Application of an innovative water-assisted ultraviolet C light technology for the inactivation of microorganisms in tomato processing industries

Maribel Abadias[a]*, Pilar Colás-Medà[a], Immaculada Viñas[b], Gloria Bobo[a], Ingrid Aguiló-Aguayo[a]

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

[b] Food Technology Department, University of Lleida, Agrotecnio Center, Lleida, Spain

***Corresponding author:** M. Abadias (isabel.abadias@irta.cat)

Abstract

We aimed to study the efficacy of a water-assisted UVC light device (WUVC) as an innovative clean technology for the disinfection of fresh sound tomatoes and processing wash water and water turbidity was evaluated as a critical parameter. First, wash waters with different turbidities (from 0.4 to 828 NTU) were inoculated with *Listeria innocua* and treated in the WUVC device at different dosages. Secondly, fresh tomatoes, inoculated with *L. innocua* and non-inoculated ones, were treated using the WUVC device containing wash water of different turbidities for different times. The reduction of *L. innocua* populations on wash water and on the surface of tomato was influenced by turbidity; lower reduction values were observed at higher turbidities. Washing tomatoes with tap water with UVC lamps off (control treatment, TW) decreased *L. innocua* population on the surface of tomatoes but did not eliminate those bacteria that went into the water. Contrarily, when UVC lights were on, *L. innocua* population in wash water after treatment significantly decreased, those in clean water being the lowest populations. Reductions of native microbiota on the clean water treated with the highest UV-C radiation dose were lower than those obtained when tomatoes were artificially inoculated. We demonstrated that high reductions of *L. innocua* population on fresh tomatoes could be achieved using the WUVC system but some drawbacks related to the increase of turbidity should be solved for its implementation in real conditions.

Keywords: water assisted UVC, *Listeria innocua*, microbial load reduction, wash water quality, water turbidity

1. Introduction

Tomato (*Lycopersicum esculentum* Miller) and its derived products have a very interesting nutritional value in addition to prominent antioxidant, anti-inflammatory, and anticancer properties (Salehi et al., 2019) and they are widely consumed as a raw, frozen or minimally processed product. In 2017, Spain produced 4,768,595 tn of tomatoes, which represented 20.6% of the total produced in the EU, being the third highest EU producer (FAOSTAT, 2019). About 54% of the tomato production was destined for processing (Lahoz-García et al., 2019). The consumption in Spain is estimated as 12.84 kg/person/year (MAPA, 2018).

Fresh and processed tomatoes have been implicated in several outbreaks worldwide. In the EU, there has been one *Salmonella* outbreak and one Norovirus outbreak associated with tomato consumption in 2007 and 2011 respectively (EFSA BIOHAZ Panel, 2014). The outbreak associated with tomatoes contaminated with *Salmonella* Strathcona was reported to have 43 human cases in Denmark and 28 cases in Germany, Italy, Austria and Belgium (Müller et al., 2016). From 1998 to 2017, in the US, there were 170 outbreaks attributed to tomatoes or tomato products, causing 8379 illnesses, 853 hospitalizations and 9 deaths (NORS, 2019), the main causative agent being *Salmonella* spp. In 2018, another outbreak involving the consumption of tomatoes contaminated with *Salmonella* affected 69 people in Kansas, without deaths reported (Foodborne Illnesses Outbreak Database, 2019). Contaminated water used in postharvest processing has been implicated in some of these tomato-associated outbreaks. Such contamination can occur when operations fail to recondition wash water effectively and maintain sufficient levels of sanitizers (Zhou et al., 2014). Recently, in August to October 2019, another outbreak of *Salmonella* infection linked to small tomatoes was reported in Sweden, with 82 people involved (Colombe et al., 2019).

Chorine in its various available forms is the most common sanitizer used in dump tanks and flume water in fruit and vegetable processing industries. However, some disadvantages related to its use include the formation of potentially hazardous disinfection by-products (Food and Agriculture Organization & World Health Organization, 2009), strong pH dependence for optimal antimicrobial function, the potential of gas emission that may affect worker comfort and health, and its environmental effects have increased the search for alternative systems. Another important point to take into account during commercial washing processes is that due to operational costs and environmental constraints on water usage, the water used in industries is reused throughout the daily operation. This causes a rapid deterioration of wash water quality manifested by an increase in COD (Chemical Oxygen Demand) and turbidity due to the entrance of waxes, soil, dust, pesticide residues, leaf debris and organic exudates from damaged fruits that deplete hypochlorous acid and promote the formation of toxic chlorinated by-products (Luo et al., 2011).

Among the alternative sanitation methods, ultraviolet light (UV) has emerged as a physical non-thermal technology for its advantages: efficacy against a broad range of spoilage and pathogenic microorganisms, a non-toxic and ‘residue-free’ status, a minimal negative effect on organoleptic and nutritional properties, and relative low costs and low energy usage compared to thermal decontamination technologies (Artés et al. 2009, Gayán et al., 2014). UV light includes wavelengths in the range of 200-400 nm of which short-wave UV (UV-C, 200-280 nm) has the most effective germicidal effects (Bintsis et al. 2000). The antimicrobial effect of UV-C light is primarily based on the formation of pyrimidine dimers in the DNA, which inhibit transcription and eventually lead to mutagenesis and cell death (Witkin, 1976). This technology has been used for the decontamination of drinking water and packages. It has also been investigated for inactivation of microorganisms in fresh-cut vegetable washing waters (Lehto et al., 2017; Mahoney et al., 2018). There are many studies on the application of UVC to reduce microorganisms on the surface of fruits, such as apples (Graça et al., 2013), melon (Manzocco et al., 2011), watermelon (Artés-Hernández et al., 2010), strawberries

(Birmpa et al., 2013) and tomatoes (Lim and Harrison, 2016; Mukhopadhyay et al., 2014). Most of these studies involved the use of cabinets or devices in which UV light is disseminated by air. However, one of the main drawbacks of UV light in air is that it is limited due to its shallow penetration ability, the shadowing effect and the potential overheating of the product, which can affect its quality (Liu et al., 2015). The innovation of the water-assisted UVC light (WUVC) technology studied is that the lamps are immersed in water. This enables fresh produce to move and rotate randomly to achieve full exposure of all surfaces of fresh produce to UVC. Water could also help remove pathogens from food surfaces, which could be more easily killed in wash water (Collazo et al., 2018; Guo et al., 2017). Another benefit of this system is that it prevents the heating and drying of fresh produce by direct UV exposure without the presence of water (Huang et al., 2018). However, increasing turbidity of wash water would impair the penetration ability of UVC light. Huang et al. (2018), Guo et al. (2017), Liu et al. (2015) and Huang and Chen (2019) have explored this technology using a UVC light chamber in which produce was immersed and agitated in a washer while exposed to UV lamps situated on the top of the chambers. Our WUVC prototype is quite different as UVC lamps are immersed in the water (Collazo et al., 2018, 2019) as well as the produce. Previous results demonstrated reductions around 2.5-log units of *L. innocua* in broccoli florets after 2 min treatment in the WUVC prototype evaluated in the present study whereas control water treatment obtained reductions of about 0.5-log units (Collazo et al., 2019).

The aim of this study was to evaluate the efficacy of an innovative WUVC technology for the sanitation of fresh sound tomatoes and processing water and to study the effect of turbidity. WUVC technology was evaluated on tomatoes artificially inoculated with *L. innocua* and on natural epiphytic microbiota.

2. Material and methods

2.1. Plant material

Plum tomatoes (*Lycopersicon esculentum*) were obtained from a local provider in the Central Market of Lleida (Catalonia, Spain) the day before the experiment. They were sorted and stored at 5±1 °C. Tomatoes with visible defects or damaged were rejected.

2.2. Bacterial strain and inoculum preparation

L. innocua strain CECT-940 (*Colección Española de Cultivos Tipo*, Burjassot, Spain) was used in this study for safety reasons as the equipment was located in a laboratory without biosafety containment measures. *L. innocua* was grown for 24 h in 50 mL of TSB (Trypto-caseine Soy Broth, Biokar Diagnostics, Beauvois, France) supplemented with 6 g/L of yeast extract (Biokar Diagnostics, Beauvois, France) (TSBYE) at 37±1 °C in a rotatory shaker set at 150 rpm. Afterwards, the culture was centrifuged at 9,800 × *g*, at 10 °C, for 10 min. The pellet was suspended in saline peptone (PS, 8.5 g NaCl (VWR Chemicals, Leuven, Belgium) and 1 g peptic digest of meat USP, (Biokar Diagnostics)) to obtain a concentrated suspension, which was approximately 10^{10} cfu/mL. *L. innocua* population of the suspension, and was checked by plating in TSAYE (TSA supplemented with 6 g/L of yeast extract, 2.5 g/L glucose (PanReac Applichem, Barcelona, Spain and Darmstadt, Germany) and 2.5 g/L K_2HPO_4 (PanReac Applichem)) and Palcam (Biokar Diagnostics) followed by incubation at 37±1 °C for 48±2 h.

2.3. WUVC equipment

Water-assisted UVC (WUVC) laboratory scale equipment (LAB-UVC-Gama, UV-Consulting Peschl España, Castellón, Spain) was used for the experiments. It consisted of a water tank (15 L) equipped with 4 UV lamps (17.2 W, 254 nm, GPH 303T5L/4,166 Heraeus Noblelight, Hanau, Germany) enclosed by a quartz tube (25 mm of outer diameter) to prevent the lamp's contact with the wash water and product (Fig.1). The tank has a water recirculating system motioned by a water pump that pressurises water through multiple sprinkles on the top. Compared to conventional UV-C chambers, the recirculating system allows the rotation of the product and improves the accessibility of UV-C light to all sides of the product and facilitate the inactivation of microorganisms that are removed from the surface to the wash water. Before and after treatments, the temperature was measured using an infrared thermometer DualTemp Pro (Labprocess Distribuciones, Barcelona, Spain). Irradiance (W/m^2) was measured through an orifice located in the lid of the equipment using a UV-Sensor EasyH1 (Peschl Ultraviolet, Mainz, Germany). The average UV-C radiation with the empty tank was 11.2 W/m^2 (11.0-11.4 W/m^2).

2.4. Effect of WUVC on the survival of L. innocua *in water*

The effect of WUVC for the reduction of *L. innocua* in water was determined at different turbidity levels (organic load). In order to prepare different wash waters, 0, 1, 2.5, 5 and 10% commercial canned chopped tomato pulp was added to wash water in the WUVC system, according to values provided by a frozen vegetable industry. Turbidity, conductivity, pH and oxido-reduction potential (ORP) were determined for each wash water. Turbidity was measured using a portable turbidimeter (TN-100, Eutech, Singapore) measuring in Nephelometric Turbidity Units (NTU). Conductivity was determined using a conductivity measurement instrument (Testo model 240, Cabrils, Barcelona, Spain). 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.

UVC lamps were switched on for 15 min and the UV-C radiation was checked to verify that it was in the desired range (11.0-11.4 W/m^2). Afterwards, the WUVC tank was filled with 12 L of cold (7±2 °C) water and the UVC lights were switched on for 12-15 min. The tomato pulp was added and the recirculation was turned on. Afterwards, the required amount of *L. innocua* inoculum was added to have an initial concentration of about 10^7 cfu/mL (7 log cfu/mL). Triplicate 10-mL samples were taken initially and after 1, 3, 5 and 7 min (UV-C radiation doses of 0.7, 2.0, 3.4 and 4.7 KJ/m^2, respectively) depending on the water turbidity. *L. innocua* was determined by plating duplicate 10-fold dilutions onto selective medium (Palcam) followed by incubation at 37 ± 1 °C for 48 h. The experiment was repeated twice.

2.5. Effect of WUVC on survival of L. innocua *on the surface of tomato*

The day before the experiment, plum tomatoes were washed with tap water, dried and put in plastic cavity tray liners inside plastic boxes. A circle of about 2.5 cm in diameter was drawn on the surface of each tomato using an indelible marker. Afterwards, 50 µL of the prepared suspension of *L. innocua* (10^{10} cfu/mL) were inoculated on the tomato peel by pipetting small droplets inside the marked surface of each tomato to simulate contamination from a point source such as contact with soil or workers' hands (Beuchat et al. 2001). They were stored at room temperature overnight to facilitate microorganism settlement.

On the day of the experiment, the equipment was set up as indicated previously. In order to evaluate the effect of organic matter (turbidity) on the efficacy of WUVC, 0, 3 and 10% of tomato pulp was added in different trials. Turbidity, conductivity, pH and ORP of water were measured. Twelve inoculated tomatoes were treated for 1, 3 and 5 min (UV-C radiation doses of 0.7, 2.0 and 3.4 KJ/m^2, respectively). The population of *L. innocua* in wash water after treatment was also determined in duplicate samples as indicated previously. Moreover, duplicate 1-mL of wash water after treatment was also added to 9-mL of Dey-Engley Neutralizing Broth (Sigma-Aldrich, St. Louis, MO, USA) to determine the presence or absence of *L. innocua* in case the counts were below detection limit (5 ufc/mL, 0.70 log cfu/mL). Dey-Engley tubes were incubated at 37 °C for 24-48 h.

Tomatoes were left to dry at ambient temperature (about 2 h). The population of *L. innocua* before and after treatments was evaluated as follows: The inoculated surface of 3 tomatoes per treatment and time were cut using a cork borer of 2.2 cm of diameter (3.80 cm^2) and only the peel (1-2 mm) was cut using a scalpel. The entire circle was put in a 80 mL-filtered bag (BagPage®, Interscience BagSystem, Saint Nom, France) and 20 mL of buffered peptone water (BPW, Biokar Diagnostics) were added and homogenized in a blender (Minimix® 100, Interscience, France) for 120 s at 12 strokes/s. Duplicate ten-fold dilutions of the homogenates were made in saline peptone (SP, 8.5 g/l NaCl) and plated in selective medium Palcam as described above. Results were expressed in log cfu/cm^2. Detection limit was 1.42 log cfu/cm^2 (26 cfu/cm^2). When the counts were below the detection limit, an arbitrary value of ½ detection limit (13 cfu/cm^2, 1.1 log cfu/cm^2) was attributed for calculation. The experiment was repeated 3 times.

2.6. Effect of WUVC on survival of native/epiphytic microbiota on the surface of tomato

In order to test the efficacy of WUVC on the survival of native microbiota, the experiment was conducted as explained before using non-inoculated unwashed tomatoes. The effect of turbidity (0, 3 and 10% tomato pulp) and time (3 and 5 min, corresponding to UV-C radiation doses of 2.0 and 3.4 KJ/m^2, respectively) were also determined. Twelve tomatoes were treated in each batch. Moreover, a control treatment, which consisted of washing tomatoes with tap water in the same device with the UVC lights off (TW), was included. The experiment was carried out three times.

The populations of total aerobic mesophilic microorganisms (TAM) and moulds and yeasts (M&Y) were determined on the surface of tomato and in wash water after treatment. In tomatoes, the populations were determined in triplicate (three tomatoes). In each tomato, 4 circles of 2.2 cm diameter (3.8 cm^2, total surface 15.2 cm^2) were made using a cork borer. The peel was taken off using a scalpel and introduced in a filtered stomacher bag with 20 mL of BPW and homogenized as described before. Duplicate ten-fold dilutions were made for each sample and 100 mL plated in PCA and DRBC medium for TAM and M&Y determination, respectively. Plates were incubated at 30 °C for 3 days for TAM and at 25 °C for 5 days for M&Y. Results were expressed in log cfu/cm^2. Detection limit was 0.82 log cfu/cm^2 (6.6 cfu/cm^2). When the counts were below the detection limit, an arbitrary value of ½ detection limit (3.3 cfu/cm^2, 0.52 log cfu/cm^2) was attributed for calculation. The populations of TAM and M&Y in wash water after treatment were also determined in duplicate samples by plating 100 µl onto PCA and DRBC plates respectively. In order to determine the presence of microorganisms in wash water samples when counts were below detection limit, duplicate 1-mL of wash water after treatment were also added to 9-mL Dey-Engley Neutralizing Broth followed by incubation at 30 and 25 °C, respectively.

2.7. Statistical analysis

All experiments were performed at least two times and included three biological replicates per treatment and sampling time. Microbial counts (cfu/cm^2 or cfu/mL) were transformed to log10. Reduction was calculated by subtracting the initial log cfu/mL or cm^2 from the log cfu/mL or cm^2 after the treatment. Data were analysed using the statistical software JMP (version 11 SAS Institute Inc., NC, USA). Means were compared by analysis of variances (ANOVA) and separated by Tukey's test ($P < 0.05$).

3. Results

3.1. Effect of WUVC on the survival of L. innocua *in water*

The properties of wash waters changed according to the tomato pulp added (Table 1). Increasing the tomato pulp in wash water increased turbidity, conductivity and ORP but decreased pH. Turbidity values ranged from 0.42 to 828 NTU.

The initial population of all experiments and tested conditions was 7.03±0.39 log cfu/mL (data not shown). The reduction of *L. innocua* in wash water depended on both UV-C radiation doses and water turbidity (Fig.2). For each UV-C radiation dose, an increase in turbidity reduced WUVC efficacy. After 1 min treatment (0.7 KJ/m^2 of UV-C radiation dose), reductions ranged from 3.5 log cfu/mL to 1.0 log cfu/mL for turbidities ranging from 0.42 to 300 NTU. After 5 min treatment (3.4 KJ/m^2 of UV-C radiation dose), wash water turbidities in the range of 0.42 and 148 NTU gave similar reduction values (around 5 log cfu/mL). When the turbidity increased up to 828 NTU, the reduction of *L. innocua* was above 1.0 and 1.7 log cfu/mL units showed non-significant differences after 5 and 7 min of treatment (3.4 and 4.7 KJ/m^2 of UV-C radiation doses), respectively.

3.2. Effect of WUVC on survival of L. innocua *on the surface of tomato*

Properties of wash water in the experiments with tomatoes (inoculated with *L. innocua* or uninoculated) are shown in Table 2. As seen before, increasing the amount of tomato pulp in wash water increased turbidity, conductivity and ORP but decreased pH. Turbidity values range from 0.88 to 664 NTU.

The initial population of *L. innocua* on the tomato surface was 6.43±0.34 log cfu/cm^2 (data not shown). Reduction of *L. innocua* on the surface of tomatoes (Fig. 3) did not depend on UV-C radiation dose, only WUVC treatment for 1 min (0.7 KJ/m^2) with wash water of 166 NTU turbidity (TW 3%) with 3.7-log reductions was significantly lower than other UV-C radiation dose (5.2 to 5.4-log reductions obtained after 3 and 5 min). The effect of turbidity was observed after 3 and 5 min of treatment (UV-C radiation doses of 2.0 and 3.4 KJ/m^2, respectively), when reduction values at the highest turbidity tested (664 NTU, TW 10%) were about 1.2 log units lower than those observed at 0.88 (TW 0%) and 166 NTU (TW 3%). Results demonstrated that control washing with water without UVC light (TW) greatly decreased *L. innocua* population on the surface of tomatoes, with reductions of 4.40 and 5.18 log cfu/cm^2 regardless of UV-C radiation dose. However, TW with no UVC light did not eliminate those bacteria that went into the water, with microbial counts above 2 log cfu/mL (Fig. 4). Contrarily, when UVC lights were on, *L. innocua* population in wash water after treatment significantly decreased, being the lowest populations when water was clean (TW 0%). In the case of using high-turbidity wash water (TW 10%) more than 3 min (UV-C radiation dose of 2.0 KJ/m^2) were required to reduce microbial contamination below 1 log cfu/mL.

3.3. Effect of WUVC on the indigenous microbiota of tomato surface

According to the results obtained in inoculated experiments, only 3 and 5 min treatments (corresponding to UV-C radiation doses of 2.0 and 3.4 KJ/m^2) were tested. Properties of wash water in non-inoculated experiments (Table 3) were similar to those obtained in *L. innocua* inoculated experiments.

Initial population of TAM on the tomato surface was 2.26 log cfu/cm^2 (Fig. 5) and the reductions observed were lower than those obtained when tomatoes were artificially inoculated (≤ 0.8 log). Organic load did not significantly affect the reduction of epiphytic microbiota after 3 and 5 min washing. The increase of UV-C radiation dose was only significant when wash water had no organic matter (WUVC 0%).

However, the population of TAM in the wash water after washing was significantly influenced by the organic load (Fig. 6); the reductions observed were significantly higher (lower population) when turbidity values were lower. High populations (>10^3 cfu/mL) remained in wash water in control (no UVC lights) washing while in WUVC 0% tomato pulp (0.91 NTU) counts were below 10 cfu/mL. For each treatment, no significant differences were observed according to UV-C radiation dose. Molds and Yeast results are not shown as they were below the detection limit in most cases.

4. Discussion

In this work, we studied the effect of a water-assisted UVC system, a novel UVC-based technology to the decontamination of fresh sound tomatoes and tomato processing wash water, in which UVC lamps are immersed in water. Studies were carried out in artificially inoculated tomatoes, using *L. innocua,* and in non-inoculated tomatoes, for the evaluation of its effect on natural epiphytic microorganisms. The effect of the turbidity of wash water was determined, as the efficacy of UVC treatment could be affected. Turbidity values were selected in a range provided by a frozen tomato company in Spain.

Results demonstrated that turbidity and time greatly affected the efficacy of the WUVC for the elimination of *L. innocua* artificially inoculated in wash water. For the same UV-C radiation dose, reduction values were lower at higher turbidities and higher UV-C radiation dose were needed at higher turbidites to obtain similar results. UV dose is the product of UV intensity and time of exposure, so longer treatment time should provide more damage to bacterial cell and, on the contrary, the presence of organic matter (increased turbidity) in the wash water would prevent the penetration ability of UVC light.

A frozen tomato processing plant provided turbidity values used in this study and they were considerably higher than others used in different studies (Liu et al., 2015, Guo et al., 2017). Our results demonstrated that to obtain *L. innocua* reductions ≥4 log cfu/mL, wash water should have turbidity values < 148 NTU and treatment times should be higher than 3 min (UV-C radiation dose > 2.0 KJ/m^2). Similarly, Liu et al. (2015) reported that UV inactivation of *Microcystis viridis* decreased when water turbidity increased from 1 to 30 NTU. Guo et al. (2017) also found an adverse effect of organic load and soil on water assisted UV inactivation, with increasing *Salmonella* survivors in wash waters of increased turbidity. In that study, wash waters from tomato (61.9 NTU), lettuce (96.7 NTU), baby-cut carrot (160 NTU) and baby spinach (232 NTU) were tested. However, in the case of tomato, they found that the single water-assisted UV treatment completely eliminated the cocktail of mutant *Salmonella* strains in wash water, regardless of water quality. For lettuce, spinach and carrots, the single water-assisted UV treatment did not completely eliminate *Salmonella* in wash water regardless of water quality, indicating the need for the combination with chemicals. Letho et al. (2016) found that the inactivation of *E. coli* and *Candida lambica* with UV-C with an interfering carrot juice (1%) decreased the inactivation and lengthened its application and suggested a water filtration to enhance the disinfection efficacy.

In the case of tomato surface, *L. innocua* populations were reduced by 3.27-5.36 log cfu/cm^2 (Fig.5) and turbidity and UV-C radiation dose had no such influence as that observed for wash water. Results demonstrated that using a water wash without UVC light (TW) greatly decreased *L. innocua* population on the surface of tomatoes, with reductions of 4.40 and 5.18 log cfu/cm^2 regardless of treatment time (radiation dose). This could be attributed to the mechanical removal of bacterial cells from the peel to the wash water caused by the agitation and waterjet through the sprinklers. However, when using water alone, about 2.5 log cfu *L. innocua*/mL were present in wash water. Liu et al. (2015) observed *E. coli* O157:H7 higher microbial reductions using longer wet UV treatments on skin-spot inoculated blueberries; the reductions achieved were 3.4, 3.9, 5.2 log cfu/g for 2, 5 and 10 min. In these conditions, they achieved undetectable levels in wash water. In a similar study, Huang et al. (2018) found that there were not significant differences on the reduction of *Salmonella* on the surfaces of grape tomatoes, blueberries and baby spinach when compared to WUV and tap water wash, with reductions between 0.88 and > 3.64 log cfu/g depending on the produce item. However, there were significant differences in the residual *Salmonella* population in turbid wash water, being significantly higher when no UV light was used. Similarly, Pangloli and Hung (2013) found that tap water reduced the number of *E. coli* O157:H7 on the surfaces of blueberries by 1.9–2.7 log cfu/g through physical or mechanical washing without killing the pathogen with pathogen populations of 3.6–3.8 log cfu/mL in wash solution. Therefore, even washing with tap water could be effective in reducing microbial contamination of produce although it might not prevent the possibility of cross-contamination as most of the microorganisms that went to water were not eliminated. In order to diminish the negative effects of organic matter in wash water, Alharbi et al. (2017) investigated a combination of electrocoagulation combined with UV light to reduce the organic content and enhance the microbiological quality of wash water derived from shredded lettuce processing.

In non-inoculated tomatoes, the efficacy of WUVC treatment was lower than that obtained in *L. innocua* inoculated samples, with values ≤ 0.8 log cfu/cm^2 of TAM after 3 min washing. Similarly, wash water turbidity did not affect the survival population on the tomato surface but it had a significant influence on the reduction of microorganisms on wash water. Increasing turbidity from 0.91 to 169 NTU increased the microbial counts in wash water from <1-log to 2.4 log cfu/mL. Similar to what happened in inoculated samples, TW was as efficient as WUVC in reducing the microorganisms on the surface of tomato, but residual population in wash water after washing was very high (>10^3 cfu/mL) compared to WUVC with 0% of tomato pulp. It was likely that the higher reduction of *L. innocua* on artificially spot inoculated samples was due to the loose attachment to the smooth surface of tomato while indigenous microbiota were strongly attached or internalized in cracks, (micro)wounds or bruises and/or protected by biofilms. It is known that biofilm could protect microorganisms from environmental changes and microbial treatments (Simões et al., 2010). According to this, Guo et al. (2017) found the highest *Salmonella* reduction using water assisted UV on grape tomatoes followed by iceberg lettuce and baby spinach, while the lowest *Salmonella* reduction was achieved on baby-cut carrots and this was attributed to the surface structure. Mukhopadhyay et al. (2014) found that inoculation site also influenced dry UVC treatment; better results of inactivation were found when *Salmonella* and *E. coli* were inoculated on tomato surface than on tomato scar. Moreover, low efficacy of WUVC on epiphytic microorganisms may also be due to a higher resistance of some of them. However, in an irregular and rough surface such as broccoli florets, Collazo et al. (2018) found higher reductions of mesophilic aerobic microorganisms (around 2-log reduction) after washing 2 min in the same equipment. Esua et al. (2018) combined UVC light treatment with ultrasound for the reduction of native microbiota on the surface of tomatoes and found higher reduction values (2-log) than those found in our studies. This could be due explained by the higher dosage level used (6.46 KJ/m^2), which was almost double than ours (3.36 KJ/m^2) and the combination with ultrasound. In this study, we have demonstrated that high reductions of *L. innocua* population on fresh tomatoes could be achieved using a novel WUVC system as it decreases microbial load on both tomatoes and wash water. Current insights show that the main expected effect of sanitizing treatments during produce washing is to reduce and control the microbial load of the water rather than produce decontamination (Van Haute et al., 2015). Constant wash water quality changes should be taken into account. Consequently, by maintaining the water quality throughout produce processing, the potential for cross-contamination during washing can be diminished (Gil et al., 2009; Parish et al., 2003; Van Haute et al., 2015). Finally, this WUVC system could be easily implemented in the existing washing lines, as UVC lights could be installed in the washing tanks and shields should be installed to protect workers from UVC exposure. However, more studies should be carried out in order to increase its efficacy in case of washing lines with high organic matter, for example, with a combination of a low risk or no residue chemical, including a prewashing step before WUVC and/or a filtration system to maintain low turbidity in the UVC washing tank.

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FIGURE LEGENDS

Figure 1. Water-assisted UV (WUVC) light equipment. (A) General overview (B) Detail of the interior of the tank. (1) Water tank 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 controlled with a power source (4). Pressurized water is introduced 100 kPa, enters through the bottom of the tank for water bubbling (7). Four equidistant UV lamps (8) (17.2 W) emitting at 254 nm are located in water proofs quartz compartments inside the tank. Radiation is measured on a hole in the lid of the tank (9).

Figure 2. Reduction of *L. innocua* (log cfu/mL) on wash water in the WUVC system according to different turbidities and treatment time. *L. innocua* was inoculated at 10^7 cfu/mL. Wash water turbidity was changed by adding different canned chopped tomato amounts (0, 1, 2.5, 5, 10 and 15%). For each time, different letters (A, B, C) indicate significant differences among turbidities according to a Tukey test ($P<0.05$). For each turbidity, different capital letters (X, Y, Z) indicate significant differences due to treatment time (radiation dose). Vertical bars indicate standard error of the mean. Nd: Not done

Figure 3. Reduction of *L. innocua* (log cfu/cm^2) on the surface of tomatoes on the WUVC system according to different turbidities and treatment time. *L. innocua* was inoculated by pipetting 50 µL of a 10^{10} log cfu/mL suspension. Wash water turbidity was changed by adding different canned chopped tomato amounts (0, 3, and 10%) to tap water (TW). For each time, different capital letters (A, B, C) indicate significant differences among turbidities according to a Tukey test ($P<0.05$). For each turbidity, different capital letters (X, Y, Z) indicate significant differences due to treatment time (radiatiation dose). Vertical bars indicate standard error of the mean.

Figure 4. Effect of WUVC system on the population of *L. innocua* (log cfu/mL) in the wash water after tomato washing according to different turbidities and treatment times. *L. innocua* was inoculated by pipetting 50 µL of a 10^{10} log cfu/mL suspension. Wash water turbidity was changed by adding different canned chopped tomato

amounts (0, 3, and 10%). For each time (radiation dose), different capital letters (A, B, C) indicate significant differences among turbidities For each turbidity, different capital letters (X, Y, Z) indicate significant differences due to treatment time. according to a Tukey test (P<0.05). Vertical bars indicate standard error of the mean

Figure 5. Effect of WUVC treatment on the population of total aerobic microorganisms (TAM) on the tomato peel (log cfu/cm^2). Values are mean of at least 6 values and bars indicate standard deviation. For each turbidity tested, different letters indicate significant differences (P<0.05) in TAM population among treatment times, according to a Tukey test. When there were no letters, no significant differences were found. Per each treatment time, no significant differences among turbidities were found. Vertical bars indicate standard error of the mean

Figure 6. Effect of WUVC treatment on the population of total aerobic microorganisms (TAM) in the wash water after 3 and 5 min treatments (log cfu/mL). Values are mean of at least 4 values and bars indicate standard deviation. For each washing time, diferent capital letters indicate significant differences (P<0.05) among treatments, according to a Tukey test. Vertical bars indicate standard error of the mean

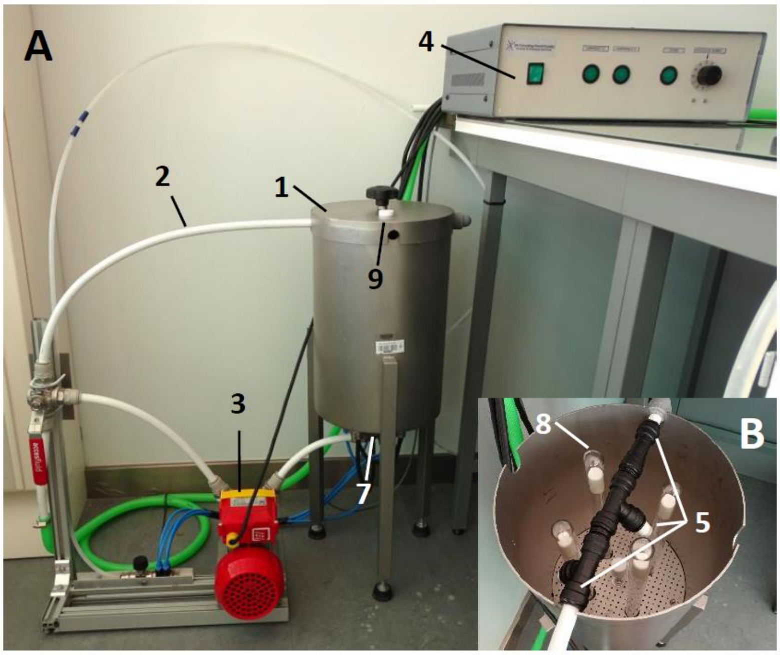

Figure 1

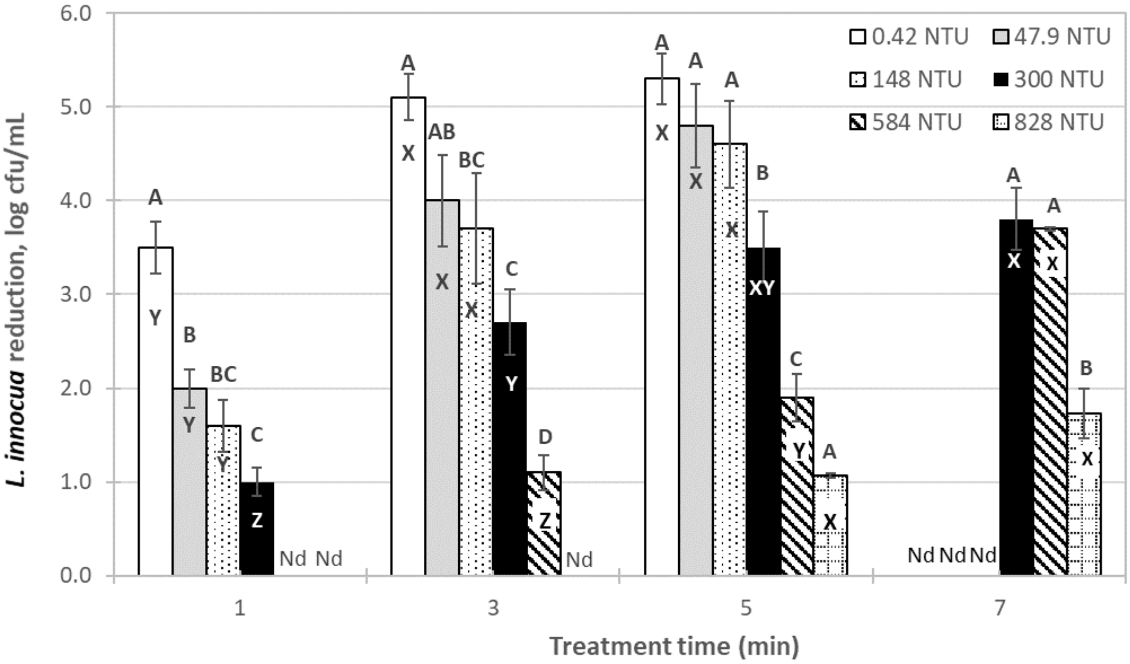
L. innocua reduction, log cfu/mL
6.0
5.0
4.0
3.0
2.0
1.0
0.0
Treatment time (min)
1
3
5
7
0.42 NTU
47.9 NTU
148 NTU
300 NTU
584 NTU
828 NTU
A
Y
B
Y
BC
Y
C
Z
Nd
Nd
A
X
AB
X
BC
X
C
Y
D
Z
Nd
A
X
A
X
A
X
B
XY
C
Y
A
X
Nd
Nd
Nd
A
X
A
X
B
X

Figure 2

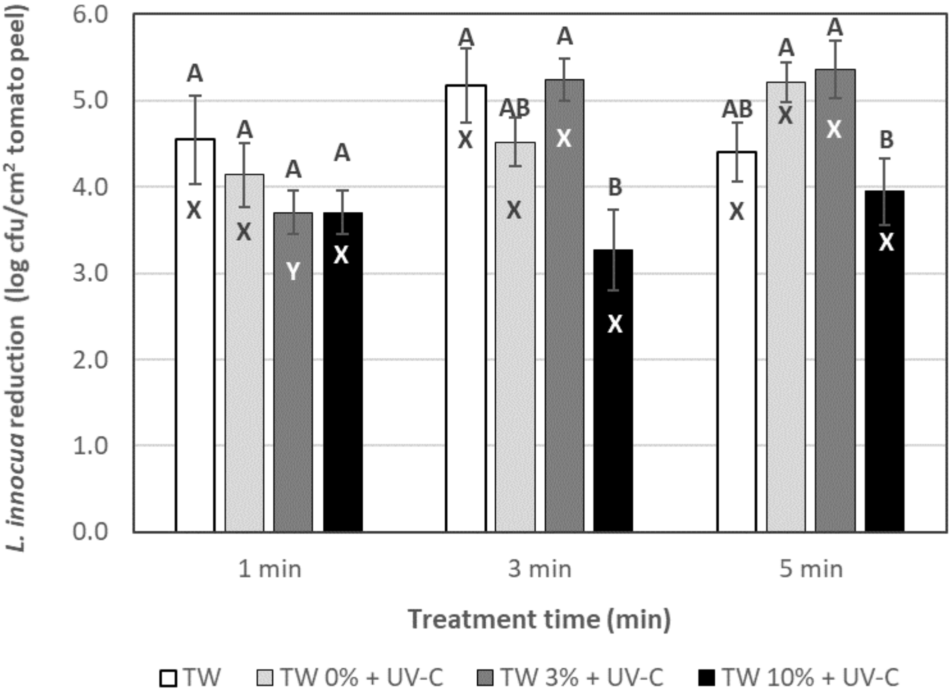
L. innocua reduction (log cfu/cm² tomato peel)
6.0
5.0
4.0
3.0
2.0
1.0
0.0
A
X
A
X
A
Y
A
X
A
X
AB
X
A
X
B
X
AB
X
A
X
A
X
B
X
1 min
3 min
5 min
Treatment time (min)
TW
TW 0% + UV-C
TW 3% + UV-C
TW 10% + UV-C

Figure 3

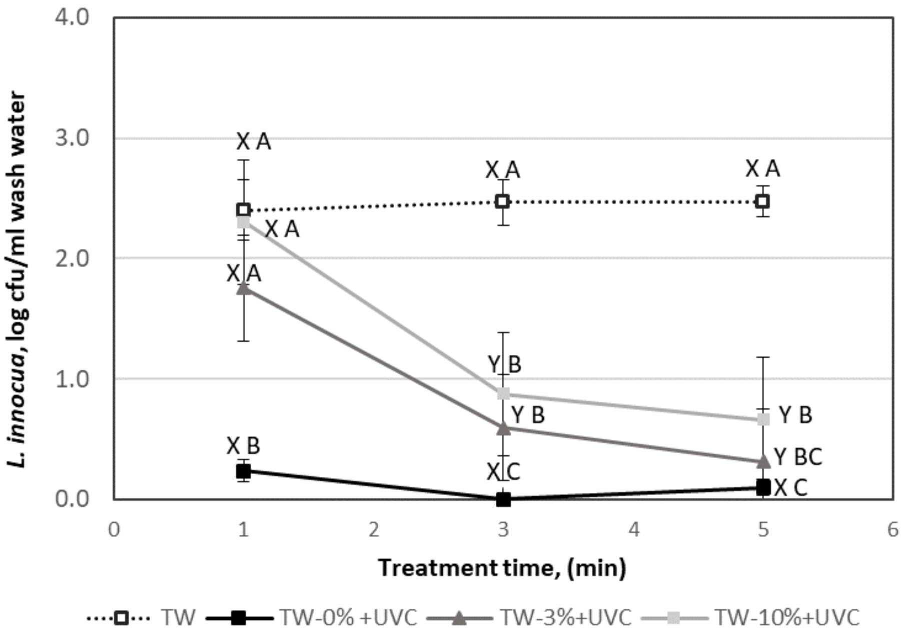
L. innocua, log cfu/ml wash water
4.0
3.0
2.0
1.0
0.0
0
1
2
3
4
5
6
Treatment time, (min)
X A
X A
X A
X A
X A
X B
Y B
Y B
X C
Y B
Y BC
X C
TW
TW-0% +UVC
TW-3%+UVC
TW-10%+UVC

Figure 4

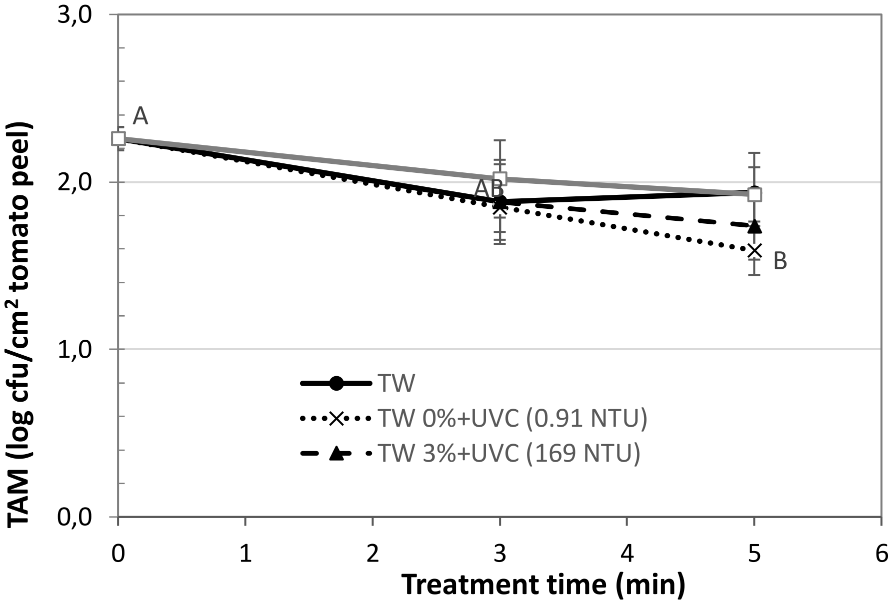
3,0
2,0
1,0
0,0
TAM (log cfu/cm² tomato peel)
0
1
2
3
4
5
6
Treatment time (min)
A
AB
B
TW
TW 0%+UVC (0.91 NTU)
TW 3%+UVC (169 NTU)

Figure 5

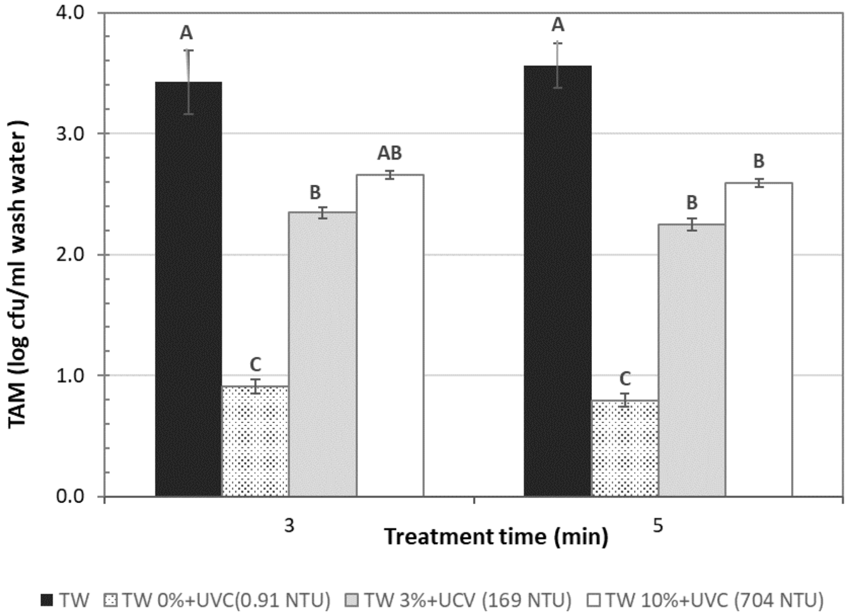
4.0
3.0
2.0
1.0
0.0
TAM (log cfu/ml wash water)
A
C
B
AB
A
C
B
B
3
5
Treatment time (min)
■ TW
▨ TW 0%+UVC(0.91 NTU)
■ TW 3%+UCV (169 NTU)
□ TW 10%+UVC (704 NTU)

Figure 6

Table 1. Properties of wash water in the experiments of *L. innocua* inactivation in water

Tomato pulp concentration (%)	Turbidity (NTU)		Conductivity (µS/cm)		pH		ORP (mV)	
	Mean	Desvest	Mean	Desvest	Mean	Desvest	Mean	Desvest
0 %	0.42	0.15	322	14	7.94	0.06	242	18
1 %	47.9	7.5	426	55	7.20	0.48	242	8
2.5 %	148	14	747	7	6.58	0.67	249	4
5 %	300	26	1219	7	5.53	0.68	284	25
10 %	584	31	2085	21	4.68	0.15	310	29
15 %	828	21	2825	120	4.49	0.10	306	28

Table 2. Properties of wash water in tomato trials, with or without *L. innocua* inoculation.

Tomato pulp concentration (%)	Turbidity (NTU)		Conductivity (µS/cm)		pH		ORP (mV)	
	Mean	Desvest	Mean	Desvest	Mean	Desvest	Mean	Desvest
0 %	0.88	0.40	322	14	7.94	0.06	243	5
3 %	166	24.7	747	7	6.58	0.67	262	30
10 %	664	36	2155	89	4.52	0.09	277	22

Table 3. Properties of wash water. Natural microbiota experiments

Tomato pulp concentration (%)	Turbidity (NTU)		Conductivity (µS/cm)		pH		ORP (mV)	
	Mean	Desvest	Mean	Desvest	Mean	Desvest	Mean	Desvest
0 %	0.91	0.45	325	5	7.96	0.12	215	21
3 %	169	34	713	68	5.72	0.32	251	19
10 %	704	69	2070	373	4.45	0.07	270	2