1   **Inactivation of *Salmonella enterica*, *Listeria monocytogenes* and murine norovirus**

2   **(MNV-1) on fresh strawberries by conventional and water-assisted ultraviolet light**

3   **(UV-C)**

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Highlights

- WUVC reduced the population of pathogenic bacteria and enteric virus on strawberries.
- At the same irradiation dose ($1.3 \ kJ/m^2$),WUVC improved the efficacy of DUVC system.
- For MNV-1, the increase in the irradiation dose did not affect their reduction.
- WUVC was effective for wash water disinfection, enabling its recirculation.
- The results obtained provide new tools to ensure the safety of strawberries.

23   **Abstract**

24   The efficacy of the water-assisted ultraviolet-C light (WUVC) strategy was evaluated as an

25   alternative to chlorine sanitization and compared to 'conventional' dry technology (DUVC) for

26   the inactivation of *Salmonella enterica, Listeria monocytogenes* and murine norovirus (MNV-1)

27   on strawberries. Strawberries were washed in a laboratory scale prototype (LAB-UVC-Gama)

28   consisting of a tank filled with water, equipped with 4 UV-C lamps emitting a dose of 0.6, 1.3,

29   3.2 and 6.3 kJ m^{-2}. For DUVC, the same doses were used. Moreover, trials with the 4 lamps off

30   with water, or with a chlorine solution (200 ppm, pH 6.5), were carried out as a control treatment.

31   Reductions of artificially inoculated *L. monocytogenes* and *S. enterica,* and the infectivity of

32   MNV-1 after WUVC treatments were comparable to those obtained with chlorine-wash, which

33   were equivalent with all irradiation doses tested for all microorganisms studied ($P < 0.05$). The

34   implementation of the WUVC strategy improved the DUVC system after 2-min exposure (1.3 kJ

35   m^{-2}), by 1.2 and 1.6 log for *S. enterica* and *L. monocytogenes*, respectively. At 3.2 kJ m^{-2} dose (5

36   min), WUVC enhanced the inactivation of *S. enterica* compared with control washing treatment

37   by 1.5 log. After 10 min, pathogenic bacteria were reduced by > 4 log by WUVC treatment and

38   chlorine sanitization. For MNV-1 reductions, we reported > 1.4 log TCID$_{50}$ with 95% certainty

39   with the different treatments and exposure times after decontamination procedures. For MNV-1,

40   the increase in the irradiation dose (kJ m^{-2}) applied did not affect their reduction on strawberries.

41   Moreover, WUVC light was effective at significantly reducing the microorganisms in wash water,

42   avoiding cross-contamination and thus, allowing water recirculation. The results obtained in the

43   present study provide new tools to ensure the safety of strawberries intended to be processed,

44   contributing to affording a more innovative and sustainable future for the food industry. However,

45   industry operation studies are needed to conclude that the treatments tested in the present study

46   are a good alternative to chlorine.

47   *Keywords:* sanitization, chlorine alternative disinfection, fruit, cross-contamination

48

1. Introduction

In recent decades, the consumption of berries has increased their cultivation in many developed countries (Assurian et al., 2020). The most commonly consumed fruits in Spain are strawberries (*Fragaria x ananassa*). Spain is 6th in the top-10 producers in the world with 344,679 tn in 2018 (FAOSTAT, 2019), 80 % of total production being destined to fresh produce. The rest are intended for industrial processing purposes, such as ice creams, yogurts, jams, jellies, dessert toppings and smoothies (frozen fruits) (Bozkurt et al., 2020; Šamec et al., 2018). Despite the high value of the strawberry industry, they are generally eaten raw and could represent a potential risk for consumers. The concern about the microbiological safety of fresh, minimally processed, or frozen berries has increased in recent years due to the huge increase in the number of people that strive to eat more healthily by increasing their consumption, and the subsequent upsurge of foodborne outbreaks linked to their intake (Lafarga et al., 2019).

The published literature reported that strawberries have been associated with several foodborne illness outbreaks caused by a broad range of biological hazards, from viruses (such as human norovirus or hepatitis A virus) to bacteria (*Escherichia coli*, *Salmonella enterica*, *Listeria monocytogenes*) (European Food Safety Authority, 2014; Hadjilouka et al., 2014). In recent years, human norovirus has been increasingly recognized as the most important agent causing outbreaks as well as sporadic cases of acute gastroenteritis worldwide (Koo et al., 2010). Among the different food types involved, frozen, processed and fresh strawberries have often been identified as vehicles of human norovirus transmission (Butot et al., 2018; Cook et al., 2018; Mäde et al., 2013; Maunula et al., 2013; Sarvikivi et al., 2012). A large gastroenteritis outbreak of human norovirus linked to the consumption of frozen strawberries from China affected nearly 11,000 people in Germany in 2012 (Bartsch et al., 2019).

Currently, the reduction of microbiological loads of produce is carried out in industry mainly with sanitation involving chlorine solutions. However, in order to reduce the health and environmental risks involved in the formation of chlorine halogenated by-products, and other disadvantages of sodium hypochlorite, alternative methods such as non-thermal physical technologies are being

76 evaluated (Collazo et al., 2018). Ultraviolet short-wave irradiation (UV-C) (200-280 nm) is an

77 excellent alternative technology to chemical sanitation, used to reduce foodborne pathogens by

78 the deleterious effect on microbial DNA structure, causing pyrimidine dimers, inhibiting DNA

79 replication, and consequently, the ability of the microorganism to survive and reproduce (Gayán

80 et al., 2014; Wallace et al., 2019). UV-C light decontamination has been studied for the

81 inactivation of bacteria, protozoan, and fungi on berries (Bhat et al., 2015; Bialka et al., 2008;

82 Kniel and Shearer, 2009), but there is limited information about the efficacy of UV-C against

83 foodborne enteric viruses in fresh and frozen strawberries (Bozkurt et al., 2020). On the other

84 hand, this technology has been widely used as an effective disinfection method in water and

85 wastewater treatment industries (Beck et al., 2015). However, conventional UV-C treatment

86 transmitted by air has several drawbacks, such as the overheating of the static fruit and its limited

87 accessibility on the surface microorganisms, which depends on the roughness of the fruit matrix.

88 UV-C light transmitted by lamps immersed in stirring water (WUVC) could be a novel technology

89 for the decontamination of strawberry and other produce, and principally for the frozen fruit

90 industry, by washing the samples before the freezing step, avoiding the problems related to mould

91 growth, and can act as a first barrier against enteric viruses so frequent in this type of market

92 (Bozkurt et al., 2020). This approach could partially avoid the disadvantages offered by air-

93 transmitted UV-C technology, preventing the shadowing effect by agitating the samples

94 homogenously in the water tank, reducing the probability of their overheating compared to

95 conventional chambers, and enhancing the removal of microorganisms from irregular surfaces of

96 strawberries (Huang et al., 2018; Huang and Chen, 2014). Previously, Collazo et al. (2018,

97 2019a,b) obtained promising results for the decontamination of *Listeria innocua* and spoilage

98 microbiota on broccoli, lettuce and spinach using the same device, without negative consequences

99 on their quality.

100 In this study, we evaluated the efficacy of 'conventional' UV-C light (transmitted by air or dry

101 DUVC) and water-transmitted UV-C (WUVC) to inactivate the foodborne pathogens, *S. enterica,*

102 *L. monocytogenes* and murine norovirus (MNV-1), a human norovirus surrogate, on strawberries.

103   To our knowledge, this is the first study to disinfect enteric viruses (MNV-1) on strawberries in

104   a system where lamps and fruits are immersed in water. Therefore, the aims of the present study

105   were first, to investigate the efficacy of both technologies (DUVC and WUVC) at different time

106   exposures for the inactivation of *S. enterica, L. monocytogenes* and MNV-1 on strawberries, and

107   second, to determine the efficacy of UV-C lamps in sanitizing washing water, in order to prevent

108   cross-contamination fruit-to-water and fruit-to-fruit. UV-C treatments were compared with a

109   standard treatment of sodium hypochlorite (NaClO).

2. Materials and Methods

2.1. Fruit

Strawberries (*Fragaria × ananassa*), were purchased the day before the experiment from local distributors in Lleida (Catalonia, Spain). Samples with visible physical damage were excluded and only healthy fruits with similar size and weight (approximately 25 g) were carefully chosen. Fruits were either inoculated on the same day or stored at 4 ± 1 °C overnight. On the day of the experiment, the peduncle of the fruit was manually removed.

2.2. Chemicals and media

Tryptone soy broth (TSB), tryptone soy agar (TSA), PALCAM base agar, yeast extract (YE), Xylose-Lysine-Deoxycholate Agar (XLD) and peptone were purchased from Biokar Diagnostics (Allonne, France). Dey-Engley broth was obtained from Honeywell Fluka (Madrid, Spain). Sodium hypochlorite 10 % w v^{-1} (NaClO) was purchased by Panreac AppliChem (Barcelona, Spain). Dulbecco's modified Eagle medium (DMEM) and fetal serum bovine (FBS) was purchased from Hyclone (Pennsylvania, US).

2.3. Microorganism preparation

2.3.1. Pathogenic bacteria

For this study, a cocktail containing five *Salmonella enterica* subsp. *enterica* strains: Agona (ATCC BAA-707), Michigan (ATCC BAA-709), Montevideo (ATCC BAA-710), Gaminara (ATCC BAA-711) and Enteritidis (CECT-4300), and five *L. monocytogenes* strains: serovar 1a (CECT-4031), serovar 3a (CECT-933), serovar 4d (CECT-940), serovar 4b (CECT-4032) and serovar 1/2a (Abadias et al., 2008), were used and prepared as described in Ortiz-Solà et al. (2020). The concentration of each inoculum was checked by plating appropriate dilutions on PALCAM agar for *L. monocytogenes* or on XLD for *S. enterica*. Plates were incubated at 37 °C $\pm$ 1 °C for 24 h (*S. enterica*) or 48 h (*L. monocytogenes*).

2.3.2. Enteropathogenic virus and human cell lines

Murine norovirus 1 (MNV-1), a surrogate of human norovirus, and murine macrophage cell line RAW 264.7 were kindly provided by Prof. H. W. Virgin (Washington University School of Medicine, US). The cell line was maintained at 37 ± 1 °C in a 5 % CO_2 humidified incubator (NU-4750, NuAire, US) in T175 flasks (Nunc, Thermo Fisher, US). MNV-1 stocks were propagated and quantified in the RAW 264.7 cell line, as described in Falcó et al. (2018). Briefly, semi-purified MNV virus was harvested 2 days after infection by three freeze-thaw cycles of infected cells followed by centrifugation at $660 \times$ g for 30 min to remove cell debris. Infectious viruses were enumerated by determining the 50% tissue culture infectious dose (TCID$_{50}$) with eight wells per dilution and 20 µL of inoculum per well using the Spearman-Karber method (Pinto et al., 1994). Stocks of MNV-1 (1 mL) were frozen until use (-80 °C). RAW 264.7 cells were cultured in DMEM supplemented as described in Ortiz-Solà et al. (2020).

2.4. Microorganism inoculation on strawberries

The day before the experiment, strawberries were inoculated with a suspension containing 10^8 CFU mL^{-1} of each cocktail (*S. enterica* and *L. monocytogenes*) by pipetting 50 µL in small droplets on the surface of one side of the fruit. Strawberries were dried at room temperature (22-25 °C) for approximately 1 – 2 h in a class II biological safety cabinet (type A, Telstar, Terrassa, Spain). Afterwards, inoculated strawberries were stored at 4 ± 1°C overnight to allow bacterial attachment and adaptation to fruit conditions.

In case of MNV-1, frozen stocks were thawed and diluted one logarithmic unit (2.8×10^7 tissue culture infective dose TCID$_{50}$ mL^{-1}) with Phosphate-Buffered Saline (PBS; ThermoFisher, US). Inoculation was done also as described above; fruits were allowed to dry and used on the same day of inoculation.

Prior to the experiments, the initial concentration of *S. enterica, L. monocytogenes* and MNV-1 in the suspensions was checked as explained below.

2.5. UV-C equipment

161   The UV-C water-assisted equipment (WUVC, LAB-UVC-Gama, UVC-Consulting Peschl

162   España, Castellón, Spain, Fig. 1A, B and C) consisted of a 15-L chamber with 4 UV-C lamps

163   (GPH303T5L/4, 254 nm), irradiating a power of 17.2 W each. The interior was fully covered with

164   a highly reflective material (Solar Bright, Fuller Ultraviolet) that increased the UV light intensity

165   and minimized the shadowing effect of irregularly shaped samples. During UV-C treatment, the

166   chamber was fully closed. Moreover, the equipment has a recirculation and ventilation system

167   that provides bubble production and makes the recirculation of the water wash, simulating the

168   industry washing tanks. This mechanism improves accessibility to UV-C light from all sides of

169   the surface of fruit. At the beginning of the experiment and before the UV-C treatments, lamps

170   were preheated for 10 min, to reach the maximum irradiance (10.5 ± 0.5 W m^{-2}). Irradiation was

171   measured in the air-filled setup with a UV-sensor Easy H1 (Peschl Ultraviolet, Mainz, Germany)

172   through a cavity located on the top of the tank. Irradiation UV-C light doses were calculated as

173   reported by Lopez-Rubira et al. (2007):

174   $$D = \frac{I \times t}{1000}$$ eq. 1

175   Where D was the irradiation dose applied (kJ m^{-2}), I was the irradiation intensity of UV-C light

176   multiplied by the area (W m^{-2}) and t was the time exposure (s).

177   The air-transmitted dry UV-C (DUVC) treatment was carried out in a biosafety laminar air cabinet

178   (class II - type A, Telstar, Terrassa, Spain) equipped with a UV-C light (30W/30G T8) with an

179   irradiating power of 10.5 ± 0.5 W m^{-2}. The UV-C lamp was located at the top of the chamber, and

180   the irradiation dose was set up by adjusting the distance between the light and the sample.

181   **2.6. Disinfection treatments**

182   Two UV-C treatments were proposed: the conventional DUVC and the WUVC treatment. Tap

183   water (W) and 200 mg L^{-1} of a free chlorine solution (prepared with NaClO) adjusted to pH 6.5

184   using citric acid 2 M were used as control treatments. For each washing treatment, 20 strawberries

185   were immersed in 12 L of cold (6 ± 2 °C) tap water with recirculation for 1, 2, 5 and 10 min. In

186   the case of WUVC treatment, these times corresponded to an irradiation dose of 0.6, 1.3, 3.2 and

187   6.3 kJ m^{-2}, respectively. For microbiological analysis, 3 fruits and 2 water samples were taken off

188   for each studied time. After the washing treatment, fruits were left to dry at room temperature in

189   the biosafety cabinet. Experiments were performed separately for each microorganism. For water

190   and NaClO treatments, the same device with the UV-C lamps switched off was used to get

191   comparable results. The free chlorine concentration was checked with an ion specific meter Hanna

192   Instruments HI 95734-11 (Rhode Island, US). After NaClO washing, strawberries were rinsed in

193   tap water for 2 min and left to dry at room temperature as described above.

194   For disinfection of the fruit with DUVC procedure, 20 inoculated fruits were arranged along a

195   stainless steel grid in a biosafety laminar air cabinet with the inoculated side upwards and just

196   below the UV light following the same conditions and time exposure mentioned above with

197   irradiation about 10.5 W m^{-2}. Three strawberries were sampled after 1, 2, 5 and 10 min for

198   microbiological analysis.

199   **2.7. Evaluation of cross-contamination of *L. monocytogenes* onto non-inoculated**

200   **strawberries**

201   *L. monocytogenes* transfer from artificially inoculated strawberries to non-inoculated fruits was

202   studied. To evaluate the efficacy of the aforementioned antimicrobial UV-C treatments in

203   preventing cross-contamination of *L. monocytogenes* during strawberry washing, the same

204   number of inoculated and non-inoculated fruits (1:1; wt:wt) was introduced in the WUVC device.

205   They were treated as described in section 2.6. Three fruits were sampled per treatment and time.

206

2.8. Microbiological analysis

2.8.1. Bacterial counts

For microbiological analysis of fruits, triplicate samples consisting of one strawberry per repetition were weighed, placed in 80 mL sterile filter bags (BagPage®, Interscience, Saint Nom, France) and diluted with buffered peptone water (BPW; Biokar Diagnostics) 1:4 (w:v). Samples were mashed in a homogenizer (MiniMix, Interscience, France) for 2 min at 9 strokes s^{-1}. Aliquots of the mixture were serially diluted in saline peptone (SP; 0.85 % w v^{-1} NaCl; 0.1 % w v^{-1} Peptone), and plated on XLD for counting *S. enterica* or on PALCAM agar for *L. monocytogenes*. The agar plates were incubated at 37 ± 1 °C for 24 h (*S. enterica*) or 48 h (*L. monocytogenes*). Homogenates were left at 37 °C to confirm the presence of the pathogens in the case that plate counts were below detection limit. Results were expressed as log CFU $fruit^{-1}$, and the detection limit was 20 CFU $fruit^{-1}$. When no colonies were counted and detection was positive, an arbitrary number of half the detection limit was estimated (10 CFU $fruit^{-1}$). For each treatment and microorganism, reduction values were calculated as $\log (N_0) - \log (N_x)$, where N_x is the population of the bacteria after each treatment and N_0 is the initial population of untreated strawberries.

After each washing treatment, the population of bacterial strains were determined in the wash water. Wash water from NaClO treatment, and PA combined treatment with UV-C (1 mL) was added to 9 mL of neutralizing Dey-Engley medium and plated as described before. Dey-Engley tubes were incubated at 37 °C for 24 h. Results were expressed as log CFU mL^{-1}, and the detection limit was 50 CFU mL^{-1}. When quantification was below the detection limit, its presence was confirmed by Dey-Engley change in colour followed by streaking onto XLD or PALCAM. In the case of wash water from water and WUVC treatments, 100 µL were directly plated in duplicate onto XLD or PALCAM and the detection limit was 5 CFU mL^{-1}.

2.8.2.Norovirus determination

Before and after disinfection treatments, the extraction of MNV-1 from the strawberries was carried out as described by Ortiz-Solà et al. (2020). The day before determination, confluent RAW 264.7 cells with DMEM 10 % were transferred to 96-well microtiter plates (ThermoFisher, US) and allowed to grow at 37 ± 1 °C in a 5 % CO_2 and 85 % of relative humidity (RH) for 24 ± 2 h. Subsequently, DMEM 10 % was removed from the 96-well plates and 20 µL/well of 10-fold dilutions with PBS of each extracted sample were inoculated into 8 wells/plate of confluent RAW 264.7 monolayers and incubated at the same temperature, RH and CO_2 conditions indicated above. After a 1h incubation, 20 µL/well of DMEM supplemented with 2 % FBS were added and incubated at 37 °C in a 5 % CO_2 incubator for 2–3 days. Over time, RAW 264.7 monolayers were observed for cytotoxicity effects by visual inspection under an optical inverse microscope. Each treatment was done in duplicate. MNV-1 from stock human norovirus was used as positive control 4 wells/plate. Negative controls were PBS, containing 2 M $NaNO_3$, 1 % beef extract, and 0.1 % Triton X-100 (pH 7.2) spread in 4 wells/plate. For each sample, the number of wells that had a cytopathic effect after 48-72 h of incubation were documented and the number of infectious viruses was calculated by determining the $TCID_{50}$. The reduction of MNV-1 on treated strawberries was calculated as log (N_x/N_0), where N_x is the infectious virus titer after each treatment and N_0 is the initial virus infect titer found in untreated strawberries (initial) (Falcó et al., 2018).

2.9. Statistical analysis

Microbiological data (reductions of population on strawberries) were analysed using JMP Statistical software (version 14.0.1 SAS Institute Inc., NC, USA). Data were verified for agreement to normal distribution and homoscedasticity of residues and accordingly, means were compared by analysis of variance (ANOVA) and separated by Tukey's Honest Significant Difference (HSD) test ($P < 0.05$).

3. Results and discussion

3.1. Effect of water-assisted UV-C treatment on *S. enterica* and *L. monocytogenes* on fresh strawberries

The initial population of *S. enterica* on artificially inoculated strawberries was ca. 7.0 ± 0.1 log CFU strawberry^{-1} (data not shown). After 1-min treatment, no significant differences among treatments were found, with reductions ≥ 1.4 log (Fig. 2). However, when washing the strawberries with water-assisted UV-C (WUVC) treatment for 2 min, the *S. enterica* population was reduced 2.7 ± 0.3 log on strawberries, which was not statistically significant to that observed after sodium hypochlorite sanitization (NaClO) (2.6 ± 0.4 log) and water wash treatment (W) (1.8 ± 0.6 log). After 2-min exposure, WUVC treatment effectively inactivated *S. enterica,* improving the efficacy of dry UV-C (DUVC) by 1.2 log. Indeed, the use of DUVC irradiation has shown low effectiveness for reducing *S. enterica* populations compared with WUVC as reduction values were significantly lower ($P < 0.05$) for each treatment time. After 5 min, WUVC treatment caused high reductions of *S. enterica* counts on strawberries (4.1 ± 0.2 log), enhancing the efficacy of water control washing by 1.5 log. The same level of reduction was observed in samples sanitized with 200 mg L^{-1} of NaClO (3.4 ± 0.3 log). Regarding the effect of time, longer treatments did not significantly affect the efficacy of water and DUVC. In contrast, for NaClO and WUVC, increasing treatment time significantly increased *S. enterica* reduction.

Regarding *L. monocytogenes*, its initial population on strawberries was ca. 7.3 ± 0.3 log CFU strawberry^{-1} (data not shown). Similarly to the *S. enterica* results, the increase of treatment time did not influence the efficacy of the water and the DUVC system and for NaClO and WUVC, longer times meant higher reduction (Fig.3). In general, the efficacy of DUVC was significantly lower than that obtained with treatments using water-immersion after 2-min treatment. In particular, WUVC improved the efficacy of DUVC by 1.6 log following 2-min treatment. After 10 min, reductions of about 4.1 ± 0.9 and 4.4 ± 0.2 log were reported for WUVC and NaClO sanitation, respectively. In some fruits, the bacterial presence on strawberries was reduced to below the detection limit (20 CFU strawberry^{-1}) during chlorine sanitization.

3.2. Efficacy of UV-C disinfection against bacterial pathogens of washing water

Foodborne bacterial counts were found in the water control treatment (W) in all tested times, with mean populations of 3.33 ± 0.46 log CFU mL^{-1} and 4.1 ± 0.1 log CFU mL^{-1} for *S. enterica* and *L. monocytogenes*, respectively (Fig. 2 and 3). In the WUVC treatment, the populations of *S. enterica* and *L. monocytogenes* were observed only in the first and second minute of the washing treatment and, in some cases, the population was below the detection limit (dl). *S. enterica* was only detected (< dl) after 1 minute of chlorine sanitation. After this time, free chlorine contributed to improving the wash water quality, with no pathogenic bacteria detected.

3.3. Assessment of the efficacy of water-assisted UV-C technology in preventing Cross-Contamination of *L monocytogenes* on strawberries

The efficacy of the aforementioned antimicrobial disinfection UV-C treatments in preventing cross-contamination of *L. monocytogenes* during strawberry washing was evaluated. Previous results (Fig. 3) indicated that during water wash ca. 4 log CFU mL^{-1} of *L. monocytogenes* were transferred from inoculated strawberries to wash solution making the contamination of non-inoculated strawberries possible at all tested times, with populations ≈ 1.9 log CFU strawberry^{-1} (Table 1). Regarding WUVC, 1-min of water wash with UV-C technology reduced ca. 1 log of *L. monocytogenes* population in wash water and 2 out of 3 strawberries presented *L. monocytogenes* with a very low population (1.1 log CFU strawberry^{-1}). After 2 min, the WUVC system had the potential to prevent cross-contamination from both fruit-to-water and fruit-to-fruit due to the bactericidal effect of this technology during the experimental time. No cross-contamination of strawberries was observed for NaClO solution.

3.4. Effect of water-assisted UV-C treatments on infectivity of MNV-1 on strawberries

307 The initial virus titer in artificially inoculated strawberries was ca. 3.7 ± 0.7 log $TCID_{50}$ mL^{-1} (data

308 not shown). Reduction values obtained for the norovirus surrogate (MNV-1) were lower than

309 those reported with the foodborne bacterial strains. The reductions obtained were between 1.4 and

310 2.5 log $TCID_{50}$ with 95% certainty with the different treatments, and time exposure (Fig.4). For

311 all the treatments tested in the present study, the increase in treatment time did not affect the

312 reduction of MNV-1 infectivity on strawberries ($P > 0.05$). When comparing treatments, DUVC

313 had lower reduction than the other studied treatments after 5 min, with no significant differences

314 at the other treatment times. As for pathogenic bacteria, no significant differences in term of

315 reduction were achieved comparing the WUVC with NaClO sanitization.

4. Discussion

317 In the present work, a novel technology consisting of UV-C light transmitted by lamps immersed

318 in stirring water (WUVC) was evaluated as an alternative to chlorine disinfection for fresh

319 strawberries at different time exposures against *S. enterica, L. monocytogenes* and murine

320 norovirus (MNV-1). According to our knowledge, this is the first investigation that has used

321 WUVC as a novel sanitizing method for the inactivation of MNV-1 on strawberries. Moreover,

322 WUVC technology was compared with conventional DUVC technology.

323 As shown by the results, the way of application of UV-C light significantly influenced its

324 effectiveness for strawberry sanitization. While multisided application of 0.6 to 6.3 kJ m^{-2} UV-C

325 dose using the WUVC effectively inactivated *S. enterica* and *L monocytogenes*, and the

326 infectivity of MNV-1, the one-sided application of dry UV-C at the same irradiation dose showed

327 itself to be less effective compared to immersed sanitization, regardless of the exposure time. One

328 of the reasons could be that the application and efficacy of UV light in air is limited by the

329 shadowing effect due to the roughness and irregular shape of the fruit (Liu et al., 2015a). Previous

330 investigations with scanning electron microscopy (SEM) observations of the fruit surface,

331 suggested that bacteria might escape from air-transmitted UV-C light by lodging in structures

332 such as stomata, trichomes or cracks (Allende and Artés, 2003). For different berries, Butot et al.

333 (2018) reported that the mean inactivation of HAV (Hepatitis A virus) and MNV-1 at different

334 doses of DUVC (2.12 to 13.31 kJ m^{-2}, 20-120 s) was greater on blueberries (2 – 3 log) than on

335 strawberries and raspberries (< 2 log). The last mentioned experiment reported no more than 1

336 log reduction of artificially inoculated strawberries with *L. monocytogenes, E. coli* O157:H7 and

337 *S. enterica*. In contrast, similar doses of air-transmitted DUVC were sufficient to inactivate 2–3

338 log of *L. monocytogenes* on the smooth surfaces of apples, pears and tomatoes compared to rough-

339 surfaced fruits such as cantaloupe, strawberry and raspberry, whose reductions were lower ($\approx$ 1.0

340 log (CFU g^{-1}) at 11.9 kJ m^{-2}) (Adhikari et al., 2015). The second reason why WUVC is more

341 effective than DUVC might be the dual action for decontaminating the fruit sample: by the

342 irradiation effect itself and by the simultaneous physical removal of microorganisms of the surface

343 or lodged in trichomes or cracks caused by the agitation with water, overcoming one of the

344 principal drawbacks of air-transmitted UV-C light. This could be observed when we used the

345 same device with water and the UV-C lamps off (control) in which the studied pathogens were

346 also reduced. Additionally, the water wash agitation reduces the increase in temperature (Liu et

347 al., 2015a). Better efficacy of WUVC compared to the dry alternative for the inactivation of

348 foodborne pathogens from fresh produce has been previously reported, although using different

349 devices (Liu et al., 2015a, b). For example, Guo et al., (2017) found an improved reduction of *S.*

350 *enterica* (by 4.2 and 1.5 log (CFU g^{-1})) in spot-inoculated blueberries and 'Iceberg' lettuce,

351 respectively using WUVC (34.8 kJ m^{-2}, 120 s) compared to D-UV-C.

352 Regarding exposure time, it did not affect the efficacy of water and DUVC treatments for any

353 microorganisms tested, but it had a beneficial effect on WUVC and NaOCl treatments, in which

354 treatment times longer than 2 min were required for better efficacy. In the case of DUVC, previous

355 research reported similar results, supporting that treatment time (20-120 s) does not have a

356 significant impact on the inactivation of *S. enterica, L. monocytogenes* and *E. coli* O157:H7 on

357 strawberries (Butot et al., 2018). This plateau is probably due to the complex surface structures

358 of the fruits which feature shadowing and cavities that protect the organisms from the germicidal

359   light source. On the other hand, for pathogenic bacteria, the efficacy of WUVC and NaClO

360   sanitization treatments was similar throughout the experiment. When the strawberries were

361   washed with the WUVC technology for 2-min exposure, the pathogenic bacteria population were

362   reduced by ≥ 2 log, which was equivalent to those observed after their respective chlorine washing

363   control disinfection ($P < 0.05$). Similarly, after 5-min exposure, WUVC decontamination

364   achieved reductions of *L. monocytogenes* and *S. enterica* similar to those obtained with chlorine

365   (4 log) at a dose used to reduce microbial contamination for fresh produce in processing industries

366   (Pangloli et al., 2013). According to previous studies in our research group, reductions obtained

367   after 2-min WUVC treatment (1.3 kJ m^{-2}) were comparable to those obtained with chlorine-wash,

368   with reductions of about ≥ 2.5 log (CFU strawberry^{-1}) of one-single strain of *Listeria innocua* and

369   *S. enterica* ser. Typhimurium on fresh strawberries (Nicolau-Lapeña et al., 2020). Similarly,

370   reductions of about 4 log units were in concordance (4.9 ± 0.6 log (CFU strawberry^{-1}) reduction

371   for *S.* Typhimurium) after washing treatments with WUVC light and NaClO sanitization on fresh

372   strawberries (Nicolau-Lapeña et al., 2020). Moreover, the last commented investigation reported

373   no significant changes in the physicochemical and nutritional quality of fresh strawberries.

374   Previous investigations based on the combination of low-dose UV light and water immersion with

375   different technologies have been carried out for the decontamination of foodborne pathogens on

376   fresh produce. These have had variable efficacy according to the methodology used for irradiation

377   and inoculation, the dose, the target microorganism and the food matrix (Collazo et al., 2018;

378   Huang et al., 2018; Liu et al., 2015). Indeed, Collazo et al. (2019a) reported that WUVC appears

379   to be a suitable technology for controlling *L. monocytogenes* populations in fresh-cut broccoli at

380   0.3 and 0.5 kJ m^{-2} by 1.7 and 2.4 log (CFU g^{-1}), respectively. Similarly, 0.1 kJ m^{-2} WUVC reduced

381   *S. enterica* initial populations in 'Iceberg' lettuce by 2.0 ± 0.6 log, improving the efficacy of water-

382   washing control by 1.7 log (Collazo et al., 2019b).

383   Even though UVC light treatment has been studied for the inactivation of bacteria, protozoan, and

384   fungi on berries (Kniel & Shearer, 2009), there is limited information about its efficacy against

385   foodborne enteric viruses in strawberries. To our knowledge, there are no studies on the efficacy

386   of WUVC against enteric viruses on strawberries. As shown by the results, reduction values

387   obtained with the norovirus surrogate (MNV-1) were lower than those reported with the

388   foodborne bacterial strains. However, WUVC disinfection could also be used for the reduction of

389   human norovirus, as we demonstrated that their efficacy was equivalent to chlorine sanitization

390   during all experimental time. Previous studies demonstrated that UVC applied in a chamber was

391   efficient at inactivating HAV, Aichi virus A, and feline calicivirus on whole strawberries (Fino

392   & Kniel, 2008), with inactivation values of the three viruses tested on fresh strawberries of (1.9

393   to 2.6 log ($TCID_{50}$ mL^{-1})) with three doses applied (0.4, 1.2, and 2.4 kJ m^{-2}).

394   Regarding the cross-contamination of fruit-to-water and water-to-fruit, we reported that

395   foodborne bacterial pathogen counts were found in the water wash treatment (W, control) in all

396   treatment times tested in the present study. This presence in wash water clearly demonstrated the

397   transfer of the microorganisms from fruit surface to water due to the physical action of water

398   pressure, agitation and aeration (bubbles), explaining the reduction of the microbial loads on

399   strawberries in the water control, as detailed above. These artificially inoculated pathogens on

400   fresh strawberries and transferred to wash water were able to contaminate contaminant-free fruits,

401   as we have shown that a single batch of strawberries harbouring *L. monocytogenes* was able to

402   contaminate pathogen-free strawberries in the absence of UV-C. Similarly, a 2-min water wash

403   transferred ca. 5.0 log CFU $tomato^{-1}$ of *S. enterica* from inoculated tomato (8.3 log CFU $tomato^{-1}$

404   ) to non-inoculated tomatoes (Gereffi et al., 2015). These data indicated that during post-harvest

405   processing, a single contaminated fresh product has the potential to compromise the whole batch

406   or entire lot of fresh produce. It is of great necessity that the antimicrobial technology used in

407   fresh produce wash can prevent cross-contamination. Therefore, bacterial cells that were washed

408   off from the strawberries were inactivated by the UV-C and/or the disinfectant in the wash

409   solution (WUV and NaClO), thereby reducing the risks for cross-contamination. Presence of *S.*

410   *enterica* and *L. monocytogenes* were reported only in the first and second minute of the washing

411   exposure, and in some cases the presence was below the detection limit. For this reason, WUVC

412   light helped to minimize remaining populations of both pathogenic microorganisms in washing

413   water compared to W control. Even when WUVC technology did not completely inactivate the

414   studied pathogens in wash water in the first minutes of the experiment, it contributed to

415   significantly reducing the pathogen population compared with the W treatment. Longer treatment

416   times (or higher UV-C dose) eliminated the pathogens from water. Therefore, the use of UV-C

417   technology assisted by water is still recommendable due to their increased effectiveness for

418   decontaminating the food matrix and maintaining the wash water free of mutagenic and

419   carcinogenic products. UV-C irradiation has been widely used as a non-thermal method of

420   disinfecting drinking, waste and recreational water to chlorine alternative, to prevent cross-

421   contamination when it is reused in the process (Beck et al., 2015). Therefore, it has to be under

422   consideration that when long processing times are not feasible for practical application, the

423   combination with environmentally friendly chemical agents, such as peracetic acid (PA), could

424   be taken into account.

425   **5. Conclusion**

426   The novel technology used in the present study for the decontamination of strawberries, consisting

427   in UV-C light transmitted by lamps immersed in stirring water (WUVC), was evaluated as a good

428   alternative to chlorine disinfection, being useful in reducing *L. monocytogenes*, *S. enterica* and

429   MNV-1 on inoculated strawberries. Moreover, the implementation of this combination device

430   enhanced the reduction effect compared with air-transmitted UV-C technologies. On the other

431   hand, washing fresh strawberries can pose a risk of mould growth, for this reason the WUVC

432   treatment could be used in the frozen strawberry industry, since the majority of the foodborne

433   disease outbreaks related to enteric virus were found on frozen produce around the world in recent

434   years (Bernard et al., 2014; Mäde et al., 2013; Maunula et al., 2009; Sarvikivi et al., 2012; Severi

435   et al., 2015).

436   In addition, low-dose WUVC did not generate toxic by-products and allowed the reusing of the

437   process water, thus enabling savings in water consumption. The amount of wastewater generated

438   per mass unit of product depends on the disinfection technique employed, so UV-C irradiation

439   being capable of disinfecting efficiently both the process water and the product, a higher ratio of

440   recycling can be achieved, with a lower impact on the environment. The results obtained herein

441   provide new tools to ensure the safety of fresh berries, contributing to the so-called " smart green

442   growth " addressed to provide a more innovative and sustainable future for the food industry.

443   However, the conditions tested in the present study are focused on a laboratory-scale prototype

444   under controlled conditions, so more studies should be carried out with the aim of improving this

445   system in the short term on production operation conditions to confirm that WUVC treatment is

446   a good alternative to chlorine sanitization for the food industry.

447

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Conflict of interests

The authors declare no conflict of interests.

459   **References**

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612

613 **Table 1** - Population of *Listeria monocytogenes* (log CFU strawberry[-1]) in **non-inoculated**
614 **strawberries** (n=3) after 1, 2, 5 and 10 min washing with inoculated ones. Water parameters: pH,
615 Oxidation-reduction potential (ORP), concentration of sanitizer are represented as the mean of
616 the 3 repetitions ± standard deviation.

| Treatment	Washing treatment time (min)	Dose	Temperature (ºC)	pH	ORP (mV)	log CFU strawberry[-1] (mean ± stdev) (positive samples*/total)
Water	1	-	7.4 ± 0.0	8.0 ± 0.1	242 ± 12	2.2 ± 0.4 (3/3)
	2	-	8.2 ± 0.2	8.1 ± 0.1	242 ± 16	1.8 ± 0.2 (3/3)
	5	-	8.6 ± 0.4	8.0 ± 0.0	240 ± 6	1.7 ± 0.0 (3/3)
	10	-	9.0 ± 0.2	8.0 ± 0.1	240 ± 13	1.8 ± 0.2 (3/3)
WUVC	1	-	7.2 ± 0.0	7.9 ± 0.1	271 ± 5	1.1 ± 0.9 (2/3)
	2	-	8.0 ± 0.2	8.1 ± 0.2	251 ± 11	0 (0/3)
	5	-	8.0 ± 0.0	8.0 ± 0.0	236 ± 12	0 (0/3)
	10	-	10.0 ± 0.2	8.0 ± 0.1	234 ± 7	0 (0/3)
NaClO	1	244 ± 5	8.2 ± 0.4	6.8 ± 0.1	875 ± 14	0 (0/3)
	2	236 ± 2	8.2 ± 0.2	7.0 ± 0.2	871 ± 17	0 (0/3)
	5	248 ± 4	8.4 ± 0.2	7.2 ± 0.3	867 ± 9	0 (0/3)
	10	252 ± 2	9.0 ± 0.2	7,2 ± 0.1	861 ± 15	0 (0/3)

617 **L. monocytogenes* positive strawberry meant presence of *L. monocytogenes* on PALCAM agar
618 plates after enrichment of the detached microbial suspension by DEY-Engley neutralizing
619 medium. Inoculated strawberries were artificially inoculated with ~7 log CFU strawberry[-1] of *L.
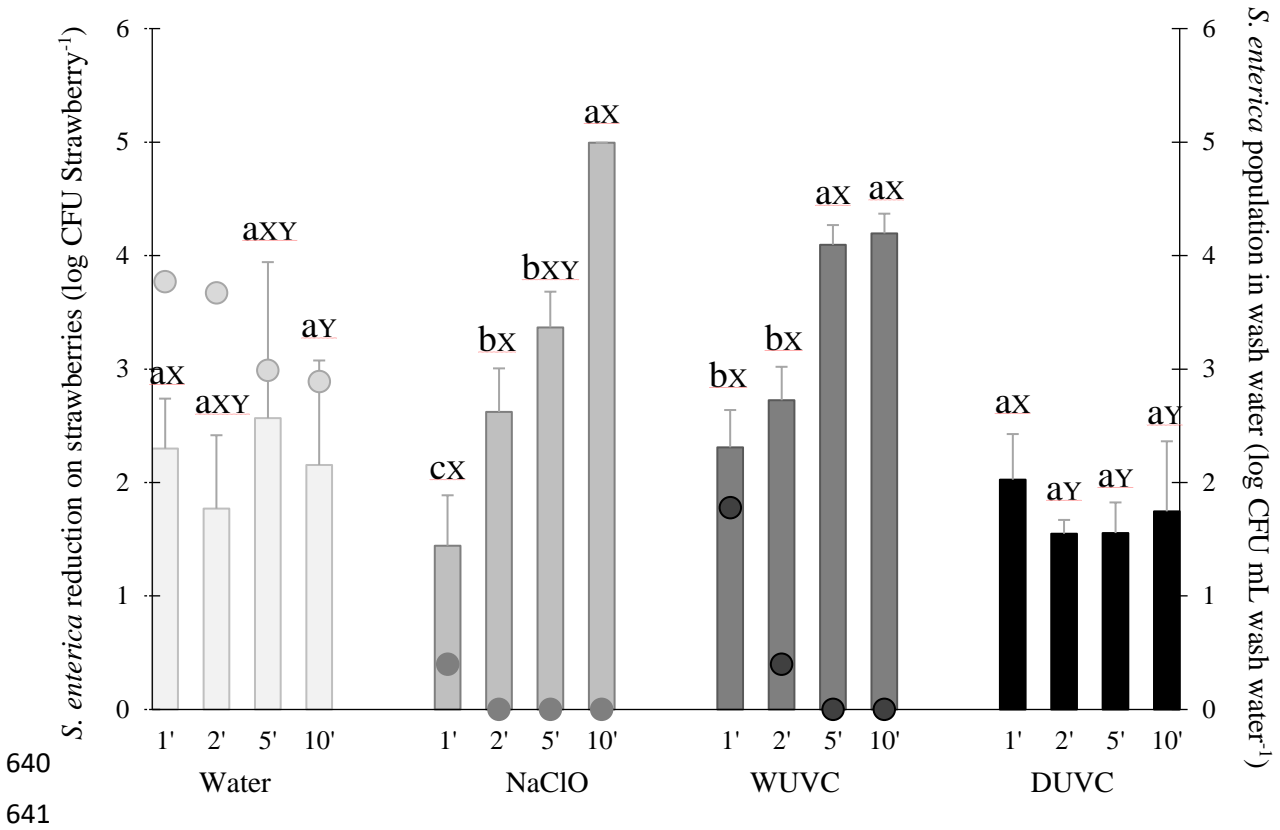620 monocytogenes*.

621

622

623 **Figure 1** – Scheme of the UV-C water-assisted device LAB-UVC-Gama with 4 UV-C lamps
624 (GPH303T5L/4, 254 nm). (A) General overview and (B) Detail of the tank: (1) Water tank
625 equipped with a recirculating water circuit (2) that is put in motion by a water pump (maximum
626 flow 1700 L/h) (3) which is controlled with a power source (4). Pressurized water is introduced
627 at 100 kPa (5 and 6), and enters through the bottom of the tank for water bubbling (7). Four
628 equidistant UV lamps (8) (17.2 W) emitting at 254 nm are located in water proofs quartz
629 compartments inside the tank. Radiation is measured on a hole in the lid of the tank (9). (C) Tank
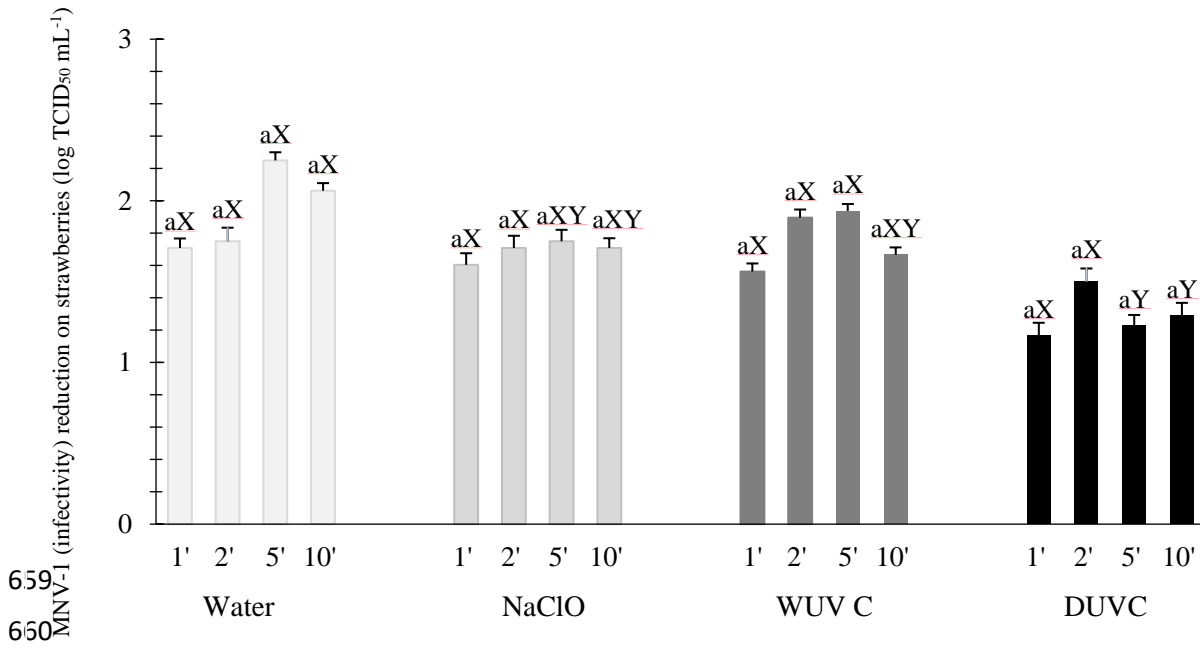630 filled with 20 strawberries.

631

632
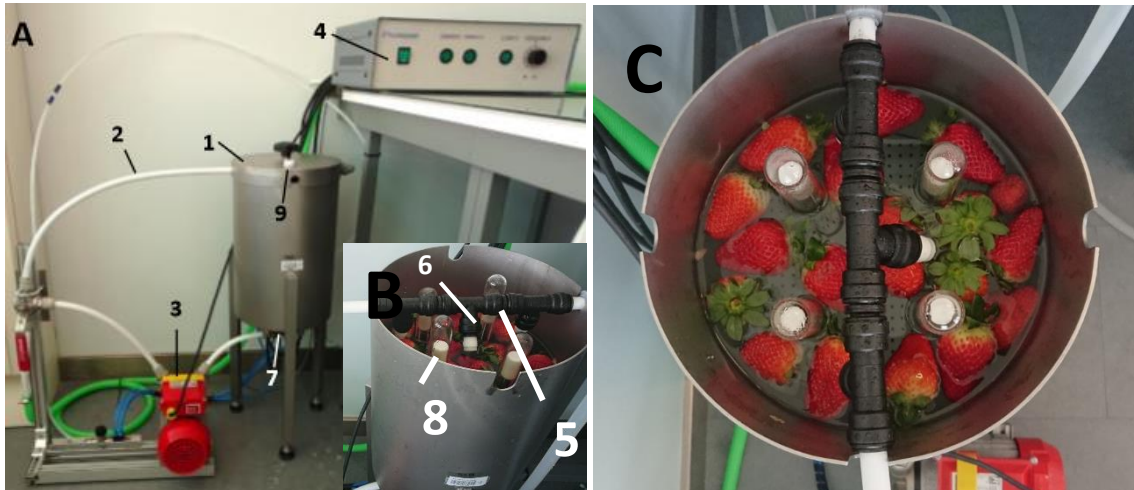
Figure 2 - Reductions of *S. enterica* population in strawberries (bars) after disinfection treatments at different times (1, 2, 5 and 10 min). Detection limit was 1.30 log CFU strawberry^{-1}. Results are the mean of 6 repetitions ± standard deviation. Remaining population of *S. enterica* in washing water (dots) were also showed. Results are the mean of 2 repetitions ± standard deviation. Different lowercase letters (a, b, c) show statistically significant differences ($P < 0.05$) among time exposure for each treatment. Different uppercase letters (X, Y, Z) show statistically significant differences ($P < 0.05$) among treatments for each time exposure.

Figure 3 – Reductions of *L. monocytogenes* population in strawberries (bars) after disinfection treatments at different times (1, 2, 5 and 10 min). Detection limit was 1.30 log CFU strawberry^{-1}. Results are the mean of 6 repetitions ± standard deviation. Remaining population of *L. monocytogenes* in washing water (dots) were also showed. Results are the mean of 2 repetitions ± standard deviation. Different lowercase letters (a, b, c) show statistically significant differences ($P < 0.05$) among time exposure for each treatment. Different uppercase letters (X, Y, Z) show statistically significant differences ($P < 0.05$) among treatments for each time exposure.
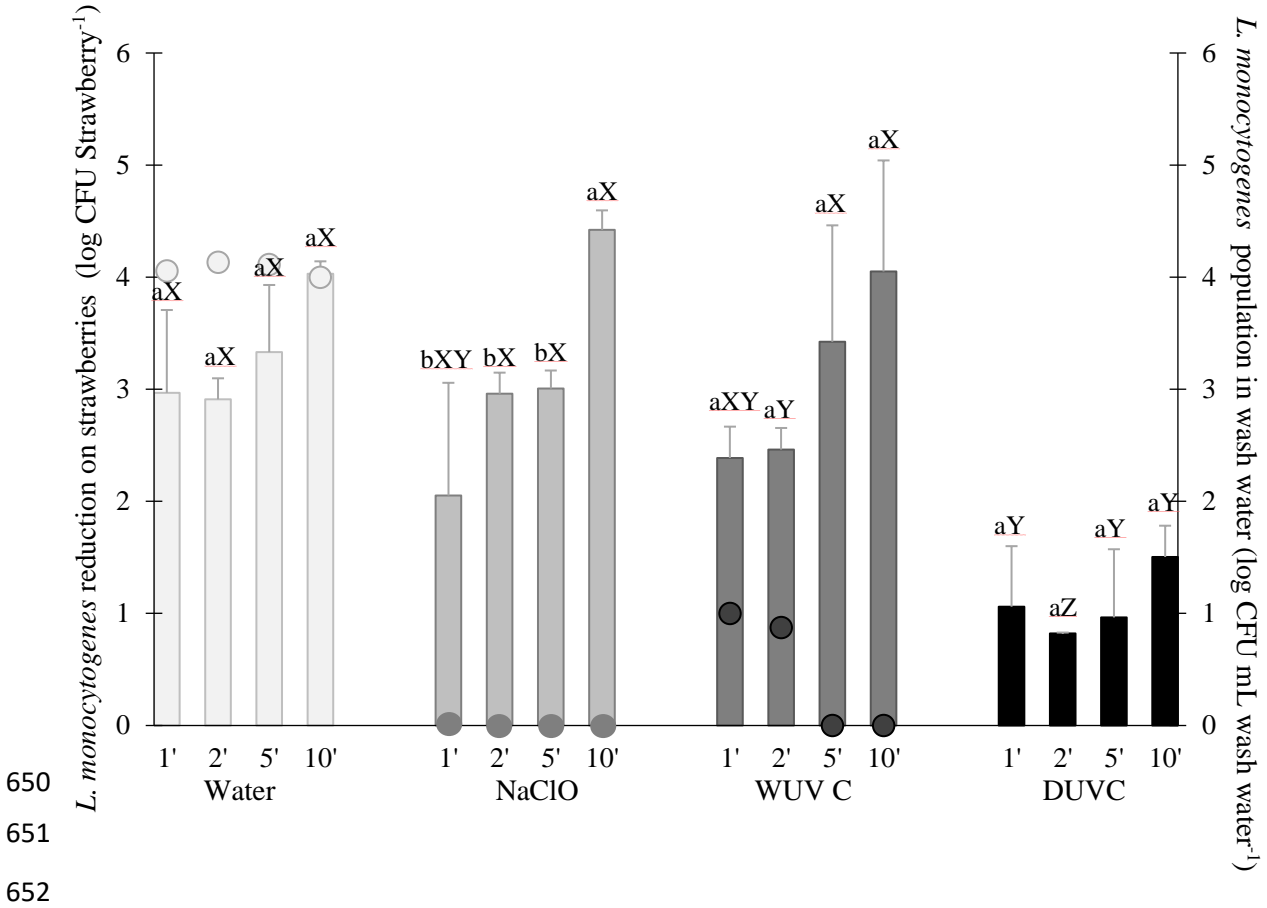
653　　**Figure 4 -** Reduction of the infectivity of murine norovirus (MNV-1) in fresh strawberries (log
654　　$TCID_{50}$ mL^{-1}) after disinfection treatments at different times (1, 2, 5 and 10 min). Detection limit
655　　was 0.8 log $TCID_{50}$ mL^{-1}. Results are the mean of 3 repetitions ± standard deviation. Different
656　　lowcase letters (a, b, c) show statistically significant differences ($P < 0.05$) among time exposure
657　　for each treatment. Different uppercase letters (X, Y, Z) show statistically significant differences
658　　($P < 0.05$) among treatments for each time exposure.

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