Highlights

- A prototype of water-assisted UV-C light was tested for strawberry sanitization
- Quality and nutritional parameters studied did not overcome major changes
- Inoculated *L. innocua* and *S. enterica* were reduced at least 3 logs after WUV-C
- WUV-C was effective for washing water disinfection, enabling its recirculation
- Combined WUV-C and peracetic acid showed more efficacy than the separate treatments

Water UV-C treatment alone or in combination with peracetic acid: A technology to maintain safety and quality of strawberries.

Iolanda Nicolau-Lapeña[a], Maribel Abadias[b], Inmaculada Viñas [a]*, Gloria Bobo[b], Tomás Lafarga[b], Albert Ribas-Agustí[c], Ingrid Aguiló Aguayo[b]*

[a] Food Technology Department, University of Lleida, XaRTA-Postharvest, Agrotecnio Center, Rovira Roure 191, 25198 Lleida, Catalonia, Spain.

[b] IRTA, XaRTA-Postharvest, Parc Científic i Tecnològic Agroalimentari de Lleida, Parc de Gardeny, Edifici Fruitcentre, 25003, Lleida, Catalonia, Spain.

[c] IRTA, Food Safety Program, Finca Camps i Armet s/n, 17121 Monells, Spain.

[*]**Corresponding authors**:

Dr. I. Aguiló-Aguayo; Phone: +34 973003431; email: Ingrid.Aguilo@irta.cat

Prof. I. Viñas; Phone +34 973702677; email: inmaculada.vinas@udl.cat

Abbreviations:

DPPH·, 2,2-diphenyl-1-picrylhydrazyl; FRAP, ferric reducing antioxidant power; GAE, gallic acid equivalents; Hº, Hue angle; PA, peracetic acid; TA, titratable acidity; TAA, total ascorbic acid; TAM, total aerobic mesophylls; TCEP, 3,3′,3″-Phosphanetriyltripropanoic acid; TCD, total color difference; TPC, total phenolic content; TPTZ, 2,4,6-tris(2-pyridyl)-s-triazine; TSS, total soluble solids; UV-C, ultra violet light C; Y&M, yeasts and molds.

Abstract

Disinfection of fruits is one of the most important steps since they are going to be eaten fresh-or minimally-processed. This step affects quality, safety, and shelf-life of the product. Despite being a common sanitizer in the fruit industry, chlorine may react with organic matter leading to the formation of toxic by-products. Alternative sustainable disinfection strategies to chlorine are under study to minimize environmental and human health impact. Water-assisted UV-C light (WUV-C) is proposed here as an alternative sanitizing method for strawberries. In this study, strawberries were washed for 1 or 5 min in a tank with 2 or 4 lamps on, each emitting UV-C light at 17.2 W/cm^2, or in a chlorine solution (200 ppm, pH 6.5). Moreover, trials with 4 lamps on, together with a washing solution consisting on peracetic acid at 40 or 80 ppm, were carried out. Overall, quality and nutritional parameters of strawberries after treatments were maintained. Changes in color were not noticeable and fruits did not lose firmness. No major changes were observed in antioxidant activity, organic acid, anthocyanin, vitamin C, and total phenolic content. Yeasts and molds were not affected by the WUV-C treatment, and 5 min were needed to significantly reduce total aerobic mesophylls population. However, reductions of artificially inoculated *Listeria innocua* and *Salmonella* Typhimurium after WUV-C treatments were comparable to those obtained with chlorine-wash, which were 3.0 log CFU / g. Moreover, WUV-C light was effective to minimize microorganisms remaining in washing water, avoiding cross-contamination and thus, allowing water recirculation. This effect was improved when combining the action of UV-C light with peracetic acid, showing the suitability of this combined treatment, understood as an alternative to chlorine sanitation, for sanitizing strawberries and keeping the populations of pathogenic bacteria in washing water lower than 0.6 ± 0.1 log CFU / mL.

Keywords: sanitization, organic acids*, Listeria innocua, Salmonella* Typhimurium, UV-C light, fruit

1. Introduction

Strawberry (*Fragaria × ananassa*) production and consumption has practically doubled over the past 15 years (Indexbox, 2017), being. Strawberries are generally considered a safe product, and some authors reported the absence of pathogenic microorganisms in the sampled fruits (Ortiz-Solà et al., 2020). However, mold growth and loss of firmness of strawberries may cause losses of 10 % to 35 % at retail and at consumer level, respectively, making the control of alterative microbiota a challenge for fruit industry (Kelly et al., 2019). Moreover, berries including strawberries have been linked to safety issues associated with foodborne pathogens, such as *Salmonella* spp. and Norovirus (European Food Safety Authority, 2014) and *Listeria monocytogenes* (Hadjilouka et al., 2014). The reported problem is mostly related to frozen strawberries, that normally are washed and disinfected with chlorine before freezing.

Chlorine is a widespread sanitizer used as a water disinfectant to reduce pathogenic and other microbiota loads in fruits and vegetables. However, its dependence on a number of factors, including pH, concentration, and presence of organic matter (Chen and Hung, 2017) along with the health concerns associated with its toxic by-products, such as chloroform and other trihalomethanes, chloramines and haloacetic acids (Meireles et al., 2016), have led to a search for safer alternatives. Moreover, a recent regulation from the European Comission (Regulation Comission (EU) 2020/685 stablishes the limits of perchlorates, which are by-products of chlorine, in some foods specially in fruits and vegetables. A chemical alternative that does not leave any residue on the food and has been proposed in the literature is peracetic acid (PA), which is effective in decreasing the native microbiota and the pathogenic contamination of produce such as strawberries, without decreasing the quality of the fruits (Méndez-Galarraga et al., 2019; Nicolau-Lapeña et al., 2019; Van de Velde et al., 2014). Ultraviolet (UV) light has also been proposed due to its inexpensiveness, efficacy on pathogen inactivation and reduced unwanted physicochemical changes (Usaga et al., 2017). UV light is the portion of the electromagnetic spectrum with wavelengths ranging between 100 to 400 nm. Within this, the fraction that has the most germicidal effect is comprised between 100 and 280 nm, and it is known as UV-C light (Pigeot-Rémy et al., 2012). Its mode of action consists of UV absorption by DNA and RNA, which in turn, origins the formation of cyclobutene-pyrimidine and pyrimidine-pyrimidone dimers, blocking the elongation of nucleic acid transcripts (Seltsam and Müller, 2011). This blocks and compromises cellular functions and replication, causing the eventual cell death (Barba et al., 2017).

This study evaluates the role of an emerging technology consisting in UV-C light transmitted by lamps immersed in stirring water (WUV-C), as an alternative to chlorine disinfection for strawberries. This approach could overcome some of the drawbacks of air-transmitted UV-C light. Shallow penetration ability, sample heating and shadowing effect of UV treatment limit its application in decontamination of fresh produce (Liu et al., 2015). Agitation of fruits conveyed by water could serve to prevent shadowing effect, which happens with static fruits. Otherwise, if the strawberries were agitated in a dry surface, an increase of mechanical damages would occur. Moreover, water may enhance the removal of microorganisms from rough surfaces or hidden in trichomes. There are studies in which UV-C irradiation assisted by water has already been used to disinfect fresh produce (Guo et al., 2017). The same WUV-C device used in this study was tested in vegetables in other investigations (Collazo et al., 2018), but to the best of authors' knowledge, this is the first approach to disinfect strawberries in a system where lamps and fruits are immersed in water.

The objective of this study was to evaluate the efficacy of this WUV-C system as a sanitizer treatment for strawberries, for both epiphytic microbiota and artificially inoculated *Listeria innocua* and *Salmonella* Typhimurium. Its efficacy was compared to that of chlorine, in order to evaluate WUV-C as a potential alternative to this well stablished sanitation method. Moreover, microorganisms that could remain in water were investigated in order to see the efficacy of UV-C lamps in sanitizing washing water, in order to prevent cross-contamination. The effect on the quality and nutritional parameters was also investigated, so as to provide a product that meets consumers' needs. Once verified the UV-C sanitizing effect and its impact on strawberry quality, this method was combined with the use of peracetic acid in solution with the washing water, so as to improve the results obtained with only UV-C irradiation while being able to diminish the washing time.

2. Materials and methods

o Materials

Strawberries (*Fragaria x ananassa*), had been harvested in Huelva between March and May in the 2019 campaign and transported and kept in a cold store. They were bought in a local supermarket the same day of the beginning of the experiment. Before the treatment, peduncle and leaves were carefully removed.

Triptone soy broth (TSB), triptone soy agar (TSA), Palcam base agar and Palcam selective supplement for *Listeria*, xilose lysine deoxycholate agar (XLD), yeast extract, plate count agar (PCA), dichloran rose bengale chloramphenicol agar (DRBC), and peptone were obtained from Biokar Diagnostics (Allonne, France). Dey-Engley broth was obtained from Honeywell Fluka (Madrid, Spain). Peracetic acid (PA) 15 % was purchased from Panreac AppliChem (Barcelona, Spain).

Ascorbic, gallic, quinic, malic, citric, tartaric and fumaric acids, 2,4,6-tris(2-pyridyl)-s-triazine (TPTZ), 2,2-diphenyl-1-picrylhydrazyl (DPPH), sodium carbonate, metaphosphoric acid, acetic acid, 3,3′,3″-phosphanetriyltripropanoic acid (TCEP), were acquired from Sigma-Aldrich (Steinheim, Germany). Catechin, cinnamic acid, coumaric acid, quercetin and kaempferol standards were obtained from Merck (Darmstadt, Germany). Pelargonidin standard was purchased from Extrasynthese (Genay, France). Methanol, acetone, chlorhidric acid (37 %), sodium acetate, sodium hydroxide, sodium chloride, potassium chloride, ferric chloride hexahydrate and Folin Ciocalteau's reagent were procured by Panreac (Llinars del Vallès, Spain).

o UV-C water-assisted equipment

Treatments were conducted in the UV-C water-assisted (WUV-C) equipment LAB-UVC-Gama (UVC-Consulting Peschl España, Castellón, Spain) (Figure 1 A and Figure 1 B). This apparatus consists of a tank where 4 UV-C lamps (GPH303T5L/4, 254 nm) are installed, emitting a power of 17.2 W each. The lamps are enclosed by a quartz tube (25 mm of outer diameter) to prevent the lamp's contact with the wash water and product. The equipment has a recirculating system connected to a water pump and an aeration system that provides bubbling, which improves accessibility to UV-C light from all sides of the fruit. Radiation, according to the simulation and calculations given by the manufacturer, is distributed inside the empty tank as shown in Figure 1 C.

Before the experiment, lamps were preheated for 10 min, to reach the maximum irradiance at the start point of washing treatments. After this time, irradiance values in the empty tank were 5.8 and 10.5 W/cm^2 with

2 and 4 lamps on, respectively, measured with a UV-sensor Easy H1 (Peschl Ultraviolet, Mainz, Germany) through an orifice located on the lid of the tank. Afterwards, the WUV-C tank was filled with 12 L of cold (6±2 °C) water and the UV-C lights were switched on for 12-15 min. Absorbance at 254 nm of the water inside the tank was measured spectrofotometrically using a GENESYS™ 10S UV–Vis spectrophotometer (Thermo Fisher Scientific, MA, USA). Turbidity was measured using a portable turbidimeter (TN-100, Eutech, Singapore) measuring in Nephelometric Turbidity Units (NTU). These measures were taken before and after each treatment, to check the ability of UV-C light to be transmitted in this media.

- **Washing treatments**

For each treatment, 20 strawberries – average weight 25 ± 5 g – were immersed in 12 L of cold (6 ± 2 °C) tap water in agitation. Experiments, with 3 repetitions each, were performed separately for non-inoculated (one replication, n=3) and inoculated (two replications, n=6) fruits. First, effect of WUV-C irradiation alone was studied. Four WUV-C treatments were proposed, combining 2 or 4 lamps on with different contact times: 1 or 5 min: 2 lamps on for 1 min (2L-1 min), 2 lamps on for 5 min (2L-5 min), 4 lamps on for 1 min (4L-1 min), and 4 on lamps for 5 min (4L-5 min). Then, the best WUV-C approach was selected for its combination with PA. For this second trial, five treatments were proposed: WUV-C alone (WUV), PA at 40 ppm (PA 40) or 80 ppm (PA 80) alone, and WUV-C with PA 40 ppm or 80 ppm (WUV + PA40 or WUV + PA 80). A 200 mg/L of free chlorine solution, adjusted to pH 6.5 using citric acid 2 M (NaOCl) was used as a reference to compare the efficacy of WUV-C treatments with that of chlorine, to check if the proposed UV-C method can be a good alternative to it. After NaOCl disinfection, strawberries were rinsed in tap water. Moreover, a tap water control was added when performing the microbial trials to determine the removal of the bacteria due to a physical effect. Water parameters including pH and oxidation-reduction potential (ORP) were measured before and after each treatment. ORP and pH were measured in a pH-meter (GLP22, Crison, Alella, Barcelona, Spain) equipped with a pH probe (ref. 52-03, Crison) or ORP probe (ref.62-51 Hach, Vésenaz, Geneva), respectively.

After the washing, fruits were let at room temperature to drain the excess of water.

- **Effect of WUV-C treatment on the quality of strawberries**

The trial to study quality and nutritional parameters was performed once, with 3 replications (n=3) in non-inoculated strawberries. Determinations on fresh-product were carried out just after the draining period, and aliquots of each treatment were frozen with liquid nitrogen, milled using a MINIMOKA GR-020

158 grinder (Taurus Group, Barcelona, Spain) and stored at -80 °C for further biochemical analysis. Finally, the
159 remaining strawberries were stored at 4 ± 1 °C for 24 h, and they were and analyzed or frozen after this
160 time.

161 ▪ **Quality analysis**

162 For determining **pH, total soluble solids** and **titratable acidity**, strawberries were smashed in a blender to
163 prepare 25 mL of juice. Each parameter was evaluated twice for each repetition (three repetitions) according
164 to Nicolau-Lapeña et al. (2019).

165 **Color** of 10 strawberries was measured on 3 sides, using a CR-200 Minolta Chrome Meter (Minolta, INC.,
166 Tokyo, Japan) with a D65 illuminant and 10° observer angle. The instrument was calibrated using a
167 standard white reflector plate. Color was expressed as CIE L* a* b* coordinates. Total color difference
168 (TCD) (Eq. 1), and Hue angle (H°) (Eq.2) were calculated.

169 $$TCD = [(L^*_f - L^*_i)^2 + (a^*_f - a^*_i)^2 + (b^*_f - b^*_i)^2]^{1/2} \quad \text{Eq. 1}$$

170 Where f = final (strawberries after each treatment) and i = initial (strawberries before any treatment)

171 $$H° = \tan^{-1}(b^* / a^*) \quad \text{Eq. 2}$$

172 **Texture** changes were evaluated on 10 strawberries halves for treatment, that were cut immediately before
173 the determination. Two textural tests using the TA.XT Plus Connect texture analyzer (Stable Micro systems
174 Ltd., Surrey, England) were performed. In the compression test, the maximum force required by 2 parallel
175 plates to compress 6.0 mm a strawberry half was recorded. Puncture test was performed with a 4 mm
176 cylindrical probe, measuring the maximum force encountered when the probe enters 8.0 mm deep into the
177 tissue. Both tests were run at 5 mm/s speed with a trigger force of 0.1 N.

178 ▪ **Biochemical analysis**

179 **Antioxidant activity** of strawberries was assessed by ferric reducing antioxidant power (FRAP) and DPPH
180 scavenging activity assays, as described in Nicolau-Lapeña et al. (2019). Results are expressed as μmol
181 ascorbic acid equivalents (AAE) / 100 g FW of 3 repetitions (n=3).

182 Content of **organic acids**, including tartaric, malic, fumaric, citric and quinic acid was determined by high-
183 performance liquid chromatography (HPLC) in a Waters 717 plus Autosampler HPLC system (Waters
184 Corp., NJ, USA) coupled to a UV detector, following the method described by Scherer et al. (2012) with
185 minor changes. Duplicate injections were performed, and average peak areas were used for quantification

(n=2). Concentrations of organic acids in samples were calculated by the area interpolation on the adequate calibration curve.

Vitamin C contents expressed as the sum of ascorbic acid and dehydroascorbic acid (TAA), was determined by high-performance liquid chromatography (HPLC) in a Waters 717 plus Autosampler HPLC system (Waters Corp., NJ, USA) coupled to a UV detector, following the method described by (Lafarga et al., 2018). Average peak areas of duplicate injections were used for quantification (n=2). Concentration of vitamin C, expressed as mg TAA / 100 g FW, was calculated by the area interpolation on the adequate calibration curve.

Anthocyanin extracts and quantification were carried out in triplicate (n=3) according to the method described by Meyers, Watkins, Pritts, and Liu (2003). Anthocyanin content was expressed as mg of cianidine-3-glucosyde / 100 g of strawberry.

The **total phenolic content** (TPC) was assessed by Folin Ciocalteau method on the same extract used for antioxidant activity determination, following the procedure described by Nicolau-Lapeña et al. (2019). Results were expressed as mg gallic acid equivalents (GAE) / 100 g FW of 3 repetitions (n=3)

For a **phenolic profile**, extracts of phenolic were analyzed according to (Aaby, et al, 2012; da Silva, et al., 2007) with minor modifications, on an Acquity UPLC system equipped with a diode array detector (DAD) (Waters, Milford, MA, USA). The peaks were tentatively identified according to chromatographic data from literature (Aaby, et al, 2012; da Silva, et al., 2007) and quantified by DAD detection and external calibration curves with pure standards.

- **Microbiological quality**

The effect of the washing treatments on total aerobic mesophylls (TAM) and yeasts and molds (Y&M) was evaluated. For this, 25 g per repetition (n=3), taken from pieces of 2 strawberries to ensure representativity, were diluted 1:4 in peptone buffered solution. The count process followed the method described in (Nicolau-Lapeña et al., 2019). Results were expressed as log CFU / g, and the detection limit was 20 CFU / g.

Remaining populations of, TAM and Y&M were also determined in wash water. Results were expressed as log CFU / mL. When counts were below the limit of detection (5 CFU / mL), and presence was confirmed by Dey-Engley color change, an arbitrary value of ½ limit of detection was assigned.

- **Effect of W-UVC system in the survival of *Listeria innocua* and *Salmonella* Typhimurium artificially inoculated on strawberries.**

  - **Strains and strawberries inoculation**

Listeria innocua strain CECT-940 (*Colección Española de Cultivos Tipo*, Burjassot, Spain) was used as a surrogate of *L. monocytogenes* in this study (Francis and O' Beirne, 1997). *Salmonella enterica* subs. *enterica* serovar Typhimurium CECT-4594 was also used to inoculate strawberries. Cultures were prepared as described in Nicolau-Lapeña et al. (2019).

The day before the experiment, strawberries designated for this purpose were inoculated with a suspension containing 10^{10} CFU / mL of *L. innocua* or *S.* Typhimurium at stationary phase, by pipetting 50 µL in small droplets on the surface. Once dried, strawberries were stored at 4 ± 1 ºC overnight. Concentration immediately after the inoculation and drying, and also after storage was checked by plating in duplicate in selective Palcam or XLD media for *L. innocua* and *S. Typhimurium*, respectively.

Washing treatments were performedº as indicated in section 2.3. The experiment was repeated twice.

  - **Determination of *L. innocua* and *S.* Typhimurium populations**

One strawberry per repetition was used for microbiological analysis (n=6). Populations were determined by plate count on selective Palcam medium for *L. innocua* or XLD for *S.* Typhimurium in duplicate, as it has been previously described in Nicolau-Lapeña et al. (2019). Results were expressed as log CFU / fruit, and detection limit was 20 CFU / fruit.

Logarithmic reductions of the pathogens were calculating by the following equation (Eq. 1)

$$\text{Log reductions (Log cfu/fruit)} = \text{Log}_{10}(\bar{N}_0) - \text{Log}_{10}(N_t) \qquad \text{Eq. 1}$$

Where $\bar{N}_0$ is the mean of the initial population (CFU / fruit), and N_t is the population after the washing treatment (CFU / fruit).

Remaining populations of *L. innocua* and *S.* Typhimurium were determined in wash water. Duplicate 1-mL samples of wash water after treatment were neutralized in 9 mL Dey-Engley medium. Results were expressed as log CFU / mL. When counts were below the limit of detection (50 CFU / mL), and presence was confirmed by Dey-Engley color change, an arbitrary value of ½ limit of detection was assigned.

2.6. Statistical analysis

241 All data were checked for significant differences by applying analysis of variance test (ANOVA). The
242 criterion for statistical significance was $p < 0.05$. When significant differences were observed, Tukey's
243 Honest Significant Difference (HSD) of the means was applied. Principal components analysis (PCA) was
244 carried out to obtain correlations among phenolic profile of strawberries. All statistical analyses were
245 carried on using JMP 13 (SAS Institute Inc., Cary, USA).

3. Results and discussion

o Properties of washing water

Water used to wash strawberries was controlled during each treatment. For those treatments with no chemical solution (control or WUV-C), pH values were 7.95 ± 0.22, while for those with PA 40, PA 80 ppm or NaOCl, pH values were, 5.79 ± 0.21, 4.64 ± 0.08, or 6.56 ± 0.11, respectively. For the same conditions, ORP values were 256 ± 22 (control or WUV-C), 444 ± 16 (PA 40 ppm), 504 ± 3 (PA 80 ppm), and 891 ± 4 (NaOCl). Washing treatments were carried out at 7.5 ± 0.5 ºC. To check that dispersion of radiation through water was not reduced by any turbidity of the media, turbidity and absorbance at 254 nm were measured. For all treatments, turbidity values were 0.9 ± 0.2 NTU, and absorbance was 0.073 ± 0.035, indicating no interference of irradiation caused by presence of particles or dirt in water.

o Quality changes in strawberries

▪ Physicochemical quality

Physicochemical quality of non-washed strawberries indicated that samples had a pH of 3.57 ± 0.07, TSS values of 6.60 ± 0.01 °Brix and TA of 6.12 ± 1.87 mg citric acid / L juice (Data not shown). When strawberries were washed, pH statistically decreased by approximately 0.2 points, reaching 3.21 ± 0.04 when NaOCl was used in water (Table 1). This treatment also showed the highest TA, reaching a concentration of 8.07 ± 0.70 mg citric acid / L juice. This was not attributed to residual chlorine on the surface of strawberries, because an additional washing step with water was added to remove any residue after this treatment. Although statistically significant differences were observed on pH, TSS and TA values, a general tendency was not detected, and changes could not be attributed to WUV-C doses or times.

▪ Color and texture

Color of strawberries, expressed as CIE L*a*b* coordinates, was L* of 43.5 ± 4.8, a* of 32.9 ± 0.3 and b* 29.1 ± 8.5 (see Supplementary material). The higher L* and b* values than those reported by other authors (Kelly et al., 2019) mean a greater luminosity and yellowish color of the strawberries of this study. There were no statistical differences in L*a*b* coordinates nor in H°, which was on average 40.92 ± 0.01, between the treatments. In strawberries, color is highly correlated with the anthocyanin content. When washed with NaOCl or 4L-1 min, TCD reached the highest values of 3.1 and 3.0, but in all cases, TCD was lower than 3.5 which, according to Mokrzycki and Tatol (2011), would not be noticed by the inexperienced viewer.

No changes in color were also reported by Liu et al. (2014), after applying 4,1 kJ/m² UV-C irradiation (in air) on strawberries.

Firmness of strawberries before and after the washing treatments was assessed by compression and pricking tests. There were no significant statistical differences in firmness immediately after the WUV-C washings, neither between the treatments or when compared to the NaOCl treatment. Average compression force was 44.6 ± 3.1 N and pricking test results were 3.6 ± 0.3 N (see Supplementary material). The preservation of firmness is important in strawberries to maintain quality through all the supply chain steps, as soft fruits are more likely to mechanical damage and waste at consumer level (Kelly et al., 2019). Liu et al. (2014) also reported no changes in strawberry firmness after WUV-C irradiation.

- **Biochemical characterization**

  - **Antioxidant activities**

Antioxidant activity of strawberries, washed or not with WUV-C, was assessed by FRAP and DPPH· free radical scavenging ability assays.

Initial antioxidant values were 797 ± 46 and 608 ± 8 μmol AAE / 100 g FW for FRAP and DPPH· assays, respectively (Data not shown). These values were in accordance with those reported in literature for 90 different strawberry cultivars (Nowicka et al., 2019). Antioxidant activity in fruits was maintained whether WUV-C was applied or not with no significant differences observed among treatment conditions (Data not shown).

  - **Acid organic contents**

Content of organic acids was determined in both WUV-C light treated and non-treated strawberries, immediately after the treatment and 24 h later (Figure 2). Initial amounts of quinic, malic, citric, tartaric and fumaric acids in strawberries were 126.6 ± 13.5, 18.5 ± 1.8, 820.5 ± 45.9, 155.0 ± 15.7 and 2.4 g / 100 g FW, respectively. No significant differences were detected in quinic and tartaric acids values between WUV-C treatments. For the other acids, changes did not show a clear tendency related with treatment times or WUV-C light dose. This independent variation has also been reported in cucumber treated with UV-C light at 8.2 W/m^2 for 1, 5 or 10 min (Erkan et al., 2001). In general, all the treatments showed the same trend in organic acid content after 24 h. The only exception was observed after applying 2L-5 min treatment to strawberries, which showed an inverse progression when compared to the other treatments of the same

acid. As far as we know, there is no study in the literature reporting the evolution of organic acids in fruit matrices depending on WUV-C treatment.

- **Vitamin C content**

Vitamin C, expressed as the sum of ascorbic acid and dehydroascorbic acid (TAA), was determined in fresh strawberries. Initial values were of 29.8 ± 1.0 mg TAA / 100 g FW (Data not shown), and immediately after the WUV-C treatments (Table 2), no statistical differences were observed when compared to NaOCl washing. A decrease in AA has been accounted (Gopisetty et al., 2018), explained by induced molecular excitation and subsequent photochemical reactions. In contrast, no changes were reported by Allende et al. (2007) when irradiating strawberries at doses of 0.28 kJ/m^2. In this study, after 24 h, TAA slightly increased in all the treatments and in the control. A similar increase was also reported by Jagadeesh et al. (2011), who applied UV-C doses of 3.7 kJ/m^2 to tomatoes, and AA increased throughout storage time.

- **Anthocyanin contents**

Initial anthocyanin content of strawberries was 12.5 ± 0.4 mg / kg FW (Data not shown). Maximum values of 22.2 ± 1.0 mg / kg FW were achieved after 2L-5 min WUV-C washing (Table 2). As has been reported by other authors (Shenget al., 2018), phenylalanine ammonia lyase (PAL) expression and activity is enhanced when hormetic doses of UV-C light are applied, suggesting a possible further increase in anthocyanins after irradiation. Nevertheless, no significant differences were found in this study, neither immediately after washing nor after 24 h of treatment. UV-C light, did not increase anthocyanin content after 1 day, comparably to Li et al. (2014) results, who irradiated strawberries with 4.1 kJ/m^2 UV-C and did not found any change on anthocyanin content.

- **TPC and phenolic profile**

Strawberries processed in this study had an initial TPC value of 113.2 ± 11.8 mg GAE / 100 g FW (Data not shown), which are in agreement with the literature (Tarola et al., 2013) and no significant differences were observed in TPC values among the treatments or within days (Table 2). WUV-C light, could induce an accumulation of phenolics during storage, as it may trigger the accumulation of WUV-C light absorbing flavonoids and other phenolic compounds (Mditshwa et al., 2017).

Xu et al. (2017) used different UV-C light doses and found an increment of 25 to 75 % of the TPC, namely cyanidin 3- glucoside, pelargonidin 3-glucoside or ellagic acid. Predominant compounds were pelargonidin derivatives, which give color to strawberries, followed by kaempferol derivatives (Table 3 and

Supplementary material), which was in accordance to (Aaby et al., 2012). No direct relationship has been found between WUV-C doses and changes in phenolic profile in the present study. To a better understanding of the variations in phenolic profile, a study of the effect of UV-C doses on enzymes related to the flavonoids and the shikimate pathway would be worth to be carried out (Tomás-Barberán and Espín, 2001).

- **Effect of WUV-C on microbial load of strawberries and wash water**

Total aerobic mesophylls (TAM) and yeasts and molds (Y&M) initial population in **strawberries** were 4.3 ± 0.3 and 4.0 ± 0.3 log CFU / g, respectively (Figure 3 A). Washing processes with 2 or 4 lamps for 5 min were needed to significantly reduce these populations in strawberry. TAM counts after 2L-5 min and 4L-5 min UV-C doses were reduced by 1.8 ± 0.4 and 1.5 ± 0.6 log CFU / g, respectively, which were equivalent to the NaOCl counts. Y&M population was maintained after all treatments except for NaOCl and 4L-5 min, in which the decrease was 1.8 ± 0.8 and 1.2 ± 0.5 log CFU / g, respectively. These results are in dissonance with those published by Collazo et al. (2018), where the highest reduction of spoilage microorganisms in broccoli did not correlate to higher WUV-C dose, applied with the same equipment. Variability in fruit or vegetable surface may be a factor that influences bacterial attachment and further removal, due to surface roughness, hydrophobicity, and presence of trichomes (Adhikari et al., 2015). Moreover, complexity and predominance of certain genres and species above others may lead to a diverse susceptibility mechanisms to UV-C light (Kim et al., 2018).

In **washing water**, population of TAM and Y&M was 3.6 ± 0.1 and 3.1 ± 0.1, log CFU / mL, respectively, in control water without NaOCl nor WUV-C light (Figure 3 B). This load could be attributed to the transference of the microorganisms from fruit surface to water due to physical action of water pressure, agitation and aeration (bubbles), explaining the reduction of microbial load in strawberries in water control, as detailed above. The most effective treatment – whose reductions comparable to the NaOCl washing – against TAM and Y&M in water was 4L-5min, which in the end, remained in water 0.2 ± 0.1 and 0.4 ± 0.1 log CFU / mL, respectively.

- **Effect of WUV-C on *L. innocua* and *S.* Typhimurium in strawberries and wash water**

Regarding pathogenic bacteria, initial *L. innocua* and *S.* Typhimurium populations in artificially inoculated **strawberries** were 6.4×10^6 and 1.7×10^7 CFU / strawberry, respectively (Data not shown). After storage at 4 ºC for 22 ± 2 h, *L. innocua* populations were maintained, while *S*. Typhimurium populations in

strawberry decreased 1 log unit. WUV-C washing procedures did not differ statistically in reductions, having counts decreased 4.5 ± 0.3 and 3.7 ± 0.5 log CFU / strawberry for *L. innocua* and *S.* Typhimurium, respectively (Figure 4 A). Moreover, reductions after washing treatments with UV-C light were similar to those after NaOCl washing, which were 3.0 ± 1.2 and 4.9 ± 0.6 log CFU / strawberry of *L. innocua* and *S.* Typhimurium, respectively. Only when 4 lamps were on, reductions of *L. innocua* were over 2 log higher from those obtained with the water control, which were 2.4 ± 0.9 log CFU / fruit. Regarding *S.* Typhimurium, no treatment, except for NaOCl, achieved statistically more reductions than control. In our study, water-assisted UV-C light acted as an effective disinfectant method whose effects could be comparable with those obtained with NaOCl at the same doses used in the food industry. In fact, its effect has been demonstrated in a number of foodborne and spoilage microorganisms, including *E. coli*, *Salmonella* Typhi, *Shigella sonnei*, *Streptococcus faecalis*, *Staphylococcus aureus* and *Bacillus subtilis* (Chang et al., 1985), *L. monocytogenes* and *Clostridium sakazakii* (Cebrián et al., 2016), in different extents. In contrast, Collazo et al. (2019) did not achieve effective inactivation for either of the pathogens studied, *L. monocytogenes* and *S. enterica*, in baby spinach leaves, when applied a 0.5 kJ/m^2 dose. Butot et al. (2018) reported no more than 1 log reduction on artificially inoculated blueberries, raspberries or strawberries with *L. monocytogenes*, *E. coli* O157:H7 and *S. enterica*. This trial was conducted with a UV-C device using intensities ranging from 2.12 to 13.31 kJ/m^2, but it was not water-assisted. In fact, water-assisted UV-C light has been more successful in reducing pathogenic loads on fruits, and this could be attributed to the higher efficacy of the water-assisted procedure, which may have overcome the limitations of UV-C light transmitted by air, such as shadowing effect (Liu et al., 2015). Water agitation can also be helpful in removing bacteria that would otherwise be lodged in trichomes or cracks (Butot et al., 2018). .

In **washing water**, counts of *L. innocua* and *S.* Typhimurium in control water without NaOCl and WUV-C light reached 4.2 ± 0.2 and 4.1 ± 0.2 log CFU / mL, respectively (Figure 4 B). *L. innocua* population in wash water after WUV-C treatments and NaOCl sanitization were not statistically different, and this microorganism persisted in concentrations of 0.2 ± 0.2 log CFU / mL in all cases except for 2L-1 min, which was 1.00 ± 0.9 log CFU / mL. Counts of *S.* Typhimurium in water did not differ between WUV-C treatments or NaOCl, averaging 0.2 ± 0.2 log CFU / mL. Indeed, UV-C irradiation has been widely used as a non-thermal method of disinfecting drinking, waste and recreational water (Beck et al., 2015) and its effectiveness has been already demonstrated in disinfecting liquid matrices of different natures (Gunter-Ward et al., 2018; Jeon and Ha, 2018). Minimizing microbial load in washing water is crucial in the fruit

industry, to prevent cross-contamination when it is reused in the process. The amount of wastewater generated per mass unit of product depends on the disinfection technique employed, so being UV-C irradiation capable of disinfecting efficiently both the process water and the product, a higher ratio of recycling can be achieved, with a lower impact on the environment (Kretzschmar, 2009).

- **Efficacy of WUV-C combined with PA on the reduction of pathogens artificially inoculated on strawberries**

To combine UV-C irradiation with the use of PA, treatments with 4 lamps were chosen. One of the reasons was because in some cases, higher reductions of microorganisms in strawberry and in water were observed with this dose. The other, is that in the trials with PA there was the intention to maximize the UV-C irradiation, to reduce treatment time in order to avoid mechanical damages that could affect strawberries during commercialization. According to previous studies of the research group (Nicolau-Lapeña et al., 2019), 2 min washing with PA at concentrations of 40 and 80 ppm were needed to exert any significant change in disinfection of strawberries, so these concentrations and time were selected to this optimization trial. It was assumed that if 2 min washing with PA was effective, the time could be decreased from 5 min to 2 min. Moreover, an additive effect was expected when using PA in combination with the UV-C lamps; so that the concentration of PA at 40 ppm with UV-C irradiation could equal the effect of 80 ppm PA without UV-C application. As Regarding strawberry quality, nutritional parameters and biochemical characterization immediately after the washing treatments, no substantial changes attributed to WUV-C irradiation or PA were observed in this or previous studies (Nicolau-Lapeña et al., 2019). Considering this, determinations of parameters other than the two pathogens counts were not carried out in the combination of WUV-C and PA trials.

In **strawberries**, the initial load of artificially inoculated *L. innocua* and *S.* Typhimurium was 6.3×10^7 and 1.7×10^7 CFU / strawberry (Data not shown). *L. innocua* was reduced 2.4 ± 0.8 and 4.3 ± 1.0 log CFU / strawberry, and *S*. Typhimurium 2.4 ± 0.1 and 4.7 ± 0.1 log CFU / strawberry, by control and by NaOCl washing, respectively (Figure 5 A). Reductions caused by WUV, PA 40 or PA 80 alone, even though similar to NaOCl values, were no statistically different from control. However, reductions after treatments with the combinations, WUV + PA 80 for *L. innocua*, and WUV + PA 40 for both pathogens, were statistically higher than control and comparable to NaClO. These results contrast with those obtained by Collazo et al. (2019) in lettuce or spinach leaves inoculated with *L. monocytogenes* or *S*. Typhimurium. They explained that the lack of synergistic effect could be related to the ability pathogens of interacting with the plant-

associated microbiota, or to their internalization and attachment to the plant tissue during overnight incubation, which could have reduced the accessibility of UV-C and PAA or led to induced resistance of bacteria against antimicrobial mechanisms. In this paper, combination of both mechanisms, physical and chemical, showed improved results than their separate applications. One possible reason could be that the attachment of the pathogens to the fruit surface is different than in the leaves surface, or that the distribution in the tank of both products may differ due their structural differences.

The efficacy of the combination of WUV-C with PA at two different concentrations was also studied in the **wash water after treatments**, focusing on the counts of the viable pathogens. After washing with water alone (control) 4.4 ± 0.5 and 4.4 ± 0.3 log CFU / mL of *L. innocua* and *S*. Typhimurium remained in water (Figure 5 B). Therefore, even the water wash was as effective as the other treatments evaluated, which could be attributed to the mechanical effect caused by water agitation that drags the microorganisms from the surface of the fruit to the water, pathogenic microorganisms remained in the water, allowing cross-contamination. As expected, NaOCl achieved absence of pathogens in washing water. *S*. Typhimurium remained at concentrations of 0.4 ± 0.1 log CFU / mL in water after all the other treatments. Meanwhile, *L. innocua* was not detected in WUV + PA 40, WUV + PA 80 and PA 80, in contrast with 0.6 ± 0.3 and 0.4 ± 0.1 log CFU / mL found in WUV and in PA 40, thus meaning and improvement in the efficacy of the combination of WUV + PA compared to those treatments applied separately. In fact, this combination has been studied for the disinfection of *E. coli*, *Enterococcus* spp., somatic coliphage, and *Cryptosporidium parvum* in waste water, and a pilot plant to escalate this application has been set up recently (Hassaballah et al., 2019, Hassaballah et al., 2020), showing promising outcomes. But it should be pointed out that, the fact that some bacteria may remain viable in the washing water must be controlled. Recirculation of this water could constitute a problem if incoming strawberries were contaminated. The potential of the lamps permanently irradiating the recirculated water should also be assessed, in order to verify if that could be reused safely after more exposure time to UV-C light.

4. Conclusions

The results of this study indicate that water-assisted UV-C light disinfection has been useful in reducing artificially inoculated *L. innocua* and *S.* Typhimurium in strawberries. Reductions could be comparable to those obtained when using a standard sodium hypochlorite treatment. WUV-C light helped minimizing remaining population of both, pathogenic and spoilage microorganisms in washing water. It would make this technique suitable for its use in the washing step in fruit industry to allow water recirculation and reduce the risk of cross-contamination. In general, the innovative WUV-C treatment evaluated did not affect physicochemical and nutritional quality of strawberries.

In order to improve the effectiveness of the WUV-C light in strawberry sanitization, this water-assisted system can be combined with other substances that can be solved in water, such as organic acids or essential oils, or with other technologies not involving high temperatures, such as ultrasound application. In this study, PA was combined with the use of WUV-C light. In this case, WUV-C light combined with PA at 40 ppm for 2 min proved to be effective in the disinfection strawberries and their washing water, with results comparable to those obtained with chlorine. This treatment allowed the reduction of treatment time from 5 to 2 min. It is important to note that, even though this study has shown the potential of UV-C light assisted by water, and the suitability of combining this treatment with PA, it was carried out at lab scale (using a low proportion strawberries:water). More studies should be carried out in order to determine the feasibility to scale up this disinfection procedure and its suitability for the actual fruit industry conditions. Normally, strawberries that will be sold in the fresh market are not washed to prevent further softening and mold growth (because these fruits are a delicate product). In this paper, only immediate effect of the proposed UV-C technique has been determined. For this, further investigations will be focused on the shelf-life of strawberries, both fresh and frozen. Also, more studies will be needed to study possible synergistic effects of combined methodologies.

Acknowledgements

The authors would like to thank M. Anguera and S. Villaró for their technical support.

Funding

The authors are thankful to 'Ministerio de Educación, Economía y Cultura' for the financial support of the project AGL2016-78086-R. I. Nicolau-Lapeña is in receipt of a predoctoral grant awarded by the 'Ministerio Español de Economía, Industria y Competitivdad' (grant number BES-2017-079779). Dr. I. Aguiló-Aguayo thanks the National Programme for the Promotion of Talent and Its Employability of the 'Ministerio de Economía, Industria y Competitividad' of the Spanish Government and the European Social Fund for her Postdoctoral Senior Grant 'Ramon y Cajal' (RYC-2016-2019 949).

Conflicts of interest

The authors declare no conflict of interests.

References

Aaby, K., Mazur, S., Nes, A., & Skrede, G. (2012). Phenolic compounds in strawberry (Fragaria × ananassa Duch.) fruits: Composition in 27 cultivars and changes during ripening. *Food Chem*, 132(1), 86-97. https://doi.org/10.1016/j.foodchem.2011.10.037

Adhikari, A., Syamaladevi, R. M., Killinger, K., & Sablani, S. S. (2015). Ultraviolet-C light inactivation of *Escherichia coli* O157: H7 and *Listeria monocytogenes* on organic fruit surfaces. *Int J Food Microbiol*, 210, 136–142. https://doi.org/10.1016/j.ijfoodmicro.2015.06.018

Alexandre, E. M. C., Santos-Pedro, D. M., Brandão, T. R. S., & Silva, C. L. M. (2011). Study on thermosonication and ultraviolet radiation processes as an alternative to blanching for some fruits and vegetables. *Food Bioproc Tech*, 4(6), 1012–1019. https://doi.org/10.1007/s11947-011-0540-8

Allende, A., Marín, A., Buendía, B., Tomás-Barberán, F., & Gil, M. I. (2007). Impact of combined postharvest treatments (UV-C light, gaseous O_3, superatmospheric O_2 and high CO_2) on health promoting compounds and shelf-life of strawberries. *Postharvest Biol Tec*, 46(3), 201–211. https://doi.org/10.1016/j.postharvbio.2007.05.007

Avalos-Llano, K. R., Martín-Belloso, O., & Soliva-Fortuny, R. (2018). Effect of pulsed light treatments on quality and antioxidant properties of fresh-cut strawberries. *Food Chem*, *264*(May), 393–400. https://doi.org/10.1016/j.foodchem.2018.05.028

Barba, F. J., Koubaa, M., do Prado-Silva, L., Orlien, V., & Sant'Ana, A. de S. (2017). Mild processing applied to the inactivation of the main foodborne bacterial pathogens: A review. *Trends Food Sci Technol*, 66, 20–35. https://doi.org/10.1016/j.tifs.2017.05.011

Beck, S. E., Wright, H. B., Hargy, T. M., Larason, T. C., & Linden, K. G. (2015). Action spectra for validation of pathogen disinfection in medium-pressure ultraviolet (UV) systems. *Water Res*, 70, 27–37. https://doi.org/10.1016/j.watres.2014.11.028

Bursać Kovačević, D., Putnik, P., Dragović-Uzelac, V., Vahčić, N., Babojelić, M. S., & Levaj, B. (2015). Influences of organically and conventionally grown strawberry cultivars on anthocyanins content and color in purees and low-sugar jams. *Food Chem*, 181, 94–100. https://doi.org/10.1016/j.foodchem.2015.02.063

508 Butot, S., Cantergiani, F., Moser, M., Jean, J., Lima, A., Michot, L., Putallaz, T., Stroheker, T., & Zuber,
509 S. (2018). UV-C inactivation of foodborne bacterial and viral pathogens and surrogates on fresh and
510 frozen berries. *Int J Food Microbiol*, 275 (oct), 8–16. https://doi.org/10.1016/j.ijfoodmicro.2018.03.016

511 Cebrián, G., Mañas, P., & Condón, S. (2016). Comparative resistance of bacterial foodborne pathogens to
512 non-thermal technologies for food preservation. *Front Microbiol*, 7(5), 1–17.
513 https://doi.org/10.3389/fmicb.2016.00734

514 Chang, J. C. H., Ossoff, S. F., Lobe, D. C., Dorfman, M. H., Dumais, C. M., Qualls, R. G., & Johnson, J.
515 D. (1985). UV inactivation of pathogenic and indicator microorganisms. *App Environ Microbiol*, 49(6),
516 1361–1365.

517 Chen, X., & Hung, Y. (2017). Effects of organic load, sanitizer pH and initial chlorine concentration of
518 chlorine-based sanitizers on chlorine demand of fresh produce wash waters. *Food Control*, 77, 96–101.
519 https://doi.org/10.1016/j.foodcont.2017.01.026

520 Collazo, C., Lafarga, T., Aguiló-Aguayo, I., Marín-Sáez, J., Abadias, M., & Viñas, I. (2018).
521 Decontamination of fresh-cut broccoli with a water–assisted UV-C technology and its combination with
522 peroxyacetic acid. *Food Control*, 93, 92–100. https://doi.org/10.1016/j.foodcont.2018.05.046

523 Collazo, C., Charles, F., Aguiló-Aguayo, I., Marín-Sáez, J., Lafarga, T., Abadias, M., & Viñas, I. (2019).
524 Decontamination of *Listeria innocua* from fresh-cut broccoli using UV-C applied in water or
525 peroxyacetic acid, and dry-pulsed light. *Inniv Food Sci Emerg Technols*, *52* (Feb), 438–449.
526 https://doi.org/10.1016/j.ifset.2019.02.004

527 Collazo, C., Noguera, V., Aguiló-Aguayo, I., Abadias, M., Colás-Medà, P., Nicolau, I., & Viñas, I.
528 (2019). Assessing water-assisted UV-C light and its combination with peroxyacetic acid and
529 *Pseudomonas graminis* CPA-7 for the inactivation and inhibition of *Listeria monocytogenes* and
530 *Salmonella Typhimurium* in fresh-cut 'Iceberg' lettuce and baby spinach leaves. *Int J Food Microbiol*,
531 *297*(Sep), 11–20. https://doi.org/10.1016/j.ijfoodmicro.2019.02.024

532 Commission Regulation (EU) 2020/685 of 20 May 2020 amending Regulation (EC) No 1881/2006 as
533 regards maximum levels of perchlorate in certain foods. *OJ L 160, 25.5.2020, p. 3–5*

534 Erkan, M., Wang, C. Y., & Krizek, D. T. (2001). UV-C irradiation reduces microbial populations and
535 deterioration in Cucurbita pepo fruit tissue. *Environ Exp*, 45(1), 1–9. https://doi.org/10.1016/S0098-
536 8472(00)00073-3

537 Escalona, V. H., Aguayo, E., Martínez-Hernández, G. B., & Artés, F. (2010). UV-C doses to reduce
538 pathogen and spoilage bacterial growth in vitro and in baby spinach. *Postharvest Biol Tech*, 56(3), 223–
539 231. https://doi.org/10.1016/j.postharvbio.2010.01.008

540 European Food Safety Authority. (2014). Scientific Opinion on the risk posed by pathogens in food of
541 non-animal origin. Part 2 (*Salmonella* and norovirus in berries). *EFSA Journal*, 12(10), 3832.
542 https://doi.org/10.2903/j.efsa.2014.3832

543 Gil, M. I., Selma, M. V, López-gálvez, F., & Allende, A. (2009). Fresh-cut product sanitation and wash
544 water disinfection: Problems and solutions. *Int J Food Microbiol*, 134(1–2), 37–45.
545 https://doi.org/10.1016/j.ijfoodmicro.2009.05.021

546 Gonzalez-Aguilar, G., Wang, C. Y., & Buta, G. J. (2004). UV-C irradiation reduces breakdown and
547 chilling injury of peaches during cold storage. *J Sci Food Agr*, 84(5), 415–422.
548 https://doi.org/10.1002/jsfa.1675

549 Gopisetty, V. V. S., Patras, A., Kilonzo-Nthenge, A., Yannam, S., Bansode, R. R., Sasges, M., Mallory
550 Burs, S., Vergne, M.J., Pan, C., & Xiao, H. (2018). Impact of UV-C irradiation on the quality, safety, and
551 cytotoxicity of cranberry-flavored water using a novel continuous flow UV system. *LWT – Food Sci*
552 *Tech*, *95*(Apr), 230–239. https://doi.org/10.1016/j.lwt.2018.04.042

553 Guerrero-Beltrán, J. A., & Barbosa-Cánovas, G. V. (2004). Review: Advantages and limitations on
554 processing foods by UV light. *Food Sci Tech Int*, 10(3), 137–147.
555 https://doi.org/10.1177/1082013204044359

556 Gunter-Ward, D. M., Patras, A., S. Bhullar, M., Kilonzo-Nthenge, A., Pokharel, B., & Sasges, M. (2018).
557 Efficacy of ultraviolet (UV-C) light in reducing foodborne pathogens and model viruses in skim milk. *J*
558 *Food Process Pres*, 42(2), 1–12. https://doi.org/10.1111/jfpp.13485

559 Guo, S., Huang, R., & Chen, H. (2017). Application of water-assisted ultraviolet light in combination of
560 chlorine and hydrogen peroxide to inactivate *Salmonella* on fresh produce. *Int J food Microbiol*,
561 257(Feb), 101–109. https://doi.org/10.1016/j.ijfoodmicro.2017.06.017

562 Gutiérrez, D. R., Chaves, A. R., & Rodríguez, S. del C. (2018). UV-C and ozone treatment influences on
563 the antioxidant capacity and antioxidant system of minimally processed rocket (*Eruca sativa* Mill.).
564 *Postharvest Biol Tec*, 138(Sep), 107–113. https://doi.org/10.1016/j.postharvbio.2017.12.014

565 Hadjilouka, A., Paramithiotis, S., & Drosinos, E. (2014). Prevalence of *Listeria monocytogenes* and
566 occurrence of listeriosis from ready-to-eat fruits and vegetables. In *Listeria monocytogenes: Food*
567 *sources, prevalence and management strategies* (pp. 283–296).

568 Hassaballah, A. H., Bhatt, T., Nyitrai, J., Dai, N., & Sassoubre, L. (2020). Inactivation of*: E. coli,*
569 *Enterococcus* spp., somatic coliphage, and *Cryptosporidium parvum* in wastewater by peracetic acid
570 (PAA), sodium hypochlorite, and combined PAA-ultraviolet disinfection. *Environ Sci – Wat Res Tec,*
571 *6(1), 197–209. https://doi.org/10.1039/c9ew00837c*

572 Hassaballah, A. H., Nyitrai, J., Hart, C. H., Dai, N., & Sassoubre, L. M. (2019). A pilot-scale study of
573 peracetic acid and ultraviolet light for wastewater disinfection. *Environ Sci – Wat Res Tec*, *5*(8), 1453–
574 1463. https://doi.org/10.1039/c9ew00341j

575 Indexbox. (2017). Strawberry: Market analysis, forecast, size, trends and insights. From:
576 https://app.indexbox.io/analyze/081010/0/ (Last acces: 2019)

577 Jagadeesh, S. L., Charles, M. T., Gariepy, Y., Goyette, B., Raghavan, G. S. V., & Vigneault, C. (2011).
578 Influence of postharvest UV-C hormesis on the bioactive components of tomato during post-treatment
579 handling. *Food Bioproc Tech*, 4(8), 1463–1472. https://doi.org/10.1007/s11947-009-0259-y

580 Jeon, M. J., & Ha, J. W. (2018). Efficacy of UV-A, UV-B, and UV-C irradiation on inactivation of
581 foodborne pathogens in different neutralizing buffer solutions. *LWT – Food Sci Tech*, 98, 591–597.
582 https://doi.org/10.1016/j.lwt.2018.09.030

583 Jin, P., Wang, H., Zhang, Y., Huang, Y., Wang, L., & Zheng, Y. (2017). UV-C enhances resistance
584 against gray mold decay caused by *Botrytis cinerea* in strawberry fruit. *Sci Hortic*, 225(Mar), 106–111.
585 https://doi.org/10.1016/j.scienta.2017.06.062

586 Kelly, K., Madden, R., Emond, J. P., & do Nascimento Nunes, M. C. (2019). A novel approach to
587 determine the impact level of each step along the supply chain on strawberry quality. *Postharvest Biol*
588 *Tech*, 147(Jul), 78–88. https://doi.org/10.1016/j.postharvbio.2018.09.012

589 Kim, M. S., Bae, J. W., & Park, E. J. (2018). Geographic and host-associated variations in bacterial
590 communities on the floret surfaces of field-grown broccoli. *App Environ Microbiol*, 84(8).
591 https://doi.org/10.1128/AEM.02837-17

592 Kretzschmar, U. (2009). Potential alternative disinfection methods for organic fresh-cut industry for
593 minimizing water consumption and environmental impact. *LWT - Food Sci Tech*, 42(3), 686–693.
594 https://doi.org/10.1016/j.lwt.2008.08.001

595 Li, D., Luo, Z., Mou, W., Wang, Y., Ying, T., & Mao, L. (2014). ABA and UV-C effects on quality,
596 antioxidant capacity and anthocyanin contents of strawberry fruit (*Fragaria* × *ananassa* Duch.).
597 *Postharvest Biol Tech*, 90, 56–62. https://doi.org/10.1016/j.postharvbio.2013.12.006

598 Liu, C., Huang, Y., & Chen, H. (2015). Inactivation of *Escherichia coli* O157: H7 and *Salmonella*
599 Typhimurium on blueberries in water using ultraviolet light. *J Food Sci*, 80(7), M1532–M1537.
600 https://doi.org/10.1111/1750-3841.12910

601 Mditshwa, A., Magwaza, L. S., Tesfay, S. Z., & Mbili, N. C. (2017). Effect of ultraviolet irradiation on
602 postharvest quality and composition of tomatoes: a review. *J Food Sci Tech*, 54(10), 3025–3035.
603 https://doi.org/10.1007/s13197-017-2802-6

604 Meireles, A., Giaouris, E., & Simões, M. (2016). Alternative disinfection methods to chlorine for use in
605 the fresh-cut industry. *Food Res Int*, 82, 71–85. https://doi.org/10.1016/j.foodres.2016.01.021

606 Meyers, K., Watkins, C., Pritts, M., & Liu, R. (2003). Antioxidant and antiproliferative activities of
607 strawberries. *J Agric Food Chem*, 51, 6887–6892. https://doi.org/10.1021/jf034506n

608 Mokrzycki, W., & Tatol, M. (2011). Color difference Delta E - A survey. *Mach Graph Vis*, 20(4), 383–
609 411. https://doi.org/1230-0535

610 Nicolau-Lapeña, I., Abadias, M., Bobo, G., Aguiló-Aguayo, I., Lafarga, T., & Viñas, I. (2019).
611 Strawberry sanitization by peracetic acid washing and its effect on fruit quality. *Food Microbiol*, *83*(Apr),
612 159–166. https://doi.org/10.1016/j.fm.2019.05.004

Nowicka, A., Kucharska, A. Z., Sokół-Łętowska, A., & Fecka, I. (2019). Comparison of polyphenol content and antioxidant capacity of strawberry fruit from 90 cultivars of *Fragaria* × *ananassa* Duch. *Food Chem*, 270, 32–46. https://doi.org/10.1016/j.foodchem.2018.07.015

Palaçiĝdem U., & Toklucu, A. K. (2013). Microbial, physicochemical and sensory properties of UV-C processed orange juice and its microbial stability during refrigerated storage. *LWT - Food Sci Tech*, 50(2), 426–431. https://doi.org/10.1016/j.lwt.2012.09.001

Pigeot-Rémy, S., Simonet, F., Atlan, D., Lazzaroni, J. C., & Guillard, C. (2012). Bactericidal efficiency and mode of action: A comparative study of photochemistry and photocatalysis. *Water Res*, 46(10), 3208–3218. https://doi.org/10.1016/j.watres.2012.03.019

Pombo, M. A., Dotto, M. C., Martínez, G. A., & Civello, P. M. (2009). UV-C irradiation delays strawberry fruit softening and modifies the expression of genes involved in cell wall degradation. *Postharvest Biol Tech*, 51(2), 141–148. https://doi.org/10.1016/j.postharvbio.2008.07.007

Pombo, M. A., Rosli, H. G., Martínez, G. A., & Civello, P. M. (2011). UV-C treatment affects the expression and activity of defense genes in strawberry fruit (*Fragaria* × *ananassa*, Duch.). *Postharvest Biol Tech*, 59(1), 94–102. https://doi.org/10.1016/j.postharvbio.2010.08.003

Scherer, R., Rybka, A. C. P., Ballus, C. A., Meinhart, A. D., Teixeira-Filho, J., & Godoy, H. T. (2012). Validation of a HPLC method for a simultaneous determination of main organic acids in fruits and juices. *Food Chem*, 135(1), 150–154.

Shama, G., & Alderson, P. (2005). UV hormesis in fruits: A concept ripe for commercialisation. *Trends Food Sci and Tech*, 16(4), 128–136. https://doi.org/10.1016/j.tifs.2004.10.001

Sheng, K., Shui, S. S., Yan, L., Liu, C., & Zheng, L. (2018). Effect of postharvest UV-B or UV-C irradiation on phenolic compounds and their transcription of phenolic biosynthetic genes of table grapes. *J Food Sci Tech*, 55(8), 3292–3302. https://doi.org/10.1007/s13197-018-3264-1

da Silva, F. L., Escribano-Bailón, M. T., Pérez Alonso, J. J., Rivas-Gonzalo, J. C., & Santos-Buelga, C. (2007). Anthocyanin pigments in strawberry. *LWT - Food Sci Tech*, 40(2), 374-382.

638 Syamaladevi, R. M., Adhikari, A., Lupien, S. L., Dugan, F., Bhunia, K., Dhingra, A., & Sablani, S. S.
639 (2015). Ultraviolet-C light inactivation of *Penicillium expansum* on fruit surfaces. *Food Control*, 50, 297–
640 303. https://doi.org/10.1016/j.foodcont.2014.09.006

641 Tomás-Barberán, F. A., & Espín, J. C. (2001). Phenolic compounds and related enzymes as determinants
642 of quality in fruits and vegetables. *J Sci Food Agric*, 81(9), 853–876. https://doi.org/10.1002/jsfa.885

643 Usaga, J., Manns, D. C., Moraru, C. I., Worobo, R. W., & Padilla-Zakour, O. I. (2017). Ascorbic acid and
644 selected preservatives influence effectiveness of UV treatment of apple juice. *LWT - Food Sci Tech*, 75,
645 9–16. https://doi.org/10.1016/j.lwt.2016.08.037

646 Wulfkuehler, S., Müller, A., Weiss, A., Mix, K., Rebmann, M., Schmidt, H., Stahl, M., & Carle, R.
647 (2015). Sanitation of process water from fresh-cut lettuce production by means of UV-C. *Acta Hort*,
648 1091, 327–334.

649 Xu, Y., Charles, M. T., Luo, Z., Mimee, B., Veronneau, P. Y., Rolland, D., & Roussel, D. (2017).
650 Preharvest ultraviolet C irradiation increased the level of polyphenol accumulation and flavonoid pathway
651 gene expression in strawberry fruit. *J Agric Food Chem*, 65(46), 9970–9979.
652 https://doi.org/10.1021/acs.jafc.7b04252

653 Yun, J., Yan, R., Fan, X., Gurtler, J., & Phillips, J. (2013). Fate of *Eschericia coli* O157:H7, *Salmonella*
654 spp. and potential surrogate bacteria on apricot fruit, following exposure to UV-C light. *Int J Food*
655 *Microbiol*, 166(3), 356–363. https://doi.org/10.1016/j.ijfoodmicro.2013.07.021

Table 1. Quality assessment of strawberries after washing treatments. Values are the mean of 3 repetitions ± standard deviation. Different letters show statistically significant differences ($p < 0.05$) between treatments.

	pH	TSS (°B)	TA (mg citric acid/L)
NaOCl	3.21 ± 0.04 ^{d}	5.67 ± 0.42 ^{c}	8.07 ± 0.70 ^{a}
UV-2L-1 min	3.36 ± 0.02 ^{bc}	6.17 ± 0.82 ^{a}	6.63 ± 0.89 ^{b}
UV-2L-5 min	3.35 ± 0.07 ^{c}	5.93 ± 0.68 ^{b}	5.26 ± 0.55 ^{c}
UV-4L-1 min	3.32 ± 0.05 ^{c}	5.90 ± 0.35 ^{d}	5.70 ± 0.43 ^{bc}
UV-4L-5 min	3.43 ± 0.00 ^{a}	5.50 ± 0.26 ^{b}	6.21 ± 0.48 ^{bc}

Table 2. Total ascorbic acid (TAA), anthocyanins and total phenolic contents of strawberries just after the treatments (0 h) or after 1 day storage at 4 ºC (24 h). Values are the mean of 3 repetitions ± standard deviation. Different lowercase letters mean statistically significant differences between treatments within the same day, and different capital letters mean statistically significant differences between days for one treatment ($p < 0.05$).

	TAA (mg ascorbic acid/100g FW)		**Anthocyanins (mg/kg)**		**TPC (mg GAE /100 g FW)**	
	0 h	24 h	0 h	24 h	0 h	24 h
NaOCl	26.4 ± 0.1 ^{abA}	30.9 ± 1.6 ^{aB}	19.1 ± 0.2 ^{aA}	20.7 ± 0.4 ^{abB}	106.5 ± 2.3 ^{aA}	126.7 ± 10.0 ^{aB}
UV-2L-1 min	25.6 ± 1.4 ^{bA}	32.5 ± 2.0 ^{aB}	15.0 ± 1.3 ^{abA}	19.2 ± 0.7 ^{bcB}	122.7 ± 4.8 ^{aA}	120.7 ± 13.6 ^{aA}
UV-2L-5 min	29.5 ± 2.2 ^{abB}	25.2 ± 0.7 ^{bA}	16.5 ± 0.9 ^{abA}	17.4 ± 0.1 ^{cB}	115.6 ± 8.0 ^{aA}	114.8 ± 8.9 ^{aA}
UV-4L-1 min	28.. ± 1.9 ^{abA}	32.4 ± 0.8 ^{aB}	14.3 ± 0.4 ^{bA}	19.5 ± 0.8 ^{bcB}	110.3 ± 1.3 ^{aA}	111.7 ± 0.4 ^{aA}
UV-4L-5 min	31.3 ± 1.6 ^{aB}	30.2 ± 0.1 ^{aA}	17.7 ± 1.6 ^{abA}	22.2 ± 1.0 ^{aB}	115.9 ± 2.1 ^{aA}	118.9 ± 11.5 ^{aA}

Table 3. Content of phenolic compounds on strawberries just after the treatments (0 h) or after 1 day of storage at 4 ºC (24 h). Values are the mean of 2 repetitions ± standard deviation. Different lowercase letters mean statistically significant differences between treatments within the same day, and different capital letters mean statistically significant differences between days for one treatment ($p < 0.05$).

	Galloyl-diHHDP-glucose (mg/100 g DW)		**(+)-Catechin (mg/100 g DW)**		**Cinnamoyl glucose (mg/100 g DW)**	
	0 h	24 h	0 h	24 h	0 h	24 h
NaOCl	31.8 ± 2.9 ^{aA}	29.2 ± 1.7 ^{aA}	18.3 ± 0.7 ^{aA}	20.1 ± 0.3 ^{aA}	42.2 ± 1.1 ^{aA}	49.5 ± 3.9 ^{aA}
UV-2L-1 min	25.4 ± 2.3 ^{aA}	33.8 ± 4.4 ^{aA}	14.9 ± 2.5 ^{aA}	16.5 ± 0.8 ^{aA}	41.6 ± 1.4 ^{aB}	37.5 ± 3.6 ^{aA}
UV-2L-5 min	28.5 ± 2.2 ^{aA}	27.6 ± 0.6 ^{aA}	18.4 ± 0.8 ^{aA}	19.6 ± 1.9 ^{aA}	45.0 ± 4.9 ^{aA}	46.1 ± 1.5 ^{aA}
UV-4L-1 min	29.3 ± 5.8 ^{aA}	25.7 ± 3.1 ^{aA}	17.3 ± 0.7 ^{aA}	16.9 ± 1.5 ^{aA}	55.1 ± 4.7 ^{aA}	53.7 ± 6.2 ^{aA}
UV-4L-5 min	27.6 ± 3.0 ^{aA}	26.7 ± 2.0 ^{aA}	19.7 ± 0.5 ^{aA}	19.2 ± 0.4 ^{aA}	50.8 ± 3.7 ^{aA}	47.9 ± 3.3 ^{aA}

	Coumaroyl hexose I (mg/100 g DW)		**Coumaroyl hexose II (mg/100 g DW)**		**Quercetin glucuronide (mg/100 g DW)**	
	0 h	24 h	0 h	24 h	0 h	24 h
NaOCl	14.7 ± 0.1 ^{aA}	14.7 ± 0.6 ^{aA}	17.8 ± 0.8 ^{aA}	18.5 ± 1.9 ^{aA}	14.0 ± 0.4 ^{aB}	8.8 ± 0.2 ^{cA}
UV-2L-1 min	11.4 ± 0.2 ^{bA}	9.9 ± 0.5 ^{bA}	13.4 ± 0.8 ^{aA}	11.8 ± 1.7 ^{aA}	8.2 ± 0.1 ^{cdA}	12.1 ± 0.0 ^{aB}
UV-2L-5 min	12.1 ± 0.9 ^{bA}	15.0± 0.6 ^{aA}	14.3 ± 1.9 ^{aA}	17.7 ± 1.3 ^{aA}	12.1 ± 0.6 ^{bA}	11.0 ± 0.1 ^{abA}
UV-4L-1 min	14.7 ± 0.8 ^{aA}	15.0 ± 0.7 ^{aA}	17.1 ± 1.6 ^{aA}	17.7 ± 2.7 ^{aA}	9.6 ± 0.3 ^{cA}	8.9 ± 0.5 ^{cA}
UV-4L-5 min	13.5 ± 0.2 ^{abA}	14.9 ± 0.2 ^{aA}	16.1 ± 1.5 ^{aA}	18.2 ± 1.5 ^{aB}	7.5 ± 0.1 ^{dA}	9.9 ± 0.5 ^{bcB}

	Kaempferol glucuronide		**Kaempferol coumaroylglucoside**	

	(mg/100 g DW)		Kaempferol malonylglucoside (mg/100 g DW)		(mg/100 g DW)	
	0 h	24 h	0 h	24 h	0 h	24 h
NaOCl	60.2 ± 1.1 ^{aB}	47.8 ± 1.4 ^{cA}	22.8 ± 1.7 ^{abA}	25.8 ± 0.3 ^{abA}	40.1 ± 3.8 ^{aA}	32.1 ± 13.9 ^{aA}
UV-2L-1 min	45.4 ± 1.9 ^{bA}	64.7 ± 0.4 ^{aB}	20.0 ± 0.1 ^{bA}	28.1 ± 1.5 ^{aB}	36.3 ± 1.0 ^{aA}	73.6 ± 7.9 ^{aB}
UV-2L-5 min	52.0 ± 2.4 ^{abA}	61.4 ± 2.3 ^{abA}	24.4 ± 0.3 ^{aA}	27.5 ± 1.0 ^{aA}	26.1 ± 8.4 ^{aA}	64.1 ± 10.2 ^{aB}
UV-4L-1 min	48.2 ± 3.7 ^{aA}	45.5 ± 2.6 ^{cA}	23.6 ± 0.0 ^{aA}	23.2 ± 0.5 ^{bA}	28.1 ± 3.3 ^{aA}	36.5 ± 8.4 ^{aA}
UV-4L-5 min	46.6 ± 2.5 ^{aA}	53.8 ± 2.9 ^{bcA}	22.2 ± 0.3 ^{abA}	25.7 ± 1.5 ^{abA}	26.6 ± 4.3 ^{aA}	53.7 ± 12.8 ^{aB}

	Pelargonidin galactoside (mg/100 g DW)		Pelargonidin glucoside (mg/100 g DW)		Pelargonidin acetylglucoside (mg/100 g DW)	
	0 h	24 h	0 h	24 h	0 h	24 h
NaOCl	205.7 ± 0.4 ^{aB}	161.1 ± 1.8 ^{bA}	418.2 ± 27.1 ^{aA}	407.2 ± 19.7 ^{aA}	109.9 ± 4.9 ^{aA}	95.4 ± 1.9 ^{bcA}
UV-2L-1 min	132.9 ± 6.9 ^{cA}	182.8 ± 2.2 ^{abB}	334.1 ± 0.6 ^{bA}	374.2 ± 31.2 ^{aA}	80.5 ± 1.3 ^{aA}	108.7 ± 2.4 ^{abB}
UV-2L-5 min	167.6 ± 1.6 ^{bA}	195.3 ± 1.3 ^{aA}	408.0 ± 36.3 ^{abA}	455.5 ± 1.9 ^{aA}	105.2 ± 7.1 ^{aA}	117.1 ± 2.6 ^{aA}
UV-4L-1 min	151.4 ± 0.3 ^{bcB}	108.6 ± 8.5 ^{cA}	418.7 ± 44.7 ^{abA}	417.3 ± 19.3 ^{aA}	97.2 ± 6.2 ^{aA}	82.7 ± 6.1 ^{cA}
UV-4L-5 min	138.7 ± 0.3 ^{bcA}	177.4 ± 13.0 ^{abA}	381.8 ± 24.7 ^{bA}	412.6 ± 1.6 ^{aA}	85.7 ± 2.2 ^{aA}	102.5 ± 2.8 ^{bB}

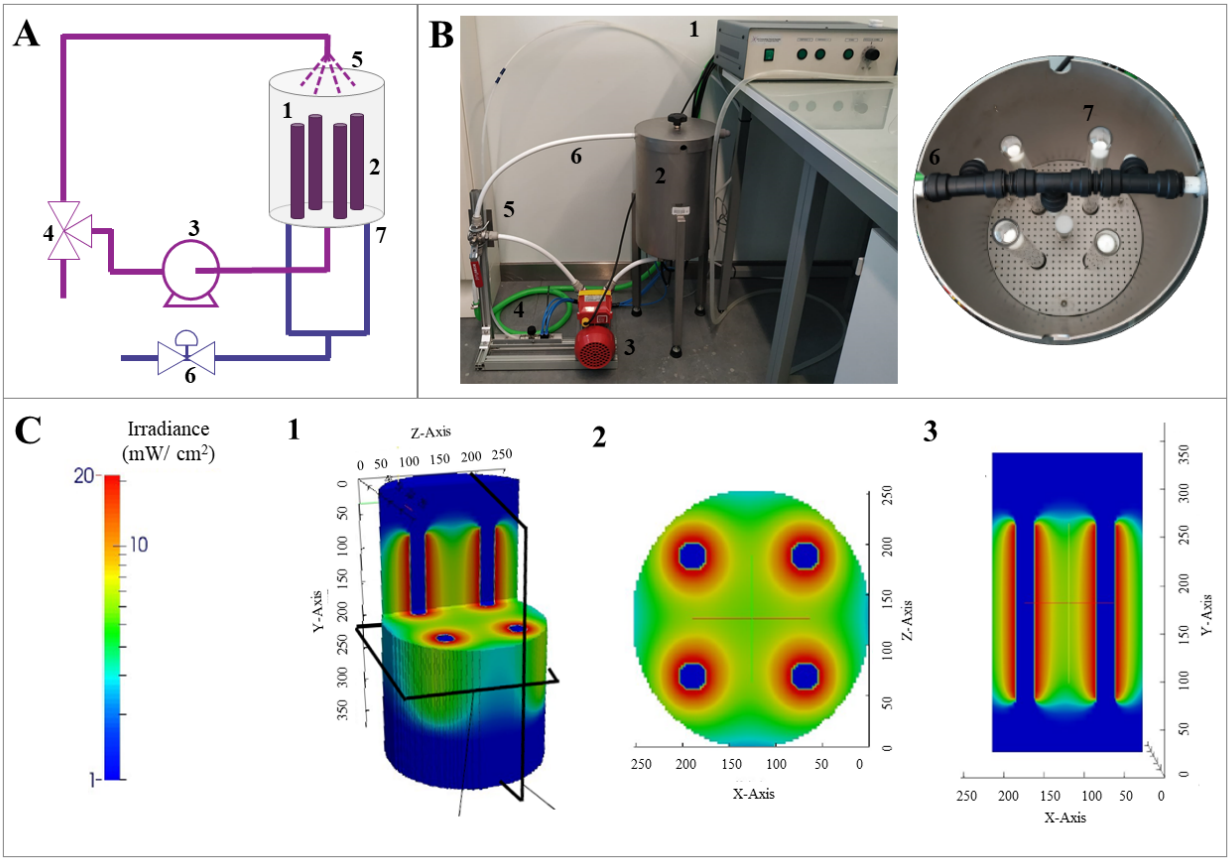


Figure 1. (**A**) Scheme of the equipment: tank (1), UV-C lamps (2), water pump (3), water circuit valve (4), water recirculation (5), air regulator valve (6), air inlet (7); (**B**) equipment: controller (1), tank (2), water pump (3), air regulator valve (4), water circuit valve (5), water recirculator (6), UV-C lamps (7); (**C**) irradiance distribution inside the tank: 3-D view (1) elevation view (2), section view (3)

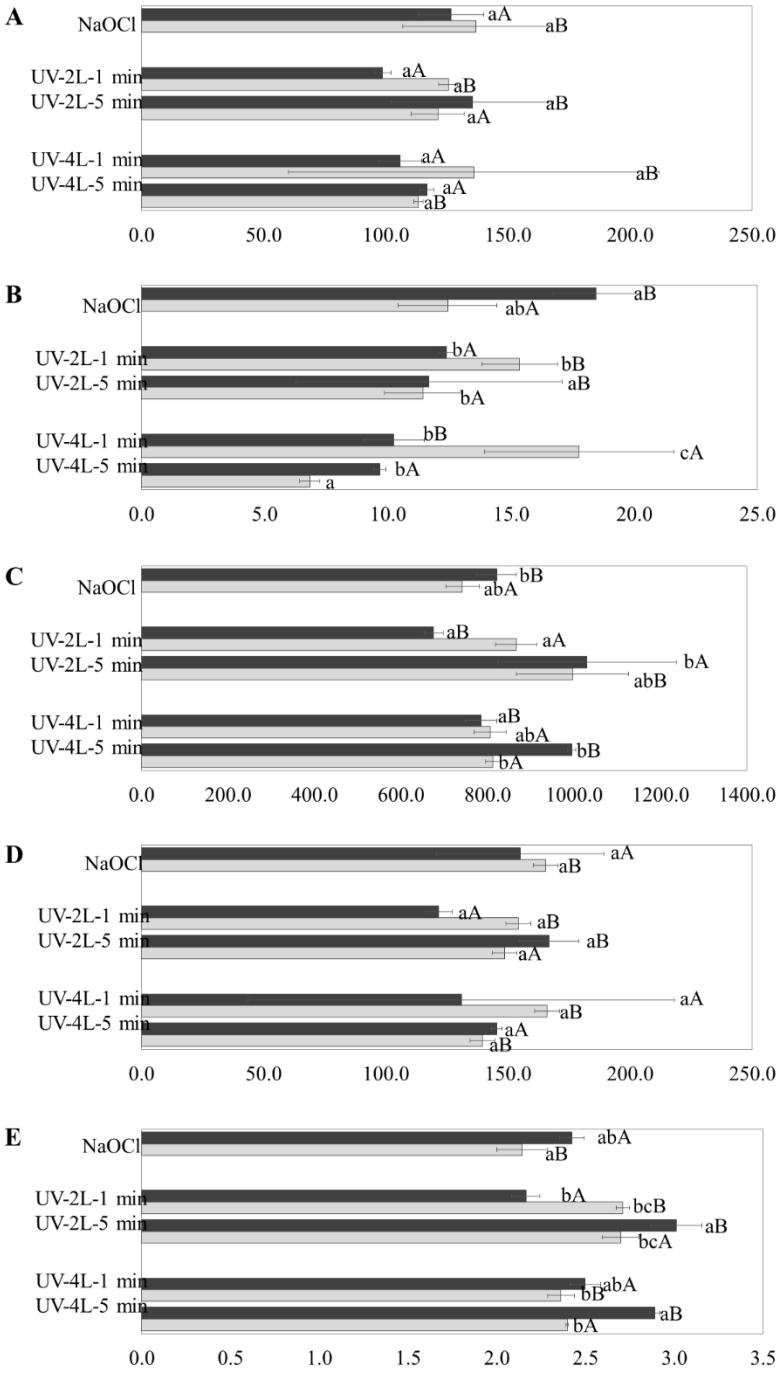


Figure 2. Organic acid content of strawberries immediately after the washing treatments (■) and 24 after storage at 4ºC (■). Contents expressed as mg quinnic acid / 100 g FW (**A**), mg mallic acid / 100 g FW (**B**), mg citric acid / 100 g FW (**C**), mg tartaric acid / 100 g FW (**D**), and mg fumaric acid / 100 g FW (**E**). Results are the mean of 2 repetitions ± standard deviation. Different letters show statistically significant differences ($p < 0.05$) between treatments and days.

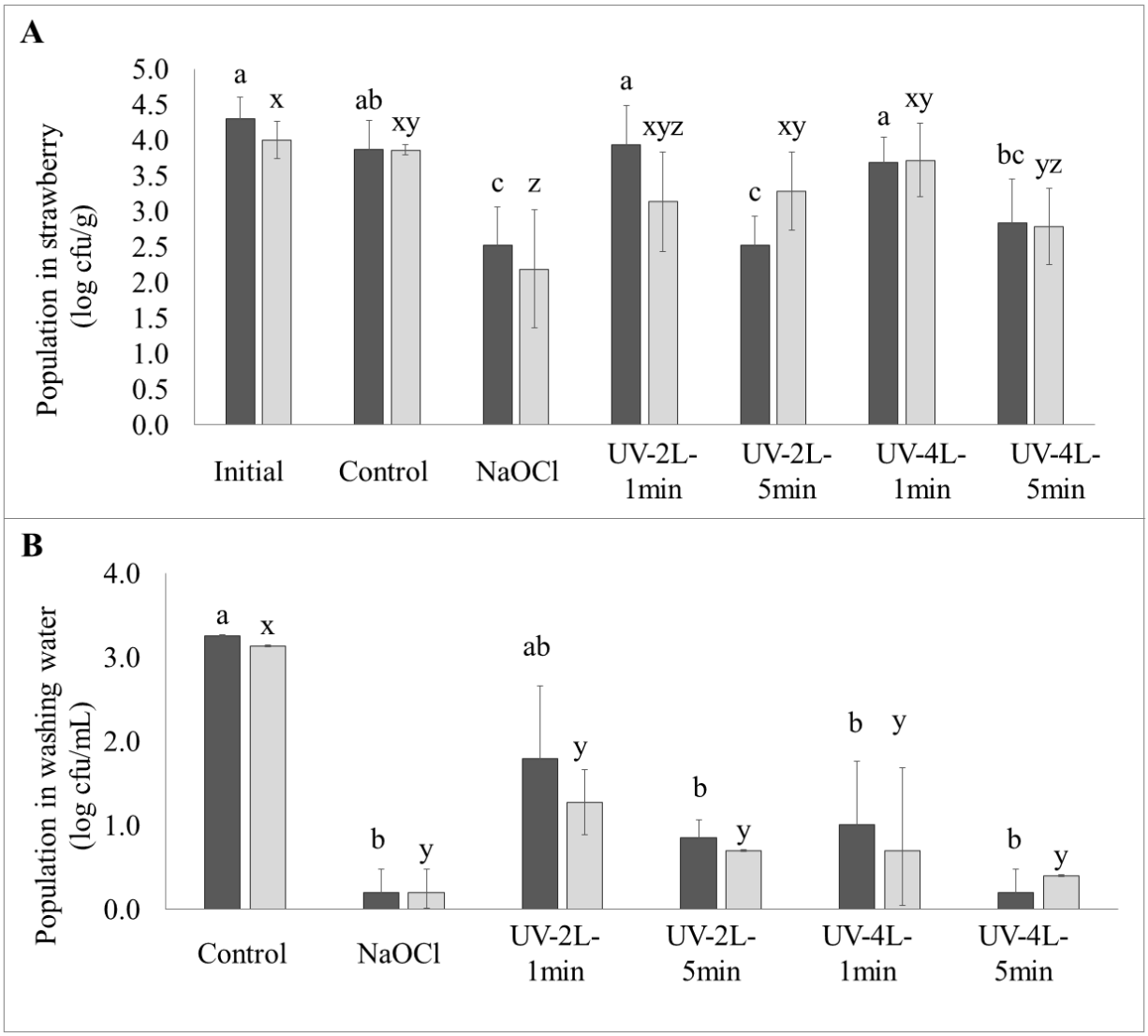


Figure 3. (**A**) Counts of total aerobic mesophylls (■) and yeasts and molds (■)in strawberries before (initial) and after washing treatments. Detection limit was 1.70 log cfu/g. Results are the mean of 6 repetitions ± standard deviation. Different letters show statistically significant differences ($p < 0.05$) between treatments. (**B**) Remaining population of total aerobic mesophylls (■) and yeasts and molds (■) in washing water. Detection limit was 0.7 log cfu/g. Results are the mean of 3 repetitions ± standard deviation. Different letters show statistically significant differences ($p < 0.05$) between treatments

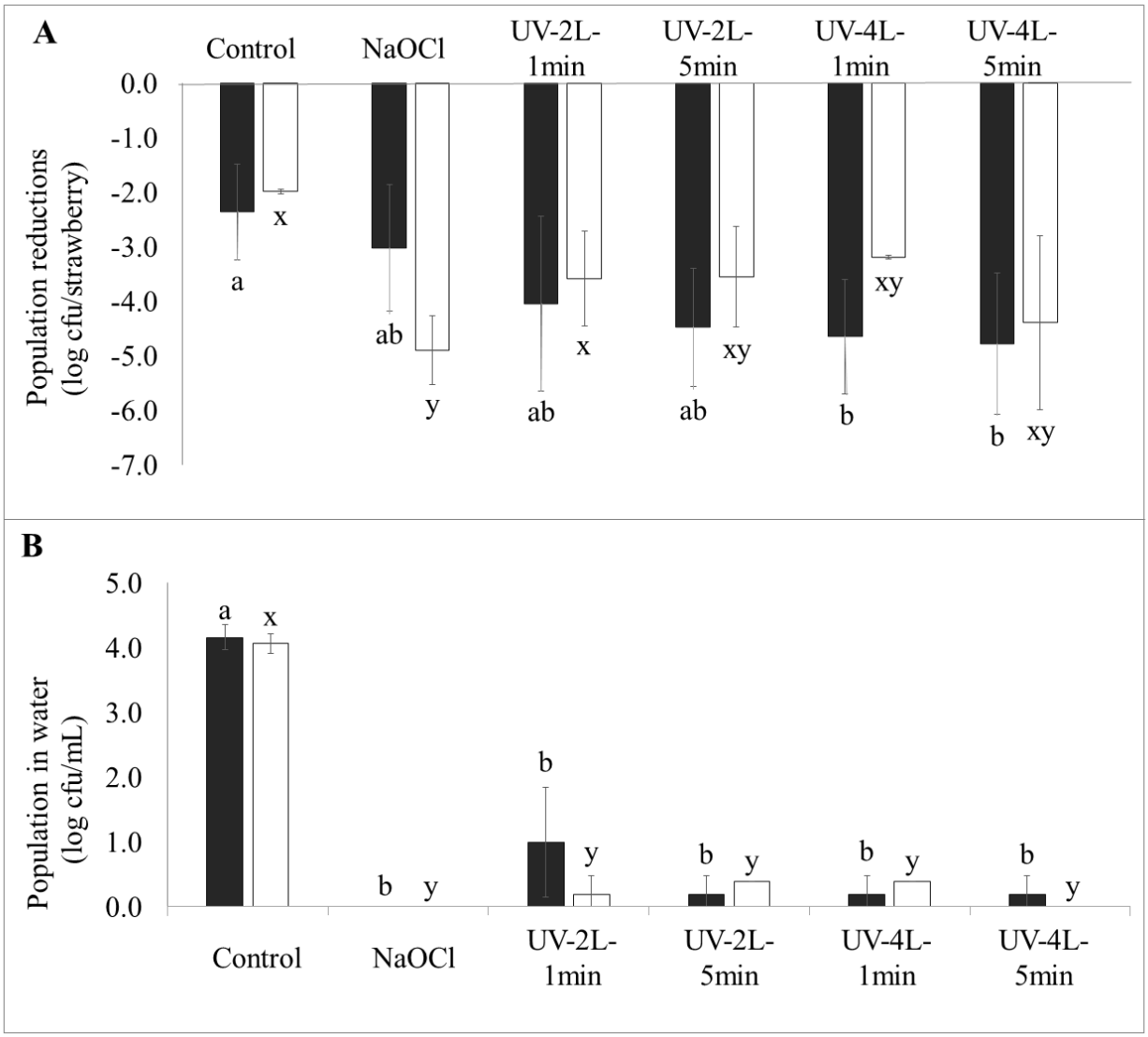


Figure 4. (**A**) Reductions of *L. innocua* (■) and *S. enterica* (□) populations in strawberries after washing treatments with WUV-C irradiation alone. Detection limit was 1.70 log cfu/g. Results are the mean of 6 repetitions ± standard deviation. Different letters show statistically significant differences ($p < 0.05$) between treatments. (**B**) Remaining population of *L. innocua* (■) and *S. enterica* (□) in washing water. Detection limit was 0.7 log cfu/g. Results are the mean of 6 repetitions ± standard deviation. Different letters show statistically significant differences ($p < 0.05$) between treatments

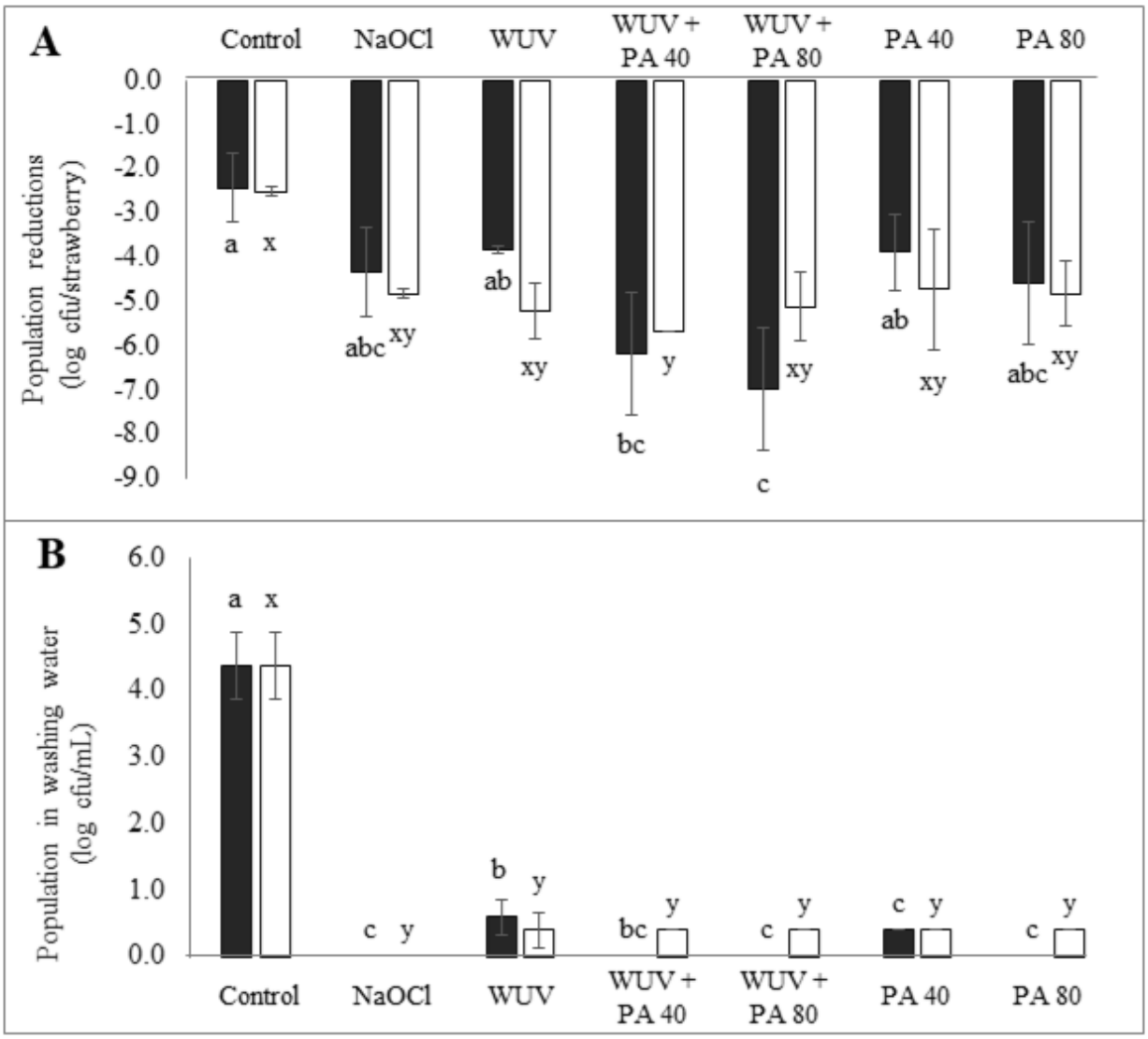
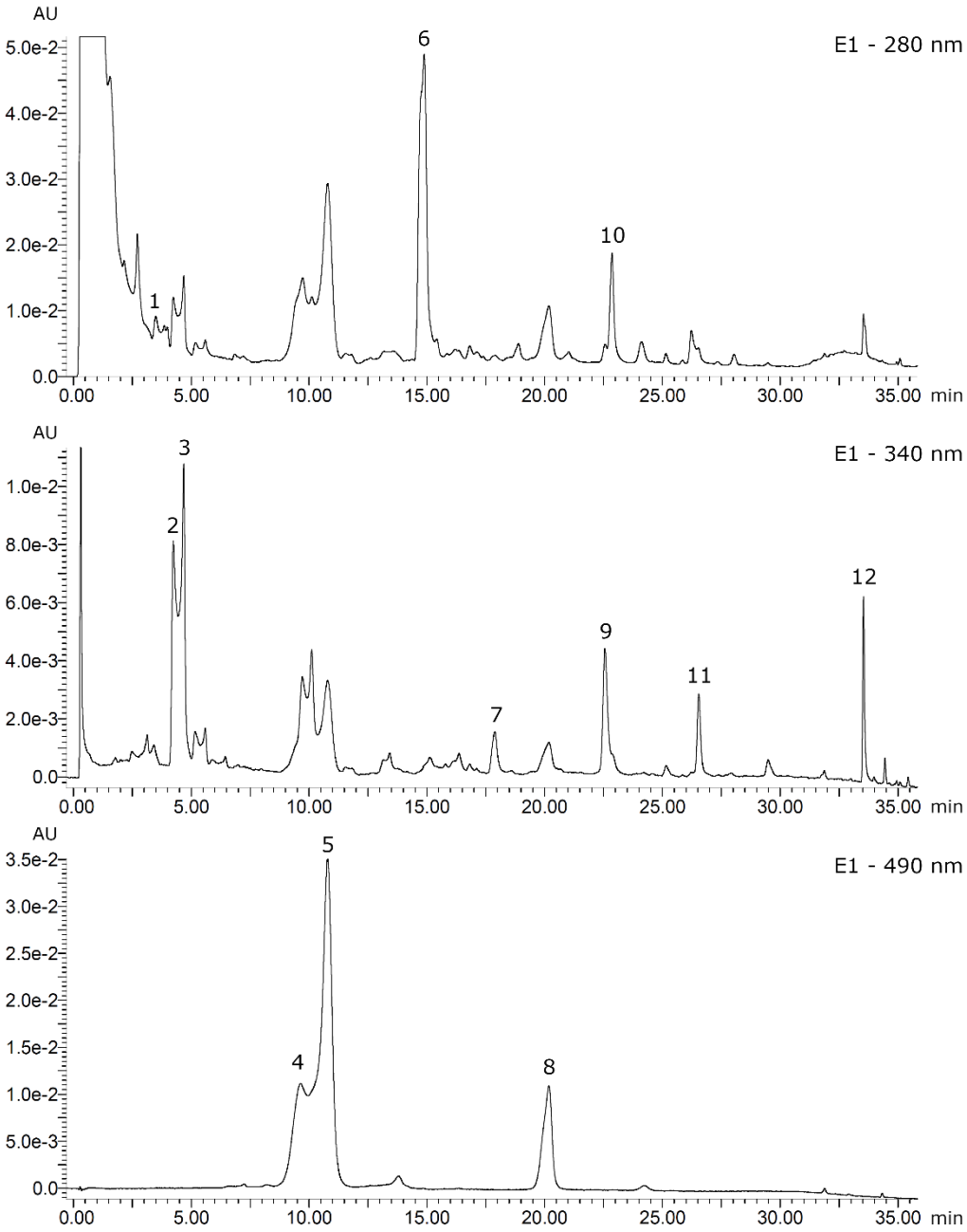


Figure 5. (**A**) Reductions of *L. innocua* (■) and *S. enterica* (□) populations in strawberries after washing treatments with WUV-C combined with PA at different doses for 2 min. Detection limit was 1.70 log cfu/g. Results are the mean of 6 repetitions ± standard deviation. Different letters show statistically significant differences ($p < 0.05$) between treatments. (**B**) Remaining population of *L. innocua* (■) and *S. enterica* (□) in washing water. Detection limit was 0.7 log cfu/g. Results are the mean of 6 repetitions ± standard deviation. Different letters show statistically significant differences ($p < 0.05$) between treatments



Supplementary figure. HPLC chromatogram of strawberry phenolic compounds, obtained at 280, 360 and 490 nm, showing (+)-catechin (1), coumaroyl hexose I (2), coumaroyl hexose II (3), pelargonidin galactoside (4), pelargonidin glucoside (5), cinnamoyl glucose (6), quercetin-3-*O*-glucuronide (7), pelargonidin acetylglucoside (8), kaempferol-3-*O*-glucuronide (9), galloyl-diHHDP-glucose (10), kaempferol-3-*O*-malonylglucoside (11), and kaempferol-3-*O*-coumaroylglucoside (12).