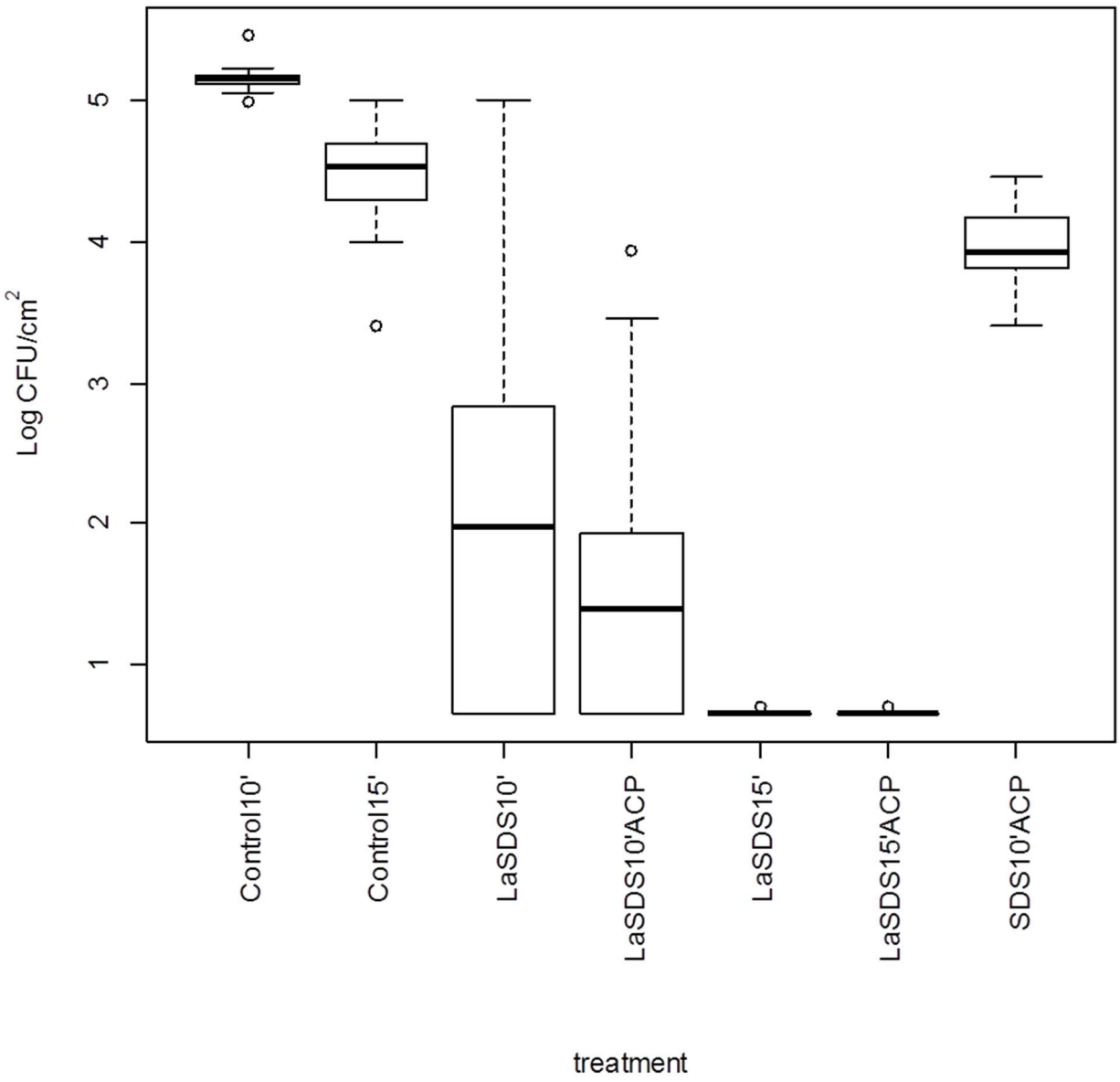
Figure

Figure 1

A



B



Figure

Figure captions

Figure 1 – Boxplot of microbial counts (Log CFU/cm^2) in treated and control samples (verotoxin-producing *E. coli* (A); *L. monocytogene*s (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

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

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[a]	5.0 ± 0[a]	5.0 ± 0[a]	5.0 ± 0[a]	9.0 ± 0[a]
		5.0 ± 0[a]	5.0 ± 0[a]	5.0 ± 0[a]	5.0 ± 0[a]	9.0 ± 0[a]
	1 day	5.0 ± 0[a]	5.0 ± 0[a]	5.0 ± 0[a]	5.0 ± 0[a]	9.0 ± 0[a]
(LA+SDS) 5'+ACP 15'	B A	5.0 ± 0[a]	5.0 ± 0[a]	5.0 ± 0[a]	5.0 ± 0[a]	9.0 ± 0[a]
		5.0 ± 0[a]	5.0 ± 0[a]	4.8 ± 0.4[b]	5.0 ± 0[a]	8.8 ± 0.4[b]
	1 day	5.0 ± 0[a]	5.0 ± 0[a]	4.8 ± 0.4[b]	5.0 ± 0[a]	8.0 ± 0[c]
(LA+SDS) 10'	B A	5.0 ± 0[a]	5.0 ± 0[a]	5.0 ± 0[a]	5.0 ± 0[a]	9.0 ± 0[a]
		5.0 ± 0[a]	5.0 ± 0[a]	5.0 ± 0[a]	5.0 ± 0[a]	9.0 ± 0[a]
	1 day	5.0 ± 0[a]	5.0 ± 0[a]	5.0 ± 0[a]	5.0 ± 0[a]	9.0 ± 0[a]
(LA+SDS) 10' +ACP 15'	B A	5.0 ± 0[a]	5.0 ± 0[a]	5.0 ± 0[a]	5.0 ± 0[a]	9.0 ± 0[a]
		5.0 ± 0[a]	5.0 ± 0[a]	5.0 ± 0[a]	5.0 ± 0[a]	9.0 ± 0[a]
	1 day	5.0 ± 0[a]	5.0 ± 0[a]	4.8 ± 0.4[b]	5.0 ± 0[a]	8.8 ± 0.4[b]
(LA+SDS) 15'	B A	5.0 ± 0[a]	5.0 ± 0[a]	5.0 ± 0[a]	5.0 ± 0[a]	9.0 ± 0[a]
		5.0 ± 0[a]	5.0 ± 0[a]	5.0 ± 0[a]	5.0 ± 0[a]	9.0 ± 0[a]
	1 day	5.0 ± 0[a]	5.0 ± 0[a]	5.0 ± 0[a]	5.0 ± 0[a]	9.0 ± 0[a]
(LA+SDS) 15' + ACP 15'	B A	5.0 ± 0[a]	5.0 ± 0[a]	5.0 ± 0[a]	5.0 ± 0[a]	9.0 ± 0[a]
		5.0 ± 0[a]	5.0 ± 0[a]	4.8 ± 0[b]	5.0 ± 0[a]	9.0 ± 0[a]
	1 day	5.0 ± 0[a]	5.0 ± 0[a]	4.8 ± 0[b]	5.0 ± 0[a]	8.8 ± 0.4[b]
SDS 10'	B A	5.0 ± 0[a]	5.0 ± 0[a]	5.0 ± 0[a]	5.0 ± 0[a]	9.0 ± 0[a]
		5.0 ± 0[a]	5.0 ± 0[a]	5.0 ± 0[a]	5.0 ± 0[a]	9.0 ± 0[a]
	1 day	5.0 ± 0[a]	5.0 ± 0[a]	5.0 ± 0[a]	5.0 ± 0[a]	9.0 ± 0[a]
SDS 10' + ACP 15'	B A	5.0 ± 0[a]	5.0 ± 0[a]	5.0 ± 0[a]	5.0 ± 0[a]	9.0 ± 0[a]
		5.0 ± 0[a]	5.0 ± 0[a]	5.0 ± 0[a]	5.0 ± 0[a]	9.0 ± 0[a]
	1 day	5.0 ± 0[a]	5.0 ± 0[a]	4.8 ± 0.4[b]	5.0 ± 0[a]	8.8 ± 0.4[b]

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, $P < 0.05$).