Assessing water-assisted UV-C light and its combination with peroxyacetic acid and *Pseudomonas graminis* CPA-7 for the inactivation and inhibition of *Listeria monocytogenes* and *Salmonella enterica* in fresh-cut 'Iceberg' lettuce and baby spinach leaves

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HIGHLIGHTS

- Water-assisted UV-C controlled *L. monocytogenes* and *S. enterica* in lettuce and spinach
- Low UV-C doses (0.1-0.3 kJ/m^2) did not reduce native mesophilic aerobic microbiota
- Pretreatment with UV-C and peroxyacetic acid inhibited *S. enterica* growth at 5 ºC
- Combined UV-C + 40 mg/L PAA inactivated both pathogens in the process solution
- Integrated UV-C, peroxyacetic acid and biopreservation was ineffective for sanitation

ABSTRACT

The effectiveness of ultraviolet C light (UV-C) delivered in water (WUV) or in peroxyacetic acid (PAA) for the inactivation and inhibition of *L. monocytogenes* and *S. enterica* in ready-to-eat 'Iceberg lettuce' and baby spinach leaves, was evaluated throughout chilled storage in modified atmosphere packaging (MAP). The inhibition of pathogen's growth by sequential pretreatments with UV-C in PAA

and then biocontrol using *Pseudomonas graminis* CPA-7 was assessed during MAP storage at 5 °C and upon a breakage of the cold-storage chain. In fresh-cut lettuce, 0.1 kJ/m^2 UV-C, in water or in 40 mg/L PAA, inactivated both pathogens by up to 2.1 ± 0.7 $\log_{10}$, which improved the efficacy of water-washing by up to 1.9 $\log_{10}$ and showed bacteriostatic effects on both pathogens. In baby spinach leaves, the combination of 0.3 kJ/m^2 UV-C and 40 mg/L PAA reduced *S. enterica* and *L. monocytogenes* populations by 1.4 ± 0.2 and 2.2 ± 0.3 $\log_{10}$ respectively, which improved water-washing by 0.8 ± 0.2 $\log_{10}$. Combined treatments (0.1 or 0.3 kJ/m^2 WUV and 40 mg/L PAA) inactivated both pathogens in the process solution from lettuce or spinach single sanitation, respectively. Pretreating lettuce with UV-C in PAA reduced *L. monocytogenes* and *S. enterica'*s growth by up to 0.9 ± 0.1 $\log_{10}$ with respect to the PAA-pretreated control after 6 d at 5 °C in MAP. Upon a cold-chain breakage, CPA-7 prevented *S. enterica* growth in PAA-pretreated lettuce, whereas showed no effect on *L. monocytogenes* in any of both matrices. Low-dose UV-C in PAA is a suitable preservation strategy for improving the safety of ready-to-eat leafy greens and reducing the risk of cross contamination.

Keywords: biological control; biopreservation; fresh-cut produce; foodborne pathogens; ready-to eat green leaves

1. INTRODUCTION

Ready-to-eat green leafy salads are growingly demanded items because they combine convenience and a wide range of nutrients and bioactive phytochemicals which are recommended for a healthy diet (Artés and Allende, 2014). Nevertheless, as they are usually consumed row, they can become vehicles for human pathogens such as *Listeria monocytogenes* and *Salmonella enterica* (Franco & Destro, 2007; Sagoo et al., 2003a; Sagoo et al., 2003b). Outbreaks caused by several strains of the mentioned pathogenic species have been associated to contaminated lettuce in the European Union and the USA in the last years (Callejón et al., 2015; EFSA, 2017). Cross-contamination with foodborne pathogens may occur during pre-harvest, through contaminated soil and irrigation water or due to

48 organic fertilizers such as manures or sewage sludge (Brandl, 2006). During postharvest processing,
49 inappropriate sanitation of tools, facilities surfaces, and process-water may also turn them into
50 contamination sources (Artés and Allende, 2014).

51 Since immersion of cut surfaces increases the probabilities for the infiltration of liquid into the
52 tissues, sanitation of fresh produce is carried out using antimicrobial solutions to reduce the
53 probability for process water to become a source of contamination (Gorny et al., 2006). The most
54 used chemical sanitizer in food industry is chlorine due to its strong antibacterial activity and low
55 costs (Hua and Reckhow, 2007). However, growing public concern about health and environmental
56 negative effects of its by-products and the advent of banning or restrictive regulations for its use in
57 several countries (EC-European Comission for Health and Consummer Protection, 2005), have
58 prompted the research and development of alternative decontamination strategies in food industry.

59 Short-wave Ultraviolet light (UV-C) has a direct deleterious effect on microbial DNA structure which
60 leads to the inactivation and death of most types of microorganisms without producing harmful
61 byproducts (Gayán et al., 2014). Therefore, UV-based technologies are being implemented for the
62 decontamination of food and food-contact surfaces, including equipment, tools, packages, liquids,
63 powders and fresh produce (Bintsis et al., 2000; Charles et al., 2013; Fine and Gervais, 2004; Ignat et
64 al., 2015; Manzocco et al., 2011). When applied at high doses, UV can damage plant tissues, being
65 counterproductive for plant products shelf-life (Kovács and Keresztes, 2002). However, at low-doses,
66 UV-C irradiation induces plant self-protective mechanisms against potential oxidative and mutagenic
67 damages. This leads to the enhancement of antioxidant mechanisms as well as to the production of
68 pathogenesis-related proteins (PR proteins), thereby eliciting the defense response to subsequent
69 pathogenic infections (indirect effect) (Allende et al., 2006; Ou et al., 2016; Scott et al., 2017).
70 Therefore, the perdurability of UV-C antimicrobial effects when applied to plant tissues can be
71 attributed to both the reduction of the multiplication capacity of irradiated surviving microorganisms
72 and to the increase of the negative pressure exerted through the elicitation of plant resistance

73 mechanisms (Shama, 2007; Yun et al., 2013). Thus, pre-treating commodities with UV-C would
74 potentially improve the safety of fresh-cut products by preventing the population increase and the
75 establishment of foodborne pathogens throughout storage in case of cross-contamination after the
76 sanitation step.

77 Water-assisted alternatives of the sanitation with UV-C light (WUV) improve the accessibility of UV-C
78 light to all sides of the product and reduce the probability of its overheating compared to
79 conventional chambers (Collazo et al., 2018b; Huang et al., 2015; Huang and Chen, 2014). They also
80 integrate the effects of irradiation and immersion by acting on microbial populations present on the
81 surface of fresh produce, while decontaminating the sanitation solution. Additionally, in order to
82 exploit the synergistic effect of the simultaneous action of UV and other sanitation methods, several
83 strategies combining UV with chemical compounds or antagonistic agents have been used to
84 improve the efficacy of these methods in several commodities (Koivunen and Heinonen-Tanski, 2005;
85 Martínez-Hernández et al., 2013; Ou et al., 2016; Park et al., 2018). Among oxidizing chemical
86 sanitizers, peroxyacetic acid (PAA) is a suitable alternative because of its wide microbial range of
87 action, its robustness against suspended organic matter, switches in pH and temperature, and the
88 non-toxicity of its by-products (water and acetic acid) (Alvaro et al., 2009). Our work group has
89 previously evaluated the efficacy of a water-assisted technology, alone and combined with
90 peroxyacetic acid for the reduction of natural microbiota in fresh-cut broccoli (Collazo et al., 2018b).
91 Results showed similar or enhanced effectiveness in respect of chlorine sanitation, depending on the
92 UV dose and the amount and type of target microorganism.

93 On the other hand, biopreservation have also been combined with physical sanitation methods for
94 attempting to reduce the incidence of fungal diseases in fresh produce during postharvest (Xu & Du,
95 2012; Ou et al., 2016). Those experiments have shown promising results regarding the control of
96 pathogens populations through the activation of the plant's defense mechanisms (Ou et al., 2016).
97 Although foodborne human pathogens are not specifically pathogenic to plants, they have developed

mechanisms allowing them to survive in intermediate plant hosts, including the use of virulence factors to promote the adhesion to plant tissues (Xicohtencatl-Cortes et al., 2009). Some of those molecules are also involved in the subsequent colonization of the human host, e. g. flagella-associated adhesins, Type 3 secretor system and surface-exposed aggregative fimbria/curli nucleator (Barak et al., 2005; Torres et al., 2005; Xicohtencatl-Cortes et al., 2009). Therefore, competition for space, inhibition of their adhesiveness to the plant surface and induction of plant's defense responses through biopreservation, could be additional mechanisms to reduce pathogens' prevalence and establishment in plant products as vehicles for transmission. We have previously assessed the antagonistic effect of the preservative strain *Pseudomonas graminis* CPA-7, originally isolated from apple surface, on the growth of *S. enterica* and *L. monocytogenes* in several fresh-cut commodities (Abadias et al., 2014; Alegre et al., 2013a; Alegre et al., 2013b; Iglesias, 2017; Iglesias et al., 2018). This effect showed to be associated to several mechanisms including the competition for ecological niche, the activation of the plant's defense mechanisms and the reduction the colonization capacities of those pathogens (Collazo et al., 2018a, 2017b).

With all this in mind, in the present work, the direct antimicrobial effect of low-dose WUV treatment for the control of the foodborne pathogens *S. enterica* and *L. monocytogenes* was evaluated in fresh-cut lettuce and baby spinach leaves during chilled MAP storage. Further improvement of WUV for the inactivation and inhibition of those pathogens in the mentioned matrices as well as in the sanitation solutions was attempted by combining WUV with peroxyacetic acid. Additionally, to assess the putative inhibitory effect on the growth of the mentioned foodborne pathogens in case of cross-contamination after the sanitation step, the sequential combination of UV-C + PAA and then inoculation of *P. graminis* CPA-7, was evaluated throughout MAP refrigerated storage and upon a breakage of the cold chain of storage.

2. MATERIALS AND METHODS

2.1 Microbial culture conditions and inocula preparation

For inoculation with foodborne pathogens, a cocktail containing five *L. monocytogenes* strains: CECT4031, ser. 1a; CECT4032, ser. 4b; CECT933, ser. 3a; CECT940, ser. 4a; and Lm203/3, ser. 1/2a (Abadias *et al*, 2008), and four *S. enterica* subesp. *enterica* strains: BAA-707, ser. Agona; BAA-709, ser. Michigan; BAA-710, ser. Montevideo; and BAA-711 ser. Gaminara, was used as inoculum. The cocktail was prepared using 5 mL of overnight cultures of each strain either in tryptone soy broth (TSB) for *S. enterica,* or in TSB supplemented with 6 g/L yeast extract (TSB-YE) for *L. monocytogenes.* After incubation at 37 °C, all cultures were mixed and centrifuged at 9800 x g for 10 min at 10 °C. The supernatant was discarded and bacterial cell pellets were suspended in 22.5 mL of aqueous saline solution (8.5 g/L NaCl). For antagonist inoculum preparation, *P. graminis* CPA-7 (deposit number CBS 136973) (Alegre et al., 2013b) was seeded onto TSA plates and incubated at 30 °C for 48 h. Single colonies were inoculated in TSB and incubated in agitation overnight at 25 °C. Antagonistic bacterial cells were harvested as previously described and suspended in sterile deionized water. All synthetic culture media, buffers and supplements were purchased from Biokar Diagnostics, Beauveais, France.

2.2 Microbial counts

For microbial viable counts, triplicate 10 g samples were homogenized with 90 mL buffered peptone water within a 400 mL whole-filter bag (Interscience, Saint Nom, France) in a Masticator (IUL, Barcelona, Spain) set at 4 strokes per s for 90 s. Appropriate 10-fold solutions in saline peptone (SP, 8.5 g/L NaCl, 1 g/L peptone) of the homogenates were plated on plate count agar (PCA), Palcam agar or xylose-lysine-desoxycholate (XLD) for the determination of total mesophilic aerobic microorganisms (MAM), *L. monocytogenes,* or *S. enterica,* respectively. PCA plates were incubated at 25 °C for 3 d and XLD and Palcam plates were incubated at 37 °C for 24 or 48 h, respectively. Viable counts of each pathogen in the process solutions after a single sanitation of each vegetable were performed as previously described. Presence/absence tests of neutralized process solutions in Dey-

146 Engley medium were also performed to corroborate the inactivation of microorganisms. All synthetic
147 culture media, buffers and supplements were purchased from Biokar Diagnostics, Beauveais, France.

148 ### 2.3 Plant material processing

149 Whole wrapped 'Iceberg' lettuce (*Lactuca sativa* var. capitata) and ready-to-use bagged baby spinach
150 leaves (*Spinacia oleracea* L.) were purchased from local retail establishments in Lleida, Spain. Before
151 treatment, the external leaves and core of 'Iceberg' lettuce were discarded and the rest was cut into
152 3-4 cm^2 pieces, washed in chlorinated tap water, drained, spin-dried using a manual centrifuge and
153 kept in trays overnight in air at 5 °C until use. Baby spinach leaves were un-bagged and stored in
154 trays overnight in air at 5 °C without any additional discarding or processing until they were
155 submitted to subsequent sanitation treatments.

156 ### 2.4 Overall quality and headspace gas composition assessment

157 Visual assessment of overall appearance was performed initially and throughout storage by 6
158 untrained panelists using a 1 to 5 hedonic corresponding to 25, 50, 75, 90 and 95 % acceptability of
159 the sample. The gas headspace composition of packages was measured using a handheld gas
160 analyzer (CheckPoint O_2/CO_2, PBI Dansensor, Denmark).

161 ### 2.5 WUV treatment preserving overall appearance: analyses and storage

162 Preliminary selection of the WUV dose was based on the visual assessment of the overall quality of
163 treated samples. For WUV treatments, batches of processed vegetables were immersed in agitated
164 cold tap water at a ratio of 0.3:10 (kg of product: L of water) using a water-assisted UV-C equipment
165 composed of deposit (15 L capacity) containing 4 UV lamps (17.2 W, 254 nm) (GPH 303T5L/4,
166 Heraeus Noblelight, Hanau, Germany). The deposit is connected to recirculating water put in motion
167 by a water pump. Pressurized water is introduced through multiple sprinklers on the top and exits

168 the tank through the bottom, while moves due to pressurized air (set at 100 kPa) that enters through
169 the bottom of the tank (Fig. 1). WUV doses were calculated as the mean of irradiance (W/m^2) * time
170 (s). Several doses were assayed by combining 4 UV-C lamps and different times of exposure. For
171 'Iceberg' lettuce, 0.1, 0.3, and 0.5 kJ/m^2 UV-C, corresponding to 1, 3 and 5 min of exposure,
172 respectively, were tested. For spinach, 0.2 and 0.3 kJ/m^2 treatments, corresponding to 2 and 3 min of
173 exposure, respectively, were tested. Before each treatment, lamps were preheated until stabilization
174 of the irradiance. Water-washing without turning on the UV lamps was performed as a control
175 treatment. Before and after WUV treatments, temperature was measured using an infrared
176 thermometer (DualTemp Pro, Labprocess distribuciones, Barcelona, Spain). Irradiance was measured
177 using a UV-sensor EasyH1, Peschl Ultraviolet, Mainz, Germany) through an orifice located in the lid.
178 After treatments, vegetables were drained, spin dried, and 15 g samples were packaged in
179 thermosealed 12 x 12 cm (lettuce) or 14 x 14 cm (spinach) polypropylene bags (PP110, ILPRA Systems
180 Espanya SL, Mataró, Spain) to achieve passive MAP conditions and were stored at 5 °C in darkness.
181 The film had a gas permeability of 1.1 and 5 cm^3/m^2 /day/KPa for O_2 of and CO_2, respectively, at 23 °C
182 and 0 % relative humidity. Visual assessment of the overall appearance was performed at day 3 and 6
183 of storage as described in Section 2.4. In the same days, measurements of the O_2/CO_2 composition
184 within packages were also performed as described in Section 2.4.

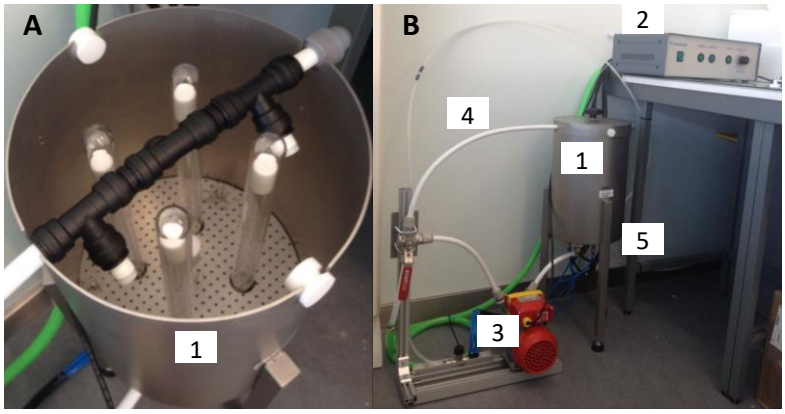

Figure 1. Water-assisted UV-C setup. (A) Tank containing four UV lamps and a multiple sprinkle device on the top. (B) The tank (1) is conected to a power source (2) a water pump (3), a water circuit (4), and a pressurised air entrance in the bottom (5).

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2.6 WUV AND PAA FOR DECONTAMINATION: INOCULATION, TREATMENT, ANALYSES, AND STORAGE

Upon selection of the optimal WUV dose preserving overall quality, the effect of UV-C in water or in PAA on *L. monocytogenes* and *S. enterica* populations was assessed. Vegetables were processed as described in section 2.3 and within the same day they were dip-inoculated for 2 min in agitation in a solution containing pathogenic inoculum (prepared as described in section 2.1) at a concentration of 10^5 CFU/mL of each strain. Inoculated samples were drained, spin-dried, and stored overnight in air at 5 °C. Afterwards, they were immersed in agitated tap water or in 40 or 80 mg/L PAA solutions (average pH 5.7 and 4.7, respectively) (average of oxidation/reduction potential 478 and 526, respectively) and submitted to 0.1 kJ/m^2 UV-C in the case of lettuce, and to 0.2 or 0.3 kJ/m^2 in the case of spinach, using the WUV device as described in section 2.5. As controls, sanitation with water or with the PAA solutions was performed using the same equipment without turning on the UV lamps. After sample draining and spin-drying, initial microbial counts were performed as described in Section 2.2. Samples were packaged (see section 2.5) and stored at 5 °C for 6 d. Analyses of overall appearance, headspace gas composition of packages (described in Section 2.4), and microbial populations (described in Section 2.2) were performed at the end of storage.

2.7 INTEGRATION OF WUV, PAA AND *P. GRAMINIS* CPA-7: TREATMENT, INOCULATION, ANALYSES, AND STORAGE

The experimental setup of this stage is showed in Figure 2. Prior inoculation, several batches of each processed vegetable were subjected to decontamination in the WUV equipment as described in section 2.5, either in agitated cold 40 mg/L PAA without turning on the UV lamps (PAA control), or with a combination of 40 mg/L PAA and UV-C: 0.1 kJ/m^2 for 'Iceberg' lettuce or 0.3 kJ/m^2 for baby spinach. After treatment, samples were stored in trays at air overnight at 5 °C, and afterwards, they were dip-inoculated for 2 min in agitation in: pathogenic inoculum containing 10^5 CFU/mL of each bacterial strain, a mixture of pathogenic + antagonist inoculum containing 10^7 CFU/mL CPA-7 and 10^5

CFU/mL of each pathogenic strain, or in antagonist inoculum containing 10^7 CFU/mL CPA-7. Then, samples were drained, spin-dried, and packaged as described in Section 2.5 and stored either for up to 6 d at 5 °C, or for 2 d at 5 °C followed by 4 d at 10 ºC, to simulate a cold-chain breakage. Before and after each treatment, concentration, pH and redox potential of PAA solutions were determined and temperature irradiance were measured as explained in Section 2.5. MAM counts were performed before initial prewashing, and before and after sanitation, as described in Section 2.2. Pathogens' population dynamics throughout storage was tracked by viable counts at 0, 2 and 6 d of storage at 5 °C and at 6 d upon a cold-chain breakage. The assessment of the overall appearance of processed vegetables as well as the O_2/CO_2 headspace composition of bags was performed at each sampling point as described in Section 2.4.

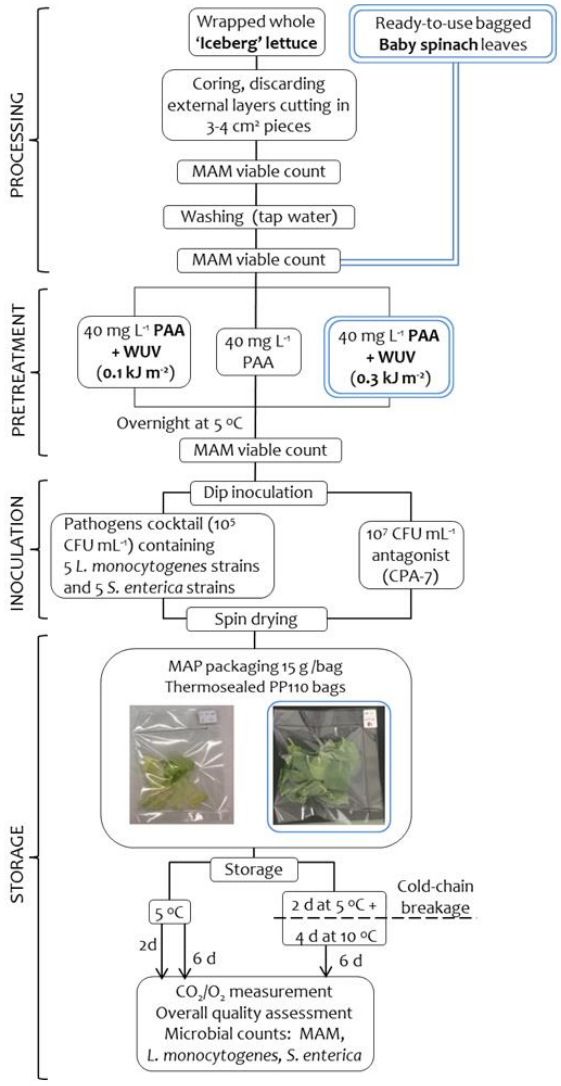


Figure 2. Experimental setup of a combined preservation strategy comprising pretreatment with UV-C + peroxyacetic acid (PAA) before inoculation with the biopreservative bacterium *P. graminis* CPA-7, for controlling the populations of *S. enterica* and *L. monocytogenes* in fresh-cut 'Iceberg' lettuce and baby spinach leaves during MAP storage at 5 °C and upon a breakage of the cold chain of storage.

2.8 Data processing and statistical analysis

All experiments were repeated two independent times and included three biological replicates per treatment and sampling time. For microbiological counts, two technical replicates per each biological replicate were analyzed and the mean of the number of colonies was used to calculate the colony forming units per milliliter (CFU/mL) and transformed to $\log_{10}$ CFU/g before means comparison. Population reductions were calculated by subtracting the count before treatment (N_0) to that obtained after treatment (N_1): $\log_{10} N_1 - \log_{10} N_0$. Statistical analyses were performed using Statistical software JMP (version 8.0.1 SAS Institute Inc., NC, USA). Categorical data were analyzed through a contingency analysis using chi-square statistic (n=12, $P < 0.05$). Microbiological and physical data were verified for agreement to normal distribution and homoscedasticity of residues and accordingly, means were compared by analysis of variance (ANOVA) and separated by Tukey's test ($P < 0.05$).

3. RESULTS AND DISCUSSION

3.1 WUV treatments preserving overall quality and respiration

Preliminary trials testing the highest WUV dose with less negative effects in the overall appearance and respiration of fresh-cut 'Iceberg' lettuce showed that samples treated with WUV in a range of 0.1-0.3 kJ/m^2 and stored in passive modified atmosphere, had a similar O_2/CO_2 composition (14 kPa / 6 kPa) that untreated controls until the end of storage (data not shown). However, at a higher dose (0.5 kJ/m^2) WUV provoked oxidative discoloration, a more marked reduction of O_2 levels (6.16 kPa) and an increase in CO_2 content (6.2 kPa) than the water-washed control (17.5 kPa O_2; 2.8 kPa CO_2) at the end of storage. The higher accumulation in CO_2 content observed in 0.5 kJ/m^2-treated samples could have been related to an enhanced respiration rate due to physiological stress in plant tissues. Similar results have been previously observed in experiments performed with 'Red Oak Leaf' and 'Lollo rosso' lettuces, that showed a positive correlation of the increase in the UV-C dose in a range of

0.4 to 8.1 kJ/m^2, with the respiration rate during MAP storage at 5 °C (Allende et al., 2006; Allende and Artes, 2003). On the other hand, in our experiments the visual assessment of overall appearance of lettuce samples treated with 0.3 and 0.5 kJ/m^2 showed unacceptability after 6 d of MAP storage (data not shown); thus, the lowest dose (1 min of exposure, 0.1 kJ/m^2) was selected for subsequent analysis. In Red Oak Leaf' lettuces, softening and browning of tissues were not detected in samples treated with conventional UV-C at doses of 1.2 and 2.4, kJ/m^2, in respect of the control (Allende et al., 2006). However, such negatives effects added to altered sensory quality were detected in samples treated with 7.1 kJ/m^2 after 7 d at 5 °C, which was associated to the production of free radicals and to a deleterious effect of UV-C on the cell wall (Allende et al., 2006). The higher browning response of crisp head lettuce varieties such as 'Iceberg' to abiotic stress compared to Romaine and other varieties with less crispiness has been previously reported (Cantos et al., 2001).

As for baby spinach leaves, no differences in the overall appearance were observed among irradiated samples regardless of the UV-C dose (data not shown). Gases analysis showed that treatment with WUV, resulted in lower O_2 (17.6 kPa) content than the water-washed control (19.5 kPa), regardless of the assayed dose (0.2 or 0.3 kJ/m^2), while no differences in the CO_2 contents (8.11 kPa) were observed among treatments. Therefore, both WUV doses were evaluated in the subsequent set of experiments. In accordance with these results, dry-UV-C treatments of spinach leaves at doses of 4.54, 7.94 and 11.35 kJ/m^2 did not affect the gases contents within packages compared to water control, and no reduction of quality was either detected for the lowest dose (Artés-Hernández et al., 2009).

3.2 WUV AND UV-C IN PAA FOR MICROBIAL DECONTAMINATION OF LETTUCE AND SPINACH

Upon the selection of 0.1 kJ/m^2 as the WUV dose better preserving the overall appearance of 'Iceberg' lettuce throughout storage, this dose was applied for microbial decontamination of native microbiota and inoculated pathogenic microorganisms. The combination of 0.1 kJ/m^2 UV-C and 40 or

80 mg/L PAA were evaluated to further improve the efficacy of the WUV. Initial counts of *L. monocytogenes* and *S. enterica* in 'Iceberg' lettuce were 3.8 ± 0.1 and 4.0 ± 0.1 $\log_{10}$ CFU/mg, respectively. WUV treatments effectively inactivated *L. monocytogenes* by 2.1 ± 0.7 $\log_{10}$ in respect of inoculated populations, improving the efficacy of water-washing by 1.9 $\log_{10}$. *L. monocytogenes'* growth was also inhibited throughout refrigerated MAP storage, showing no population increase in respect of the initial levels (Fig. 3 A). Combining 0.1 kJ/m^2 UV-C and PAA did not improve the inactivation but enhanced the inhibition of *L. monocytogenes'* growth during storage at 5 °C, in respect of the PAA control. Similarly, 0.1 kJ/m^2 WUV reduced *S. enterica* initial populations in 'Iceberg' lettuce by 2.0 ± 0.6 $\log_{10}$ (Fig. 3 B), which improved the efficacy of water-washing by 1.7 $\log_{10}$. Combining UV-C and PAA, regardless of the PAA dose, achieved the same efficacy as WUV, maintaining *S. enterica's* populations 1.9 ± 0.7 $\log_{10}$ below the inoculated levels until the end of storage. Final populations of this pathogen reached 3 ± 0.7 $\log_{10}$ below those present in the water-washed samples. Similarly, Huang et al. (2018) obtained a reduction by 2 $\log_{10}$ of *Salmonella* spp. populations in fresh-cut 'Iceberg' lettuce using a water-assisted device equipped with stirrers (10 L capacity, at doses of 26.7 to 33.6 kJ/m^2 UV-C). In agreement with our results, they obtained no synergistic effect when combining UV-C with 80 mg/L PAA, compared to WUV. In the same way, the sequential application of UV-C at fluencies > 1.5 kJ/m^2 and PAA (80 mg/L) for the sanitation of *S.* Typhimurium in 'Iceberg' lettuce achieved > 2 $\log_{10}$ reduction of the internalized bacteria regardless of the chemical decontamination step (Ge et al., 2013). Similarly, sequential application of 60 mg/L PAA (90 s) and WUV (10 s, unspecified dose) did not improve the inactivation (≈2.2 $\log_{10}$ reduction) or inhibition (1.7 $\log_{10}$) of inoculated *Citrobacter freundii* (7.1 $\log_{10}$/g) in 'Romaine' lettuce pieces compared to the WUV treatment.

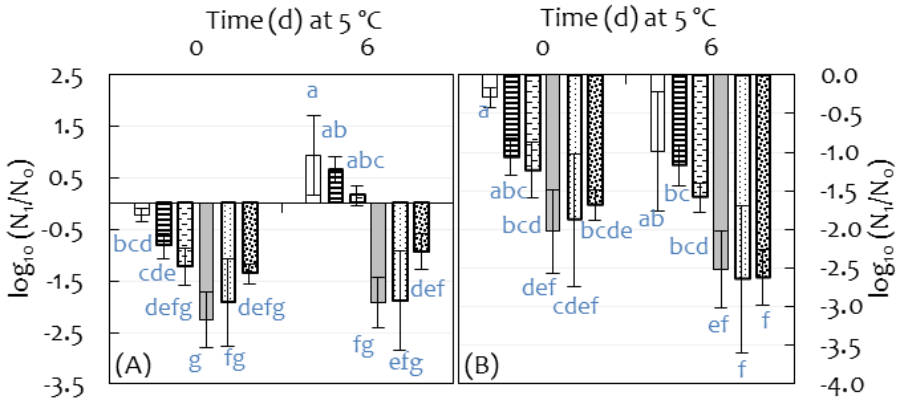


Figure 3. Efficacy of WUV in combination with PAA for the decontamination of (A) *L. monocytogenes* and (B) *S. enterica* in fresh-cut 'Iceberg' lettuce: Columns are means of the logarithmic reductions (log_{10} (N_1/N_0)) of the population levels of the treated samples (N_1) in relation to initial inoculated microbial populations (N_0): (☐) water control, (▤) 40 mg L^{-1} PAA: PAA40, (▦) 80 mg L^{-1} PAA: PAA80, (▩) 0.1 kJ m^{-2} WUV, (▨) 0.1 kJ m^{-2} WUV + PAA40, (▩) UV-C + PAA80. Error bars represent standard deviations (n=6). Different letters represent significant differences at each sampling time according to analysis of variances (ANOVA) and Tukey's test ($p < 0.05$).

As shown by our results, in baby spinach leaves, processing with WUV even at a higher dose (0.2 kJ/m^2) than that applied to lettuce, was not effective for inactivation of any of both pathogens (initial levels 4.3 ± 0.2 log_{10} CFU/g) compared to water-washing (Fig 4 A and C). Higher effectiveness of WUV in lettuce than in baby spinach leaves for the inactivation of *Salmonella* spp. has previously been obtained (Guo et al., 2017; Huang et al., 2018). However, in the present work this limitation was overcome by combining 0.2 kJ/m^2 UV-C with 40 mg/L PAA. This combination inactivated both *S. enterica* and *L. monocytogenes* by up to 0.9 ± 0.1 and 2 ± 0.1 log_{10}, respectively, which in the latter case was significantly better than the correspondent WUV treatment. However, processing with 0.2 kJ/m^2 UV-C whether applied in water or in PAA, was ineffective for inhibiting *L. monocytogenes* throughout storage. Contrastingly, treatment with 0.2 kJ/m^2 UV-C and 80 mg/L PAA inhibited *S. enterica* growth, maintaining its populations 1.4 ± 0.2 log_{10} below the inoculated levels at the end of storage, which was significantly better than water-washing. Furthermore, increasing the UV-C dose to 0.3 kJ/m^2 did not improve the efficacy of WUV (0.9 ± 0.2 log_{10}) at inactivating *S. enterica* (Fig. 4 D) in baby spinach but it enhanced the inactivation of *L. monocytogenes* to 2.0 ± 0.1 log_{10} (Fig. 4 B). Higher UV-C doses (2.4 kJ/m^2) applied in a conventional chamber have shown to be effective at

inactivating *L. monocytogenes*'s (by 2.2 $\log_{10}$) and at inhibiting its growth (by 0.9 $\log_{10}$) in spinach leaves during 14 d of refrigerated storage in air (Escalona et al., 2010). We obtained no further improvement by combining 0.3 kJ/m^2 UV-C with PAA. This result agreed with those obtained by Martínez-Hernández et al. (2015) upon sequential treatment of fresh-cut broccoli with 100 mg/L PAA and then dry-UV-C irradiation (7.5 kJ/m^2), when no synergistic effect at inhibiting *S. enterica* or *E. coli* populations were observed after 7 d of MAP storage at 5 °C, compared to the single treatments. Using the combination of 0.3 kJ/m^2 UV-C in 40 mg/L PAA, the initial reductions of *S. enterica*'s population in baby spinach leaves was 0.7 ± 0.2 $\log_{10}$ more than those achieved by water-washing, and the inhibition of *L. monocytogenes'* populations were maintained during storage at 5 °C, keeping them 1.1 ± 0.4 $\log_{10}$ below the inoculated levels, although it was not significantly better than the WUV treatment. Similarly, no significant improvement of the water-assisted technology at inactivating *S. enterica* (up to 2 $\log_{10}$) in baby spinach leaves was obtained by Huang et al., (2018) using the combination of 80 mg/L PAA and a considerably higher WUV dose (33.6 kJ/m^2) than that used in the present study.

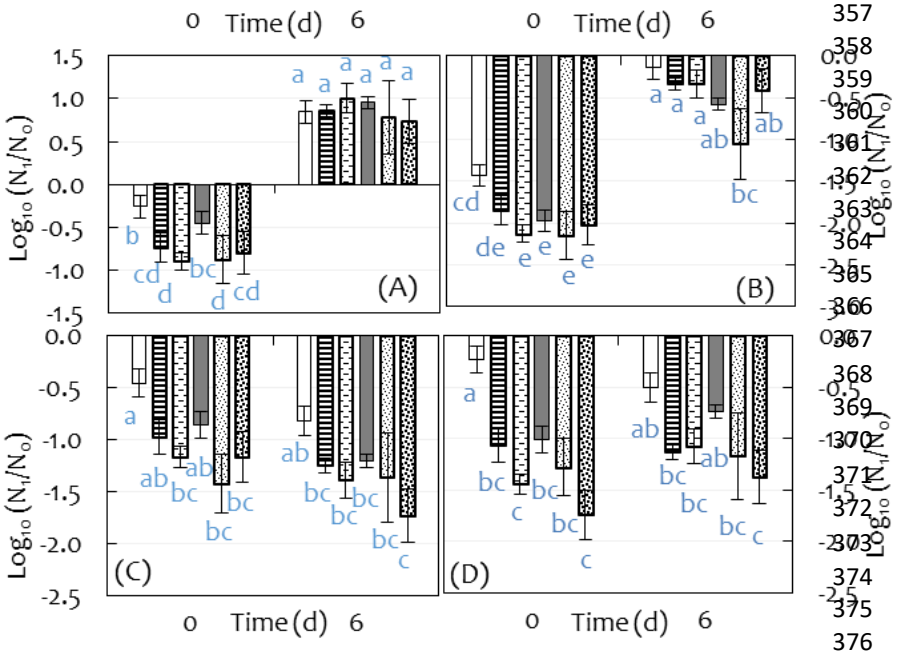


Figure 4. Efficacy of WUV in combination with PAA for the decontamination of (A and B) *L. monocytogenes* and (C and D) *S. enterica* in baby spinach leaves: Columns represent means of the logarithmic reductions of microbial populations in sanitized samples (N_1): (□) water control, (▤) 40 mg L^{-1} PAA control, (▦) 80 mg L^{-1} PAA control, (■)UV-C -treated: 0.2 kJ m^{-2} in A and C or 0.3 kJ m^{-2} in B and D, (▨) 40 mg L^{-1} PAA + UV-C: 0.2 kJ m^{-2} in A and C or 0.3 kJ m^{-2} in B and D, (▩) 80 mg L^{-1} PAA + UV-C: 0.2 kJ m^{-2} in A and C) or 0.3 kJ m^{-2} in B and D, in relation to inoculated populations (N_0). Error bars represent standard deviations (n=6). Different letters represent significant differences among treatments at each sampling time according to analysis of variances (ANOVA) and Tukey's test ($p < 0.05$).

However, the reason for the lack of enhanced antimicrobial effect in lettuce or spinach is still unclear. It could be related to the ability of foodborne pathogens of interacting with the plant-associated microbiota, or to their internalization and attachment to the plant tissue during overnight incubation, which could have reduced the accessibility of UV-C and PAA or led to induced resistance of bacteria against antimicrobial mechanisms (Brandl et al., 2013; Gayán et al., 2014; Kroupitski et al., 2009; Takeuchi et al., 2000; Takeuchi and Frank, 2001; Vandekinderen et al., 2009) In this sense, H_2O_2 sprayed upon continuous application of UV-C (0.378 kJ/m^2 for 60 s) at 50 °C in a conventional chamber achieved 4 and 0.9 $\log_{10}$ reductions of internalized *S. enterica* in 'Iceberg' lettuce and spinach leaves, respectively, which contrasted with the lack of reduction obtained with 200 mg/L calcium hypochlorite washing and the UV or H_2O_2 treatments alone (Hadjok et al., 2008). It was speculated that the higher efficacy was due to the penetration of free radicals in a vapor form as opposed to the liquid phase (Hadjok et al., 2008). In general, the synergistic effect of integrated strategies involving UV-C irradiation and chemicals for the decontamination of inoculated pathogens or native microbiota in fresh produce has shown to be depend not only on the UV-C and the chemical compound doses and their ways of application, but also on the topography of the food matrix and its indigenous microbiota, the target microorganism, and the inoculation method (Fan et al., 2017; Guo et al., 2017). For example, high synergism has been observed when using the combination of 10 mg/L PAA and UV-C (0.1 kJ/m^2) for the inactivation of *S. enteritidis* (6.2 $\log_{10}$ reduction) in peptone water compared to the single treatments (1.9 and 2.6 $\log_{10}$ reduction for PAA and UV-C, respectively) (Ou et al., 2016). Combining UV-C (0.378 kJ/m^2) and sprayed 1.5 % H_2O_2 at 50 °C for 30 s achieved a synergistic reduction of *Salmonella* spp. 'Iceberg' lettuce (Hadjok et al., 2008). However, the enhanced effect showed to be related with an increased free radical generation upon high temperature, since no improvement was observed when the 1.5 % H_2O_2 was applied at 20 °C. However, in blueberries, combining WUV with hydrogen peroxide, sodium dodecyl sulfate, levulinic acid, or chlorine using a water-assisted UV-C device, achieved no improvement in the inactivation of *S. enterica* and *E. coli*, compared to the WUV control (Liu et al., 2015). Furthermore, several

406 chemicals including 1 % H_2O_2, and 100 mg/L chlorine has recently been assessed using a water-
407 assisted UV-C technology (4 L capacity, 34.8 kJ/m^2) for the inactivation of a cocktail of *Salmonella*
408 spp. on fresh-cut 'Iceberg' lettuce, and baby spinach leaves showing no improvement of the WUV
409 treatment (Guo et al., 2017). In the last mentioned experiment, when comparing different food
410 matrices and inoculation methods they obtained higher reductions in spot-inoculated samples than
411 in dip-inoculated ones using the combined methods, and a decrease in effectiveness with the
412 increase of roughness and cut surfaces of the matrix (grape tomatoes > lettuce > baby spinach).

413 In the present work, the UV-C + 40 mg/L PAA combined treatment showed no significant effect on
414 the overall appearance of 'Iceberg' lettuce compared to the single treatments (data not shown).
415 However, the combined application of UV-C + 80 mg/L PAA resulted in diminished quality ($P < 0.001$)
416 of the fresh-cut product at the end of storage, with the 60 % of the evaluations falling in the category
417 3 due to discoloration of the samples. The action of the oxidant agent at a higher concentration
418 combined to the mechanical damages of the membranes related to cutting could account for this
419 result (Dai et al., 2012). Ge et al., (2013) found no differences in firmness of 'Iceberg' lettuce
420 between the single treatments: 4.5 or 9 kJ/m^{2}UV-C and PAA (80 mg/L for 10 min), and the sequential
421 combinations thereof. However, they reported a slight color change when a dose of 9 kJ/m^2 UV-C
422 was used. In contrast, we found no significant differences in the overall appearance of spinach leaves
423 ($P > 0.05$) among treatments, which could be related to the less extent of physical damage inflicted
424 by cutting during processing (only detached). Similarly, no differences in color or gas headspace
425 content was found among UV-C-treated (4.54 kJ/m^2) and non-treated baby spinach samples at 6 d of
426 storage at 5 °C in a closed system (Artés-Hernández et al., 2009).

427 Regarding the effect of the assayed technologies on microbial populations in the process water, the
428 combination of UV-C with PAA inactivated both pathogens in the process solutions showing no viable
429 cells (< 5 CFU/mL) after single-use sanitation of either inoculated spinach or 'Iceberg' lettuce, while
430 10 UFC/mL could be detected upon the WUV treatment. This enabled solutions for reutilization,

improving the efficacy of the decontamination step and lowering the production costs and sustainability due to water consumption. In according with our results, the decontamination with 0.1 kJ/m^2 UV-C of the sanitation water resulting from lamb's lettuce washing, achieved > 5 log_{10} (below a detection limit of 10 CFU/mL) of both *L. monocytogenes* and *S. enterica,* when inoculated at 10^6-10^7 CFU/mL (Ignat et al., 2015). Similarly, combining 33.6 kJ/m^2WUV and 80 mg/L PAA reduced *Salmonella* spp. levels under the detection limit after the sanitation of 'Iceberg' lettuce improving the result obtained with the WUV and PAA single treatments (Huang et al., 2018). In a previous work, a sequential sanitation with cold (4 ºC) or warm (45 ºC) water after pre-treatment with 1.2 kJ/m^2 UV-C significantly improved the efficacy of UV-C for the inactivation of viable MAM cells in the water after single sanitation of fresh-cut endive (Hägele et al., 2016). The combination of O_3 with UV-C have also improved the efficacy of the UV-treatment alone by up to 3 log_{10} regarding the reduction of the mesophilic microbiota populations in the water, after sanitation of fresh-cut escarole and onion for 20 min (Selma et al., 2008). To sum up, even when PAA + UV-C combinations did not enhance the inactivation of inoculated pathogens in the evaluated food matrices, combined chemical-physical treatments are still recommendable due their increased effectiveness at decontaminating the process water, thereby reducing the risks for cross-contamination during processing workflow, as it has been reported by other authors (Huang et al., 2018; Petri et al., 2015; Singh et al., 2018).

3.3 Integration of WUV, PAA and biopreservation

3.3.1 Effect of UV-C + PAA on native microbiota

Pretreatment with UV-C + PAA was expected to reduce initial MAM levels and subsequently inhibit their growth throughout storage. The subsequent inoculation with the biopreservative bacterium CPA-7 was hypothesized to synergistically act with the physical-chemical treatment through direct antagonistic activity and indirect mechanisms involving the activation of the plant's defense response, thereby controlling pathogens and MAM's populations throughout storage (Xu and Du, 2012; Urban et al., 2016). For example, combining conventional UV-C (6.0 kJ/m^2) with super-

456 atmospheric O_2 packaging and electrolyzed water showed enhanced effects at inactivating MAM in
457 fresh-cut broccoli compared to the single treatments, which was linked to the induced activities of
458 APX, SOD and the increased total antioxidant capacity after 5 days of storage (Martínez-Hernández et
459 al., 2013).

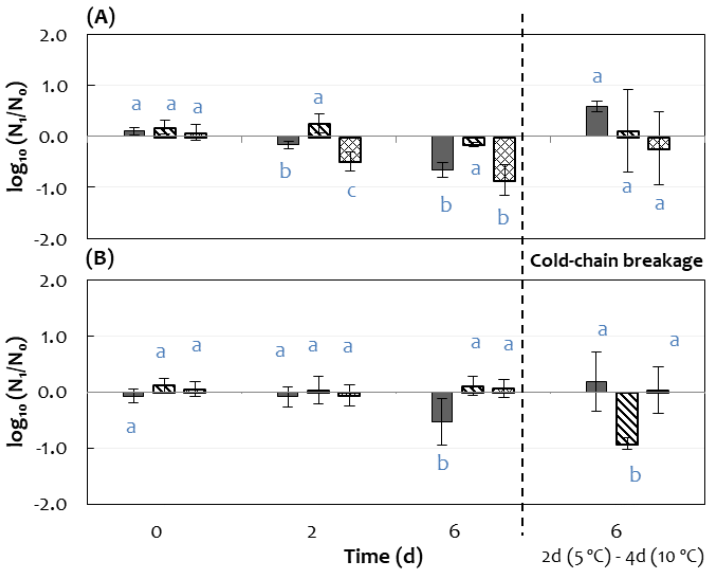
460 Native initial populations of mesophilic microbiota in lettuce and baby spinach leaves were 4.9 ± 0.2
461 and 6.9 ± 0.4 $\log_{10}$ CFU/g, respectively. Pretreating fresh-cut 'Iceberg' lettuce with UV-C in PAA
462 resulted in a similar reduction of initial populations of MAM to that obtained after PAA sanitation (by
463 1.3 ± 0.2 $\log_{10}$). Modelling PAA decontamination of 'Iceberg' lettuce showed a linear relation
464 between the PAA concentration (0, 25, 80, 150 and 250 mg/L), the time of exposure and the
465 reduction of native microbiota (Vandekinderen et al., 2009). However, the reduction levels were
466 limited (0.4–2.4 $\log_{10}$) probably because native microbiota was already adapted and attached to plant
467 surfaces even forming biofilms showing an increased resistance towards sanitizers (Vandekinderen et
468 al., 2009). As observed for lettuce, UV-C in PAA reduced initial MAM populations in baby spinach
469 leaves with similar efficacy to that of PAA sanitation. However, reductions were less than a half to
470 those observed for lettuce (by 0.5 ± 0.2 $\log_{10}$), which showed that the efficacy of sanitation methods
471 is influenced by the initial levels of indigenous microbiota. No inhibition of MAM's growth was
472 observed throughout storage for 6 d at 5 °C either in lettuce or spinach samples pretreated with UV-
473 C in PAA, reaching populations of 6.9 ± 0.4 and 7.7 ± 0.3 $\log_{10}$ CFU/g, respectively. In agreement with
474 our results, Escalona et al., (2010) obtained a slight initial reduction of MAM (by 1.4 $\log_{10}$) in baby
475 spinach leaves treated with 2.4 kJ/m^2 UV-C while no inhibition of growth was observed after 6 d of
476 refrigerated storage. Similarly, significantly higher UV-C doses (4.54 to 11.35 kJ m^{-2}) have shown to
477 be effective for reducing mesophilic microorganisms from 0.5 to 1 $\log_{10}$ in spinach leaves whereas no
478 inhibition was observed throughout storage, compared to chlorine sanitation (Artés-Hernández et al.,
479 2009).

As shown by the results, upon a breakage of the cold chain of storage, no differences among treatments regarding the MAM's final populations were either detected in lettuce or spinach leaves (7.6 ± 0.3 and 8.8 ± 0.1 $\log_{10}$ CFU/g, respectively). The dose-dependent UV-C effect on native microbiota and its synergistic improvement by other chemical and physical factors seems to be largely linked to the food matrix. UV-C treatments at higher doses than those used in the present study achieved significant reductions of MAM's titers in other lettuce varieties, i.e. by > 1 $\log_{10}$ using 4.06 or 8.14 kJ/m^2 in 'Lollo Rosso' lettuce and by 0.5-2 $\log_{10}$ using 0.8 to 8.14 kJ/m^2 in 'Red Oak Leaf' lettuce (Allende & Artés, 2003). However, in our case the detrimental effect on quality did not compensate the putative improvement of microbiological quality which would have putatively achieved a higher UV-C dose. Thus, perhaps it would be interesting to test the combination of UV-C with other chemical compounds or physical treatments such as ultrasounds to accomplish this objective.

3.3.2 Effect of the sequential treatment with WUV + PAA and then CPA-7 on the pathogens' growth

Initial populations of CPA-7 in lettuce and spinach were 5.9 ± 0.2 $\log_{10}$ CFU/g and were not significantly affected by the UV-C + PAA pretreatment. At the end of storage, they reached 6.2 CFU/g at 5 °C and 6.8 $\log_{10}$ CFU/g upon a cold-chain breakage in both matrices, regardless of the pretreatment with UV-C in PAA. Inoculated populations of *L. monocytogenes* were 3.9 ± 0.1 and 4.1 ± 0.1 $\log_{10}$ CFU/g in 'Iceberg' lettuce and spinach, respectively. Initial populations of *S. enterica* were 4.1 ± 0.1 $\log_{10}$ CFU/g in both matrices. Results showed that *L. monocytogenes'* growth was inhibited by 0.5 ± 0.2 and 0.9 ± 0.2 $\log_{10}$ in respect of the 40 mg/L PAA-pretreated control after 2 d and 6 d of MAP storage at 5 °C, in samples treated with WUV + PAA + CPA-7 (Fig. 5 A). At day 6 of storage, *L. monocytogenes'* populations were also reduced by 0.7 ± 0.2 $\log_{10}$ in samples treated either with UV-C in PAA. In a previous experiment, CPA-7 inhibited *L. monocytogenes'* growth in 'Romaine' lettuce by 1.5 $\log_{10}$ compared to the untreated control after 6 d at 10 °C in MAP (Oliveira et al., 2015). Although the UV-C + PAA and the WUV + PAA + CPA-7 treatments inhibited *L. monocytogenes'* growth in

respect of the PAA treatment, the inhibitory effect of CPA7 was not significantly different from that obtained using the double combination, which could have been due to the interference of the native microbiota of the vegetable. In this sense, the incidence and severity of blue and gray molds in pear fruits, caused by *Penicillium expansum* and *Botrytis cinerea*, respectively, was reduced throughout storage at 20 °C for 15 d upon dip-inoculation with the antagonistic yeast *Candida guilliermondii* (5 x 10^7 CFU/mL) after 5 kJ/m^2 (15 min) UV-C pretreatment, but before inoculation with the pathogens and the antagonist, fruit surfaces had been disinfected with 2 % (v/v) sodium hypochlorite for 2 min (Xu and Du, 2012). Similarly, the integrated application of 4 kJ/m^2 UV- C and the antagonistic yeast *Candida tropicalis* increased the resistance of pineapple to the phytopathogenic fungus *Chalara paradoxa* and preserved the firmness of the fruit (Ou et al., 2016). Again, fruit have been disinfected with 2 % (v/v) sodium hypochlorite before wound inoculation. However, the increased resistance to pathogens of fruit treated with UV and biocontrol agents has been previously correlated with lower activities of cell-wall degrading enzymes (pectin methylesterase, polygalacturonase, and cellulase) and enhancement of both non-enzymatic (total phenolic content via PAL activation) and enzymatic antioxidant mechanisms (catalase, superoxide dismutase and peroxidase) as well as with the increase of PR protein activities (β-1,3-glucanase and CHT) (El Ghaouth et al., 2003; Ou et al., 2016; Pombo et al., 2011). We have previously observed that CPA-7 induced the activities of defense-related enzymes in fresh-cut 'Golden' apple (Collazo et al., 2018a). However, its effect on green leaves remains to be investigated. In general, there is a lack of information about green leaves' response to plant or human pathogens and this is a matter worthy to be studied. Pretreatment with UV-C in PAA did not show a significant inhibitory effect on *L. monocytogenes'*s growth in 'Iceberg' lettuce upon a cold-chain breakage. In such conditions, CPA-7's antagonistic activity against *L. monocytogenes* was inconsistent among independent repetitions of the experiment and therefore, it was not statistically significant.



531

532 Figure 5. Logarithmic reductions (log_{10} (N_1/N_0)) of (A) *L. monocytogenes'* and (B) *S. enterica'*s populations in 'Iceberg'
533 lettuce-treated samples (N_1) using the combination of 0.1 kJ m^{-2} UV-C + 40 mg L^{-1} PAA (■), sequential treatment with
534 40 mg L^{-1} PAA and then CPA-7 (▨) or sequential treatement with 0.1 kJ m^{-2} UV-C + 40 mg L^{-1} PAA and then CPA-7 (▩
535) in respect of microbial populations in the 40 mg L^{-1} PAA-washed control (N_0). Columns represent means and and
536 error bars represent standard deviations (n=6). Different letters represent significant differences according to
537 analysis of variances (ANOVA) and Tukey's test ($p < 0.05$).

538 Regarding the effect on *S. enterica*, a slight inhibition (by 0.5 ± 0.3 log_{10}) compared to the PAA-
539 pretreated control was observed at day 6 of storage at 5 °C, in lettuce samples pretreated with UV-C
540 in PAA but no inhibitory effect was observed in CPA-7-treated samples (Fig. 5 B). This agreed with the
541 lack of inhibition shown by CPA-7 against a six-strains cocktail of *Salmonella* spp. in a previous
542 experiments performed in 'Romaine' lettuce for 6 d at 10 °C in MAP (Oliveira et al., 2015). In contrast
543 to that observed for *L. monocytogenes*, at day 6 of storage upon a cold-chain breakage, CPA-7 was
544 able to reduce *S. enterica* populations by 0.9 ± 0.1 log_{10} in respect of the control samples pre-treated
545 with 40 mg/L PAA. This result suggested that the use of the antagonist would contribute to maintain
546 the safety of this product in case of that event.

547 In baby spinach leaves, no differences among the double (0.3 kJ/m^2UV-C + 40 mg/L PAA) and triple
548 combination (UV-C + PAA + CPA-7) were observed before 6th day of refrigerated storage. At that day,
549 samples pre-treated with UV-C in PAA showed a slight inhibition of *L. monocytogenes'* growth in
550 respect of the PAA-pretreated control. At the same time of analysis, the sequential application of

PAA + WUV and then inoculation with CPA-7 inhibited *L. monocytogenes'* growth by 0.4 ± 0.1 $\log_{10}$ in respect of the non-inoculated PAA-pretreated control (Fig. 6 A). No significant differences in *L. monocytogenes'* growth were observed upon a cold-chain breakage whether the biopreservative agent was present or not. Similarly, any of the evaluated treatments controlled *S. enterica'*s growth after 6 d of storage at 5 °C (Fig. 6 B). However, *S. enterica'*s growth was inhibited upon a breakage of the cold chain of storage in UV-C + PAA-pretreated spinach samples compared to the PAA-pretreated control, regardless of the presence of the antagonist. This contrasted with the significant growth of *L. monocytogenes* (up to 2 $\log_{10}$ CFU/g) in this food matrix. As observed for lettuce, no synergistic effect of the integration of UV-C, PAA and CPA-7 was observed in spinach leaves throughout storage at 5 °C or upon a breakage of the cold-chain of storage compared to the CPA-7 + PAA and the UV-C + PAA double combinations.

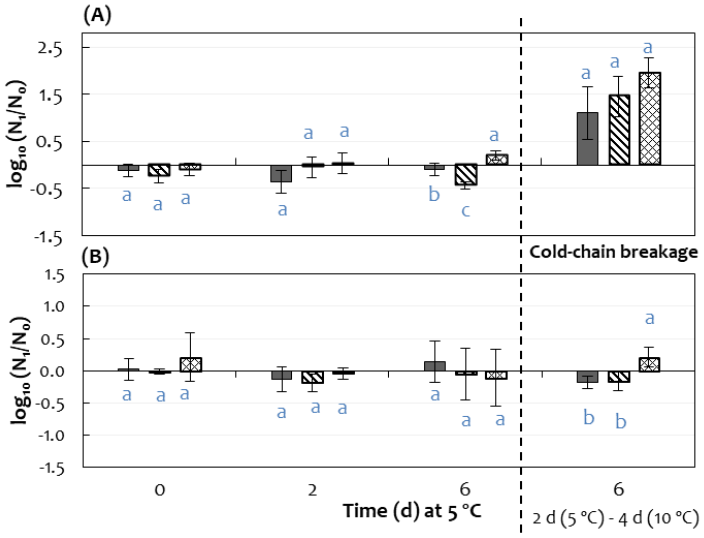


Figure 6. Logarithmic reductions ($\log_{10}$ (N_1/N_0)) of (A) *L. monocytogenes'* and (B) *S. enterica'*s populations in baby spinach leaves samples treated (N_1) using the combination of 0.3 kJm^{-2} UV-C + 40 mg L^{-1} PAA (■), sequential treatment with 40 mg L^{-1} PAA and then CPA-7 (▨) or ssequential treatment with 0.3 kJ m^{-2} UV-C + 40 mg L^{-1} PAA and then CPA-7 (▩) (N_1) in respect of the populations in the 40 mg L^{-1} PAA-washed control (N_0). Columns represent means and and error bars represent standard deviations (n=6). Different letters represent significant differences according to analysis of variances (ANOVA) and Tukey's test ($p < 0.05$).

3.3.3 Effect on overall appearance and respiration

Pretreatment with PAA and UV-C and subsequent inoculation with CPA-7 showed no significant differences among treatments in the O_2/CO_2 contents of the packages headspace at the end of refrigerated storage in any of the analyzed matrices. Final gases contents in the bags containing baby spinach leaves were 19.8 kPa O_2/11.1 kPa CO_2, regardless of breakage of the cold-chain. In 'Iceberg' lettuce, the O_2 content of packages at the end of storage was 19.5 kPa, regardless of the storage conditions. However, the CO_2 content was lower at the end of storage at 5 °C (11.0 kPa) than upon a breakage of the cold-chain (16.9 kPa), probably due to increased respiration rate and enzymatic activity in the vegetables. The overall quality of inoculated samples from both matrices was unacceptable at the end of storage but that was expected because of the high initial microbial populations that were inoculated in order to obtain detectable levels after decontamination using the viable count method.

2. CONCLUSIONS

WUV at doses ranging from 0.1 to 0.3 kJ/m^2 achieved effective inactivation and growth inhibition of both *S. enterica* and *L. monocytogenes* in fresh-cut 'Iceberg' lettuce and baby spinach leaves. The combined treatment of UV-C at such doses and 40 mg/L PAA showed no synergistic reduction of the pathogens' populations in respect of the individual control treatments in the evaluated food matrices but it is still recommendable since it improved the efficacy of the single treatments at inactivating both pathogens in the process solutions. Sequential treatment with UV-C + PAA and CPA-7 inhibited *L. monocytogenes'* growth in 'Iceberg' lettuce after 6 d of refrigerated storage in respect of the PAA-pretreated control but it did not show a synergistic improvement in respect of the PAA + UV-C treatment. In samples pretreated with PAA, CPA-7 inhibited the growth of *S. enterica* in 'Iceberg' lettuce upon a cold-chain breakage. The potential usefulness of the bacteriostatic activity of this biocontrol agent for reducing the risks of cross-contamination throughout refrigerated storage and upon an eventual breakage of these conditions is promising, but further study is needed to improve

596 the conditions for a more stable performance in green leaves, focusing on the prior reduction of
597 indigenous microbiota. Low-dose UV-C combined with PAA could be a suitable preservation strategy
598 for improving the safety of ready-to-eat leafy greens.

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