Decontamination of fresh-cut broccoli with a water–assisted UV-C technology and its combination with peroxyacetic acid

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Highlights

- 0.5 kJ/m^2 reduced mesophilic bacteria by 2 $\log_{10}$ in fresh-cut conventional broccoli
- 0.3 kJ/m^2 + 50 mg/L peracetic acid reduced mesophils by 2 $\log_{10}$ in organic broccoli
- WUV reduced the microbial load in the water wash to undetectable levels
- WUV processing enhanced the sulforaphane content in fresh-cut broccoli

Abstract

The effectiveness of a water-assisted UV-C (WUV) technology for the decontamination of fresh-cut broccoli from conventional and organic agricultural practices was evaluated as an alternative to chlorine sanitation. Several WUV doses (0.3 - 1.8 kJ m^{-2}) were tested alone or combined with peroxyacetic acid (PAA). Results showed that 0.5 kJ m^{-2} was sufficient to reduce natural total aerobic mesophilic microorganisms by 2 $\log_{10}$ in conventional broccoli without

negative consequences on the physical quality. However, in order to achieve the same effect on organic broccoli, a combined application of at least 0.3 kJ m^{-2} and 50 mg L^{-1} PAA was required. Total antioxidant capacity (TAC) was enhanced by 42, 90 and 81% in conventional broccoli 24 h after treatment with 0.3, 0.5 and 1.8 kJ m^{-2}, respectively, compared to water-control. A similar trend was observed in organic broccoli, although the increase in TAC (by 22%) compared to the water-control was only significant when a dose of 1.8 kJ m^{-2} was used. Similarly, 0.5 kJ m^{-2} enhanced the sulforaphane content in conventional broccoli by 1.5 and 4-fold compared to water and chlorine-controls, respectively. WUV is a promising alternative technology to improve the microbiological and nutritional quality of fresh-cut broccoli.

Key words: **sanitation technologies, fresh-cut vegetables, nutritional properties, glucosinolates, minimally processed vegetables**

1. INTRODUCTION

Broccoli is a vegetable belonging to the Brassicaceae family which contains high levels of phytochemicals including vitamins, minerals, flavonoids, and glucosinolates (Herr & Büchler, 2010). Major glucosinolates present in broccoli are glucoraphanin and glucobrassicin, which are precursors of the isothiocyanates sulforaphane and indole-3-carbinol, respectively (Roy, Juneja, Isobe, & Tsushida, 2009; Song & Thornalley, 2007). Isothiocyanates have been widely studied for their anticancer, anti-inflammatory, and antimicrobial properties (Conaway et al., 2005; Munday et al., 2008). Specifically, sulforaphane and indole-3-carbinol have shown chemoprotective activity against several cancer types (colon, bladder, breast, and lung among others) by stimulating cellular antioxidant systems, interfering with cytokine production and activity or by restricting tumor progression through the induction of cell cycle arrest and apoptosis (Cheung & Kong, 2010; Radošević et al., 2017; Tortorella, Royce, Licciardi, & Karagiannis, 2015). However, studies with animal models have shown that some glucosinolates and its degradation products might also have genotoxic effects (Latté, Appel, & Lampen, 2011).

Public awareness about the healthy properties of food includes not only the nutritional properties but the agricultural practices due to the belief that organic products have higher nutritional content, less pesticides residues, and reduced environmental impact than conventional ones; therefore the preference for this kind of products is growing among vegetable consumers (Fess & Benedito, 2018; Hoefkens et al., 2010). In this regard, several studies have shown that organic vegetables have higher and more varied resident microbiota as well as increased amounts of some nutrients (e.g. certain glucosinolates) than conventional ones, but depending on several factors including the cultivar, the physiological stage of the commodity at harvest, and the growing conditions (Hoefkens et al., 2010; Maffei, Silveira, & Catanozi, 2013; Maggio, De Pascale, Paradiso, & Barbieri, 2013; Pace et al., 2013).

Commercialized as a fresh-cut product, the health-promoting properties of broccoli are added to its convenience, which is highly appreciated in current society. However, during processing the perishability of this vegetable increases due to the loss of integrity of the plant cell's physical barriers, allowing the leakage of nutrients and the mixing of cellular components, thereby improving the conditions for microbial activity and triggering physiological processes that are detrimental to the product quality (Artés, Gómez, Aguayo, Escalona, & Artés-Hernández, 2009; Toivonen & Dell, 2002). In order to counteract these effects, sanitation techniques and preservation methods are included in the processing workflow (Leistner, 2000). The microbial load of fresh produce is usually reduced through washes with chlorine, but growing concern about its side-products such as trihalomethanes, which are harmful to humans and environment, has urged researchers and producers to search for alternative sanitation methods (Parish et al., 2003).

Among non-chlorinated chemicals peroxyacetic acid (PAA), also known as peracetic acid, is one of the bio-friendly alternative sanitizers used in the fresh-cut industry since acetic acid, water and oxygen are the only formed side-products (Abadias, Alegre, Usall, Torres, & Viñas, 2011;

74 Artés & Allende, 2014). Furthermore, it is effective in a broader range of temperatures (0 – 40
75 °C) and pH (3.0 - 7.5) than chlorine (Vandekinderen et al., 2007). The action mechanism of PAA
76 is mainly based on the oxidation of proteins and lipids of cell walls and membranes of bacterial
77 cells, endospores, yeasts, and mold spores, thereby disrupting their permeability, inactivating
78 key enzymes and inhibiting DNA-synthesis (Finnegan et al., 2010). Furthermore, after its
79 decomposition in acetic acid it can diffuse through the cell membrane of microorganisms
80 reducing cytoplasmic pH, which in turn affects the functionality of enzymes, structural
81 proteins, and DNA (Mani-López, García, & López-Malo, 2012; Rodgers, Cash, Siddiq, & Ryser,
82 2004; Rossoni & Gaylarde, 2000).

83 In addition to chemical methods, physical non-thermal technologies such as ultraviolet light
84 (UV) have emerged in the food industry because of their many advantages. These include the
85 effectiveness against a broad range of spoilage and pathogenic microorganisms, a non-toxic
86 and 'residue free' status, minimal negative effect on organoleptic and nutritional properties,
87 and relative low costs and energy consumption compared to thermal decontamination
88 technologies (reviewed by Gayán, Condón, & Álvarez, 2014). UV light includes wavelengths in
89 the range of 200 to 400 nm, from which short UV-C waves (200 - 280 nm) have the most
90 effective germicidal effects (Bintsis, Litopoulou-Tzanetaki, & Robinson, 2000). Antimicrobial
91 effect of UV-C light is primarily based on the formation of pyrimidine dimers in the DNA which
92 inhibit transcription and eventually lead to mutagenesis and cell death (Witkin, 1984). This
93 technology has been mostly used for the decontamination of water and packages. Direct
94 exposure of commodities to UV-C light in a dose range of 0.2 to 20 kJ m^{-2} has also been
95 assessed for the sanitation of several fresh-cut fruit and vegetables with variable effectiveness
96 depending on the dose applied and on factors intrinsic to the commodity (its constituents,
97 physiological stage, surface topography, and number of cell layers) (Civello, Vicente, &
98 Martinez, 2006; Gayán et al., 2014). UV-C light has also shown several hormetic effects which
99 improve the nutritional properties of broccoli, including the increase in glucosinolates,

phenolic compounds and ascorbic acid contents and the delay of chlorophyll degradation (Costa, Vicente, Civello, Chaves, & Martínez, 2006; Formica-Oliveira, Martínez-Hernández, Díaz-López, Artés, & Artés-Hernández, 2017; Gamage, Heyes, Palmer, & Wargent, 2016; M. Lemoine, Civello, Martínez, & Chaves, 2007; Ginés Benito Martínez-Hernández, Artés-Hernández, Gómez, Formica, & Artés, 2013). However, the application and efficacy of UV light in air is limited by the shadowing effect and the potential overheating of the product which can affect its quality (Liu, Huang, & Chen, 2015). In an attempt to address those problems, the aim of the present study was to evaluate the effectiveness of a water–assisted UV-C light (WUV) technology, which allows the tridimensional application of UV-C light to the product, for the sanitation of fresh-cut broccoli and the improvement of its nutritional quality. This technology also integrates the decontamination of the product by irradiation and by water washing, while simultaneously decontaminating the water bath. A combined strategy using WUV and PAA was assessed in organic broccoli, to further improve the effectiveness of WUV in a product potentially containing higher microbial load and more varied microbiome.

2. MATERIALS AND METHODS

2.1 Plant material processing

Broccoli (*Brassica oleracea* L var. *Italica* cv. Parthenon) heads from conventional and organic agricultural practices were purchased from a local distribution warehouse or farm, respectively, in Catalonia, Spain. Heads were stored in wrapped boxes at 4 ^{o}C for up to 2 d until they were cut into 2 - 3 cm diameter florets with a sharpened knife on the day of the experiment.

2.2 Water–assisted UV-C equipment

A water-assisted laboratory scale equipment LAB-UVC-Gama (UV-Consulting Peschl España, Castellón, Spain) composed of a water tank equipped with a recirculating system and

connected to a water pump (Fig. 1) was used in order to improve the accessibility of UV-C light to all sides of the product in respect of conventional UV-C chambers. Before WUV treatments, lamps were preheated for 15 min. 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).

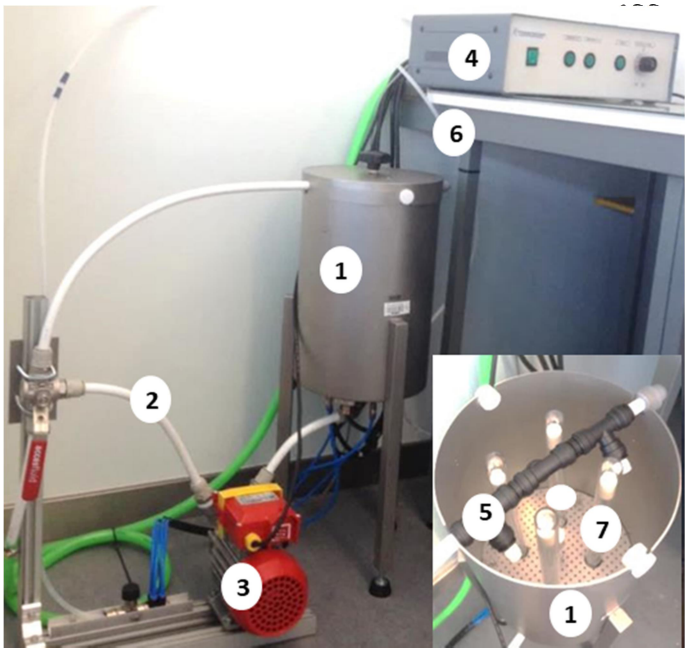


Figure 1. Water-assisted UV (WUV) light equipment setup: water tank (1) equipped with a recirculating water circuit (2) that is put in motion by a water pump (maximum flow 1700 L h^{-1}) (3) which is connected to a power source (4). Pressurized water is introduced through an adjustable device with multiple water sprinklers on the top (5), and pressured air, set at 1 bar, enters through the bottom of the tank for water bubbling (6). Four equidistant UV lamps (7) (17.2 W) emitting at 254 nm are located in water proofs quartz compartments inside the tank.

2.3 Sanitation of fresh-cut broccoli using WUV; dose optimization

To optimize the sanitation procedure, approximately 300 g of conventional fresh-cut broccoli florets were immersed in 10.5 L of cold (5 °C) tap water in agitation and submitted to four UV-C doses (0.3, 0.5, 0.9 and 1.8 kJ m^{-2}) by combining treatments with 2 or 4 lamps for 120 or 360 s of exposure. Doses were calculated as: the mean values of irradiance (W m^{-2}) of the several repetitions of the treatment x time of exposure (s). Washing broccoli florets for 120 s in agitated tap water or in 100 mg L^{-1} sodium hypochlorite solution with pH adjusted to 6.5 with ortho-phosphoric acid (Merck Millipore, Darmstadt, Germany), in the same proportion used for WUV treatments, were included as controls. After draining the excess of water and air-drying on the bench, some samples were immediately submitted to microbial analysis. For the

155 analysis of the effect of treatments on biochemical parameters, processed florets were let at
156 room temperature for a gap time of 6 h before freezing and storage. The rest of processed
157 broccoli was stored at 5 °C for 24 h in wrapped polystyrene trays before sampling and freezing
158 for biochemical analysis.

159 ## 2.4 Sanitation of fresh-cut broccoli using WUV and its combination with
160 peroxyacetic acid (PAA)

161 Considering the results obtained during the optimization phase, two UV-C doses (0.3 and 0.5 KJ
162 m^{-2}) were selected based on their better suitability for industrial purposes (lower time of
163 exposure and effectiveness regarding the control of microbial populations) and subsequently
164 evaluated for the sanitation of organic broccoli following the procedure described in the
165 previous section. Additionally, combined treatments including the selected UV-C doses and
166 two doses of PAA (50 and 80 mg L^{-1}) were also tested. Sanitation with 50 or 80 mg L^{-1} PAA
167 solutions, cold tap water or 100 mg L^{-1} hypochlorite solution without lighting the UV lamps,
168 were included as control treatments.

169 ## 2.5 Analysis of physical quality parameters

170 Physical quality parameters were evaluated in non-treated broccoli and in treated florets 6 h
171 and 24 h after treatment. Superficial color of floret heads was determined by measuring CIE
172 parameters L*, a* and b* with a chromameter (CR400, Minolta, Osaka, Japan) on two
173 positions of 5 florets heads per treatment. Color results were interpreted according to the
174 International Commission on Illumination (CIE) parameters: **L*** defines lightness (black to
175 white) with values within 0 and 100; **a*** indicates redness when positive and greenness when
176 negative; and **b*** represents yellowness to blueness corresponding to positive to negative
177 values. Parameters a* and b* were expressed as hue angle (^{o}) calculated as: 180 + arc
178 tan(b*/a*) (McLellan, Lind, & Kime, 1995). Firmness evaluation was performed using a TA-XT2
179 Texture Analyzer (Stable Micro Systems Ltd., England, UK) by measuring the maximum force

180 required for a compression platform (75 mm diameter) to cause a 10% deformation of a
181 broccoli floret at 5 mm s^{-1}, for an activation threshold of 10 N. Overall visual assessment of
182 quality in a 7 point hedonic scale (from **1**: dislike to **7**: like very much) was evaluated two
183 independent times by 23 untrained panelists. The evaluation panel was composed by 76% of
184 women and 24% of men within the age ranges of 18-30 (69%) and 31-45 (31%).

185 ## 2.6 Microbial analysis

186 Microbial populations were estimated before sanitation and immediately after. For this, three
187 replicates of 25 g of florets were homogenized in 225 mL of buffered peptone water (BPW,
188 Biokar, Beauvais, France) within a 400 mL sterile full-page filter bag (Bagpage, Interscience,
189 Saint Nom, France) in a Masticator (IUL, Barcelona, Spain) set at 4 strokes per s for 90 s. Total
190 mesophilic aerobic microorganisms (MAM) were determined by plating the appropriate ten-
191 fold dilutions in saline peptone (8.5 g L^{-1} NaCl, 1 g L^{-1} peptone) on Plate Count Agar plates
192 (PCA, Biokar, Beauvais, France) after incubation at 30 °C for 72 h. Native yeasts and molds
193 were enumerated on Dichloran Rose Bengal Chloramphenicol agar plates (DRBC, Biokar,
194 Beauvais, France) after incubation at 25 °C for 5 d. Viable counts of MAM, yeasts, and molds in
195 water and chlorine baths were also performed. The analysis of chlorine baths was preceded by
196 a neutralization step in Dey-Engley Neutralizing Broth (Sigma-Aldrich, Madrid, Spain). Bath
197 samples were plated as previously described. Microbiological data were expressed as $\log_{10}$ of
198 the colony forming units per gram of fresh weight of broccoli (CFU g^{-1} FW). Microbial
199 reductions were calculated as: $\log_{10}$ (N_1/N_0), where N_1 is the microbial count of sanitized
200 broccoli and N_0 is the microbial count of untreated broccoli.

201 ## 2.7 Biochemical analysis

202 Approximately 70 g of florets per replicate, per treatment and per sampling time were frozen
203 with liquid nitrogen, grinded using a commercial grinder (Minimoka 6R-020, Coffeemotion,
204 Lleida, Spain), and stored at -80 °C until biochemical analysis.

205 Total antioxidant capacity (TAC) and total phenolic content (TPC) were measured in the
206 supernatants resulting from the centrifugation at 24 000 x g for 20 min (at 4 °C) of the extracts
207 obtained from a mix containing 3 g of frozen broccoli powder and 10 mL of extraction solution
208 (19.7 mol L^{-1} methanol, 0.05 mol L^{-1} HCl), after agitation at 20.94 rad s^{-1} for 2 h. TAC was
209 quantified using a spectrophotometer (EONC, Biotek Instruments, Highland Park, VT, USA) by
210 the Ferric Reducing Antioxidant Power (FRAP) method and the DPPH (2,2 – diphenyl – 1 –
211 picrylhydrazyl) free radical-scavenging activity method. The FRAP method was performed
212 following the protocol of Benzie and Strain (1996) with some modifications (Giné-Bordonaba &
213 Terry, 2016); OD was measured at 593 nm. The DPPH method, based on the described by
214 Brand-Williams et al., (1995), was performed by measuring OD at 515 nm of a 1.5 mL reaction
215 containing 1.4 mL of 1 mmol L^{-1} DPPH and 0.1 mL broccoli extract after 1 h incubation at room
216 temperature in darkness. TPC was quantified by the Folin-Ciocalteu method (Singleton, Rossi
217 Jr., & Rossi J A Jr., 1965), by measuring OD at 765 nm of a reaction containing 0.7 mL of each
218 sample extract, 4.3 mL water and 0.5 mL Folin-Ciocalteu reagent, incubated for 5 min in
219 darkness before adding 2 mL of 200 g L^{-1} Na_2CO_3 solution. Non-enzymatic antioxidant activities
220 were expressed as g of the measured analyte (i.e. Gallic acid, GAE or ascorbic acid, AA) per
221 kilogram of fresh weight of broccoli (g kg^{-1} FW).

222 Chlorophyll pigments were extracted from 2 g of frozen fresh broccoli using N,N dimethyl-
223 formamide following the method of Moran (1982) and expressed as (mg kg^{-1} FW).

224 For glucosinolates extraction, 150 mg of processed broccoli, lyophilized after storage at 4 °C
225 for 24 h, was mixed with 3 mL of a solution containing methanol: water (80:20, v:v) following
226 the protocol described by Alarcón-Flores et al. (2013). Glucosinolates content was determined
227 by ultra-high performance liquid chromatography coupled to tandem mass spectrometry
228 (UHPLC–MS/MS) using an Agilent series 1290 RRLC instrument (Agilent, Santa Clara, CA, USA)
229 coupled to an Agilent triple quadrupole mass spectrometer (6460A) with a Jet Stream ESI ion

source (G1958-65138) (Alarcón-Flores et al., 2013). Results were expressed as mg kg^{-1} of dry weight (DW). For the identification and quantification of glucosinolates, a multi-compound (5 mg L^{-1} of each standard) methanolic solution containing sulforaphane (Sigma-Aldrich, Steinheim, Germany), proigonitrin, gluconasturtin, glucoraphanin (PhytoLab GmbH & Co., Vestenbergsgreuth, Germany), glucotropaeolin, glucoerucin, and glucoiberin (Scharlab, Barcelona, Spain) was used.

2.8 Statistical analysis

All experiments were performed twice and included three biological replicates per treatment and sampling time. Physical, microbiological and biochemical data were analyzed using the statistical software JMP (version 11 SAS Institute Inc., NC, USA). All data were verified for normal distribution and homoscedasticity of residues and accordingly, means were compared by analysis of variances (ANOVA) and separated by Tukey's test ($P < 0.05$). Categorical data from overall quality assessment were analyzed by a logistic regression analysis ($P < 0.05$).

3. RESULTS AND DISCUSSION

3.1 Analysis of physical quality parameters

The evaluated WUV doses (0.3, 0.5, 0.9 and 1.8 kJ m^{-2}) did not affect the firmness of conventional broccoli when compared to the control treatments (Table 1). Similar results were obtained for organic broccoli when treated with 0.3 or 0.5 kJ m^{-2} (Table 2). Sanitation with PAA caused a reduction in the firmness of organic broccoli 24 h post-treatment when compared to the water control ($p < 0.05$).

Similarly, WUV had no effect on the color of conventional broccoli (Table 1). Regarding organic broccoli, WUV or its combinations with PAA showed no effect on lightness (L*) (Table 2).

Table 1. Physical quality parameters of fresh-cut conventional broccoli after sanitation with different UV-C doses using WUV, compared to water and chlorine washing.

				WUV (kJ m^{-2})			
Treatment		**Water**	**NaClO**	**0.3**	**0.5**	**0.9**	**1.8**
L*	0 h	42±2a	40±3a	42±3a	41±3a	42±3a	41±2a
Hue (°)	0 h	129±5a	127±6a	128±5a	127±5a	128±5a	127±4 a
Firmness (N)	0 h	13±5a	9±3a	9±3a	10±5a	12±3a	12±5 a
L*	24 h	43±2a	42±3a	43 ± 2 a	42±2a	42±2a	42±2a
Hue (°)	24 h	128±4a	129±3a	129±5a	124±8ab	125±9ab	128±4a
Firmness (N)	24 h	12±3a	20±7a	16±4a	13±2a	16±4a	19±3a

Values are means ± standard deviations (n=20). Different letters represent significant differences among treatment at each sampling time according to analysis of variances (ANOVA) and Tukey's test ($P < 0.05$)

Table 2. Physical quality parameters of fresh-cut organic broccoli after sanitation with several UV-C doses using WUV or its combination with peroxyacetic acid, compared to water and chlorine (NaClO) washing.

		WUV (kJ m^{-2}), Peroxyacetic acid (PAA) (mg L^{-1})									
		water	**Cl**	**50 PAA**	**80 PAA**	**0.3 kJ m^{-2}**	**0.3 + PAA 50**	**0.3 + PAA 80**	**0.5 kJ m^{-2}**	**0.5 + PAA 50**	**0.5 + PAA 80**
L*	6h	46 ± 4 bc	44 ± 3 c	46 ± 2 bc	47 ± 5 abc	45 ± 3 bc	45 ± 4 bc	45 ± 4 bc	48 ± 2 a	49 ± 2 a	51 ± 4 a
Hue (°)	6h	124±6 a	124±4 ab	117±7 b	119±5 b	124±5 a	125±4 a	122±7 ab	121±5 a	121±2 a	117±5 b
Firmness (N)	6h	19 ± 8 bc	22 ± 8 abc	14 ± 4 c	14 ± 7 c	20 ± 6 bc	26 ± 7 ab	22 ± 9 bc	33 ± 7 a	29±11 a	23±9 ab
L*	24h	45 ± 2 ab	43 ± 2 b	46 ± 3 ab	48 ± 3 a	43 ±2 b	45 ± 4 ab	45 ± 3 ab	45 ± 4 ab	47 ± 5 a	47 ± 3 a
Hue (°)	24h	127±3 a	126±3 a	117±6 c	120±5 bc	124±5 a	127±4 a	126±4 a	120±2 b	118±3 bc	119±3 bc
Firmness (N)	24h	23 ± 7 a	22 ± 9 a	16 ± 6 b	15 ± 8 b	23 ± 7 a	20 ± 6 ab	26 ± 7 a	29 ± 5 a	30 ± 4 a	25 ± 8 a

Numbers are means ± standard deviations (n = 20). Different letters represent significant differences among treatments at each sampling time according to an analysis of variances (ANOVA) and a Tukey's test ($P < 0.05$). H_2O: water control, NaClO: 100 mg L^{-1} sodium hypochlorite

However, 24 h after processing, the hue angle was slightly reduced ($p < 0.05$) in samples treated with 0.5 kJ m^{-2} and its combinations with PAA compared to the water control, although the highest reductions were observed for the PAA control treatments. Nevertheless, color changes were not visually detected by the panelists during the analysis of the overall visual quality of samples collected at 6 or 24 h after treatment (data not shown). Hue angle has previously shown to better fit as a color parameter for measuring the progression of yellowing

in broccoli florets during storage at 5 ^{o}C (Argüello et al., 2017). UV-C doses ranging from 0.9 to 1.5 kJ m^{-2} have previously shown to contribute to color preservation in several broccoli varieties during a storage period of up to 23 days at 4 ^{o}C (Costa et al., 2006; Duarte-Sierra, 2015; G. B. Martínez-Hernández, Gómez, Pradas, Artés, & Artés-Hernández, 2011).

3.2 Microbial analysis

3.2.1 Sanitation using WUV; dose selection

Initial populations of MAM, molds and yeasts on fresh-cut conventional broccoli were 4.1 ± 0.1, 2.2 ± 0.1, and 2.3 ± 0.1 $\log_{10}$ CFU g FW^{-1}, respectively. After sanitation with 0.5 kJ m^{-2} using WUV, a significant reduction of MAM by 2 ± 0.1 $\log_{10}$ compared to the untreated control was obtained ($p < 0.05$) (Fig. 2).

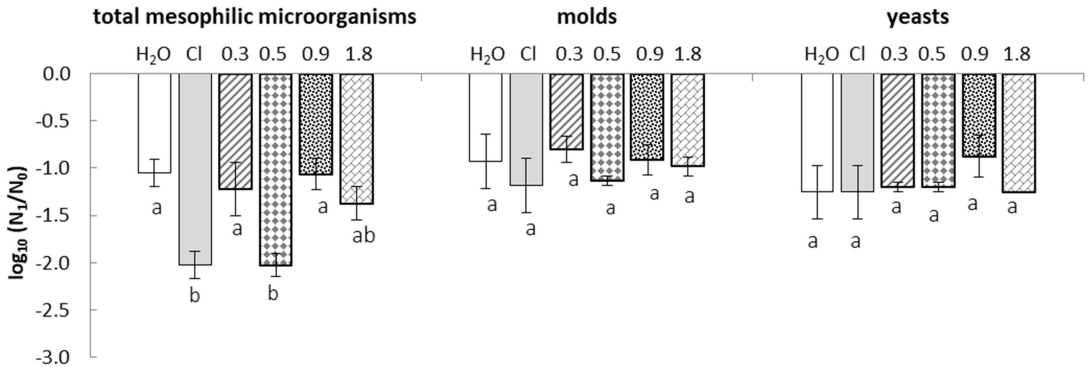


Figure 2. Logarithmic reductions of native microbial populations on conventional fresh-cut broccoli sanitized (N_1) with: tap water in agitation (H_2O), a 100 mg L^{-1} chlorine solution (Cl) or with different UV doses (0.3, 0.5, 0.9, 1.8 kJ m^{-2}) using WUV, in respect of untreated broccoli (N_0). Columns represent means and error bars represent standard error of the mean (n=6). Different letters represent significant differences for each type of microorganism according to an analysis of variances (ANOVA) and a Tukey's test ($P < 0.05$).

Similar results were obtained after chlorine washing. No significant differences compared to the water control were observed after processing using higher doses (0.9 or 1.8 kJ m^{-2}). These results agreed with previous reports showing that the highest reduction of spoilage mesophilic microorganisms or pathogenic bacteria on fresh produce does not always correlate to higher UV doses. For example, Martínez-Hernández et al., (2015) after testing doses up to 15 kJ m^{-2} using a dry UV technology, found that the maximum inactivation rate of *E. coli*, *S. enterica* and

287 *L. monocytogenes* in inoculated Kaylan-hybrid broccoli was obtained while operating in the
288 range from 0 to 2.5 kJ m^{-2}. Such lack of correlation between the dose and the extent of
289 microbial reduction may has been due to putative structural changes occurred during
290 treatments which may have influenced the response to UV or improved the conditions for
291 microbial penetration into the plant tissue (Escalona et al., 2010; Graça et al., 2017).

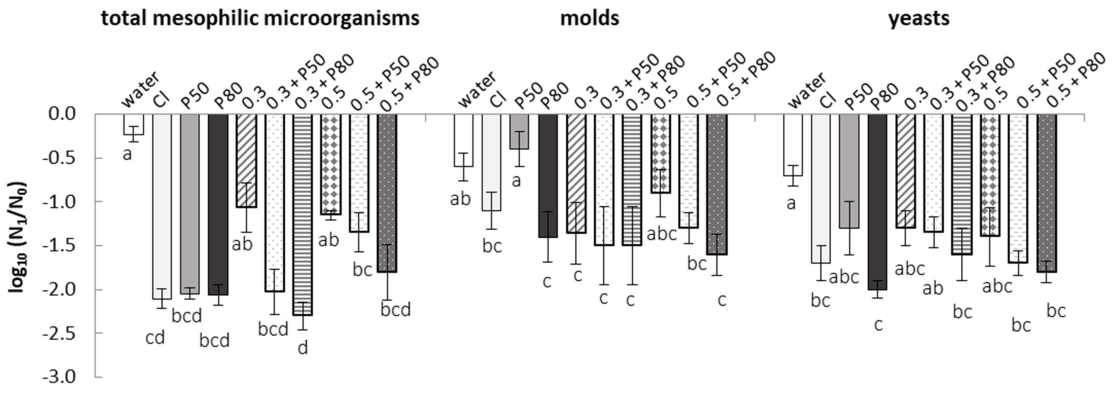
292 The results obtained showed that water washing was enough to reduce the initial native molds
293 and yeasts populations on conventional broccoli to values close to the detection limit (5 CFU
294 mL^{-1}); thus the efficacy of WUV beyond water sanitation could not be established. The low
295 initial levels of molds and yeasts populations compared to those previously reported for
296 conventional broccoli from several varieties (4.9 to 6.5 $\log_{10}$ CFU g^{-1}) might be conditioned by
297 the growing location, season and management system which influence the composition and
298 population levels of microbial communities (Argüello et al., 2017; Martínez-Hernández, Artés-
299 Hernández, Gómez, & Artés, 2013; Wang et al., 2016).

300 MAM, yeasts and molds populations present in the water wash after the sanitation of
301 conventional broccoli are shown in Table 3. No viable cells of yeasts and molds were detected
302 after any of the assayed treatments. MAM populations in the water wash after treatment with
303 0.3 kJ m^{-2} WUV were 1.4 $\log_{10}$ lower that those present in the water wash after broccoli
304 sanitation without switching on the UV lamps. Processing using 0.5 to 1.8 kJ m^{-2}, resulted in
305 reductions ranging between 2.8 $\log_{10}$ (non-detected cells) and 1.3 ± 0.2 $\log_{10}$, compared to the
306 process water without UV. After performing a UV treatment of the water bath for 2 additional
307 minutes after broccoli sanitation, using 2 or 4 UV lamps, no viable cell was detected. This
308 suggested that the viable cells detected in the water after broccoli sanitation could represent a
309 combination of the cells that were circulating in the system during the UV treatment and failed
310 to be exposed to the light and those that were protected within the product structure and
311 passed to the water during the gap time from the light switching off until the withdrawing of

312 the product from the equipment. A similar situation was described by Huang et al. (2015)
313 when using a water-assisted pulsed light device for the decontamination of berries.
314 Nevertheless, the use of 4 lamps instead of 2 would be advisable to better counteract the
315 added effect of organic matter in suspension which is likely to occur at an industrial level when
316 a higher amount of vegetal product is used.

317 ### *3.2.2 Sanitation using WUV and its combination with PAA.*

318 In order to assess a worse-case scenario implying higher and more varied microbial load, fresh-
319 cut broccoli from organic practices was used and the combination of WUV and PAA was tested
320 to improve the efficacy of WUV (Lupatini, Korthals, de Hollander, Janssens, & Kuramae, 2017;
321 Renaud et al., 2014; Wang et al., 2016). The initial populations of MAM, molds and yeasts on
322 fresh-cut organic broccoli were 4.9 ± 0.1, 3.9 ± 0.3, and 3.4 ± 0.1 $\log_{10}$ CFU g FW^{-1}, respectively.
323 The pH of the baths did not vary after treatments, being 5.2 ± 0.1 and 4.6 ± 0.1 for the 50 and
324 80 mg L^{-1} PAA solutions, respectively and 6.5 ± 0.1 for the chlorine solution. For the reduction
325 of native MAM populations, the combined application of 0.3 kJ m^{-2} (2 lamps for 2 min) and 50
326 or 80 mg L^{-1} PAA were the most efficient treatments, with reductions of 2 ± 0.2 $\log_{10}$,
327 compared to the untreated control (Fig. 3).



328

329 Figure 3. Logarithmic reductions of native microbial populations on organic fresh-cut broccoli sanitized (N_1) with
330 different UV doses (0.3 and 0.5 kJ m^{-2}) using WUV or its combination with 50 or 80 mg L^{-1} peroxyacetic acid (P50 and
331 P80, respectively), in respect of untreated broccoli (N_0), as compared with tap water (H_2O) or 100 mg L^{-1} chlorine (Cl)
332 washes. Columns represent means and error bars represent standard error of the mean (n=6). Different letters
333 represent significant differences for each type of microorganism according to an analysis of variances (ANOVA) and a
334 Tukey's test ($P < 0.05$).

335 However, the same efficacy was obtained with PAA and chlorine control treatments. Similarly,
336 using a small scale laboratory version of a water-assisted UV-C technology, Liu et al. (2015) did
337 not obtain a significant improvement of the UV treatment (7.9 mW cm^{-2} for 10 min) when
338 combining it with 10 ppm chlorine, 100 ppm SDS, or 0.5% levulinic acid + 100 ppm SDS, for the
339 reduction of *E. coli* and *Salmonella* spp. in dip-inoculated blueberries. Those researchers
340 neither obtained differences among the dry, the wet UV technology and the chlorine control
341 (100 mL L^{-1} NaClO, for 1 min). Other researchers obtained a reduction of MAM populations by
342 1.6 log_{10} in fresh-cut endives by washing them in cold water (4 ^{o}C) and then irradiating them
343 with 1.2 kJ m^{-2} UV-C (Hägele et al., 2016). That reduction efficacy was improved to 2.1 log_{10}
344 when warm water (45 ^{o}C) was used instead of cold water but, in both cases it was similar to
345 that obtained with chlorine sanitation. The results obtained in the present study concerning
346 the effectiveness of PAA compared to chlorine for microbial control contrasted to those
347 obtained in previous experiments using a similar ratio of vegetal weight: volume of bath and
348 time of exposure, when 100 mg L^{-1} PAA showed higher effectiveness than 100 mL L^{-1} chlorine
349 for reducing *E. coli* and *Salmonella enteritidis* (reductions by 2–3 log) in fresh-cut kailan-hybrid
350 broccoli (Martínez-Hernández, Navarro-Rico, et al., 2015). Such disagreement may be
351 explained by the higher PAA concentration, different sensitivities to the sanitizers of the
352 microorganisms tested or by a deeper colonization and establishment of the native microbiota
353 compared to the inoculated one.

354 The results showed reductions of yeast populations ranging from 1.5 to 2.0 ± 0.1 log_{10} using 0.3
355 kJ m^{-2} + 80 mg L^{-1} PAA or the combination of 0.5 kJ m^{-2} + 50 or 80 mg L^{-1} PAA, which was
356 significantly higher than the water control but similar to those obtained with the chemical
357 control treatments. Molds populations were reduced by 1.0 to 1.6 ± 0.1 log_{10} when WUV
358 treatments were combined with PAA, regardless of the dose applied. A similar reduction was
359 obtained with 80 mg L^{-1} PAA (1.4 ± 0.1 log_{10}) and chlorine (1.1 ± 0.2 log_{10}) which was
360 significantly higher than that obtained with 50 mg L^{-1} PAA (0.4 ± 0.2 log_{10}).

Comparing the efficacy of WUV for the decontamination of conventional and organic broccoli, results showed that WUV, at doses of 0.3 and 0.5 kJ m^{-2}, it was 50% less effective for the reduction of MAM in organic broccoli than in conventional broccoli compared to untreated controls. As expected for organic broccoli, reduced effectiveness of WUV for decontamination might be related to a higher and more heterogeneous initial microbial population, which could comprise various microbial species or strains with different sensitivity to UV (Lupatini et al., 2017); to a different physiological stage of broccoli at harvest or to the stressful agricultural conditions which might influence the plant hormetic response (Hassenberg, Huyskens-Keil, & Herppich, 2012).

Viable counts of the water baths after a single-use sanitation of organic broccoli showed that all of the studied WUV conditions reduced mesophilic microbial populations in a range of 2.3 ± 0.5 to 3.0 ± 0.5 log_{10} compared to water-washing, showing the same efficacy as chlorine-sanitation (Table 3).

Table 3. Populations (log_{10} CFU mL^{-1}) of total mesophilic aerobic microorganisms, yeasts and molds present in the wash after broccoli sanitation with several UV doses using WUV or its combination with peroxyacetic acid, compared to water and chlorine (NaClO) washing.

	Treatment		MAM	Yeasts	Molds
Conventional broccoli	water		2.8 ± 0.2	0.4 ± 0.2	0.9 ± 0.3
	NaClO (100 mg L^{-1})		nd - 0.7 ± 0.01	nd	nd
	WUV (kJ m^{-2})	0.3	1.4 ± 0.2	nd	nd
		0.5	nd - 1.1 ± 0.1	nd	nd
		0.9	nd - 1.2 ± 0.2	nd	nd
		1.8	nd - 1.1 ± 0.2	nd	nd
Organic broccoli	water		3.1 ± 0.4	2.5 ± 0.3	2.2 ± 0.4
	NaClO (100 mg L^{-1})		nd - 0.7± 0.2	nd	nd
	WUV (kJ m^{-2})	0.3	1.0 ± 0.4	nd	nd
		0.5	nd	nd	nd
	PAA (mg L^{-1})	50	nd - 1.0 ± 0.4	nd	nd
		80	nd	nd	nd
	WUV (kJ m^{-2}) + PAA (mg L^{-1})	0.3 + 50	nd - 1.2 ± 0.5	nd	nd
		0.3 + 80	nd	nd	nd
		0.5 + 50	nd	nd	nd
		0.5 + 80	nd	nd	nd

MAM: mesophilic aerobic microorganisms, PAA: peroxyacetic acid (PAA). nd: not detected, below detection limit (5 CFU mL^{-1}). Values are means ± standard deviations (n=6).

377 The populations of yeasts and molds present in the water wash after sanitation of organic
378 broccoli were reduced to below the detection limit (5 CFU mL^{-1}) regardless of the essayed
379 treatment. In agreement to our results, UV doses as low as 0.4 kJ m^{-2} achieved a 3.8 log_{10}
380 reduction of total mesophilic bacteria in the water bath after sanitation of lamb's lettuce in
381 semi-industrial conditions (Ignat, Manzocco, Bartolomeoli, Maifreni, & Nicoli, 2015). In the
382 present work the efficacy of chlorine for decontamination was evaluated only after a single use
383 of the water bath. Since free chlorine concentration was reduced by 10% after sanitation, the
384 efficacy of this treatment for the decontamination of a subsequent set of samples, as often
385 occur at an industrial level, could also be diminished (Rodgers et al., 2004). Furthermore, we
386 observed that two additional minutes of WUV were enough to reduce the residual populations
387 of MAM populations to below the detection limit, enabling water for potential reuse.
388 However, a filtration step should be considered in order to reduce organic matter for up-scaled
389 workflows (Fan, Huang, & Chen, 2017). In this sense, despite the increasing turbidity and
390 microbial load observed after several cycles of processing fresh-cut onions and endives at an
391 industrial level, reductions from 0.6 to 1.3 log_{10} of bacterial populations in the water baths,
392 have been recorded for each product, respectively, corroborating the usefulness of UV-C for
393 reducing water consumption (Hägele et al., 2016; Selma et al., 2008). In addition to the UV
394 dose, the water column thickness is an important factor to take into account in obtaining
395 better results (Hägele et al., 2016). Unlike previous experiments testing the combination of
396 PAA and UV-C for wastewater disinfection at a pilot plant level, we observed no synergistic
397 effect of UV-C and PAA (Caretti & Lubello, 2003).

398 ## 3.3 Biochemical analysis

399 ### *3.3.1 Total antioxidant capacity (TAC)*

400 As measured by the DPPH method, the TAC of conventional broccoli 6 h after treatment with
401 0.3 kJ m^{-2} WUV was enhanced by 16% compared to the water-washed control (Fig. 4A). This

402 difference increased to 42% at 24 h post-treatment. When compared to chlorine washing,
403 irradiation with 0.3 kJ m^{-2} WUV increased TAC by 70 and 65%, after 6 h and 24 h, respectively.
404 Increasing the UV-C dose to 1.8 kJ m^{-2} resulted in an increase in TAC by 22 and 80% in the WUV
405 treated samples compared to the water-washed control, at 6 and 24 h post-treatment,
406 respectively. Compared to the chlorine control, the difference was higher (by 80% at 6 h post-
407 treatment), duplicating its value after 24 h. Treatment with 0.5 kJ m^{-2} showed no immediate
408 effect on TAC but it duplicated the value observed for the water control, 24 h post-processing.
409 In agreement with these results, Martínez-Hernández et al. (2011) obtained that in a certain
410 range, higher UV-C doses (1.5 < 4.5 > 9; 4.5 > 15 kJ m^{-2}) correlated with higher TAC in Bimi®
411 broccoli immediately after treatment. Higher antioxidant capacity was also detected in cv
412 'Cicco' broccoli florets 6 d after treatment with 10 kJ m^{-2} UV-C and storage at 20 ^{o}C, as
413 measured by the DPPH method, although those differences were not significant at initial time
414 (Costa et al., 2006).

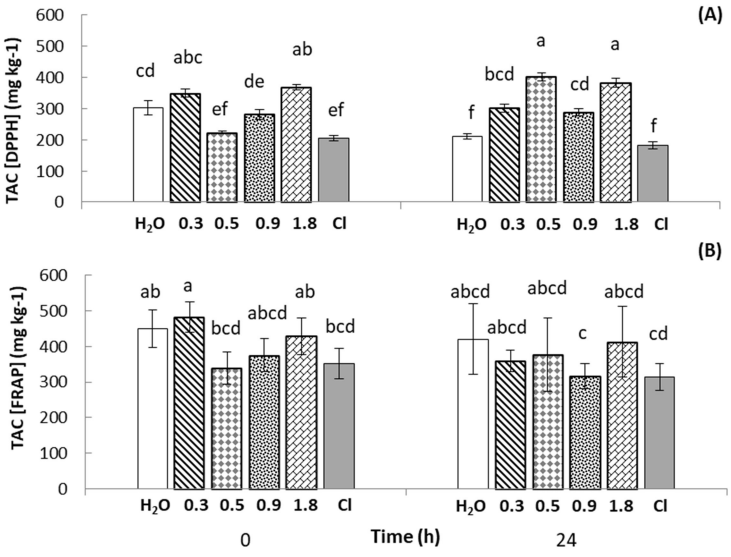


Figure 4. Total antioxidant capacity in fresh-cut conventional broccoli treated with different UV-C doses (0.3, 0.5, 0.9, and 1.8 kJ m^{-2}) using WUV as compared to sanitation with tap water (H_2O) or 100 mg L^{-1} chlorine (Cl). (A) Measured by the DPPH method, (B) measured by the FRAP method. Columns represent means and 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 test ($P < 0.05$).

432 Using the DPPH method, total antioxidant capacity in organic broccoli showed no variation
433 (333 ± 11 mg kg^{-1}) after treatment with 0.3 or 0.5 kJ m^{-2} WUV compared to the water and
434 chlorine controls, at 6 or 24 h post-treatment (Fig. 5A). No significant differences were
435 observed between the application of 0.3 kJ m^{-2} WUV alone and its combinations with 50 or 80

436 mg L^{-1} PAA. In contrast, the combined application of 0.5 kJ m^{-2} and 50 or 80 mg L^{-1} PAA resulted
437 in poorer total antioxidant capacities than this WUV treatment alone, showing reductions by
438 27 and 35%, respectively, at 6 h post-treatment. However, such differences in TAC vanished
439 after 24 h of incubation. Differential effect of UV-C in TAC, as measured by the DPPH method,
440 have previously been observed according to the broccoli variety and cultural practices
441 (Martínez-Hernández, Artés-Hernández, Gómez, Formica, et al., 2013). The observed higher
442 antioxidant capacity might has been due to an increase in the vitamin C and glutathione
443 contents or to higher activities of antioxidant enzymes, as previously observed in UV—treated
444 fresh-cut broccoli and red cabbage (Lemoine, Chaves, & Martínez, 2010; Zhang et al., 2017),
445 but this hypothesis cannot be corroborated by our results.

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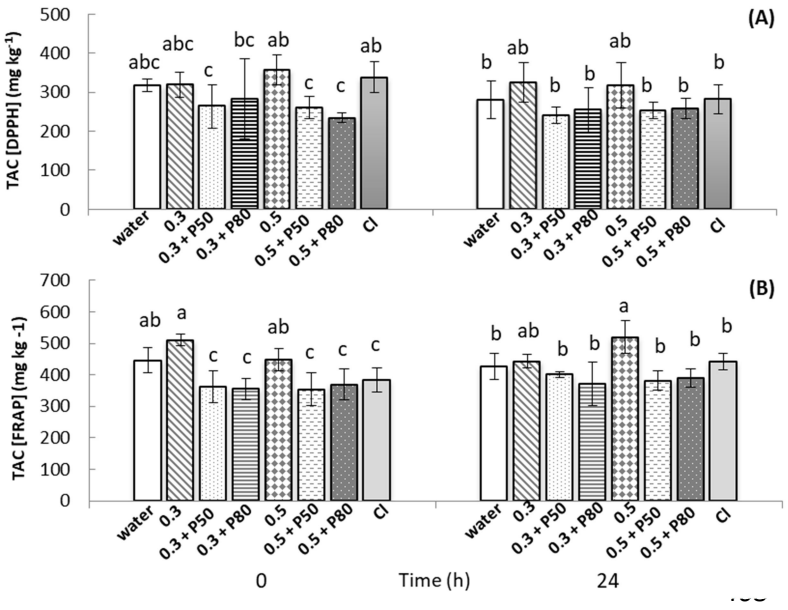

Figure 5. Total antioxidant capacity in fresh-cut organic broccoli treated with different UV-C doses (0.3 and 0.5 kJ m^{-2}) using WUV or its combination with 50 or 80 mg L^{-1} peroxyacetic acid (P50 or P80, respectively) as compared to sanitation with tap water (H_2O) or 100 mg L^{-1} chlorine (Cl) washes. (A) Measured by the DPPH method, (B) measured by the FRAP method. Columns represent means and 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 test ($P < 0.05$).

469

470 When assessed using the FRAP method, differences in TAC were not so evident. An increase by
471 37% was observed in conventional broccoli 6 h after treatment wih 0.3 kJ m^{-2} WUV when

472 compared to the chorine-washed control (Fig. 4B). However, differences between those WUV-
473 treated samples and the water control were not significant at any of the analyzed times. In the
474 same way, no differences were observed between the 1.8 kJ m^{-2}—treated samples and the
475 water or the chlorine controls probably because of the higher variability obtained with this UV-
476 C dose. In organic broccoli, treatments with WUV alone were the best of all of the assayed
477 since maintained fresh-cut broccoli TAC (469 ± 11 mg kg^{-1}) similar to the water-washed control
478 6 h after treatment (Fig. 5B). The broccoli samples treated with 0.5 kJ m^{-2} showed an increased
479 TAC (by 22%) compared to the water control, 24 h post-treatment.

480 ### *3.3.2 Total phenolic content (TPC)*

481 No significant differences were observed in the phenolic compounds content of conventional
482 broccoli among the evaluated treatments. Stable values around 77 ± 12 mg kg^{-1} remained
483 during the analyzed period (data not shown). As observed for conventional broccoli, no
484 variation in TPC could be detected in the samples treated with WUV or with the combined
485 alternatives with PAA compared to the water-washed samples (48 ± 6 mg kg^{-1}) at 6 or 24 h
486 posty-processing. The putative induction of the phenylpropanoid metabolism could have taken
487 longer than 24 h after UV-C radiation (Duarte-Sierra, 2015), as observed in Bimi® broccoli
488 when using UV-C doses from 1.5 to 15 kJ m^{-2} (Martínez-Hernández et al., 2011). Lower TPC in
489 organic broccoli compared to the conventional one, was unexpected since organic practices
490 usually result in higher content in bioactive compounds (Valverde et al., 2015). This could be
491 due to a more advanced physiological stage at harvest of the conventional broccoli used in the
492 present studies. Contradictory results regarding the TPC in broccoli florets after UV-C
493 irradiation have been obtained by other authors, i.e. a reduction was observed using 10 kJ m^{-2}
494 while an increase was obtained with 8 kJ m^{-2} (Costa et al., 2006; Lemoine et al., 2007).

3.3.2 Chlorophylls content

Total chlorophyll content in conventional broccoli was 134 ± 21 µg kg $^{-1}$ FW, with 67 ± 10 µg kg $^{-1}$ FW of chlorophyll a, and 44 ± 8 µg kg $^{-1}$ FW of chlorophyll b, regardless of the treatment applied. Thus, WUV did not affect the chlorophylls contents compared to the fresh-cut non-treated broccoli (data not shown) which is in line with the color retention discussed above and agree with previous results using similar UV-C doses (Duarte-Sierra, 2015; Martínez-Hernández et al., 2011). In the same way, no differences in the total chlorophyll content in organic broccoli (141 ± 19 µg kg $^{-1}$ FW) nor in the chlorophyll a (69 ± 7 µg kg $^{-1}$ FW) or b (47 ± 8 µg kg $^{-1}$ FW) contents were observed among the analyzed treatments (data not shown).

3.3.3 Glucosinolate content

Although more than 120 different glucosinolates have been identified in cruciferous vegetables, only some of these are present in high quantities. From the glucosinolates tested (glucoiberin, glucoraphanin, glucotropaeolin, proigonitrin, glucoerucin, and gluconasturtin), glucoraphanin was the only glucosinolate detected with contents ranging from 320 to 527 mg kg^{-1} DW (data not shown). Glucoraphanin is the predominant glucosinolate in broccoli sprouts from several varieties (Verkerk, Tebbenhoff, & Dekker, 2010; Westphal et al., 2017), although in a previous work glucobrassicin and glucobrassicanapin were the most abundant found in 'Parthenon' variety (Fernández-León, Fernández-León, González-Gómez, Ayuso, & Bernalte, 2017). The glucosinolates composition and contents vary not only at the inter-variety level but also depending on the physiological stage, agricultural practices, pre-harvest treatments, and processing styles at the intra-variety level (Torres-Contreras et al., 2017; Valverde et al., 2015). For instance, Valverde et al. (2015) observed that the content in certain glucosinolates (i.e. glucobrassicin and neoglucobrassicin) was higher in organically than in conventionally produced broccoli, although those practices did not influence the content in glucoraphanin and its derived products. Factors intrinsic to the vegetal product such as those mentioned

above, also influence the effect of UV-C irradiation on specific bioactive compounds, which is added to the effect of the dose applied (Reviewed by Civello, Vicente, & Martinez, 2006).

Treatment with 0.5 kJ m^{-2} WUV resulted in a 1.5-fold increase in sulforaphane content when compared to the water control and a 4-fold increase when compared to the chlorine-sanitized control. Higher WUV doses (2.3 kJ m^{-2}) enhanced the content in sulforaphane by 2-fold when compared to the water control, and by 5.5-fold when compared to the chlorine control, but at expenses of an increase in exposure time, from 2 to 10 min. Although high UV-C doses have shown to enhance the glucosinolates content in broccoli, they can entail a reduction of the product quality throughout storage (Duarte-Sierra, 2015).

In addition, a 0.6- and 0.7-fold decrease was observed in the glucoraphanin content in the 0.5 and 2.3 kJ m^{-2} WUV-treated samples when compared to the water-washed samples. No reduction was observed after chlorine sanitation. The conversion of glucoraphanin into sulforaphane can be enhanced by modulating physical factors such as temperature, pressure and pH during processing due to the activation of myrosinase, the enzyme that catalyzes this reaction; in this way, positive results have been obtained with mild heat and high pressure treatments (Hanschen et al., 2017; Liu et al., 2017; Matusheski et al., 2004; Westphal et al., 2017). However, we found no reference regarding the effect of UV-C on this enzyme, which could explain the increase in the sulforaphane content in detriment of the glucoraphanin one. Thus, further studies on factors affecting the hydrolysis of glucosinolates would be helpful to clarify this issue. In others broccoli varieties, dry UV-C irradiation induced the content in several glucosinolates according to the dose, ranging from hormetic to high levels, and to the period of storage. For example, glucoraphanin levels increased in cv 'Everest' broccoli florets 24 h after treatment with 1.2 kJ m^{-2} UV; however no variation was obtained with a higher dose (3.6 kJ m^{-2}) (Nadeau, Gaudreau, Angers, & Arul, 2012). In cv 'Diplomat' broccoli florets, the application of 1.2 and 3 kJ m^{-2} UV-C enhanced the titers of glucoraphanin and reduced those

of glucobrassicin immediately after treatment, but afterwards the levels of the first one remained stable while those of the latter increased at 72 h (Duarte-Sierra, 2015). In Bimi® broccoli the increase of a glucoraphanin levels occurred 72 h after treatment with a higher UV-C dose (9 kJ m^{-2}) or with a UV-B + UV-C combination (9 + 15 kJ m^{-2}, respectively) (Formica-Oliveira et al., 2017). However, in those experiments, the quantification of sulforaphane was not included.

4. CONCLUSIONS

The results obtained suggested that 0.5 kJ m^{-2} is a hormetic effective dose for the decontamination of natural microbiota and the enhancement of the nutritional quality of fresh-cut conventional broccoli, enabling water, energy and time savings, which are relevant to an upscale level. This UV-C dose significantly preserved nutritional quality associated with antioxidant and glucosinolates content in conventional broccoli compared to water and chlorine treatments. Therefore, this is a promising and economic technology to preserve the microbiological, physicochemical and nutritional quality of fresh-cut conventional broccoli at an industrial level. Furthermore, sanitation using 0.5 kJ m^{-2} WUV in combination with 50 mg L^{-1} PAA was an effective strategy alternative to chlorine for reducing the potentially higher microbial load and the more varied native microbiota from organic broccoli, showing better efficacy than water-washing and WUV alone. However, current regulations do not allow the use of biocidal chemicals for organic produce. Therefore, the combination of WUV with mild heat treatments could be an alternative to be tested for this purpose.

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