



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

Cyrellys Collazo^a, Violeta Noguera^a, Ingrid Aguiló-Aguayo^b, Maribel Abadias^b, Pilar Colás-Medà^b, Iolanda Nicolau^a, Inmaculada Viñas^{a,*}

^a Food Technology Department, University of Lleida, XaRTA-Postharvest, Agrotecnio Center, Rovira Roure 191, 25198 Lleida, Spain

^b Institut de Recerca i Tecnologia Agroalimentàries, XaRTA-Postharvest, Edifici Fruïtcentre, Parc Científic i Tecnològic Agroalimentari de Lleida, Parc de Gardeny, 25003 Lleida, Spain

ARTICLE INFO

Keywords:

Biological control
Biopreservation
Fresh-cut produce
Foodborne pathogens
Ready-to eat green leaves

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/m² UV-C, in water or in 40 mg/L PAA, inactivated both pathogens by up to 2.1 ± 0.7 log₁₀, which improved the efficacy of water-washing by up to 1.9 log₁₀ and showed bacteriostatic effects on both pathogens. In baby spinach leaves, the combination of 0.3 kJ/m² UV-C and 40 mg/L PAA reduced *S. enterica* and *L. monocytogenes* populations by 1.4 ± 0.2 and 2.2 ± 0.3 log₁₀ respectively, which improved water-washing by 0.8 ± 0.2 log₁₀. Combined treatments (0.1 or 0.3 kJ/m² 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 log₁₀ 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.

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 raw, they can become vehicles for human pathogens such as *Listeria monocytogenes* and *Salmonella enterica* (Franco and 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 organic fertilizers such as manures or sewage sludge (Brandl, 2006). During postharvest processing, inappropriate sanitation of tools, facilities surfaces, and process-water may also turn them into contamination sources (Artés and Allende, 2014).

Since immersion of cut surfaces increases the probabilities for the infiltration of liquid into the tissues, sanitation of fresh produce is carried out using antimicrobial solutions to reduce the probability for process water to become a source of contamination (Gorny et al., 2006). The most used chemical sanitizer in food industry is chlorine due to its strong antibacterial activity and low costs (Hua and Reckhow, 2007). However, growing public concern about health and environmental negative effects of its by-products and the advent of banning or

* Corresponding author.

E-mail address: ivinas@tecal.udl.cat (I. Viñas).

<https://doi.org/10.1016/j.ijfoodmicro.2019.02.024>

Received 27 September 2018; Received in revised form 22 January 2019; Accepted 28 February 2019

Available online 01 March 2019

0168-1605/ © 2019 Elsevier B.V. All rights reserved.

restrictive regulations for its use in several countries (EC-European Commission for Health and Consumer Protection, 2005), have prompted the research and development of alternative decontamination strategies in food industry.

Short-wave Ultraviolet light (UV-C) has a direct deleterious effect on microbial DNA structure which leads to the inactivation and death of most types of microorganisms without producing harmful byproducts (Gayán et al., 2014). Therefore, UV-based technologies are being implemented for the decontamination of food and food-contact surfaces, including equipment, tools, packages, liquids, powders and fresh produce (Bintsis et al., 2000; Charles et al., 2013; Fine and Gervais, 2004; Ignat et al., 2015; Manzocco et al., 2011). When applied at high doses, UV can damage plant tissues, being counterproductive for plant products shelf-life (Kovács and Keresztes, 2002). However, at low-doses, UV-C irradiation induces plant self-protective mechanisms against potential oxidative and mutagenic damages. This leads to the enhancement of antioxidant mechanisms as well as to the production of pathogenesis-related proteins (PR proteins), thereby eliciting the defense response to subsequent pathogenic infections (indirect effect) (Allende et al., 2006; Ou et al., 2016; Scott et al., 2017). Therefore, the durability of UV-C antimicrobial effects when applied to plant tissues can be attributed to both the reduction of the multiplication capacity of irradiated surviving microorganisms and to the increase of the negative pressure exerted through the elicitation of plant resistance mechanisms (Shama, 2007; Yun et al., 2013). Thus, pre-treating commodities with UV-C would potentially improve the safety of fresh-cut products by preventing the population increase and the establishment of foodborne pathogens throughout storage in case of cross-contamination after the sanitation step.

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

On the other hand, biopreservation have also been combined with physical sanitation methods for attempting to reduce the incidence of fungal diseases in fresh produce during postharvest (Xu and Du, 2012; Ou et al., 2016). Those experiments have shown promising results regarding the control of pathogens populations through the activation of the plant's defense mechanisms (Ou et al., 2016). 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 biopreservative 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 of the colonization capacities of those pathogens (Collazo et al., 2017a, 2017b, 2018a).

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* subsp. *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 ×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-Engley

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

2.3. Plant material processing

Whole wrapped ‘Iceberg’ lettuce (*Lactuca sativa* var. capitata) and ready-to-use bagged baby spinach leaves (*Spinacia oleracea* L.) were purchased from local retail establishments in Lleida, Spain. Before treatment, the external leaves and core of ‘Iceberg’ lettuce were discarded and the rest was cut into 3–4 cm² pieces, washed in chlorinated tap water, drained, spin-dried using a manual centrifuge and kept in trays overnight in air at 5 °C until use. Baby spinach leaves were unbagged and stored in trays overnight in air at 5 °C without any additional discarding or processing until they were submitted to subsequent sanitation treatments.

2.4. Overall quality and headspace gas composition assessment

Visual assessment of overall appearance was performed initially and throughout storage by 6 untrained panelists using a 1 to 5 hedonic corresponding to 25, 50, 75, 90 and 95% acceptability of the sample. The gas headspace composition of packages was measured using a handheld gas analyzer (CheckPoint O₂/CO₂, PBI Dansensor, Denmark).

2.5. WUV treatment preserving overall appearance: analyses and storage

Preliminary selection of the WUV dose was based on the visual assessment of the overall quality of treated samples. For WUV treatments, batches of processed vegetables were immersed in agitated cold tap water at a ratio of 0.3:10 (kg of product: L of water) using a water-assisted UV-C equipment composed of deposit (15 L capacity) containing 4 UV lamps (17.2 W, 254 nm) (GPH 303T5L/4, Heraeus Noblelight, Hanau, Germany). The deposit is connected to recirculating water put in motion by a water pump. Pressurized water is introduced through multiple sprinklers on the top and exits the tank through the bottom, while moves due to pressurized air (set at 100 kPa) that enters through the bottom of the tank (Fig. 1). WUV doses were calculated as the mean of irradiance (W/m²) * time (s). Several doses were assayed by combining 4 UV-C lamps and different times of exposure. For ‘Iceberg’ lettuce, 0.1, 0.3, and 0.5 kJ/m² UV-C, corresponding to 1, 3 and 5 min of exposure, respectively, were tested. For spinach, 0.2 and 0.3 kJ/m² treatments, corresponding to 2 and 3 min of exposure, respectively, were tested. Before each treatment, lamps were preheated

until stabilization of irradiance. Water-washing without turning on the UV lamps was performed as a control treatment. Before and after WUV treatments, temperature was measured using an infrared thermometer (DualTemp Pro, Labprocess distribuciones, Barcelona, Spain). Irradiance was measured using a UV-sensor EasyH1, Pechl Ultraviolet, Mainz, Germany) through an orifice located in the lid of the tank. After treatment, vegetables were drained, spin dried, and 15 g samples were packaged in thermosealed 12 × 12 cm (lettuce) or 14 × 14 cm (spinach) polypropylene bags (PP110, ILPRA Systems Espanya SL, Mataró, Spain) to achieve passive MAP conditions and were stored at 5 °C in darkness. The film had a gas permeability of 1.1 and 5 cm³/m²/day/KPa for O₂ and CO₂, respectively, at 23 °C and 0% relative humidity. Visual assessment of the overall appearance was performed at day 3 and 6 of storage as described in Section 2.4. In the same days, measurements of the O₂/CO₂ composition within packages were also performed as described in Section 2.4.

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 10⁵ 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/m² UV-C in the case of lettuce, and to 0.2 or 0.3 kJ/m² 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.

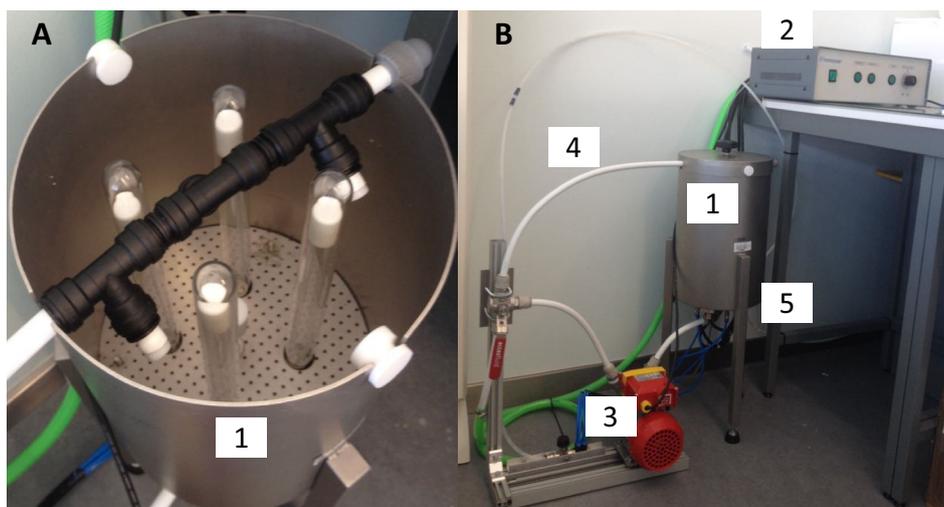


Fig. 1. Water-assisted UV-C setup. (A) Tank containing four UV lamps and a multiple sprinkle device on the top. (B) The tank (1) is connected to a power source (2) a water pump (3), a water circuit (4), and a pressurized air entrance in the bottom (5).

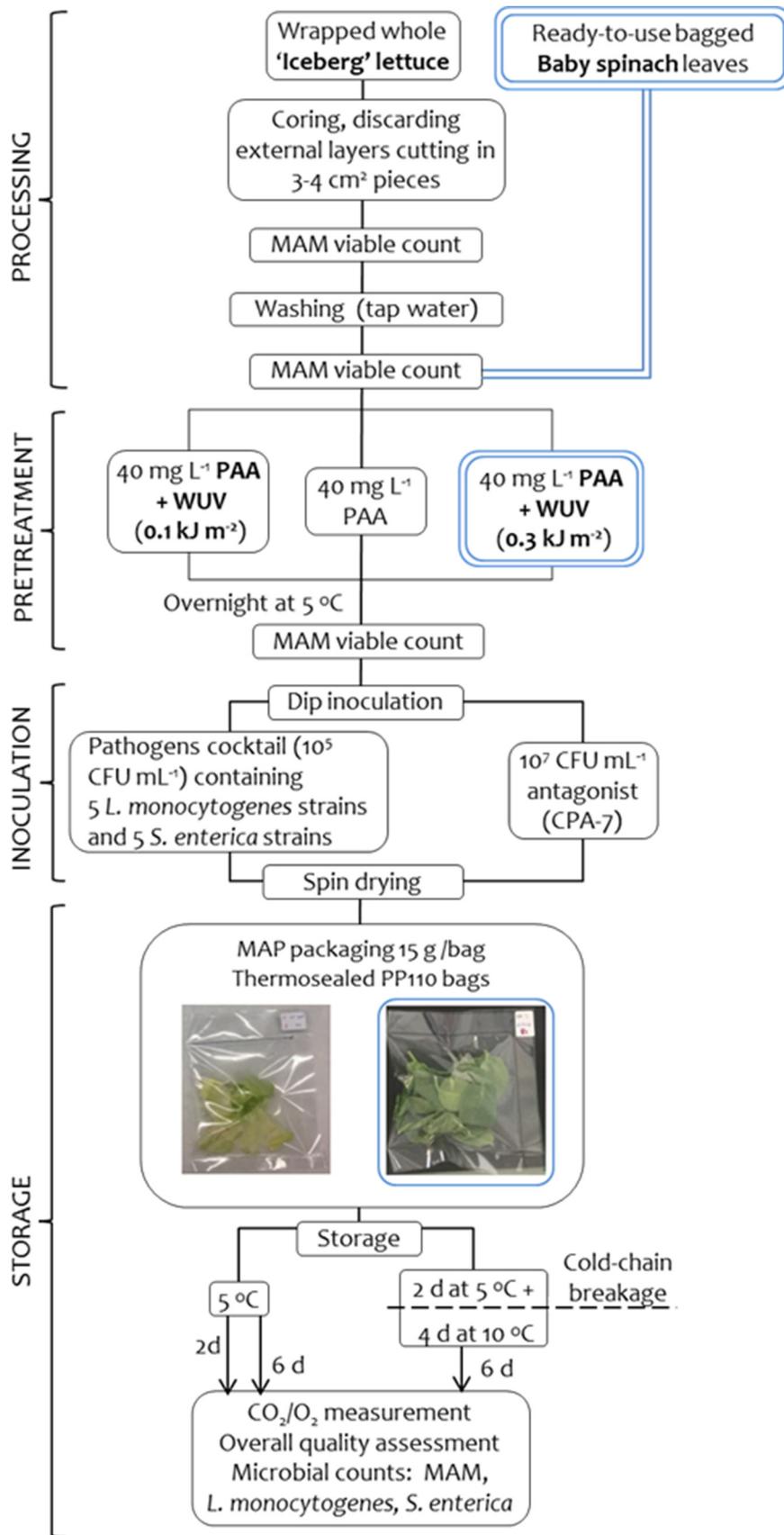


Fig. 2. Experimental setup of a combined preservation strategy comprising pretreatment with immersion-assisted UV-C + peroxyacetic acid (PAA) before inoculation with the biopreservative bacterium *P. graminis* CPA-7, for controlling the populations of *S. enterica* and *L. monocytogenes* in fresh-cut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

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 Fig. 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/m² for 'Iceberg' lettuce or 0.3 kJ/m² 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 10⁵ CFU/mL of each bacterial strain, a mixture of pathogenic + antagonist inoculum containing 10⁷ CFU/mL CPA-7 and 10⁵ CFU/mL of each pathogenic strain, or in antagonist inoculum containing 10⁷ 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 °C, 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 O₂/CO₂ headspace composition of bags was performed at each sampling point as described in Section 2.4.

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 log₁₀ CFU/g before means comparison. Population reductions were calculated by subtracting the count before treatment (N₀) to that obtained after treatment (N₁): log₁₀ N₁ – log₁₀ N₀. 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/m² and stored in passive modified atmosphere, had a similar O₂/CO₂ composition (14 kPa/6 kPa) that untreated controls until the end of storage (data not shown). However, at a higher dose (0.5 kJ/m²), WUV provoked oxidative discoloration, a more marked reduction of O₂ levels (6.16 kPa) and an increase in CO₂ content (6.2 kPa) than the water-washed control (17.5 kPa O₂; 2.8 kPa CO₂) at the end of storage. The higher accumulation in CO₂ content observed in 0.5 kJ/m²-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/m², 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/m² showed unacceptability after 6 d of MAP storage (data not shown); thus, the lowest dose (1 min of exposure, 0.1 kJ/m²) 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/m², 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/m² 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 O₂ (17.6 kPa) content than the water-washed control (19.5 kPa), regardless of the assayed dose (0.2 or 0.3 kJ/m²), while no differences in the CO₂ 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/m² 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/m² 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/m² 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 log₁₀ CFU/mg, respectively. WUV treatments effectively inactivated *L. monocytogenes* by 2.1 ± 0.7 log₁₀ in respect of inoculated populations, improving the efficacy of water-washing by 1.9 log₁₀. *L. monocytogenes*' growth was also inhibited throughout refrigerated MAP storage, showing no population increase in respect of the initial levels (Fig. 3A). Combining 0.1 kJ/m² 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/m² WUV reduced *S. enterica* initial populations in 'Iceberg' lettuce by 2.0 ± 0.6 log₁₀ (Fig. 3B), which improved the efficacy of water-washing by 1.7 log₁₀. 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 log₁₀ below the inoculated levels until the end of storage. Final populations of this pathogen reached 3 ± 0.7 log₁₀ below those present in the water-washed samples. Similarly, Huang et al. (2018) obtained a reduction by 2 log₁₀ 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/m² 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/m² and PAA (80 mg/L) for the sanitation of *S. Typhimurium* in 'Iceberg' lettuce achieved > 2 log₁₀ 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 log₁₀ reduction) or inhibition (1.7 log₁₀) of inoculated *Citrobacter freundii* (7.1 log₁₀/g) in 'Romaine' lettuce pieces compared to the WUV treatment (Lacasse and Holcroft, 2016).

As shown by our results, in baby spinach leaves, processing with

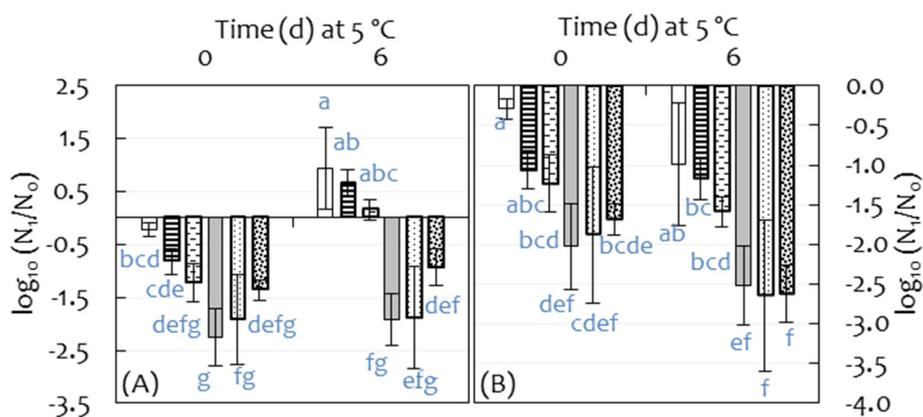


Fig. 3. Efficacy of WUV in combination with PAA for the decontamination of (A) *L. monocytogenes* and (B) *S. enterica* in fresh-cut 'Iceberg' lettuce: Columns are means of the logarithmic reductions ($\log_{10}(N_t/N_0)$) of the population levels of the treated samples (N_t) in relation to initial inoculated microbial populations (N_0): (□) water control, (▨) 40 mg/L PAA: PAA40, (▩) 80 mg/L 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 ($p < 0.05$).

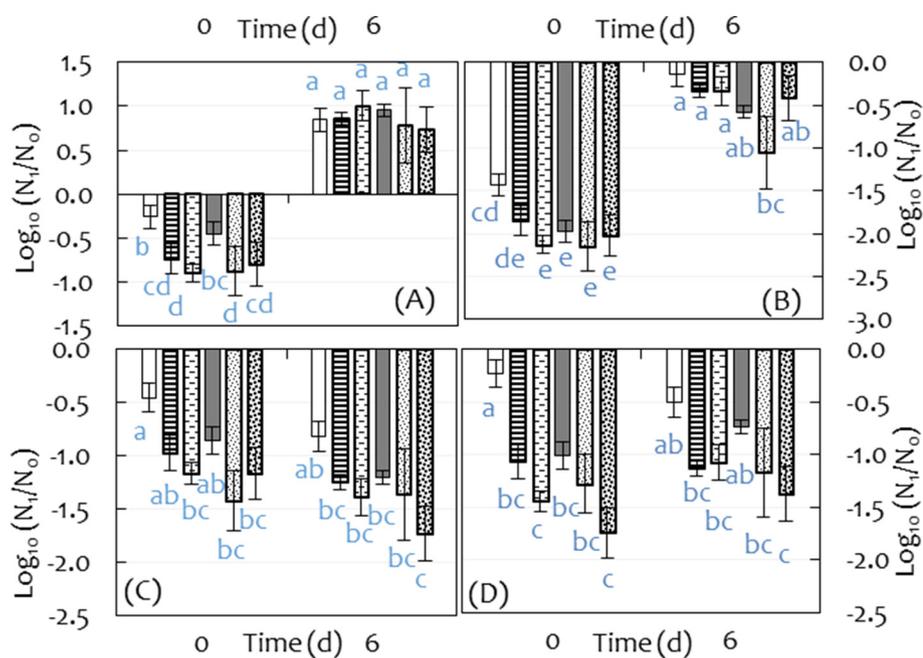


Fig. 4. Efficacy of WUV in combination with PAA for the decontamination of (A and B) *L. monocytogenes* and (C and D) *S. enterica* in baby spinach leaves: Columns represent means of the logarithmic reductions of microbial populations in sanitized samples (N_t): (□) water control, (▨) 40 mg/L PAA control, (▩) 80 mg/L 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 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 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 ($p < 0.05$).

WUV even at a higher dose (0.2 kJ/m²) than that applied to lettuce, was not effective for inactivation of any of both pathogens (initial levels $4.3 \pm 0.2 \log_{10}$ 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/m² UV-C with 40 mg/L PAA. This combination inactivated both *S. enterica* and *L. monocytogenes* by up to 0.9 ± 0.1 and $2 \pm 0.1 \log_{10}$, respectively, which in the latter case was significantly better than the correspondent WUV treatment. However, processing with 0.2 kJ/m² UV-C whether applied in water or in PAA, was ineffective for inhibiting *L. monocytogenes* throughout storage. Contrastingly, treatment with 0.2 kJ/m² UV-C and 80 mg/L PAA inhibited *S. enterica* growth, maintaining its populations $1.4 \pm 0.2 \log_{10}$ 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/m² did not improve the efficacy of WUV ($0.9 \pm 0.2 \log_{10}$) at inactivating *S. enterica* (Fig. 4 D) in baby spinach but it enhanced the inactivation of *L. monocytogenes* to $2.0 \pm 0.1 \log_{10}$ (Fig. 4 B). Higher UV-C doses (2.4 kJ/m²) applied in a conventional chamber have shown similar effectiveness than that obtained with the presently used dose for the inactivation of *L. monocytogenes*'s (by $2.2 \log_{10}$) and the inhibition of its growth (by $0.9 \log_{10}$) in spinach leaves for up to 14 d of refrigerated storage in air (Escalona

et al., 2010). We obtained no further improvement by combining 0.3 kJ/m² 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/m²), 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/m² UV-C in 40 mg/L PAA, the initial reductions of *S. enterica*'s population in baby spinach leaves was $0.7 \pm 0.2 \log_{10}$ more than those achieved by water-washing, and the inhibition of *L. monocytogenes*' populations were maintained during storage at 5 °C, keeping them $1.1 \pm 0.4 \log_{10}$ 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/m²) than that used in the present study.

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, H₂O₂ sprayed upon continuous application of UV-C (0.378 kJ/m² for 60 s) at 50 °C in a conventional chamber achieved 4 and 0.9 log₁₀ 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 H₂O₂ 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/m²) for the inactivation of *S. enteritidis* (6.2 log₁₀ reduction) in peptone water compared to the single treatments (1.9 and 2.6 log₁₀ reduction for PAA and UV-C, respectively) (Ou et al., 2016). Combining UV-C (0.378 kJ/m²) and sprayed 1.5% H₂O₂ 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% H₂O₂ 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 chemicals including 1% H₂O₂, and 100 mg/L chlorine has recently been assessed using a water-assisted UV-C technology (4 L capacity, 34.8 kJ/m²) for the inactivation of a cocktail of *Salmonella* spp. on fresh-cut 'Iceberg' lettuce, and baby spinach leaves showing no improvement of the WUV treatment (Guo et al., 2017). In the last mentioned experiment, when comparing different food matrices and inoculation methods they obtained higher reductions with the combined methods in spot-inoculated samples than in dip-inoculated ones, and a decrease in effectiveness with the increase of roughness and cut surfaces of the matrix (grape tomatoes > lettuce > baby spinach).

In the present work, the UV-C + 40 mg/L PAA combined treatment showed no significant effect on the overall appearance of 'Iceberg' lettuce compared to the single treatments (data not shown). However, the combined application of UV-C + 80 mg/L PAA resulted in diminished quality ($P < 0.001$) of the fresh-cut product at the end of storage, with the 60% of the evaluations falling in the category 3 due to discoloration of the samples. The action of the oxidant agent at a higher concentration combined to the mechanical damages of the membranes related to cutting could account for this result (Dai et al., 2012). Ge et al. (2013) found no differences in firmness of 'Iceberg' lettuce between the single treatments: 4.5 or 9 kJ/m²UV-C and PAA (80 mg/L for 10 min), and the sequential combinations thereof. However, they reported a slight color change when a dose of 9 kJ/m² UV-C was used. In contrast, we found no significant differences in the overall appearance of spinach leaves ($P > 0.05$) among treatments, which could be related to the less extent of physical damage inflicted by cutting during processing (only detached). Similarly, no differences in color or gas headspace content was found among UV-C-treated (4.54 kJ/m²) and non-treated baby spinach samples at 6 d of storage at 5 °C in a closed system (Artés-Hernández et al., 2009).

Regarding the effect of the assayed technologies on microbial populations in the process water, the combination of UV-C with PAA inactivated both pathogens in the process solutions showing no viable cells (< 5 CFU/mL) after single-use sanitation of either inoculated spinach or 'Iceberg' lettuce, while 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 accordance with our results, decontamination with 0.1 kJ/m² UV-C of the sanitation water resulting from lamb's lettuce washing, achieved > 5 log₁₀ (below a detection limit of 10 CFU/mL) of both *L. monocytogenes* and *S. enterica*, from samples inoculated with 10⁶–10⁷ CFU/mL (Ignat et al., 2015). Similarly, combining 33.6 kJ/m²WUV and 80 mg/L PAA reduced *Salmonella* spp. to 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 °C) or warm (45 °C) water after pre-treatment with 1.2 kJ/m² 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 O₃ with UV-C have also improved the efficacy of the UV-treatment alone by up to 3 log₁₀ 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/m²) with super-atmospheric O₂ packaging and electrolyzed water showed enhanced effects at inactivating MAM in fresh-cut broccoli compared to the single treatments, which was linked to the induced activities of APX, SOD and the increased total antioxidant capacity after 5 d of storage (Martínez-Hernández et al., 2013).

Native initial populations of mesophilic microbiota in lettuce and baby spinach leaves were 4.9 ± 0.2 and 6.9 ± 0.4 log₁₀ CFU/g, respectively. Pretreating fresh-cut 'Iceberg' lettuce with UV-C in PAA resulted in a similar reduction of initial populations of MAM to that obtained after PAA sanitation (by 1.3 ± 0.2 log₁₀). Modelling PAA decontamination of 'Iceberg' lettuce showed a linear relation between the PAA concentration (0, 25, 80, 150 and 250 mg/L), the time of exposure and the reduction of native microbiota (Vandekinderen et al., 2009). However, the reduction levels were limited (0.4–2.4 log₁₀) probably because native microbiota was already adapted and attached to plant surfaces even forming biofilms which may have resulted in an increased resistance towards sanitizers (Vandekinderen et al., 2009). As observed for lettuce, UV-C in PAA reduced initial MAM populations in baby spinach leaves with similar efficacy to that of PAA sanitation. However, reductions were less than a half to those observed in lettuce (by 0.5 ± 0.2 log₁₀), which showed that the efficacy of sanitation methods is influenced by the initial levels of indigenous microbiota. No inhibition of MAM's growth was observed throughout storage for 6 d at 5 °C either in lettuce or spinach samples pretreated with UV-C in PAA, reaching populations of 6.9 ± 0.4 and 7.7 ± 0.3 log₁₀ CFU/g, respectively. In agreement with our results, Escalona et al. (2010) obtained a slight initial reduction of MAM (by 1.4 log₁₀) in baby spinach leaves treated with 2.4 kJ/m² UV-C while no inhibition of growth was observed after 6 d of refrigerated storage. Similarly, significantly higher

UV-C doses (4.54 to 11.35 kJ m^{-2}) have shown limited effectiveness for reducing mesophilic microorganisms from (0.5 to $1 \log_{10}$ reduction) in spinach leaves whereas no inhibition was observed throughout storage, compared to chlorine sanitation (Artés-Hernández et al., 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 $8.8 \pm 0.1 \log_{10} \text{ CFU/g}$, 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 8.4 kJ/m^2 in 'Lollo Rosso' lettuce and by 0.5 – $2 \log_{10}$ using 0.8 to 8.4 kJ/m^2 in 'Red Oak Leaf' lettuce (Allende and Artes, 2003). However, in our case the detrimental effect on quality did not compensate the putative improvement of microbiological quality which might have achieved a higher UV-C dose. Alternatively, the combination of UV-C with other chemical compounds or physical treatments such as ultrasounds could be tested 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 $5.9 \pm 0.2 \log_{10} \text{ CFU/g}$ 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 $6.8 \log_{10} \text{ CFU/g}$ 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 $4.1 \pm 0.1 \log_{10} \text{ CFU/g}$ in 'Iceberg' lettuce and spinach, respectively. Initial populations of *S. enterica* were $4.1 \pm 0.1 \log_{10} \text{ CFU/g}$ in both matrices. Results showed that *L. monocytogenes*' growth was inhibited by 0.5 ± 0.2 and $0.9 \pm 0.2 \log_{10}$ 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 $0.7 \pm 0.2 \log_{10}$ in samples treated

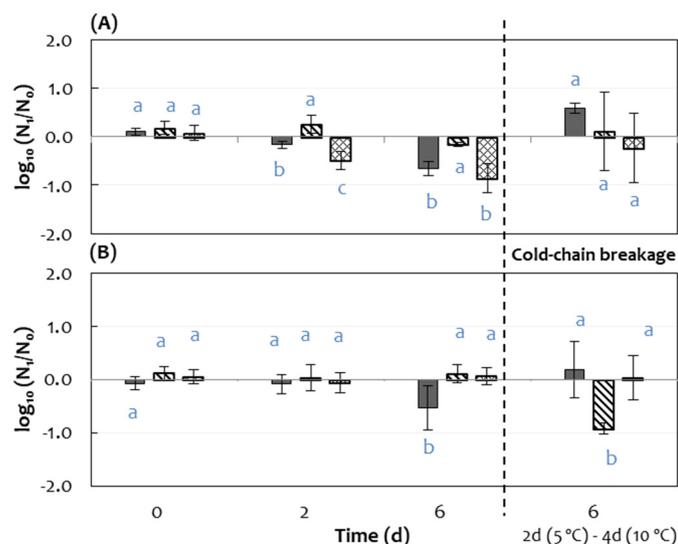


Fig. 5. Logarithmic reductions ($\log_{10}(N_1/N_0)$) of (A) *L. monocytogenes*' and (B) *S. enterica*'s populations in 'Iceberg' lettuce-treated samples (N_1) using the combination of 0.1 kJ m^{-2} UV-C + 40 mg/L PAA (■), sequential treatment with 40 mg/L PAA and then CPA-7 (▨) or sequential treatment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

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 CPA-7 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* ($5 \times 10^7 \text{ CFU/mL}$) 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*' 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.

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

In baby spinach leaves, no differences among the double (0.3 kJ/m^2 UV-C + 40 mg/L PAA) and triple combination (UV-C + PAA + CPA-7) were observed before the 6th day of refrigerated storage. At that day, samples pre-treated with UV-C in PAA showed a slight inhibition of *L. monocytogenes*' growth in 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 $0.4 \pm 0.1 \log_{10}$ 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*

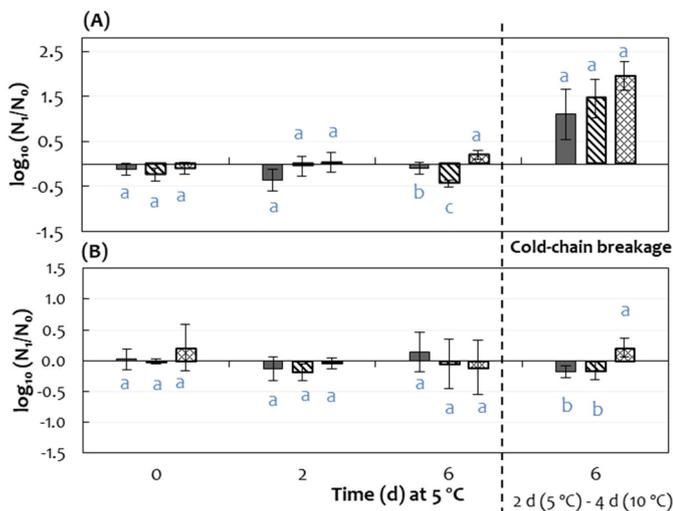


Fig. 6. Logarithmic reductions ($\log_{10}(N_1/N_0)$) of (A) *L. monocytogenes*' and (B) *S. enterica*'s populations in baby spinach leaves samples treated (N_1) using the combination of 0.3 kJ m^{-2} UV-C + 40 mg/L PAA (□), sequential treatment with 40 mg/L PAA and then CPA-7 (▨) or sequential treatment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

(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.

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 $\text{O}_2/11.1 \text{ kPa CO}_2$, 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.

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

Acknowledgements

This work has been supported the CERCA Programme/Generalitat de Catalunya, Spain; the Secretaria d'Universitats i Recerca del Departament d'Economia i Coneixement/ Generalitat de Catalunya; the European Social Fund (grant FI-DGR-2015-0004); and the Spanish Ministry of Economy, Industry, and Competitiveness (National Project of the Spanish Foundation for Science and Technology: FECYT CC/2013105 (2013-16) and grant: RYC-2016-19949). Thanks are given to Marina Anguera for her technical assistance.

References

- Abadias, M., Usall, J., Anguera, M., Solsona, C., Viñas, I., 2008. Microbiological quality of fresh, minimally-processed fruit and vegetables, and sprouts from retail establishments. *Int. J. Food Microbiol.* 123, 121–129.
- Abadias, M., Altisent, R., Usall, J., Torres, R., Oliveira, M., Viñas, I., 2014. Biopreservation of fresh-cut melon using the strain *Pseudomonas graminis* CPA-7. *Postharvest Biol. Technol.* 96, 69–77. <https://doi.org/10.1016/j.postharvbio.2014.05.010>.
- Alegre, I., Viñas, I., Usall, J., Anguera, M., Altisent, R., Abadias, M., 2013a. Antagonistic effect of *Pseudomonas graminis* CPA-7 against foodborne pathogens in fresh-cut apples under simulated commercial conditions. *Food Microbiol.* 33, 139–148. <https://doi.org/10.1016/j.fm.2012.09.007>.
- Alegre, I., Viñas, I., Usall, J., Teixido, N., Figge, M.J., Abadias, M., 2013b. Control of foodborne pathogens on fresh-cut fruit by a novel strain of *Pseudomonas graminis*. *Food Microbiol.* 34, 390–399. <https://doi.org/10.1016/j.fm.2013.01.013>.
- Allende, A., Artes, F., 2003. UV-C radiation as a novel technique for keeping quality of fresh processed 'Lollo Rosso' lettuce. *Food Res. Int.* 36, 739–746. [https://doi.org/10.1016/S0963-9969\(03\)00054-1](https://doi.org/10.1016/S0963-9969(03)00054-1).
- Allende, A., McEvoy, J.L., Luo, Y., Artes, F., Wang, C.Y., 2006. Effectiveness of two-sided UV-C treatments in inhibiting natural microflora and extending the shelf-life of minimally processed "Red Oak Leaf" lettuce. *Food Microbiol.* 23, 241–249. <https://doi.org/10.1016/j.fm.2005.04.009>.
- Alvaro, J.E., Moreno, S., Dianez, F., Santos, M., Carrasco, G., Urrestarazu, M., 2009. Effects of peracetic acid disinfectant on the postharvest of some fresh vegetables. *J. Food Eng.* 95, 11–15. <https://doi.org/10.1016/j.jfoodeng.2009.05.003>.
- Artés, F., Allende, A., 2014. Minimal processing of fresh fruit, vegetables, and juices. In: *Emerging Technologies for Food Processing*. CRC Press, Boca Raton, FL, pp. 583–597. <https://doi.org/10.1016/B978-0-12-411479-1.00031-0>.
- Artés-Hernández, F., Escalona, V.H., Robles, P.A., Martínez-Hernández, G.B., Artés, F., 2009. Effect of UV-C radiation on quality of minimally processed spinach leaves. *J. Sci. Food Agric.* 89, 414–421. <https://doi.org/10.1002/jsfa.3460>.
- Barak, J.D., Gorski, L., Naraghi-arani, P., Charkowski, A.O., 2005. *Salmonella enterica* virulence genes are required for bacterial attachment to plant tissue. *Appl. Environ. Microbiol.* 71, 5685–5691. <https://doi.org/10.1128/AEM.71.10.5685>.
- Bintsis, T., Litopoulou-Tzanetaki, E., Robinson, R.K., 2000. Existing and potential applications of ultraviolet light in the food industry - a critical review. *J. Sci. Food Agric.* 80, 637–645. [https://doi.org/10.1002/\(SICI\)1097-0010\(20000501\)80:6<637::AID-JSFA603>3.0.CO;2-1](https://doi.org/10.1002/(SICI)1097-0010(20000501)80:6<637::AID-JSFA603>3.0.CO;2-1).
- Brandl, M.T., 2006. Fitness of human enteric pathogens on plants and implications for food. *Annu. Rev. Phytopathol.* 44, 367–392. <https://doi.org/10.1146/annurev.phyto.44.070505.143359>.
- Brandl, M.T., Cox, C.E., Teplitski, M., 2013. *Salmonella* interactions with plants and their associated microbiota. *Phytopathology* 103, 316–325. <https://doi.org/10.1094/PHYTO-11-12-0295-RVW>.
- Callejón, R., Rodríguez-Naranjo, M., Ubeda, C., Hornedo-Ortega, R., García-Parrilla, M., Troncoso, A., 2015. Reported foodborne outbreaks due to fresh produce in the United States and European Union: trends and causes. *Foodborne Pathog. Dis.* 12, 32–38. <https://doi.org/10.1089/fpd.2014.1821>.
- Cantos, E., Espín, J.C., Tomás-Barberán, F.A., 2001. Effect of wounding on phenolic enzymes in six minimally processed lettuce cultivars upon storage. *J. Agric. Food Chem.* 49, 322–330. <https://doi.org/10.1021/jf000644q>.
- Charles, F., Vidal, V., Olive, F., Filgueiras, H., Sallanon, H., 2013. Pulsed light treatment as new method to maintain physical and nutritional quality of fresh-cut mangoes. *Innov. Food Sci. Emerg. Technol.* 18, 190–195. <https://doi.org/10.1016/j.ifset.2013.02.004>.
- Collazo, C., Abadias, M., Aguiló-Aguayo, I., Alegre, I., Chenoll, E., Viñas, I., 2017a. Studies on the biocontrol mechanisms of *Pseudomonas graminis* strain CPA-7 against food-borne pathogens *in vitro* and on fresh-cut melon. *LWT Food Sci. Technol.* 85, 301–308. <https://doi.org/10.1016/j.lwt.2017.02.029>.
- Collazo, C., Abadias, M., Colás-Medà, P., Iglesias, M.B., Granado-Serrano, A.B., Serrano, J., Viñas, I., 2017b. Effect of *Pseudomonas graminis* strain CPA-7 on the ability of

- Listeria monocytogenes* and *Salmonella enterica* subsp. *enterica* to colonize Caco-2 cells after pre-incubation on fresh-cut pear. *Int. J. Food Microbiol.* 262, 55–62. <https://doi.org/10.1016/j.ijfoodmicro.2017.09.003>.
- Collazo, C., Giné-Bordonaba, J., Aguiló-Aguayo, I., Povedano, I., Bademunt, A., Viñas, I., 2018a. *Pseudomonas graminis* strain CPA-7 differentially modulates the oxidative response in fresh-cut 'Golden delicious' apple depending on the storage conditions. *Postharvest Biol. Technol.* 138, 46–55. <https://doi.org/10.1016/j.postharvbio.2017.12.013>.
- Collazo, C., Lafarga, T., Aguiló-Aguayo, I., Marín-sáez, J., Abadías, M., Viñas, I., 2018b. Decontamination of fresh-cut broccoli with a water-assisted UV-C technology and its combination with peroxyacetic acid. *Food Control* 93, 92–100. <https://doi.org/10.1016/j.foodcont.2018.05.046>.
- Dai, X., Luo, H., Jiang, L., Ling, L., Xue, Y., Yu, Z., 2012. Efficacy of different sanitizing agents and their combination on microbe population and quality of fresh-cut Chinese chives. *J. Food Sci.* 77, 348–353. <https://doi.org/10.1111/j.1750-3841.2012.02770.x>.
- EC-European Commission for Health and Consumer Protection, 2005. Regulation (EU) No 396/2005 on maximum residue levels of pesticides in or on food and feed of plant and animal origin, and amending Council Directive 91/414/EEC. *Off. J. Eur. Union* L70, 28–39.
- EFSA, 2017. The European Union summary report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in 2016. *EFSA J.* 15. <https://doi.org/10.2903/j.efsa.2017.5077>.
- El Ghaouth, A., Wilson, C.L., Callahan, A.M., 2003. Induction of Chitinase, β -1,3-Glucanase, and phenylalanine ammonia lyase in peach fruit by UV-C treatment. *Phytopathology* 93, 349–355. <https://doi.org/10.1094/PHYTO.2003.93.3.349>.
- Escalona, V.H., Aguayo, E., Martínez-Hernández, G.B., Artés, F., 2010. UV-C doses to reduce pathogen and spoilage bacterial growth in vitro and in baby spinach. *Postharvest Biol. Technol.* 56, 223–231. <https://doi.org/10.1016/j.postharvbio.2010.01.008>.
- Fan, X., Huang, R., Chen, H., 2017. Application of ultraviolet C technology for surface decontamination of fresh produce. *Trends Food Sci. Technol.* 70, 9–19. <https://doi.org/10.1016/j.tifs.2017.10.004>.
- Fine, F., Gervais, P., 2004. Efficiency of pulsed UV light for microbial decontamination of food powders. *J. Food Prot.* 67, 787–792. <https://doi.org/10.4315/0362-028X-67.4.787>.
- Franco, B.D.G.M., Destro, M.T., 2007. Minimally processed vegetable salads: microbial quality evaluation. *J. Food Prot.* 70, 1277–1280.
- Gayán, E., Condón, S., Álvarez, I., 2014. Biological aspects in food preservation by ultraviolet light: a review. *Food Bioprocess Technol.* 7, 1–20. <https://doi.org/10.1007/s11947-013-1168-7>.
- Ge, C., Bohrerova, Z., Lee, J., 2013. Inactivation of internalized *Salmonella typhimurium* in lettuce and green onion using ultraviolet C irradiation and chemical sanitizers. *J. Appl. Microbiol.* 114, 1415–1424. <https://doi.org/10.1111/jam.12154>.
- Gorny, J.R., Giclas, H., Gombas, D., Means, K., 2006. Commodity Specific Food Safety Guidelines for the Lettuce and Leafy Greens Supply Chain [WWW Document]. <http://www.fda.gov/downloads/Food/GuidanceRegulation/UCM169008.pdf>, Accessed date: 6 January 2018.
- Guo, S., Huang, R., Chen, H., 2017. Application of water-assisted ultraviolet light in combination of chlorine and hydrogen peroxide to inactivate *Salmonella* on fresh produce. *Int. J. Food Microbiol.* 257, 101–109. <https://doi.org/10.1016/j.ijfoodmicro.2017.06.017>.
- Hadjok, C., Mittal, G.S., Warriner, K., 2008. Inactivation of human pathogens and spoilage bacteria on the surface and internalized within fresh produce by using a combination of ultraviolet light and hydrogen peroxide. *J. Appl. Microbiol.* 104, 1014–1024. <https://doi.org/10.1111/j.1365-2672.2007.03624.x>.
- Hägele, F., Nübling, S., Schweiggert, R.M., Baur, S., Weiss, A., Schmidt, H., Menegat, A., Gerhards, R., Carle, R., 2016. Quality improvement of fresh-cut endive (*Cichorium endivia* L.) and recycling of washing water by low-dose UV-C irradiation. *Food Bioprocess Technol.* 9, 1979–1990. <https://doi.org/10.1007/s11947-016-1782-2>.
- Hua, G., Reckhow, D.A., 2007. Comparison of disinfection byproduct formation from chlorine and alternative disinfectants. *Water Res.* 41, 1667–1678. <https://doi.org/10.1016/j.watres.2007.01.032>.
- Huang, Y., Chen, H., 2014. A novel water-assisted pulsed light processing for decontamination of blueberries. *Food Microbiol.* 40, 1–8. <https://doi.org/10.1016/j.fm.2013.11.017>.
- Huang, Y., Sido, R., Huang, R., Chen, H., 2015. Application of water-assisted pulsed light treatment to decontaminate raspberries and blueberries from *Salmonella*. *Int. J. Food Microbiol.* 208, 43–50. <https://doi.org/10.1016/j.ijfoodmicro.2015.05.016>.
- Huang, R., de Vries, D., Chen, H., 2018. Strategies to enhance fresh produce decontamination using combined treatments of ultraviolet, washing and disinfectants. *Int. J. Food Microbiol.* 283, 37–44. <https://doi.org/10.1016/j.ijfoodmicro.2018.06.014>.
- Iglesias, M., 2017. Bioconservación de pera mínimamente procesada mediante el uso de *Pseudomonas graminis* CPA-7 y *Lactobacillus rhamnosus* GG y su efecto en la calidad de la fruta y en la modificación del potencial patogénico de *Listeria monocytogenes* María Belén Iglesias Val. (WWW Document).
- Iglesias, M.B., Abadías, M., Anguera, M., Viñas, I., 2018. Efficacy of *Pseudomonas graminis* CPA-7 against *Salmonella* spp. and *Listeria monocytogenes* on fresh-cut pear and setting up of the conditions for its commercial application. *Food Microbiol.* 70, 103–112. <https://doi.org/10.1016/j.fm.2017.09.010>.
- Ignat, A., Manzocco, L., Bartolomeoli, I., Maifreni, M., Nicoli, M.C., 2015. Minimization of water consumption in fresh-cut salad washing by UV-C light. *Food Control* 50, 491–496. <https://doi.org/10.1016/j.foodcont.2014.09.036>.
- Koivunen, J., Heinonen-Tanski, H., 2005. Inactivation of enteric microorganisms with chemical disinfectants, UV irradiation and combined chemical/UV treatments. *Water Res.* 39, 1519–1526. <https://doi.org/10.1016/j.watres.2005.01.021>.
- Kovács, E., Keresztes, 2002. Effect of gamma and UV-B/C radiation on plant cells. *Micron* 33, 199–210. [https://doi.org/10.1016/S0968-4328\(01\)00012-9](https://doi.org/10.1016/S0968-4328(01)00012-9).
- Kroupitski, Y., Golberg, D., Belausov, E., Pinto, R., Swartzberg, D., Granot, D., Sela, S., 2009. Internalization of *Salmonella enterica* in leaves is induced by light and involves chemotaxis and penetration through open stomata. *Appl. Environ. Microbiol.* 75, 6076–6086. <https://doi.org/10.1128/AEM.01084-09>.
- Lacasse, S.T., Holcroft, D.M., 2016. Novel Use of UV-C Sanitation of Fresh and Fresh-Cut Produce. [WWW Document]. IUVA news URL. http://iuvanews.com/stories/pdf/archives/190203-IUVA_News_Summer2017_final_Lacasse.pdf, Accessed date: 9 October 2018.
- Liu, C., Huang, Y., Chen, H., 2015. Inactivation of *Escherichia coli* O157: H7 and *Salmonella enterica* on blueberries in water using ultraviolet light. *J. Food Sci.* 80, M1532–M1537. <https://doi.org/10.1111/1750-3841.12910>.
- Manzocco, L., Da Pieve, S., Maifreni, M., 2011. Impact of UV-C light on safety and quality of fresh-cut melon. *Innov. Food Sci. Emerg. Technol.* 12, 13–17. <https://doi.org/10.1016/j.ifset.2010.11.006>.
- Martínez-Hernández, G.B., Artés-Hernández, F., Gómez, P.A., Formica, A.C., Artés, F., 2013. Combination of electrolysed water, UV-C and superatmospheric O₂ packaging for improving fresh-cut broccoli quality. *Postharvest Biol. Technol.* 76, 125–134. <https://doi.org/10.1016/j.postharvbio.2012.09.013>.
- Martínez-Hernández, G.B., Navarro-Rico, J., Gómez, P.A., Otón, M., Artés, F., Artés-Hernández, F., 2015. Combined sustainable sanitising treatments to reduce *Escherichia coli* and *Salmonella enteritidis* growth on fresh-cut kailan-hybrid broccoli. *Food Control* 47, 312–317. <https://doi.org/10.1016/j.foodcont.2014.07.029>.
- Oliveira, M., Abadías, M., Colás-Medà, P., Usall, J., Viñas, I., 2015. Biopreservative methods to control the growth of foodborne pathogens on fresh-cut lettuce. *Int. J. Food Microbiol.* 214, 4–11. <https://doi.org/10.1016/j.ijfoodmicro.2015.07.015>.
- Ou, C., Liu, Y., Wang, W., Dong, D., 2016. Integration of UV-C with antagonistic yeast treatment for controlling post-harvest disease and maintaining fruit quality of *Ananas comosus*. *BioControl* 61, 591–603. <https://doi.org/10.1007/s10526-016-9740-5>.
- Park, J.B., Kang, J.H., Song, K. Bin, 2018. Combined treatment of cinnamon bark oil emulsion washing and ultraviolet-C irradiation improves microbial safety of fresh-cut red chard. *Lwt* 93, 109–115. <https://doi.org/10.1016/j.lwt.2018.03.035>.
- Petri, E., Rodríguez, M., García, S., 2015. Evaluation of combined disinfection methods for reducing *Escherichia coli* O157:H7 population on fresh-cut vegetables. *Int. J. Environ. Res. Public Health* 12, 8678–8690. <https://doi.org/10.3390/ijerph120808678>.
- Pombo, M.A., Rosli, H.G., Martínez, G.A., Civallo, P.M., 2011. UV-C treatment affects the expression and activity of defense genes in strawberry fruit (*Fragaria × ananassa*, Duch.). *Postharvest Biol. Technol.* 59, 94–102. <https://doi.org/10.1016/j.postharvbio.2010.08.003>.
- Sagoo, S.K., Little, C.L., Mitchell, R.T., 2003a. Microbiological quality of open ready-to-eat salad vegetables: effectiveness of food hygiene training of management. *J. Food Prot.* 66, 1581–1586.
- Sagoo, S., Little, C.L., Ward, L., Gillespie, I.A., Mitchell, R.T., 2003b. Microbiological study of ready-to-eat salad vegetables from retail establishments uncovers a National Outbreak of salmonellosis. *J. Food Prot.* 66, 403–409.
- Scott, G., Rupar, M., Fletcher, A.G.D., Dickinson, M., Shama, G., 2017. A comparison of low intensity UV-C and high intensity pulsed polychromatic sources as elicitors of hormesis in tomato fruit. *Postharvest Biol. Technol.* 125, 52–58. <https://doi.org/10.1016/j.postharvbio.2016.10.012>.
- Selma, M.V., Allende, A., López-Gálvez, F., Conesa, M.A., Gil, M.I., 2008. Disinfection potential of ozone, ultraviolet-C and their combination in wash water for the fresh-cut vegetable industry. *Food Microbiol.* 25, 809–814. <https://doi.org/10.1016/j.fm.2008.04.005>.
- Shama, G., 2007. Process challenges in applying low doses of ultraviolet light to fresh produce for eliciting beneficial hormetic responses. *Postharvest Biol. Technol.* 44, 1–8. <https://doi.org/10.1016/j.postharvbio.2006.11.004>.
- Singh, P., Hung, Y.C., Qi, H., 2018. Efficacy of Peracetic acid in inactivating foodborne pathogens on fresh produce surface. *J. Food Sci.* 83, 432–439. <https://doi.org/10.1111/1750-3841.14028>.
- Takeuchi, K., Frank, J.F., 2001. Quantitative determination of the role of lettuce leaf structures in protecting *Escherichia coli* O157:H7 from chlorine disinfection. *J. Food Prot.* 64, 147–151. <https://doi.org/10.4315/0362-028X-64.2.147>.
- Takeuchi, K., Matute, C.M., Hassan, A.N., Frank, J.F., 2000. Comparison of the attachment of *Escherichia coli* O157:H7, *Listeria monocytogenes*, *Salmonella Typhimurium*, and *Pseudomonas fluorescens* to lettuce leaves. *J. Food Prot.* 63, 1433–1437.
- Torres, A.G., Jeter, C., Langley, W., Matthyse, A.G., 2005. Differential binding of *Escherichia coli* O157:H7 to alfalfa, human epithelial cells, and plastic is mediated by a variety of surface structures. *Appl. Environ. Microbiol.* 71, 8008–8015. <https://doi.org/10.1128/AEM.71.12.8008>.
- Urban, L., Charles, F., de Miranda, M.R.A., Aarouf, J., 2016. Understanding the physiological effects of UV-C light and exploiting its agronomic potential before and after harvest. *Plant Physiol. Biochem.* 105, 1–11. <https://doi.org/10.1016/j.plaphy.2016.04.004>.
- Vandekinderen, I., Devlieghere, F., De Meulenaer, B., Ragaert, P., Van Camp, J., 2009. Optimization and evaluation of a decontamination step with peroxyacetic acid for fresh-cut produce. *Food Microbiol.* 26, 882–888. <https://doi.org/10.1016/j.fm.2009.06.004>.
- Xicohtencatl-Cortes, J., Sánchez Chacón, E., Saldaña, Z., Freer, E., Girón, J.A., 2009. Interaction of *Escherichia coli* O157:H7 with leafy green produce. *J. Food Prot.* 72, 1531–1537.
- Xu, L., Du, Y., 2012. Effects of yeast antagonist in combination with UV-C treatment on postharvest diseases of pear fruit. *BioControl* 57, 451–461. <https://doi.org/10.1007/s10526-011-9400-8>.
- Yun, J., Yan, R., Fan, X., Gurtler, J., Phillips, J., 2013. Fate of *E. coli* O157:H7, *Salmonella* spp. and potential surrogate bacteria on apricot fruit, following exposure to UV-C light. *Int. J. Food Microbiol.* 166, 356–363. <https://doi.org/10.1016/j.ijfoodmicro.2013.07.021>.