Decontamination of *Listeria innocua* from fresh-cut broccoli using UV-C applied in water or peroxyacetic acid, and dry-pulsed light

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Abstract

The efficacy of two irradiation technologies: Ultraviolet-C light (UV-C), applied in water or in peroxyacetic acid, and dry-pulsed light (PL), for the inactivation and growth inhibition of *Listeria innocua* populations in fresh-cut broccoli, were evaluated. Water-assisted UV-C (WUV) (0.3 and 0.5 kJ m^{-2}) reduced *L. innocua* initial populations by 1.7 and 2.4 $\log_{10}$, respectively; the latter dose also inhibited the growth for 8 d at 5 ºC. Replacing water with 40 or 80 mg L^{-1} peroxyacetic acid did not improve this efficacy. Pulsed light (5, 10, 15, and 20 kJ m^{-2}) showed no effect on broccoli's native microbiota. Neither did 15 kJ m^{-2} PL inactivate *L. innocua* or inhibit its growth. Nonetheless, 24 h post-processing, PL (15 kJ m^{-2}) increased total phenolic content by 25 % in respect of chlorine-sanitation, and enhanced total antioxidant capacity by 12 and 18 % compared to water and chlorine controls, respectively. Unlike dry-PL, WUV is a suitable technology for controlling *L. monocytogenes* populations in fresh-cut broccoli.

Industrial relevance

The present work provides relevant information to the fresh-cut food industry regarding a suitable decontamination alternative to chlorine sanitation. Low-dose immersion-assisted UV-C allows inactivation and inhibition of native and pathogenic microbiota while generates non-toxic byproducts and allows reusing the process water thereby enabling savings in water consumption. The results obtained herein provide new tools to ensure both quality and safety of minimally processed products, contributing to the so-called "smart green growth" addressed to provide a more innovative and sustainable future for the food industry.

Key words: antioxidant capacity; glucosinolates; non-thermal sanitation technologies

1. Introduction

Fresh-cut broccoli is a convenient source of health-promoting compounds including flavonoids such as kaempferol and quercetin, as well as of carotenoids, glucosinolates, minerals and dietary fiber (Latté, Appel, & Lampen, 2011). Among these bioactive compounds, glucosinolates, which are sulfur-containing secondary metabolites, are highly valued by their chemopreventive and anticancer effects, as are its break-down products indoles and isothiocianates. These glucosinolate-derived products have shown to increase the activity of the phase II enzymes, and induce the cell cycle arrest and apoptosis of tumor cells in several cancer types such as lung, colorectal, breast and prostate (Brown & Hampton, 2011; Clarke, Dashwood, & Ho, 2008).

To counteract the negative implications of processing such as cross-contamination with foodborne pathogens, the enhanced proliferation of native microbial populations, as well as the activation of metabolic processes detrimental to the product quality, several preservation methods must be implemented during the production workflow (Toivonen 2009). The reduction of microbial load is currently carried out in industry mainly by sanitation with chlorine solutions. However, in order to reduce the health and environmental risks entailed by the formation of chlorine halogenated side-products, alternative methods such as non-thermal physical technologies are being evaluated for different commodities (Artés-Hernández, Martínez-Hernández, Aguayo, Gómez, & Artés, 2017).

As one of these chlorine-alternative methods, UV-C irradiation is a non-ionizing technology comprising wavelengths in the range 200 to 280 nm that is being used in the food industry for microbial inactivation of surfaces (e.g. food, liquids or packaging materials) (Gayán, Condón, & Álvarez, 2014). UV-C provokes the formation of DNA pyrimidine dimers, which hinder transcription and replication and eventually lead to mutagenesis and microbial cell death (Vishwakarma, Singh, & Kewat, 2013). In addition, facing the potential generation of free radicals upon UV light irradiation, antioxidant mechanisms are activated in the plant including the production of shield molecules. This leads to the accumulation of secondary metabolites with antioxidant, plant-defense promoting or antimicrobial activities such as glutathione, phenolic compounds, phytoalexins, and glucosinolates (Lemoine, Chaves, & Martínez 2010). Therefore, as hormetic effects, low-dose UV-C indirectly acts on microorganisms through the induction of the plant defense mechanisms and the accumulation of secondary metabolites with antimicrobial activity, thereby

priming the response against subsequent attacks and improving the content in bioactive compounds (Gamage, Heyes, Palmer, & Wargent, 2016; Shama, 2007). The use of two-sided dry UV-C radiation at doses from 0.4 to 15 kJ/m^2 has shown effectiveness for reducing native microbial load and inoculated foodborne pathogens populations in fresh-cut broccoli from different varieties (Gamage et al., 2016; Martínez-Hernández, Huertas, et al., 2015). However, at higher doses, UV-C can provoke overheating, changes in plant cell structure and permeability, increased leakage of nutrients, softening, and browning (Allende & Artés, 2003). Thus, the application of low UV- C doses in water is an alternative that contributes to improve the efficacy of this technology by increasing accessibility and limiting temperature rise, thereby maintaining the product's quality. Previous attempts of combining low-dose UV and immersion have been carried out for the decontamination of natural mesophilic bacteria and foodborne pathogens (*Salmonella* spp. and *Escherichia coli*) on fresh produce with variable efficacy according to the methodology used for irradiation and inoculation, the dose, the target microorganism and the food matrix (Hägele et al., 2016; Huang, de Vries, & Chen, 2018; C. Liu, Huang, & Chen, 2015).

Pulsed light (PL) is another emerging non-thermal technology alternative to chlorine which involves the use of intense light pulses in a broad wavelength range (200– 1100 nm), comprising ultraviolet (200–400 nm), visible (400–700 nm) and near-infrared region (700–1100 nm). Its application on the surface of either foods or packaging materials results in the inactivation of pathogenic and spoilage microorganisms (Kramer, Wunderlich, & Muranyi 2017b; Oms-Oliu, Martín-Belloso, & Soliva-Fortuny 2010). The advantages of this method include the very short treatment times, the relatively low energy expense and cost of treatments compared to thermal technologies, and the lack of residual compounds (Ignat, Manzocco, Maifreni, Bartolomeoli, & Nicoli, 2014). Microbial inactivation by PL is mainly due to the photochemical effect of UV light on microbial DNA, but photo-thermal and photo-physical effects damaging cell wall and cytoplasmic membrane have also been reported (Krishnamurthy, Tewari, Irudayaraj, & Demirci, 2010). PL efficacy is influenced by the pulse width and the peak of power, since both modulate the amount of UV-C radiation and the intensity of energy that are applied to the samples (Elmnasser et al., 2007). PL has been used for the decontamination of several fresh-cut fruit and vegetables (Ignat et al., 2014; Ramos-Villarroel, Aron-Maftei, Martín-Belloso, & Soliva-Fortuny, 2012), but no information was found in the body of literature about its use in fresh-cut broccoli.

In previous experiments our work group assessed the effect of a water-assisted UV-C (WUV) technology on the populations of natural mesophilic bacteria, yeasts and molds as well as on the accumulation of

antioxidant compounds and glucosinolates in fresh-cut broccoli (Collazo et al., 2018). Results from those experiments showed a significant reduction of native mesophilic microbiota upon treatments as well as a hormetic increase in the contents of bioactive compounds without negatively affecting the physical quality. In this context, the aims of the present work were i) to further evaluate the effectiveness of the immersion-assisted technology for the inactivation and growth inhibition of artificially inoculated *Listeria innocua* on fresh-cut broccoli as a surrogate of the foodborne pathogen *Listeria monocytogenes*, ii) to test whether the increase of the UV-C dose or the substitution of water by peroxyacetic acid (PAA) improve the efficacy of WUV and iii) to compare the efficacy of aforementioned technology with dry-pulsed light (PL) at both inactivating and inhibiting the growth of native total mesophilic aerobic microbiota and artificially inoculated *L. innocua*. Additionally, the effects of PL on the physical quality and on the accumulation of antioxidant compounds and glucosinolates in fresh-cut broccoli, as it has been previously assessed by our work group using WUV, were evaluated.

2. MATERIALS AND METHODS

2.1 Plant material

Broccoli (*Brassica oleracea* L var. *Italica*) heads were purchased from local farms from Lleida, Spain (used for WUV experiments), or from Avignon, France (used for PL experiments) and cut into 2 - 3 cm diameter florets with a sharpened knife.

2.2 Bacterial culture and inoculation

L. innocua Seeliger strain CECT-910, a non-pathogenic species originally isolated from cow brain, was selected as a surrogate of *L. monocytogenes* as it has shown a similar resistance to PL to that of six *L. monocytogenes* strains belonging to five different serotypes, isolated either from food products or from food processing environments (Lasagabaster & Martínez de Marañón, 2012; Lasagabaster & Martínez- de Marañón, 2014). Moreover, it has effectively been used for the validation of both static and fluid PL systems (Artíguez & Martínez de Marañón, 2015; Lasagabaster & Martínez de Marañón, 2013). For inoculum preparation, *L. innocua* was grown overnight in agitation at 37 °C in tryptone soy broth (TSB, Biokar, Beauveais, France) supplemented with 6 g L^{-1} yeast extract (TSB-YE). The bacterial pellet obtained by centrifugation at 9800 x g for 10 min at 10 °C was diluted in saline solution (8.5 g L^{-1} NaCl). The concentration of the bacterial solution was determined by plate count in TSB-YE agar plates (15 g L^{-1} agar)

after overnight incubation at 37 °C. For inoculation, a solution of *L. innocua* in deionized water was used to immerse broccoli florets for 2 min in agitation, at a ratio of 1:5 (g of broccoli: mL of bath). After draining the excess of water, samples were air- dried on the bench for 2 h before the decontamination treatments.

2.3 UV-C applied in water or in peroxyacetic acid

2.3.1 Immersion-assisted UV-C equipment

UV-C treatments were performed in a laboratory scale equipment LAB-UVC-Gama (UV-Consulting Peschl España S.L., Spain) (Fig. 1) composed of a deposit containing four equidistant UV-C lamps (GPH 303T5L/4, Heraeus Noblelight, Hanau, Germany) vertically located throughout the tank. Water is driven by a water pump through a recirculating circuit and sprinkled from the top of the tank while pressured air enters through the bottom for water bubbling. Two opposite lamps or the four lamps can be activated in order to change irradiance. Different doses were assessed by combining 120 s of exposure with two (2.46 W m^{-2}) or four lamps (3.98 W m^{-2}), based in previous dose evaluation (Collazo et al., 2018). Doses were calculated as: the mean values of irradiance (W m^{-2}) * time of exposure (s). Lamps were preheated for 15 min before each treatment. Before and after each treatment, temperature was measured using an infrared thermometer DualTemp Pro (Labprocess distribuciones, Barcelona, Spain) and irradiance was measured through an orifice located in the lid of the equipment using a UV-sensor EasyH1 (Peschl Ultraviolet, Mainz, Germany).

2.3.2 Treatment and packaging

Fresh-cut broccoli florets (300g) previously dip-inoculated in a $2*10^8$ CFU mL^{-1} *L. innocua* solution as described in section 2.2, were immersed for 120 s in 10.5 L of agitated cold (5 °C) tap water and concomitantly submitted to 0.3 kJ m^{-2} UV-C, by activating two lamps for 120 s of exposure. In order to test the synergistic effect of the combination of UV-C and a chemical compound alternative to chlorine, the same procedure was performed except for the substitution of water by 50 or a 80 mg L^{-1} peroxyacetic acid (PAA) solutions. In the same way, control treatments using tap water, PAA or 100 mg L^{-1} sodium hypochlorite (pH 6.5) without turning on the UV-C lamps, were performed. In view of the results obtained using the combination of UV-C and PAA, the increase of the UV-C dose was assessed. Doses of 0.3 and 0.5 kJ m^{-2} were applied in water by combining two or four lamps and 120 s of treatment, following the same procedure. Control treatments using tap water or 100 mg L^{-1} sodium hypochlorite without UV-C, were performed. To evaluate the inhibitory effect of WUV on *L. innocua's* growth throughout storage as

well as the population recovery due to DNA-repair systems, broccoli florets were treated with 0.5 kJ m^{-2} or sanitized with water or 100 mg L^{-1} sodium hypochlorite without UV-C as controls. Some broccoli samples (120 g) were analyzed after treatment and the rest were packaged within 20 x 10 cm thermosealed bags made of a 20 µm thick oriented polypropylene (OPP) microperforated film (PDS group, Murcia, Spain) and stored for 8 d at 5 ºC either exposed to daylight or in darkness before analyses.

2.3.3 Microbiological analysis

For *L. innocua* viable counts, 25 g of treated broccoli florets were homogenized with 225 mL of buffered peptone water (BPW) (Oxoid, Basingstoke, Hampshire, England) within a 400 mL sterile full-page filter bag (Bagpage, Interscience, Saint Nom, France) in a Masticator (IUL, Barcelona, Spain) set at 4 strokes per second for 90 s. Appropriate ten-fold dilutions of the homogenates in saline peptone (8.5 g L^{-1} NaCl and 1 g L^{-1} peptone) were plated onto selective Palcam agar (Biokar, Beauveais, France) and incubated at 37 ºC for 48 h before counting.

2.3.4 Headspace gas composition

Headspace gas composition (O_2 and CO_2) of each replicate stored in the bags was measured at 4 and 8 d of refrigerated storage using a handheld gas analyzer (CheckPoint O_2/CO_2, PBI Dansensor, Denmark).

2.4 Dry-Pulsed light (PL)

2.4.1 Pulsed light equipment

Pulsed light treatments were performed in a laboratory scale system (CLARANOR S.A., Avignon, France) equipped with two automatic flash xenon lamps situated on the top of a closed chamber above a 47 * 95 mm platform adjusted to leave a 100 mm height from the top of the sample to the lamps. Xenon flash lamps of the PL unit emitted short-time pulses (300 µs) of broad spectrum (200 – 1100 nm) light. At that distance from the sample, each pulse delivered 5 kJ m^{-2} containing approximately 10 % of UV-C, for an input of 3000 V. Temperature measurements were performed after each treatment using a non-contact infrared thermometer (TempTestr® IR, Oaklon, USA). Irradiance was measured using a joulemeter UP17P-6S-H5-D0 (GENTEC-EO, Meudon, France).

2.4.2 Physical quality parameters

Overall quality was visually assessed. Superficial color of floret heads was determined by measuring CIE parameters L∗, a∗ and b∗ with a chromameter (CR400, Minolta, Osaka, Japan) on two positions of 5 florets

per treatment. Parameters a* and b* were expressed as hue angle (°) calculated as: 180 + arc tan (b*/a*) (McLellan, Lind, & Kime, 1995). Fluorescence emitted by chlorophyll a was measured at each sampling time in the center of the heads of five florets using a handheld fluorimeter (Pocket-PEA, Hansatech Instrument, United Kingdom). Measurements were performed after a 1 s of light induction with 3000 µmol m^{-2} s^{-1} following a 30 min dark adaptation allowing the electron acceptor center of photosystems II (PSII) to be gradually re-oxidized while redoing photochemistry (Stirbet & Govindjee, 2011). Results were expressed as the maximal quantum yield of photosystem II (Fv/Fm ratio), where Fv = Fm − Fo; Fm: maximum fluorescence yield in darkness, Fo: minimum fluorescence yield in darkness (Toivonen & DeEll, 2001).

2.4.3 Microbiological analysis

For native total mesophilic aerobic microorganisms (MAM) viable counts, 25 g of broccoli florets were homogenized in 225 mL of BPW as described in Section 2.3.3 and plated onto Plate Count Agar plates (PCA) after incubation at 25 ºC for 72 h. Yeasts and molds populations were counted on Dichloran Rose Bengal Chloramphenicol agar plates (DRBC) after incubation at 25 ºC for 3 to 5 d. Culture media and its additives were purchased from Biokar (Beauvais, France) and the rest of chemical compounds were purchased from Sigma-Aldrich (Darmstadt, Germany). *L. innocua* counts were performed as described in Section 2.3.3.

2.4.4 Setting up the optimal PL dose

The selection of the optimal PL dose was based on the reduction of native total MAM populations in broccoli (as described in Section 2.4.3) and on the maintenance of its overall quality (visually assessed), color and fluorescence (measured as described in Section 2.4.2) throughout storage at 5 ºC (at 0, 1, 7 and 14 d after treatment). Prior to PL treatments, broccoli florets were immersed in agitating tap water in a proportion 1:5 (g of broccoli: mL of bath) during 2 min in a tabletop orbital shaker and air-dried on the bench. Six broccoli florets were evenly distributed on the platform of the PL device and submitted to 1, 2, 3 or 4 light pulses on two opposite sides. The first dose was selected similar to the highest dose assayed in the immersion-assisted UV-C technology. As a control treatment, broccoli florets were immersed for 2 min in agitation in 100 mg L^{-1} sodium hypochlorite in the same proportion. Samples were analyzed in the day of the experiment or stored within 250 mL polypropylene (PP) trays (Befor Technitrans, France) thermosealed with a six-hole macro-perforated polyethylene terephthalate -polyethylene (PETPE) film

(Befor Technitrans, France). The headspace gas composition of trays was measured at 1, 7 and 14 d after treatment using a gas analyzer (CheckPoint O_2/CO_2, PBI Dansensor, Denmark).

2.4.5 Decontamination of *L. innocua* and native microbiota: treatment and packaging

Based on the results of the optimization phase, 3 light-pulses (15 kJ m^{-2}, 1.5 kJ m^{-2} UV-C) were selected as a dose for further analyses (Fig. 2). To test the effectiveness of this technology for the decontamination of native microbiota, broccoli florets were washed in agitated tap water and submitted to PL on two opposite sides as described in section 2.4.4. Untreated, water-washed and chlorine-sanitized samples were included as controls. To assess the effect of PL on *L. innocua* populations, broccoli florets were dip-inoculated in a $3*10^7$ CFU mL^{-1} solution (see section 2.2) prior PL treatments. Non-sanitized and chlorine-sanitized (100 mg L^{-1}) *L. innocua*-inoculated samples were included as controls. To test the antimicrobial hormetic effect of PL, via the activation of the plant defense response upon a subsequent infection with *L. innocua*, some pre water-washed broccoli florets were treated with 3 light pulses following the same procedure, and after 24 h of incubation at 5 °C, they were dip-inoculated with *L. innocua* as described in Section 2.2. Samples were either analyzed in the same day of sanitation or at 1, 8 or 15 d of storage at 5 ºC as described in the section 2.4.2. For viable counts, 10 g of each sample were homogenized in 90 mL of BPW and processed as described in section 2.3.3. O_2 and CO_2 measurements of the headspace of each tray were performed at 1, 8 and 15 d post-treatment as described in Section 2.4.2.

2.4.6 Biochemical analysis

Approximately 50 g of florets per replicate, per treatment and per sampling time was frozen with liquid nitrogen, grinded to powder (Grindomix GM 200, Retsch, Germany), and stored at -80 °C until biochemical analysis. Extracts for total antioxidant capacity (TAC) and total phenolic content (TPC) determinations were obtained by centrifugation at 24 000 x g for 20 min at 4 °C of a mixture containing 3 g of frozen broccoli powder and 10 mL of an aqueous solution (19.7 mol L^{-1} methanol and 0.05 mol L^{-1} HCl) previously agitated for 2 h at 20.94 rad s^{-1} in an tabletop orbital shaker. TAC was quantified by measuring optical density (OD) at 593 nm and 515 nm, respectively, following the Ferric Reducing Antioxidant Power (FRAP) method described by Benzie and Strain (1996) with some modifications (Giné-Bordonaba & Terry, 2016) and by the DPPH (2,2 – diphenyl – 1 – picrylhydrazyl) free radical-scavenging activity method (Brand-Williams et al. 1995). Total phenolic content was determined using the Folin-Ciocalteu method

(Singleton and Rossi, 1965). Non-enzymatic antioxidant activities were expressed as g of the measured analyte, i.e. Gallic acid (GAE) or ascorbic acid per kilogram of fresh weight (FW) of broccoli (g kg^{-1}).

Chlorophylls content was determined by measuring OD at 662, 645 and 670 nm of the supernatant resulting from the centrifugation at 15 000 x g for 5 min of a mixture containing 1 mL acetone and 100 mg of broccoli powder, previously incubated for 15 min at 4 °C (Lichtenthaler and Buschmann, 2001). Standard analytes and reagents were purchased from Sigma-Aldrich. Results were expressed as mg kg^{-1} of fresh weight.

For glucosinolates extraction, broccoli samples were stored for 24 h at 4 ºC, frozen with liquid nitrogen, lyophilized, and 150 mg of powder were subsequently mixed with 3 mL of a extraction solution (80:20, volume of methanol: volume of water) (Alarcón-Flores et al., 2013). Glucosinolates quantification was performed by ultra-high performance liquid chromatography coupled to tandem mass spectrometry (UHPLC–MS/MS) analysis using an Agilent series 1290 RRLC instrument (Agilent, Santa Clara, CA, USA) coupled to an Agilent triple quadrupole mass spectrometer (6460A) with a Jet Stream ESI ion source (G1958-65138 using a multi-compound standard methanoic solution containing 5 mg/L of sulforaphane (Sigma-Aldrich, Steinheim, Germany), gluconasturtin and glucoraphanin (PhytoLab GmbH & Co., Vestenbergsgreuth, Germany), glucotropaeolin, glucoerucin, glucoiberin and proigonitrin (Scharlab, Barcelona, Spain). Results were expressed as mg kg^{-1} of dry weight (DW).

2.5 Statistical analysis and expression of results

All experiments were repeated two independent times and included three biological replicates per treatment and sampling time. Resulting data were analyzed using the Statistical software JMP (version 8.0.1 SAS Institute Inc., NC, USA). All data were tested for agreement to normal distribution and homoscedasticity of residues. The significance of the differences and interactions among factors were determined by analysis of variances (ANOVA) and separated by Tukey's test ($P < 0.05$). Microbiological data were calculated as colony forming units per milliliter (CFU mL^{-1}) and transformed to $\log_{10}$ CFU per gram of fresh weight of fruit ($\log_{10}$ CFU g FW^{-1}) before means comparison.

3. RESULTS

3.1 UV-C applied in water and in peroxyacetic acid

3.1.1 Microbiological analysis of fresh-cut broccoli and process water

Initial *L. innocua* populations on broccoli florets ranged from 6.6 to 7.3 ± 0.4 log_{10} CFU g^{-1}. UV-C treatments at a dose of 0.3 kJ m^{-2} reduced *L. innocua* populations by 1.7 ± 0.2 log_{10} in respect of untreated broccoli (Fig. 3). Substituting water by 50 or 80 mg L^{-1} peroxyacetic acid solutions in the washing deposit did not improve the efficacy of UV-C in respect of the controls. Reductions obtained with UV-C, regardless of the combination with peroxyacetic acid, were up to 2.8-fold higher than that achieved by water-washing the samples, and similar to that obtained by chlorine or peroxyacetic acid sanitation (by 1.4 ± 0.2 log_{10}).

Increasing the UV-C dose to 0.5 kJ m^{-2} improved the effectiveness of the technology, with reductions by 40 % higher than that obtained with 0.3 kJ m^{-2} and by 75 % higher than that obtained by chlorine sanitation (Fig. 4). *L. innocua* populations in 0.5 kJ m^{-2}-irradiated samples were inhibited by 1.5 ± 0.4 and 1 ± 0.2 log_{10} after 4 and 8 d of storage, respectively, in respect of the water-washed control (data not shown). The same level of growth inhibition was observed in samples sanitized with chlorine. No differences *L. innocua* populations in UV-C-irradiated samples were observed after 8 d of exposure to daylight in refrigerated storage compared to the populations stored in darkness.

No viable *L. innocua* cells were detected upon treatment with 0.3 or 0.5 kJ m^{-2} UV-C in the process water or in the PAA solutions after a single-sanitation of inoculated broccoli for a detection limit of 5 CFU mL^{-1}. This represented a 3 log_{10} reduction in respect of the single-used control process water.

3.1.2 Gases analysis

The initial gas headspace composition of bags was 20.6 kPa O_2, 0 kPa CO_2 (data not shown). After 4 d of MAP storage at 5 ºC in darkness, packages' headspace gas composition of UV-C-treated broccoli was similar to that of the water and chlorine controls (16.1 kPa O_2; 4.1 kPa CO_2) and 8 d (17.9 kPa O_2; 2.8 kPa CO_2). No differences ($P > 0.05$) in the gas composition within the bags were observed between samples after storage for 8 d whether they were exposed to daylight or stored in darkness.

3.2 Dry-Pulsed light

3.2.1 Setting up PL treatment conditions

Microbiological analysis: Effect on native microbiota

Initial total MAM populations on fresh broccoli were 5.5 ± 0.4 log_{10} CFU g^{-1} FW (data not shown). No reduction beyond that achieved by water-washing the samples (1 ± 0.1 log_{10}) was obtained using PL,

288 regardless of the applied dose. In the same way, PL treatments showed no growth inhibition of MAM during
289 the first 7 d of refrigerated storage, since populations reached 6.3 ± 0.2 $\log_{10}$ CFU g^{-1} FW in all samples.
290 However on day 14, samples treated with 4 light pulses (containing 2 kJ m^{-2} UV-C) showed a reduced
291 growth (by 1.79 ± 0.04 $\log_{10}$) compared to the rest of the samples ($P < 0.05$) (data not shown).

292 ***Analysis of physical parameters***

293 Color was maintained during the first 7 d of storage in all samples (Table 1). Lightness showed a trend to
294 increase after 14 d of storage without significant differences among treatments. In samples treated with 3
295 and 4 light pulses the hue angle was reduced after 14 d compared to initial time, which was similar to that
296 observed for the water and chlorine controls. The visual analysis of overall quality showed some browning
297 of the nervure at the cut surface of irradiated sides and slight signs of dehydration in all PL-treated samples
298 after 7 and 14 d of storage. However, the poorest overall appearance was observed in 4 pulse-treated
299 samples (data not shown). Analysis of fluorescence showed an initial stress in samples treated with 3 and
300 4 pulses one day after treatment, as suggested by lower Fv/Fm ratio values (0.79 ± 0.01) compared to the
301 water control (0.82 ± 0.01) ($P < 0.05$). However, after 7 d of storage, the Fv/Fm ratios of all samples were
302 similar showing a recovery of the photosynthetic capacity.

303 ***Gases headspace composition***

304 Headspace composition within trays remained close to ambient air (20.6 kPa O_2, 0 kPa CO_2) during the
305 whole storage. During the first 24 h there was a decrease in O_2 content and an increase in CO_2 content in
306 all samples (Fig. 5). Afterwards, the concentration of both gases stabilized during the rest of storage in all
307 samples. Samples treated with 4 light pulses showed the lowest O_2 content and the highest CO_2 content
308 after 1 d of storage, compared to the chlorine control, although no significant differences were observed in
309 respect of the rest of the treatments.

310 In summary, results from the optimization phase suggested that 4 light pulses was an excessive dose to
311 maintain the physical quality parameters of broccoli. Thus, the immediate lower dose (3 light pulses,
312 containing 1.5 kJ m^{-2} UV-C) was selected to subsequently test the effect of PL on artificially inoculated *L.*
313 *innocua* and on naturally present yeasts and molds populations in fresh-cut broccoli.

314 **3.2.2 Decontamination with the selected PL dose**

315 ***Microbiological analyses***

316 Initial populations of *L. innocua* on broccoli were 5.7 ± 0.3 $\log_{10}$ CFU g^{-1} (Fig. 6). No inactivation was
317 achieved by PL treatments and no inhibition of growth was observed at any of the analyzed sampling points,
318 regardless of the dose and the moment of inoculation (before or after PL treatment). Significant reductions
319 of *L. innocua* populations (by 0.5 ± 0.3, 0.8 ± 0.5, and 1.0 ± 0.3 $\log_{10}$) compared to water-washing, were
320 only observed for the chlorine-treated control at 0, 1 and 8 d of storage, respectively.

321 Native yeasts populations on broccoli were initially 3.3 ± 0.5 $\log_{10}$ CFU g^{-1}. Immediately after sanitation,
322 a reduction by 0.7 ± 0.4 $\log_{10}$ in respect of untreated broccoli was accomplished by water-washing the
323 samples, regardless of PL treatment afterwards (Fig. 7A). A similar reduction was obtained at initial time
324 by chlorine-sanitizing (1.1 ± 0.3 $\log_{10}$) the samples compared to untreated broccoli Chlorine sanitation had
325 an inhibitory effect on yeasts populations which was maintained during the whole storage period, showing
326 reductions by 1 ± 0.2, 1.3 ± 0.4 and 0.6 ± 0.4 $\log_{10}$ compared to the water control after 1, 8, and 15 d,
327 respectively. No effect of PL on yeasts populations was observed at any of the analyzed sampling points.
328 Initial molds populations naturally present on broccoli were 3.1 ± 0.2 $\log_{10}$ CFU g FW^{-1}. Reductions
329 obtained by water-washing (by 0.5 ± 0.2 $\log_{10}$) the samples did not differ from those achieved by PL
330 treatments (by 0.6 ± 0.4 $\log_{10}$) or chlorine sanitation (by 0.9 ± 0.5 $\log_{10}$) (Fig. 7B). However, a inhibitory
331 effect in respect of the water control (by 0.5 ± 0.2 and 1.1 ± 0.5 $\log_{10}$) were observed in chlorine-sanitized
332 samples at 1 and 8 d of storage, which contrasted with that observed in PL treated samples.

333 ***Analysis of physical parameters***

334 Color (L* and hue angle) did not show significant differences ($P > 0.05$) among treatments at any of the
335 analyzed sampling points (data not shown). In general, lightness increased throughout the incubation
336 period: 42 ± 1, 47 ± 1, and 50 ± 1 at 1, 8, and 15 d, respectively. Hue angle decreased in all samples from
337 132 ± 2 to 127 ± 2 during the first 8 d of storage and then remained stable up to 15 d (125 ± 2). Off odors
338 and poor appearance of samples were detected in all samples at 15 d of storage. Fv/Fm ratios were similar
339 in all samples during the first 24 h post-processing (0.81 ± 0.02). Afterwards, Fv/Fm ratio showed a trend
340 to decrease throughout storage in all samples, but this reduction was only significant ($P < 0.05$) in the case
341 of the *L. innocua*-untreated control in which it reached 0.6 ± 0.2 at day 15 of storage.

342 ***Biochemical analysis***

343 Twenty four hours after processing, total phenolic content (TPC) in 3-pulse-treated samples increased by
344 15 % compared to unprocessed broccoli (65 ± 4 mg kg^{-1} FW). Although this increase was not significantly

345 different ($P > 0.05$) from the water-washed control, it exceeded by 25 % the TPC content of the chlorine
346 control (Fig. 8A). However, this effect did not remain throughout storage.

347 As measured by the DPPH method, total antioxidant capacity (TAC) increased by 12 and 18 % in 3-pulse-
348 treated samples compared to the water and chlorine-washed controls, respectively, 24 h post-processing
349 (Fig. 8B). When using the FRAP method no significant difference in TAC was observed for any of the
350 treatments at 1 or 8 d of storage (Fig. 8C). PL treatment did not alter the contents in carotenoids and
351 chlorophylls a or b compared to the control samples, regardless of the storage time (Table 2).

352 From the detected glucosinolates including two aromatic (gluconasturtiin and glucotropaeolin), one
353 aliphatic (glucoraphanin), and one alkenyl (progoitrin) glucosinolates, glucoraphanin was the most
354 abundant 24 h after processing (Table 3). Processing resulted in an increase of the glucoraphanin content
355 by 32 % in respect of unprocessed broccoli. Treatment with PL preserved this value unlike the reduction
356 observed for chlorine-sanitized samples. In the same way, the content in the thiosulfate derived from
357 glucoraphanin: sulforaphane was duplicated upon processing. This increase was maintained in PL-treated
358 broccoli. No significant differences were observed for the rest of the analyzed glucosinolates as their
359 content was very close to the detection limit (100 µg kg DW^{-1} for all glucosinolates except for gluconasturtin
360 which was 25 µg kg DW^{-1}).

361 ***Gases headspace composition***

362 Analysis of the headspace composition of packages showed O_2 and CO_2 levels close to ambient air (20.6
363 kPa O_2, 0 kPa CO_2) during the whole period of storage (data not shown). In non-inoculated samples, no
364 differences in the O_2 or CO_2 contents among treatments were observed during the storage period except for
365 24 h after treatment, when the chlorine control showed a reduced CO_2 level (0.7 ± 0.2%) compared to both
366 PL-treated and water-washed samples (1.2 ± 0.2%) while the O_2 content was similar. In the samples
367 inoculated with *L. innocua*, the O_2 or CO_2 contents were similar for all treatments.

368 # 4. DISCUSSION

369 In the present work we compared the efficacy of two UV-C-based technologies: immersion-assisted
370 continuous UV-C at low doses (0.3 and 0.5 kJ m^{-2}) and dry-pulsed light at higher doses (5 to 20 kJ m^{-2},
371 containing 10 % of UV-C) for the decontamination of *L. innocua* in fresh-cut broccoli. Continuous dry-
372 UV-C at doses ranging from 2.5 to 15 kJ m^{-2} had previously been evaluated for the inactivation of native

microbiota as well as of different strains of *L. monocytogenes* in fresh-cut broccoli from several varieties (Formica-Oliveira, Martínez-Hernández, Díaz-López, Artés, & Artés-Hernández, 2017b; Gamage, 2015; Lemoine, Civello, Martínez, & Chaves, 2007; Martínez-Hernández, Navarro-Rico, et al., 2015). In contrast, no previous attempts of using pulsed light for this purpose were found in the body of literature. Thus, we considered more interesting to compare immersion-assisted UV-C with conventional PL rather than comparing it with conventional UV-C. Nevertheless, previous findings obtained using the last mentioned technology for broccoli's decontamination, are discussed.

As shown by the results, the way of application of UV-C light significantly influenced its effectiveness for broccoli's sanitation. While continuous and multisided application of 0.5 kJ m^{-2} UV-C using the immersion-assisted technology effectively inactivated and inhibited the growth of *L. innocua*, the two-sided application of a three-fold higher UV-C dose (1.5 kJ m^{-2}) using the dry pulsed light technology showed to be ineffective. Similarly, in previous experiments the continuous application of 2.5 kJ m^{-2} UV-C in a conventional chamber also failed to inactivate *L. monocytogenes* CETC 935 and to inhibit its growth in fresh-cut Kaylan broccoli during storage in air for 13 d at 5 °C (Martínez-Hernández, Huertas, et al., 2015). Continuous dry-UV-C at doses of 0.56 and 1.1 kJ m^{-2} has previously shown reduced effectiveness at inactivating *L. monocytogenes* in matrices with rough surfaces as compared to those with smoother ones, i.e. 1.0, 0.9, 0.6 and 0.4 $\log_{10}$ reductions in apple, pear, strawberry and Cantaloupe discs, respectively (Adhikari, Syamaladevi, Killinger, & Sablani, 2015).

The dual action for decontaminating the plant product, by irradiation and by immersion, and the simultaneous decontamination of the process water could account for the higher efficacy of immersion-assisted UV-C. Although broccoli florets do not completely submerge in the water/disinfection solution within the tank of the WUV device, the pressurized liquid that is sprinkled from the top of the tank joint to the water/disinfectant column in movement due to the air that enters through the bottom of the deposit, makes the product to rotate and move, increasing the accessibility of UV-C light to a higher amount of microorganisms. Moreover, bacterial cells that are washed off from the plant product are inactivated by the UV-C and/or the disinfectant in the wash solution, thereby reducing the risks for cross contamination. Better efficacy of WUV compared to the dry alternative for the inactivation of foodborne pathogens from fresh produce has been previously reported, although using different setups (Guo, Huang, & Chen, 2017; Liu et al., 2015; Liu, Li, & Chen, 2015). An improved reduction of *S. enterica* (by 4.2 and 1.5 $\log_{10}$) was achieved in spot-inoculated blueberries and 'Iceberg' lettuce, respectively using WUV (34.8 kJ m^{-2}, 2 min) compared

to dry-UV-C (Guo et al., 2017). However, for dip-inoculated samples, the improvement of WUV in respect of conventional UV-C in blueberries was less marked (by 2 $\log_{10}$) while no improvement was obtained in lettuce samples. The inactivation efficacies of WUV treatments has also shown to decrease with the increase in roughness and intricate topography of the food matrix, i.e. reductions of *S. enterica* by 3.6 > 2.6 > 2.0 ≈ 1.7 were obtained using 27.6 kJ m^{-2} WUV in whole grape tomatoes, fresh-cut lettuce, fresh-cut baby carrot, and whole blueberry, respectively (Huang et al., 2018).

In line with this thought, the observed lack of efficacy of PL (15 kJ m^{-2} containing 1.5 kJ m^{-2} UV-C) at reducing *L. innocua* populations may have been partly due to the shadowing effect caused by the complicated topography of the plant material, and to a high resistance of this strain to PL (Lasagabaster & Martínez de Marañón, 2013). At a molecular level, chromosomal DNA analyses performed by Cheigh, et al. (2012), revealed that the application of continuous UV-C light caused more double-strand breaks in the DNA of *L. monocytogenes* KCCM40307 than did pulsed light.

We found that small increases of the UV-C dose (from 0.3 to 0.5 kJ m^{-2}) applied in water resulted in an improvement of the efficacy for *L. innocua* inactivation by 0.7 ± 0.2 $\log_{10}$. Contrastingly, when using PL, increases in the UV-C dose from 0.5 to 2.0 kJ m^{-2} (corresponding to total fluences from 5 to 20 kJ m^{-2}) resulted in no significant inactivation of this microorganism or of native microbiota. In previous experiments, we observed that increasing the WUV dose from 0.3 to 0.5 kJ m^{-2} increased the efficacy of WUV for the decontamination of total mesophilic microbiota in fresh-cut conventional broccoli, whereas no improvement was obtained for the same purpose in organic one (Collazo et al., 2018). In agreement with our results regarding WUV, other researchers have reported that the dose-responses for the inactivation of foodborne pathogens on fresh-cut broccoli using dry-UV-C in a range below 2.0 kJ m^{-2}, fitted a Weibull model (Martínez-Hernández, Huertas, et al., 2015). Similar dose-response curves of *L. monocytogenes* CETC 935 were observed in fresh-cut Kaylan broccoli upon treatment with continuous dry-UV-C at doses of 2.5, 5.0, 7.5, 10 and 15 kJ m^{-2} (Martínez-Hernández, Huertas, et al., 2015). In those experiments, higher inactivation rates were observed at 2.5 kJ m^{-2}, whereas the increase of the dose up to 15 kJ m^{-2} only led to slight but not significant improvement (from 0.72 $\log_{10}$ to 1 $\log_{10}$ reduction).

On the other hand, previous experiments testing the effectiveness of PL at total fluences from 0.5 to 12 kJ m^{-2} (containing 20 % of UV-C) for the inactivation of *L. innocua* CECT 910 in potassium phosphate buffered saline suspension, showed a shoulder at fluences below 0.5 kJ m^{-2} and a maximum inactivation level (4 $\log_{10}$ reduction) at 1.6 kJ m^{-2} (Lasagabaster & Martínez de Marañón, 2013). The use of PL in a

433 higher range of fluences (from 1.9 to 120 kJ m^{-2}) showed that the inactivation of MAM in fresh-cut shredded
434 white cabbage followed a Weibull model: significant effect on populations at very low fluence levels, and
435 small increases of efficacy for high increases of the dose (upward concavity and tail) (Izquier & Gómez-
436 López, 2011; Levy, Aubert, Lacour, & Carlin, 2012). It is possible that using the present set up at higher
437 doses would result in a similar dose-response behavior. However, since we noticed a significant
438 diminishment of broccoli's quality at the presently essayed dose range, higher doses would not be suitable
439 for a shelf-life of 7 d, which is already shorter than that of currently commercialized products.

440 The selection of the effective PL dose for microbial inactivation should be contingent to the maintenance
441 of the physical and chemical quality of the target plant product. In this regard, although several reports have
442 stated the usefulness of PL for the decontamination of native or inoculated microorganisms from fruits and
443 vegetables, the effectiveness of high PL doses is sometimes at the expense of negatively affecting the
444 product's quality. For example, in fresh-cut mushrooms 120 kJ m^{-2} intense pulsed light (IPL) reduced *L.*
445 *innocua* populations by 2 $\log_{10}$ on but also caused extensive damage of cytoplasm and cytoplasmic
446 membrane of the product (Ramos-Villarroel et al. 2012). In the same food matrix, doses of 48 kJ m^{-2} were
447 ineffective to reduce native MAM (Oms-Oliu, Aguiló-Aguayo, Martín-Belloso, & Soliva-Fortuny 2010).
448 Increasing the dose to 120 and 480 kJ m^{-2} accomplished reductions by 1.4 $\log_{10}$ but also caused browning
449 and diminished texture. In contrast, doses closer to those used in the present study (20 kJ m^{-2}), have achieved
450 2 $\log_{10}$ reductions of mesophilic microbiota in whole spinach leaves (Aguero, Jagus, Martin-Belloso, &
451 Soliva-Fortuny, 2016). The smoother surface topography of spinach and the limited physical damage
452 applied during processing (only detached) could account for the better results due to a lower surface for
453 internalization of native microbiota into the tissue and the lower shadowing effect. Supporting this
454 hypothesis, much lower effectiveness of PL (675 light pulses of 7 kJ each, at a distance of 12.3 cm from
455 the platform) at reducing MAM (by 0.34 $\log_{10}$) obtained in shredded spinach leaves treated with PL
456 (Gómez-López, Devlieghere, Bonduelle, & Debevere, 2005). Taking previous results as a base for dose
457 selection is un-accurate because the instrumental and experimental set-ups significantly influence the output
458 of the technology, i.e. shorter distances from the light source, longer pulse widths as well as higher inputs
459 (Kramer, Wunderlich, & Muranyi, 2017a). Besides of physical factors, the effectiveness of PL for microbial
460 reduction also depends on many other biological factors such the composition and topography of the matrix,
461 the levels of the initial inoculated population and/or the levels and composition of the native microbiome
462 and their sensitivity to UV-C (Kramer, Wunderlich, & Muranyi, 2017c; Ramos-Villarroel et al., 2012).

In the present study, a decline in the Fv/Fm ratio, which indicates stress at values below 0.83, was observed upon 15 and 20 kJ m^{-2} PL application, probably due to an increase in protective non-radiative energy dissipation or to photo-inhibitory damage to the PSII reaction center (Maxwell & Johnson, 2000). In both cases the photosynthetic capacity of the plant recovered from initial stress after 7 d of storage as previously been described (Hägele et al., 2016). However, the undesirable effects on overall physical quality shown by the 20 kJ m^{-2}-treated samples as well as the increased respiration rate, also indicator of physiological stress caused by mechanical wounding and PL application, motivated the exclusion of this dose (Mattos, Moretti, & Yosino Da Silva, 2013). Treatments with continuous UV-C at a dose of 8 kJ m^{-2} have previously shown to extend shelf-life of fresh-cut broccoli through the reduction of the respiration rate and degradation of chlorophyll during storage at 4 ºC for up to 21 d (Costa, Vicente, Civello, Chaves, & Martínez 2006; Lemoine, Civello, Martínez, & Chaves 2007). In contrast, we observed no change in the headspace composition or in the chlorophylls contents in samples treated with WUV (0.5 kJ m^{-2}) or PL (15 kJ m^{-2}) compared to the water control during storage for 8 or 15 d, respectively. These results agreed with our previous results in this matrix (Collazo et al., 2018). In contrast, increased respiration and negative changes in color parameters were eventually observed in chlorine-treated samples, implying that both WUV and PL technologies contributed better to preserve color and overall quality than chlorine sanitation.

Regarding the reversibility of the antimicrobial effect of UV-C, no population recovery of *L. innocua* throughout storage was observed irradiated samples compared to the water control whether they were exposed to daylight or darkness. This suggested that no photorreactivation or dark DNA-repair occurred in irradiated cells (Kramer, Wunderlich, & Muranyi, 2015; Lim & Harrison, 2016) or at least, that they entered in a non-cultivable stage as it has been previously reported for *Listeria* spp. (Kramer & Muranyi, 2014; Lasagabaster & Martínez- de Marañón, 2014). Moreover, WUV inhibited the growth of *L. innocua* after 8 d of storage suggesting that the activation of the defense mechanisms within the plant host may have prevented the population increase compared to the water-washed samples (Scott, Dickinson, Shama, & Rupar, 2018). In PL-treated samples, only those exposed to 4 pulses showed a hormetic inhibition of MAM after 14 d of storage. However, this effect was not observed for *L. innocua* in 3 pulse-treated samples. *Listeria* spp. have previously shown to be more resistant than other microorganisms to both continuous UV-C light and PL treatments in fresh-cut produce (Lasagabaster & Martínez de Marañón, 2013; Martínez-Hernández, Huertas, et al., 2015; Ramos-Villarroel et al., 2012).

The combination of UV-C (0.3 kJ m^{-2}) with PAA (50 or 80 mg L^{-1}) did not enhance the efficacy of WUV or PAA single treatment for the inactivation of *L. innocua*, regardless of the PAA dose. In contrast, Martínez-Hernández et al., (2015) observed a synergistic effect (up to 2-fold more effectiveness than the single treatments) when combining 100 mg L^{-1} PAA for 1 min with subsequent treatment with 7.5 kJ m^{-2} UV-C for the control of *E. coli* and *S. enterica* on fresh-cut 'Kaylan' broccoli. Discrepancies among the results could be explained by the higher doses of both PAA and UV-C used in that experiment (by 10-fold in case of UV-C) compared to those used in our trials as well as different target microorganisms. In accordance with our results, no enhancement of *S. enterica* inactivation was observed when combining WUV (27.6 kJ m^{-2}) and 80 mg L^{-1} PAA (2 min) in baby spinach leaves, fresh-cut 'Iceberg' lettuce, whole blueberries or whole grape tomatoes (Huang et al., 2018). Nevertheless, even when no enhanced microbial reduction was obtained in those food matrices, the combination of WUV and PAA significantly improved microbial inactivation in process water containing high organic matter loads (turbidities ranging from 64.2 to 258, and chemical oxygen demand (COD) ranging from 1750 to 2300 mg L^{-1}) compared to WUV alone. This justified the recommendation of the combined strategy as a way of reducing the risks for cross-contamination (Huang et al., 2018). WUV (27.6 kJ m^{-2}) has also been tested in combination with other chemicals (100 mg L^{-1} sodium dodecyl sulfate, 5 mg L^{-1} levulinic acid or 10 mg L^{-1} free chlorine) for inactivating *Escherichia coli* O157:H7 or *S. enterica* in blueberries showing no improvement in respect of the single treatments (Liu et al., 2015). In previous experiments we observed that the combination of UV-C with 80 mg L^{-1} PAA enhanced the efficacy of WUV at a lower dose (0.3 kJ m^{-2}) while showed no improvement at a higher dose (0.5 kJ m^{-2}). Altogether those results showed that several factors can influence the outcome of these technologies including the doses and the way of application of UV-C and the chemical compound selected for the combination, the topography and characteristics of the matrix, as well as the amount and tolerance of target microorganisms, being inoculated or indigenous microbiome, to irradiation and/or to the chemical sanitizer.

The hormetic effect of continuous UV-C light regarding the activation of secondary metabolism and the delay of ripening and senescence has been demonstrated in several commodities (Formica-Oliveira, Martínez-Hernández, Díaz-López, Artés, & Artés-Hernández, 2017a; Martínez-Hernández, Gómez, Pradas, Artés, & Artés-Hernández, 2011). In fresh-cut broccoli, continuous UV-C at a dose of 8 kJ m^{-2} increased total antioxidant activity as well as phenolic compounds and ascorbic acid contents. We observed that total phenolic content was maintained after PL treatment compared to water-washing while it was

522 reduced after chlorine sanitation as it was observed in previous experiments performed by our work group
523 in fresh-cut broccoli using the same technology (Collazo et al., 2018). Furthermore, we observed and
524 enhanced accumulation in total antioxidant capacity in PL-treated samples during the first 24 h, as measured
525 by DPPH method, showing certain hormetic effect as has been previously reported in this commodity
526 (Martínez-Hernández et al., 2011). TAC showed the same trend using the FRAP method but the variability
527 of measures did not allow to detect significant differences, probably because of the interference of the
528 elevated ascorbic acid content of broccoli with the activity of the standard used (Koh, Wimalasiri, Chassy,
529 & Mitchell, 2009). Higher values and less interference were observed through DPPH method using gallic
530 acid as standard (Stratil, Klejdus, & Kubáň, 2006). We observed a similar trend in our previous experiments
531 using the water-assisted UV-C in broccoli: an increase in TAC by 90 % compared to the water control
532 was detected by the DPPH method, 24 h after treatment, whereas no significant differences were observed
533 when the FRAP method was used (Collazo et al., 2018).

534 We observed that glucoraphanin (GP) was the most abundant glucosinolate in the broccoli samples, as it
535 has been previously reported for several broccoli varieties (Jones, Faragher, & Winkler, 2006; Torres-
536 Contreras, Nair, Cisneros-Zevallos, & Jacobo-Velázquez, 2017). Both GP and sulforaphane content
537 increased 24 h after processing. The activation of the glucosinolates synthesis pathways is one of many
538 plant responses to wounding (Mikkelsen, 2003). Different cutting styles have shown to induce variations
539 in specific glucosinolates as well as their transformation into thiocyanates due to the releasing of
540 myrosinase enzyme from specialized cells after cutting (Chen & Andreasson, 2001; Torres-Contreras et al.,
541 2017). UV radiation (specifically the wavelengths in the UV-B spectrum) at low doses (0.45 kJ m^{-2}) has
542 also been demonstrated to elevate glucosinolates levels in broccoli sprouts which has been associated to the
543 up-regulation of genes involved the biosynthetic pathway of aliphatic glucosinolates as well as of genes
544 related to the response of pathogens and herbivores (Mewis et al., 2012). We observed that glucosinolates
545 and sulforaphane contents of broccoli did not increase any further 24 h after PL treatment that what they
546 did after cutting and water washing. This differed from our previous results using WUV, when the content
547 of sulforaphane increased by 1.5-fold in respect to water-washed control and by 4-fold in respect to the
548 chlorine-sanitized control (Collazo et al., 2018). Continuous UV-C application (1.2 kJ m^{-2}) have previously
549 shown to differentially activate the synthesis of certain glucosinolates (e.g. 4-methoxyglucobrassicin, 4-
550 hydroxyglucobrassicin and GP) while reducing others (glucobrassicin) in broccoli florets. The optimal
551 moment to detect the significant accumulation of each glucosinolate compared to untreated control is also

552 variable (from 3 d to 14 d after treatment) depending on the dose and the specific analyzed compound
553 (Formica-Oliveira et al., 2017a; Nadeau, Gaudreau, Angers, & Arul, 2012). The application of PL in order
554 to improve the nutritional properties could be adjusted not only in function of the type of commodity but
555 of the processing step. For instance, the application of low PL doses (6 kJ m^{-2}) have shown to be more
556 suitable than higher doses (up to 48 kJ m^{-2}) for improving quality of whole mangoes, by decreasing pectin-
557 methylesterase activity, thereby maintaining firmness, which later resulted in an increased total phenolic
558 content (TPC) in fresh-cut product (Lopes et al., 2016). Contrastingly, no increase in TPC was observed in
559 the same commodity when 80 kJ m^{-2} PL was applied during the processed stage (Charles, Vidal, Olive,
560 Filgueiras, & Sallanon, 2013).

561 CONCLUSIONS

562 The present study showed the usefulness of low-dose immersion-assisted UV-C (0.5 kJ m^{-2}) for the
563 inactivation of *L. innocua*'s by more than 2 $\log_{10}$ in fresh-cut broccoli compared to water washing, leaving
564 no viable cells in single-used process water. Based on those results this is a promising sanitation residue-
565 free technology, alternative to chlorine, for inactivating and inhibiting pathogenic *Listeria* spp. in infected
566 broccoli as well as for reducing the risks of cross-contamination by the process solutions. Since no further
567 improvement of the effectiveness of this technology was obtained by combining it with peroxyacetic acid,
568 increasing the temperature of the water or a selected chemical could be an alternative worth of being
569 essayed. Low dose dry-PL was ineffective for the decontamination *L. innocua* or native mesophilic
570 populations in fresh-cut broccoli. Nonetheless, it showed better results than chlorine-washing regarding the
571 maintenance of total phenolic and glucosinolates contents and the increased total antioxidant capacity.

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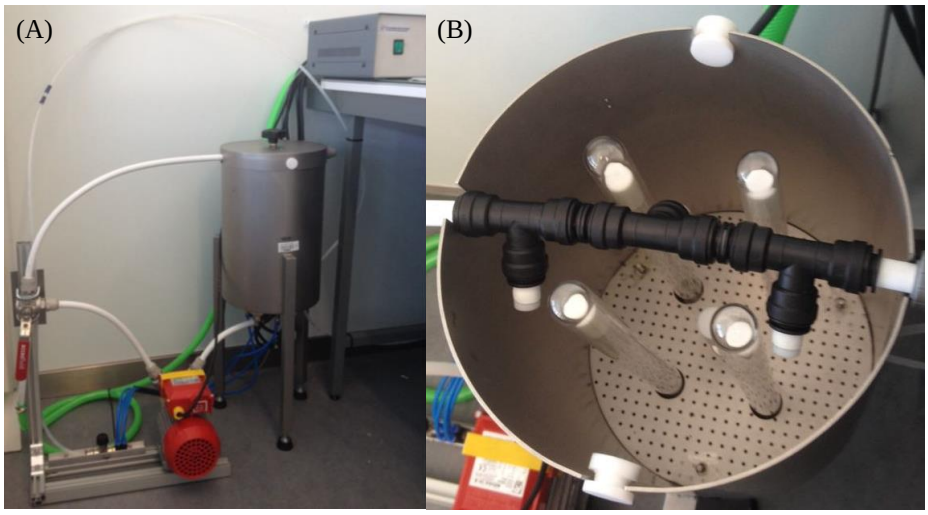
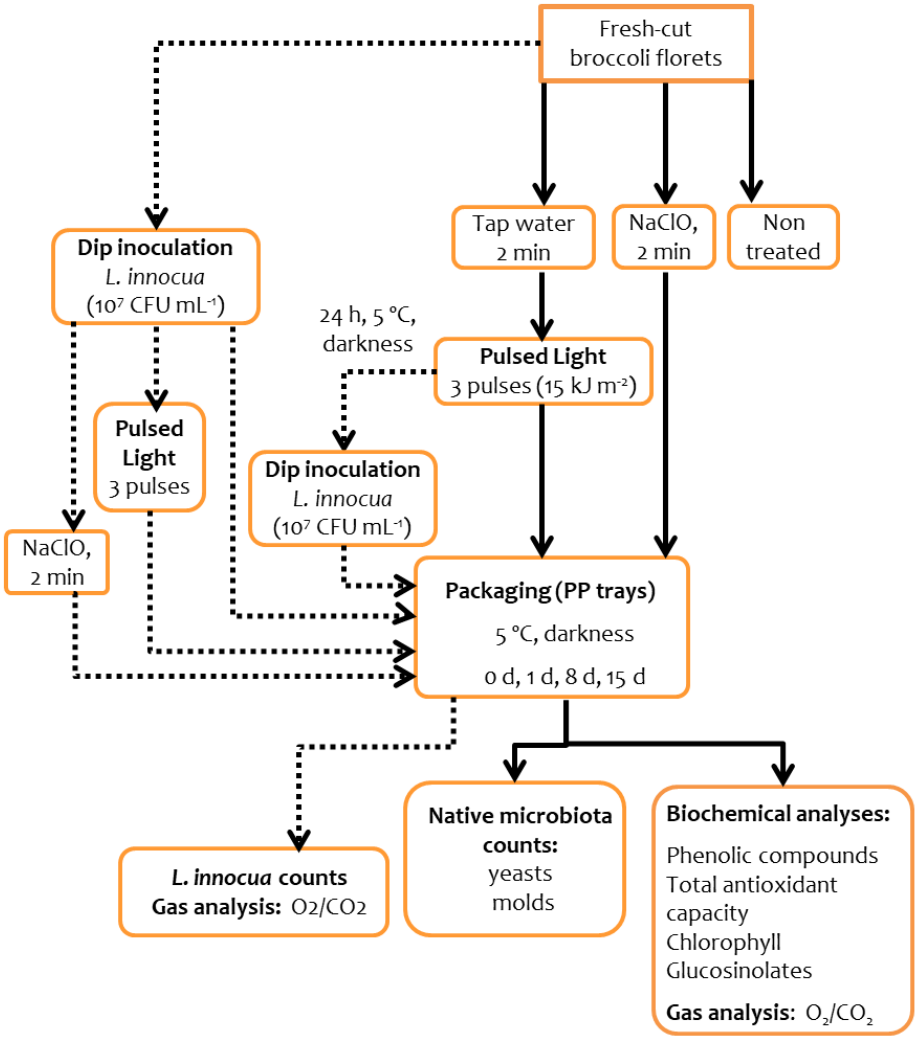


Fig. 1 (A) Water-assisted UV-C setup which consists of a tank (15 L) that is connected to a power source, a water pump (maximum flow 1700 L/h), a recirculating water circuit, and adjustable device with three sprinklers on the top and an exit on the bottom, besides of four entrances for pressurized air (100 kPa) on the bottom. (B) The tank contains four vertical UV-C lamps (17.2 W, 254 nm) located within waterproof quartz compartments



798

799 **Fig. 2** Experimental setup testing the direct and indirect (**.....**) antimicrobial effects of pulsed light (3 pulses,

800 1.5 kJ/m^2 UV-C) on *L. innocua* populations, as well as its effects on native yeasts and molds populations

801 and biochemical properties (**____**) of fresh-cut broccoli florets

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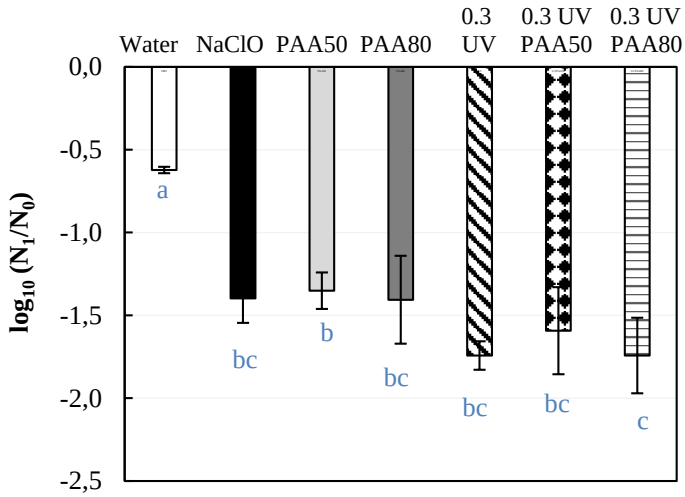


Fig. 3 Logarithmic reductions of *L. innocua* populations after decontamination with UV-C (0.3 kJ/m^2) in water or in 50 or 80 mg/L^1 peroxyacetic acid (PAA) , compared to water-washing or chlorine-sanitizing (NaClO). N_0: initial populations, N_1: final populations. Columns represent means and error bars represent standard deviation (n=6). Different letters represent significant differences according to Tukey's test ($P < 0.05$)

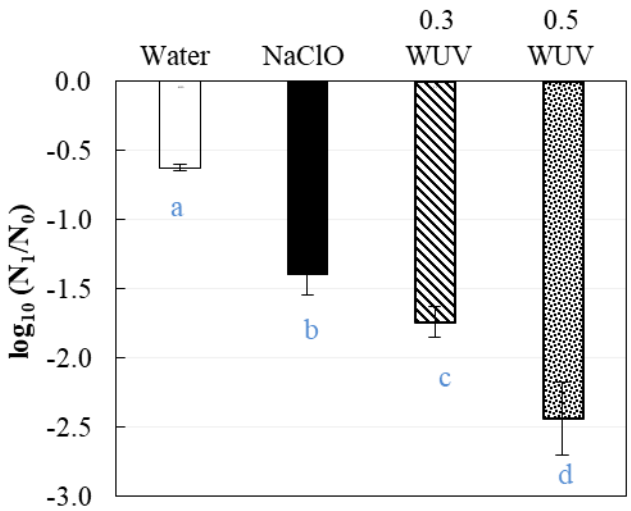
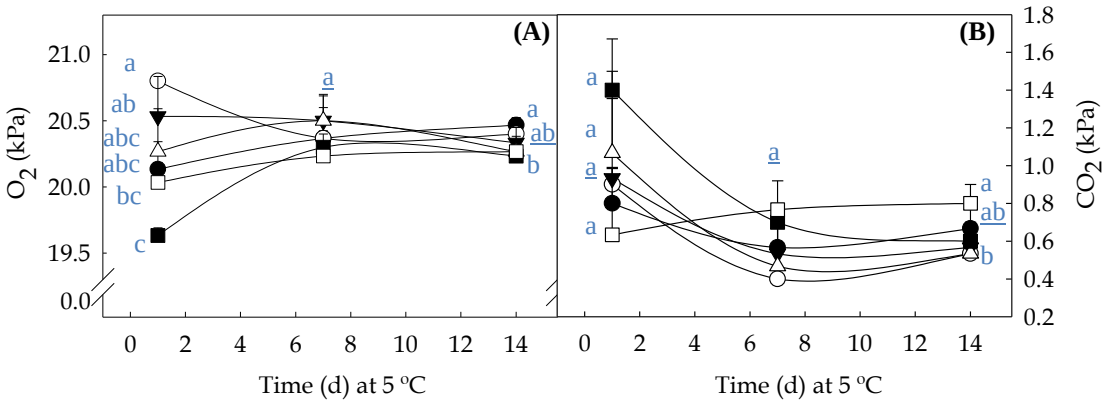
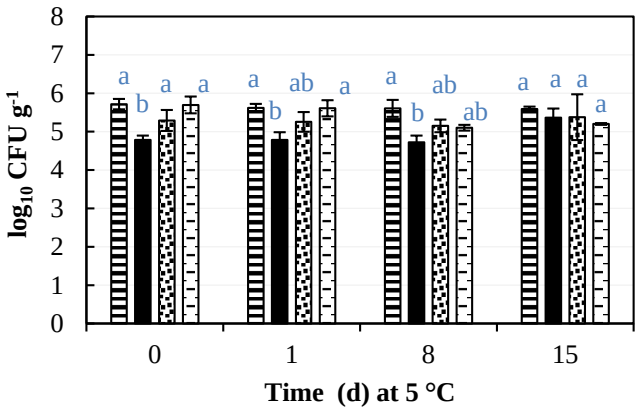


Fig. 4 Logarithmic reductions of *L. innocua* populations after decontamination with WUV (0.3 or 0.5 kJ/m^2) compared to the water-washed or chlorine-sanitized (NaClO) controls. N_0: initial populations, N_1: final populations. Columns represent means and error bars represent standard deviations (n=6).



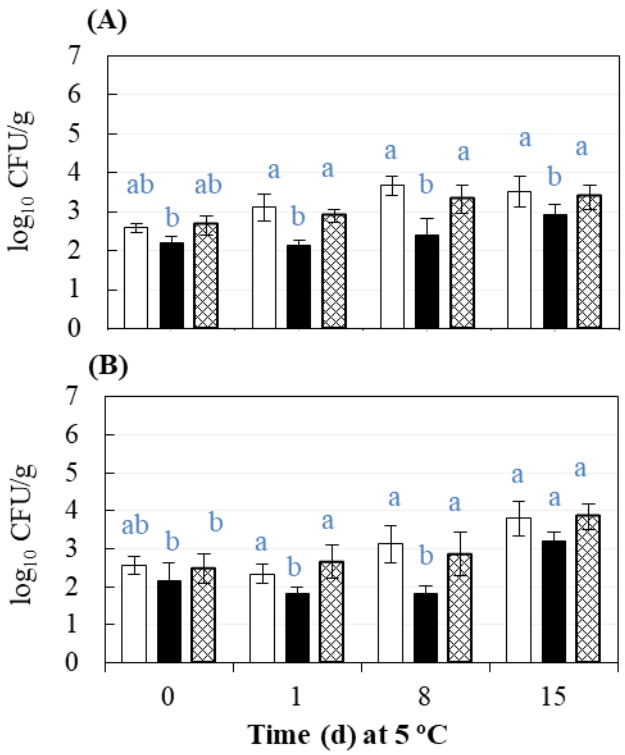
816

817 **Fig. 5** Headspace gas composition (A) O_2 and (B) CO_2 of trays containing fresh-cut broccoli treated with
818 different pulsed light doses throughout refrigerated storage: 1 pulse (○), 2 pulses (▼), 3 pulses (△) and 4
819 pulses (■) compared to water-washed (●) or chlorine-sanitized (□) controls. Symbols represent means and
820 error bars represent standard deviations (n=3). Different letters represent statistical differences according
821 to Tukey's test ($P < 0.05$). Underlined letters represent equal means corresponding to overlapped symbols



822

823 **Fig. 6** Changes in *L. innocua* populations in fresh-cut broccoli throughout refrigerated storage upon PL
824 treatments. (▤) *L. innocua* non-sanitized control, (■) chlorine-sanitized control, (▩) PL-treated (3 pulses,
825 15 kJ/m^2) after inoculation with *L. innocua*, (▨) PL-treated before inoculation with *L. innocua*. Columns
826 represent means and error bars represent standard deviations (n=6). Different letters represent significant
827 differences according to Tukey's test ($P < 0.05$).



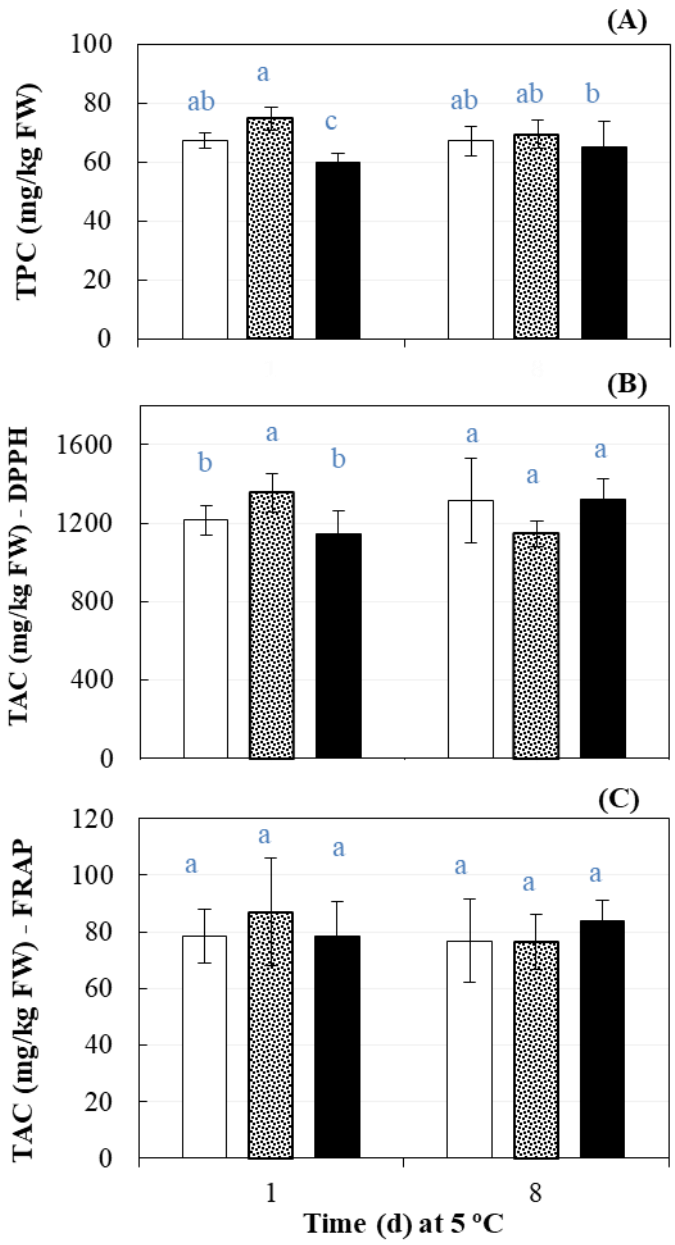
828

829 **Fig. 7** Changes in native microbiota (A) yeasts and (B) molds of fresh-cut broccoli throughout storage upon

830 PL treatments: (☐) water-washed control, (■) chlorine-sanitized control, (☒) pulsed light-treated (3 pulses,

831 1.5 kJ/m^2 UV-C). Columns represent means and error bars represent standard deviations (n=6). Different

832 letters represent significant differences according to Tukey's test ($P < 0.05$)



833

834 **Fig. 8** Changes in (A) total phenolic content (TPC) and total antioxidant capacity (TAC) in fresh-cut
835 broccoli throughout refrigerated storage upon PL treatments as measured by the DPPH (B) or FRAP (C)
836 methods: (□)water-washed control, (▨) pulsed light-treated (3 pulses), and (■) chlorine-sanitized control.
837 Columns represent means and error bars represent standard deviations (n=6). Different letters represent
838 significant differences according to Tukey's test ($P < 0.05$)

839

840

841 **Table 1.** Color changes in fresh-cut broccoli treated with different pulsed light doses throughout
842 refrigerated storage compared to water-washed and chlorine-sanitized samples

843

Color parameter		H_2O	100 mg L^{-1} Chlorine	1 pulse	2 pulses	3 pulses	4 pulses
L*	1 d	41 ± 3Ab	40 ± 2Ab	39 ± 3Ab	40 ± 3Aa	41 ± 1Ab	41 ± 2Ab
Hue (°)		135 ± 7Aa	134 ± 6Aa	130 ± 4Aa	134 ± 6Aa	133 ± 4Aa	131 ± 4Aa
L*	7 d	41 ± 1Ab	43 ± 2Aab	40 ± 2Ab	42 ± 3Aa	40 ± 3Ab	41 ± 2 Ab
Hue (°)		132 ± 4Aab	127 ± 6Aab	130 ± 4Aa	128 ± 5Aa	129 ± 4Aa	126 ± 2Aab
L*	14 d	45 ± 3Aa	48 ± 5Aa	43 ± 1Aa	43 ± 2Aa	44 ± 2Aa	46 ± 4Aa
Hue (°)		126 ± 3Ab	120 ± 10Ab	129 ± 4Aa	129 ± 4Aa	128 ± 5Aa	122 ± 7ab

Values are means ± standard deviations (n = 6). Different letters represent significant differences among treatments at each sampling time according to an analysis of variances (ANOVA) and a Tukey's test ($P < 0.05$). Each pulse of light contained 0.5 kJ m^{-2} UV-C. Water control (H_2O), 100 mg L^{-1} sodium hypochlorite control (Chlorine)

844

845 **Table 2.** Chlorophylls and carotenoids contents in fresh-cut broccoli treated with pulsed light (3 pulses, 1.5
846 kJ/m^2 UV-C) throughout refrigerated storage compared to water-washed or chlorine-sanitized samples

847

		Chlorophyll a (mg kg FW^{-1})	Chlorophyll b (mg kg FW^{-1})	Carotenoids (mg kg FW^{-1})
	H_2O	0.036 ± 0.007 a	0.011 ± 0.003 abc	3.9 ± 0.8 ab
	LP	0.034 ± 0.006 a	0.011 ± 0.002 abc	3.7 ± 0.7 ab
	Cl	0.018 ± 0.007 a	0.009 ± 0.005 abc	2.5 ± 0.1 b
1 d	Li	0.033 ± 0.004 a	0.011 ± 0.002 ab	3.6 ± 0.4 b
	Li- LP	0.030 ± 0.004 a	0.009 ± 0.002 abc	3.5 ± 0.5 b
	Li- Cl	0.026 ± 0.003 a	0.008 ± 0.001 bc	2.8 ± 0.3 ab
	LP- Li	0.036 ± 0.008 a	0.010 ± 0.001 abc	4.1 ± 0.6 ab
	H_2O	0.033 ± 0.005 a	0.011 ± 0.001 abc	4.1 ± 0.7 ab
	LP	0.028 ± 0.003 a	0.009 ± 0.002 abc	3.3 ± 0.4 ab
	Cl	0.035 ± 0.001 a	0.012 ± 0.002 abc	4.4 ± 0.4 a
8 d	Li	0.028 ± 0.005 a	0.007 ± 0.004 c	3.4 ± 0.6 b
	Li- LP	0.028 ± 0.009 a	0.009 ± 0.003 abc	3.4 ± 0.7 b
	Li- Cl	0.030 ± 0.007 a	0.008 ± 0.002 bc	3.7 ± 0.4 ab
	LP- Li	0.028 ± 0.004 a	0.008 ± 0.002 bc	3.5 ± 0.4 ab

Treatments: H_2O (non-inoculated water-washed), Cl (non-inoculated chlorine-washed), PL (non-inoculated pulsed light-treated), Li (inoculated with *L. innocua*), Li- LP (PL-treated after inoculation with *L. innocua*), Li Cl (inoculated with *L. innocua* and chlorine-sanitized), (LP-Li (PL-treated before inoculation with *L. innocua*). Values are means ± standard deviations. Different letters represent different treatments according to analysis of variances (ANOVA) and Tukey's test (n=6)

848

849 **Table 3.** Glucosinolates and sulforaphane contents in fresh-cut broccoli 24 h after treatment with 3 light
850 pulses (1.5 kJ/m^2 UV-C) compared to water-washed or chlorine-sanitized samples

Treatment	Glucoraphanin (mg kg DW^{-1})	Gluconasturtin (mg kg DW^{-1})	Progoitrin (mg kg DW^{-1})	Sulforaphane (mg kg DW^{-1})
NP	635 ± 32 b	20 ± 8 a	5 ± 1 a	18 ± 4 b
H_2O	842 ± 113 a	27 ± 3 a	5 ± 1 a	31 ± 12 ab
PL	837 ± 63 a	11 ± 3 a	5 ± 1 a	40 ± 9 a
Cl	572 ± 34 b	15 ± 4 a	4 ± 1 a	18 ±10 ab

Treatments: NP: non-processed, H_2O (water-washed), Cl (chlorine-washed), PL (pulsed light-treated). Values are means ± standard deviations (n=6). Different letters represent differences among treatments according to analysis of variances (ANOVA) and Tukey's test ($P < 0.05$)

851