Evaluation of a sanitizing washing step with different chemical disinfectants for the strawberry processing industry

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Highlights

- Chemical sanitizers selected may be a feasible alternative to chlorine (100 ppm) sanitization.
- Inoculated *L. monocytogenes* and *S. enterica* were reduced at least 2–log units.
- MNV-1 infectivity was decreased by ≥ 1.7 log $TCID_{50}$.
- Physio-chemical parameters studied did not overcome major changes.
- Peracetic acid (PA) was effective for washing water and fruit disinfection.

23   **Abstract**

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25   Strawberries are often consumed fresh or only receive minimal processing, inducing a significant health

26   risk to the consumer if contamination occurs anywhere from farm to fork. Outbreaks of foodborne illness

27   associated with strawberries often involve a broad range of microbiological agents, from viruses (human

28   norovirus) to bacteria (*Salmonella spp.* and *Listeria monocytogenes*). The addition of sanitizers to water

29   washes is one of the most commonly studied strategies to remove or inactivate pathogens on berries as well

30   as avoid cross contamination due to reuse of process wash water. The risk posed with the safety issues of

31   by-products from chlorine disinfection in the fruit industry has led to a search for alternative sanitizers. We

32   evaluated the applicability of different chemical sanitizers (peracetic acid (PA), hydrogen peroxide (H_2O_2),

33   citric acid (CA), lactic acid (LA) and acetic acid (AA)) for the inactivation of *S. enterica*, *L. monocytogenes*

34   and murine norovirus (MNV-1) on strawberries. A control treatment with chlorine (NaClO) (100 ppm) was

35   included. For each sanitizer, different doses (40, 80 and 120 ppm for PA and 1, 2.5 and 5 % for H_2O_2, LA,

36   AA and CA) and time (2 and 5 min) were studied in order to optimize the decontamination washing step.

37   The best concentrations were 80 ppm for PA, 5 % for H_2O_2 and 2.5 % for organic acids (LA, AA and CA)

38   after 2 min treatment. Results indicate that the sanitizers selected may be a feasible alternative to chlorine

39   (100 ppm) for removing selected pathogenic microorganisms ($P > 0.05$), with reductions about ≥ 2 log for

40   bacterial strains and ≥ 1.7 log for MNV-1. As the washing water may also increase the microbial counts by

41   cross-contamination, we observed that no pathogenic bacteria were found in wash water after 5 % H_2O_2

42   and 80 ppm PA after 2 min treatment. On the other hand, we also reported reductions about total aerobic

43   mesophyll (TAM) (0.0 – 1.4 log CFU/g) and moulds and yeasts (M&Y) (0.3 - 1.8 log CFU/g) with all

44   alternative sanitizers tested. Strawberries treated did not shown significant differences about physio-

45   chemical parameters compared to the untreated samples (initial). For this study, the optimal sanitizer

46   selected was PA, due to the low concentration and cost needed and its microbiocidal effect in wash water

47   and fruit. Notwithstanding the results obtained, the effect of PA in combination with other non-thermal

48   technologies such as water-assisted ultraviolet (UV-C) light should be studied in future research to improve

49   the disinfection of strawberries.

50   *Keywords: peracetic acid, organic acids, Listeria monocytogenes, Salmonella enterica, fruit*

51

1. Introduction

Strawberries are one of the most important fruits in the Mediterranean diet. They are highly appreciated for their unique fragrance, nutritional value and antioxidant activity due to their vitamin C and polyphenol contents with nutraceutical properties (Mezzetti et al., 2014). Fresh strawberries are generally cultivated, hand-picked, packaged and commercialized in the fresh market, but are not subjected to any step that can eliminate postharvest pathogens. However, they are subjected to a washing process before processing (Janowicz et al., 2007; Velickova et al., 2018).

Foodborne illness outbreaks have been linked with the consumption of fresh or frozen strawberries that were contaminated with pathogenic viruses, parasites, or bacteria. The majority of outbreaks have been caused by enteric viruses, and many of the virus-associated outbreaks have been associated to frozen strawberries (Palumbo et al. 2013). In fact, Bozkurt et al. (2020) documented that human norovirus in soft red fruits was the most common and implicated pathogen in 46 foodborne outbreaks globally with over 15.000 cases during 1983-2018. Against this background, the EFSA (European Food Safety Authority, 2014) emitted a scientific opinion on the risk posed by human norovirus and *Salmonella* spp. in berries. Even though no bacterial pathogenic microorganisms have usually been found on field and sold retail strawberries (Delbeke et al., 2015; Ortiz-Solà et al., 2019a), *Salmonella* spp. and *Listeria monocytogenes* were able to survive on the fruit surface at different stored temperatures (Ortiz-Solà et al., 2019b; Sreedharan et al., 2015), and *L. monocytogenes* could grow in the conditions in which strawberries are stored (Siro et al., 2006). For this reason, EFSA Panel on Biological Hazards concluded that improper fruit handling practices and the use of contaminated washing water should be considered as sources of contamination, and it recommended that a decontamination step treatment should be integrated into the strawberry production chain in order to avoid possible foodborne outbreaks related to this fruit.

The addition of sanitizers to water wash is one of the most upsurge studied strategies to remove or inactivate pathogens on fresh and pre-cut fruits (Lafarga et al., 2019; Ramos et al., 2013). As

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79   the washing water may also increase the bacterial counts by cross-contamination, it is important

80   that the washing step not only removes bacteria but also maintains water quality (Pablos et al.,

81   2018). For example, norovirus or norovirus RNA could persist in some type of waters for 60 to

82   728 days and in fruits and vegetables for longer than product's shelf life (Cook et al., 2016).

83   Chlorine is the first choice as a disinfectant due to its low price, simplicity of use and effectiveness

84   against vegetative bacteria (Ölmez and Kretzschmar 2009). However, its action is highly pH-

85   dependent and it reacts with organic matter, producing undesirable by-products such as

86   trihalomethanes. Due to this, it has been banned as a wash for produce in some European

87   countries, including Germany, the Netherlands, Switzerland, Denmark and Belgium (Artes et al.,

88   2009; Rico et al., 2007). These drawbacks have encouraged the search for alternatives to chlorine

89   in wash water (Meireles et al., 2016). Organic acids, which are considered 'Generally Recognized

90   as Safe' (GRAS) by FDA, have been described as strong antimicrobial agents due to environment

91   pH reduction, disturbance of membrane transport and/or permeability, anion accumulation, or a

92   reduction in internal cellular pH (Beuchat, 1995; Harris et al., 2003; Miller et al., 2009). These

93   sanitizers are stable compounds that may persist on produce surfaces for a long period, avoiding

94   bacterial attachment (De Villiers et al., 1997). Citric (CA) and acetic acids (AA) are commonly

95   used in fruit and vegetable washing and added in fruit juices, such as vinegar and lemon juice

96   (Lynch et al., 2019). Lactic acid (LA) is also frequently used in the food industry to reduce

97   microbial populations and previous studies with produce models such as chicory, tomatoes, and

98   lettuce have demonstrated that lactic acid is an effective antimicrobial treatment (del Carmen

99   Velazquez et al., 2009; Trevisani et al., 2017). However, no studies have demonstrated yet the

100 antimicrobial effectiveness of LA on berries. Indeed, there are numerous studies in reference to

101 the effectiveness of all these organic acids against pathogenic bacteria and the microbiota of some

102 fruits and vegetables. Though, there is much less information about its effectiveness against

103 viruses (Lafarga et al., 2019).

104 Hydrogen peroxide (H_2O_2) and peracetic acid (PA) have also been evaluated as potential

105 substitutes for sodium hypochlorite (NaClO), which bacteriostatic and bactericidal activity caused

106   by a strong oxidizing power of C-C bonds and the maintaining of water washing quality (Wessels

107   and Ingmer, 2013). Their mode of action would imply a poor chance for the development of

108   resistance in microorganisms, as borne out by the absence of such reports in the literature

109   (Nicolau-Lapena et al., 2019).

110   Alternative disinfection methods to chlorine must be found in order to provide consumers with

111   safe fresh-cut, frozen and processed strawberries. Hence, the objective of this study was to

112   establish the optimal concentration/time procedure of different sanitizers: organic acids (LA, AA

113   and CA), H_2O_2 and PA as sanitizers in strawberry washing processes on artificially inoculated

114   strawberries with *Salmonella, L. monocytogenes* and murine norovirus, a norovirus surrogate.

115   The effects of these products on native microbiota and their effects in the physico-chemical

116   quality of strawberries were also determined.

2. Materials and Methods

2.1. Samples and materials

Strawberries (*Fragaria × ananassa*), were purchased from local distributors in Lleida (Catalonia, Spain) the day before the experiment and stored at 4 ± 1 °C for 24 ± 2 h.

For the disinfection, acetic acid 99-100 % w/v (AA) was purchased from Normapur VWR (Llinars del Vallés, Spain). Peracetic acid 15 % w/v (PA), sodium hypochlorite 10 % w/v (NaClO), hydrogen peroxide 33 % w/v (H_2O_2), pure anhydrous citric acid (CA) and pure lactic acid (LA) were procured by Panreac AppliChem (Barcelona, Spain). Triptone soy broth (TSB), triptone soy agar (TSA), plate count agar (PCA), dichloran rose bengale chloramphenicol agar (DRBC), PALCAM base agar, yeast extract (YE), Xylose-Lysine-Desoxycholate Agar (XLD) and peptone were purchased from Biokar Diagnostics (Allonne, France).

2.2. Microorganism preparation

2.2.1. Pathogenic bacteria

The bacterial strains used in this work included the serovars of *Salmonella enterica* subsp. *enterica*: Agona (ATCC BAA-707), Michigan (ATCC BAA-709), Montevideo (ATCC BAA710), Gaminara (ATCC BAA-711) and Enteritidis (CECT-4300) in addition to the *L. monocytogenes* serovar 1a (CECT 4031), serovar 3a (CECT 933), serovar 4d (CECT 940), serovar 4b (CECT 4032) and serovar 1/2a, which was previously isolated in our laboratory from a fresh-cut lettuce sample (Abadias et al., 2008). For each strain of selected bacteria, a single *S. enterica* colony from a streak in TSA medium (20-24 h, 37 ± 1 °C) was inoculated in 5 mL of TSB for 20-24 h at 37 ± 1 °C. *L. monocytogenes* strains obtained from a streak in tryptone soy agar plus 6 g/L yeast extract (TSAYE) were grown individually in TSB supplemented with 6 g/L of yeast extract, 2.5 g/L glucose and 2.5 g/L K_2HPO_4 (TSBYE) for 20-24 h at 37 ± 1 °C. Bacterial cells were harvested by centrifugation at $9800 \times$ g for 10 min at 10 °C (Sorvall Legend XTR Centrifuge, Thermo Fischer, US) and then resuspended in sterile saline solution (SS; 0.85 % w/v NaCl). Equal volumes of the five *S. enterica* concentrated suspensions were mixed to produce a

143   single suspension, and equal volumes of the five *L. monocytogenes* concentrated suspensions

144   were mixed to provide the 5-strain concentred cocktail. Afterwards, a volume of the concentrated

145   bacterial suspension was added to saline peptone (SP; 8.5 g/L and 1 g/L peptone) and mixed to

146   obtain approximately 10^8 colony-forming units (CFU)/mL. The inoculum concentration was

147   checked by plating appropriate dilutions on Palcam agar for *L. monocytogenes* (Palcam Agar Base

148   with selective supplement, Biokar Diagnostics) or on XLD for *S. enterica*. Plates were incubated

149   at 37 ± 1 °C for 24 ± 2 h (*S. enterica*) or 48 ± 2 h (*L. monocytogenes*).

2.2.2. Virus and cell culture

151   Murine norovirus 1 (MNV-1), a surrogate of human norovirus, and murine macrophage cell line

152   RAW 264.7 were kindly provided by Prof. H. W. Virgin (Washington University School of

153   Medicine, US). MNV-1 stocks were propagated and quantified in the murine macrophage cell

154   line RAW 264.7. Semi-purified MNV-1 virus was harvested at 2 days after infection by three

155   freeze-thaw cycles of infected cells followed by centrifugation at $660 \times g$ for 30 min to remove

156   cell debris (Sánchez et al., 2011). Infectious MNV-1 virus was enumerated by determining the 50

157   % tissue culture infectious dose ($TCID_{50}$). Stocks of MNV-1 (1 mL) were frozen until use (-80

158   °C).

159   RAW 264.7 cells were cultured in Dulbecco's modified Eagle medium (DMEM; Hyclone,

160   Pittsburgh, PA) supplemented with 10 % fetal bovine serum (FBS; Hyclone) previously

161   inactivated for 30 min at 56 °C in a water bath, 2 mM glutamine, 10 mM N-2-

162   hydroxyethylpiperazine-N0-2-ethanesulfonic acid, 2 mM Glutamine, and 1 % Penicillin-

163   Streptomycin (all from Biowest, US). The cell line was maintained at 37 ± 1 °C in a 5 % CO_2

164   humidified incubator (NU-4750, NuAire, US) in T175 flasks (Nunc, Thermo Fisher, US).

2.3. Microorganism inoculation on strawberries

166   The day before the experiment, strawberries were inoculated with 50 μL of *S. enterica* and

167   *L. monocytogenes* suspensions at 10^8 CFU/mL. Inoculated strawberries were stored at 4 ± 1 °C

168   overnight until the assay to facilitate bacterial establishment on fruits. In case of MNV-1, frozen

169 stocks were defrosted (1 h at room temperature 20-22 °C) and ten-fold diluted (10^7 tissue culture

170 infective dose $TCID_{50}/mL$) with Phosphate-Buffered Saline (PBS; ThermoFisher, US) before

171 inoculation. Then, 50 µL of MNV-1 were inoculated by pipetting small droplets on the surface of

172 each strawberry and allowing them to dry for approximately 1-2 h in a biosafety laminar air

173 cabinet (class II - type A, Telstar, Terrassa, Spain) at room temperature. Prior to the experiments,

174 the initial concentration of *S. enterica, L. monocytogenes* and MNV-1 was checked as explained

175 below.

2.4. Experimental design

177 Three types of experiments were carried out as indicated below. For all experiments, strawberries

178 were removed from storage at 4 °C before the disinfection washing step.

2.4.1. Evaluation of different chemical sanitizers on the population of bacterial strains.

181 The first set of experiments consisted in determining the effect of different sanitizers' dose and

182 exposition time on the survival of *L. monocytogenes* and *S. enterica* on strawberries. Products and

183 doses tested were 1, 2.5 and 5 % for H_2O_2, CA, LA and AA, and 40, 80 and 120 ppm for PA. A

184 control treatment, 100 ppm of NaClO adjusted to pH (6.5) using citric acid 2 M, was included.

185 Each treatment was tested for 2 and 5 min. One single sanitizer at different concentrations and

186 times was evaluated each day, using different strawberry batches. For washing, strawberries were

187 immersed into 2 L beaker containing 1 L of distilled water (8-12 °C) and the sanitizer at the tested

188 dose. The washing step was performed with constant agitation at 150 rpm on an orbital shaker

189 (Heidolph unimax 1010). After 100 ppm NaClO treatment, strawberries were rinsed in 1 L of

190 cold tap water (4 °C) for 2 min. Fruits were left to dry at room temperature. Free chlorine

191 concentration was checked with an ion specific meter (model HI 95734-11, Hanna Instruments,

192 Spain) and PA concentration was determined by iodometric titration with potassium

193 permanganate and sodium hydroxide (NaOH) 2M (Panreac AppliChem, Barcelona, Spain).

194   Furthermore, pH and ORP (Oxidation Reduction Potential) values were measured using pH meter

195   (Crison GLP-22, Barcelona, Spain).

196   In the second set of experiments, optimum concentration and time selected for each sanitizer were

197   tested all together in the same trial (using the same batch of strawberries and inoculum), in order

198   to minimize the experimental variability arising from the heterogeneity of these variables. The

199   procedures were the same that we commented above. Experiments were repeated twice.

200   To determine *S. enterica* and *L. monocytogenes* population, one artificially inoculated strawberry

201   before and after disinfection was weighted, placed in a sterile filter bag (80 mL BagPage®,

202   Interscience BagSystem, Saint Nom, France) and diluted with buffered peptone water (BPW;

203   Biokar Diagnostics) 1:4 (w:v). The content of the bag was mashed in a paddle blender (MiniMix,

204   Interscience, France) for 2 min at 9 strokes/s. Aliquots of the mixture were serially diluted in

205   saline peptone (SP; 0.85 % w/v NaCl; 0.1 % w/v Peptone), and plated in duplicate on XLD for

206   enumerating *S. enterica* or on Palcam agar for *L. monocytogenes*. The agar plates were incubated

207   at 37 ± 1 °C for 24 ± 2 h (*S. enterica*) or 48 ± 2 h (*L. monocytogenes*). Three replications (three

208   strawberries *per* treatment) were made at each sampling point. The data was transformed to log

209   CFU/g strawberry. The limit of detection was 1.30 log CFU/g strawberry. When no colonies were

210   counted and detection was positive, an arbitrary number of half detection limit was estimated (1

211   log CFU/g). Moreover, after each washing treatment, the population of *S. enterica* and

212   *L. monocytogenes* were determined in the wash water, by adding 1 mL of wash water to

213   neutralizing Dey-Engley medium (Fluka, Madrid, Spain) and were incubated at 37 ± 1 °C for 24

214   ± 2 and 48 ± 2 h. Other $100 \mu L$ of wash water was plated in duplicate as described before. Results

215   were expressed as log CFU/mL, and the detection limit was 0.70 log CFU/mL. When

216   quantification was below the detection limit, *S. enterica and L. monocytogenes* presence were

217   confirmed by Dey-Engley change in colour followed by streaking onto XLD or Palcam.

218   **2.4.2. Assessment of the sanitization washing step with the different optimal**

219   **sanitizers on MNV-1 infectivity**

220 The optimal dose and exposition time for each sanitizer was selected from previous experiments

221 (section 2.4.1). Treatments and procedures were performed as mentioned previously. For MNV-

222 1 extraction, one fruit after disinfection per treatment was placed in a small sterile filter bag (80

223 mL BagPage®, Interscience, France) with 10 mL of Tris-Glycine Beef Extract buffer (TGBE;

224 Biokar Diagnostics) in triplicate. The content was mixed with a homogenizer (MiniMix,

225 Interscience, France) for 2 min at normal speed (7 strokes/s). The homogenate obtained were

226 placed in 15 mL sterile tubes and centrifuged at $3000 \times g$ for 10 min at 4 °C. Supernatant was

227 positioned in 2 mL Eppendorf and stored at -80 °C until analysis.

228 Enumeration of MNV-1 on cell monolayers was done by the Spearman-Karber method as

229 indicated above. Briefly, the day before determination, confluent RAW 264.7 cells grown in T175

230 flasks with DMEM 10 % were transferred to 96-well microtiter plates with Hydrocell™ surface

231 (ThermoFisher, US). Cell lines were stained with trypan blue (Biowest, US) and observed under

232 the optical inverted microscope. The concentration needed per plate (1.3×10^5 cell/mL) was

233 determined with a Bürker chamber (1 mm^2 surface $\times$ 0.1 mm depth). Plates were incubated at 37

234 $\pm$ 1 °C in a 5 % CO_2 for 24 h. Subsequently, DMEM 10 % was removed out the 96-well plates

235 and 20 µL/well of ten-fold dilutions of treated virus extract (sample) in PBS were inoculated into

236 8 wells/plate of confluent RAW 264.7 monolayers. Then, 96-well plates were incubated at same

237 conditions commented above. After 1 h incubation, 150 µL/well of DMEM supplemented with 2

238 % FBS were added and incubated again at 37 °C in a CO_2 incubator for 2–3 days. Cell monolayers

239 were observed for cytotoxicity effects by visual inspection under the optical inverse microscope.

240 Positive sample for MNV-1 was used as reference material in 4 wells/plate. Negative control was

241 PBS, containing 2 M NaNO3, 1 % beef extract, and 0.1 % Triton X-100 (pH 7.2) spread in 4

242 wells/plate.

243 Number of infectious viruses was enumerated by determining the 50 % tissue culture infectious

244 dose (TCID$_{50}$) with eight wells per dilution and 20 µL of inoculum per well using the Spearman-

245 Karber method (Pinto et al., 1994). The number of wells that had cytopathic effect after 48-72 h

246 of incubation were recorded. The $TCID_{50}$/mL value was calculated with Spearman-Karber

247 formula:

248 $$M = xk + d [0.5 - (1/n)(r)] \qquad \text{eq. 1}$$

249 Where xk was the dose of the highest dilution; r was the sum of the number of "-" responses; d

250 was the spacing between dilutions; and n was the wells per dilution.

251 The reduction of MNV-1 on strawberries was calculated as $\log(N_x/N_0)$, where N_x is the infectious

252 virus titer after each treatment and N_0 is the initial virus infect titer (Falcó et al., 2018).

2.4.3. Effect of different sanitizers on the microbiological quality of non-inoculated strawberries

255 In experiments with epiphytic microbiota, the optimal dose and exposition time selected for each

256 disinfectant was performed as discussed earlier in this report. This experiment was done once,

257 with 3 determinations (repetitions). Three strawberries per treatment were weighed, placed in a

258 sterile filter bag and diluted and homogenized as explained above. A 10-fold serial dilutions were

259 made in SP and plated in duplicate on PCA for total aerobic mesophilic counts (TAM) and in

260 DRBC for molds and yeasts (M&Y). Plates were incubated at 30 ± 1 °C for 3 days for TAM and

261 at 25 ± 1 °C for 3–5 days for M&Y. Results were expressed as log CFU/g and the detection limit

262 was 1.70 log CFU/g. Moreover, after each washing treatment, the population of TAM and M&Y

263 was determined in the wash water. One milliliter of water was added to neutralizing Dey-Engley

264 medium and plated as described before. Results were expressed as log CFU/mL, and the detection

265 limit was 0.70 log CFU/mL. When quantification was below the detection limit, its presence was

266 confirmed by Dey-Engley variation in colour and followed by streaking onto PCA or DRBC.

2.4.4. Physicochemical Quality Analysis

268 Physicochemical quality analyses were performed the same day of the experiments in non-

269 inoculated strawberries, before (initial) and immediately after treatments. This experiment was

270 done once, with 6 determinations (repetitions).

271 For pH, titratable acidity (TA) and total soluble solids (TSS) determination, strawberries were

272 crushed in a paddle blender (MiniMix, Interscience, France). For each replication (n=6 fruits per

273 treatment), 25 mL of strawberry juice were needed, and analysed twice. pH was determined using

274 an electrode in a pHmeter (Crison GLP-21, Barcelona, Spain) equipped with a pH probe (ref. 52-

275 03, Crison). TA was measured by diluting 10 mL of strawberry juice with 10 mL of distilled water

276 and titrated with 0.1 M NaOH. Results were expressed as g of citric acid per L. TSS was measured

277 at 20 °C with a refractometer (Atago Co. Ltd., Tokyo, Japan), and the results expressed as °Brix.

278 Regarding to the colour, 6 strawberries per treatment was measured on 3 points of each strawberry

279 by using a CR-200 Minolta Chroma Meter (Minolta, INC., Tokyo, Japan). Colour was expressed

280 as CIE L*, a*, and b* coordinates, using the D65 illuminant and a 10° angle.

281 Changes in texture (firmness) was measured by the maximum penetration force using the TA. XT

282 Plus Connect texture analyser (Stable Micro systems Ltd., Surrey, England). The firmness test

283 was performed using the cylindrical probe of 4 mm. Tests were run at 5 mm/s speed and using a

284 trigger force of 0.1 N, permitting the probe to enter 8.0 mm deep into the matrix tissue.

285 **2.5. Statistical Analysis**

286 All data were checked for significant differences by applying variance analysis (ANOVA) using

287 the JMP14.0 (SAS Institute Inc., Cary, USA) statistical package. They were subjected to mean

288 separation by least significant differences by Tukey's Honest Significant Difference (HSD) test

289 ($P < 0.05$).

3. Results and Discussion

3.1. Evaluation of chemical sanitizers on the population of *S. enterica* and *L. monocytogenes*

3.1.1. Optimum concentration and time exposition

Concentrations of sanitizers, pH and ORP values are detailed in Table 1. In the PA and organic acids (LA, AA and CA) washing solutions, pH and ORP values were lower than those observed in NaClO treatment, which ranged from 6.6 to 6.8 and 873–898 mV, respectively. The H_2O_2 treatments had the highest pH values and the lower ORP units, due to the basic nature of the product.

Regarding the washing time, 2 or 5 min, no significant differences ($P < 0.05$) were observed between washing 2 or 5 min with all sanitizers tested (data not shown). Therefore, 2 min treatment was selected for subsequent experiments. Previous publications have used 2 min as optimal time for their experiments and achieved > 3.0 log CFU/g reduction, showing efficacy for significant removal of bacterial strains (includes *Salmonella* spp. *and L. monocytogenes*), as well as three tested virus strains (murine norovirus (MNV-1), hepatitis A virus (HAV) and bacteriophage MS2) on strawberries (Huang et al., 2015; Nicolau-Lapena et al., 2019; Wang and Ryser, 2014).

S. enterica and *L. monocytogenes* average reductions obtained for each treatment after 2 min are shown in Table 2. Peracetic acid (PA) treatments achieved higher significant average reductions of pathogenic bacteria in comparison to the NaClO (100 ppm) control treatment ($P < 0.05$). *L. monocytogenes* and *S. enterica* were below detection limit after washing in all doses tested, with reductions ca. 3.8 and 4.1 log units, respectively. There were not significant differences ($P > 0.05$) among PA doses studied. In fact, reductions of about 4 log units observed in study conducted by Singh et al. (2018) were in accordance with the present investigation, which also found no statistical differences between different concentrations of 45 or 85 ppm PA washings for 5 min on lettuce, cantaloupe, tomato, lemon, and blueberry.

315 Results of microorganisms in the water washing are also shown in Table 2. In the case of PA, the

316 intermediate concentration of 80 ppm was chosen, since this concentration preserved the wash

317 water quality, avoiding the possibly subsequent cross-contamination of the fruit. Moreover, the

318 80 ppm dose is permitted for the washing of fruits and vegetables in the United States (FDA

319 CFR173.315).

320 On the other hand, reductions of $\geq$ 4.9 log CFU/g were reported for *L. monocytogenes* and

321 *S. enterica* with 5 % of hydrogen peroxide (H_2O_2) (Table 2). The highest concentration (5 %)

322 showed the greatest activity against both microorganisms ($P < 0.05$) compared with 1 and/or 2 %

323 dose and compared with the effect of NaClO (100 ppm). Moreover, the effectiveness of the

324 different doses was equal for both microorganisms ($P > 0.05$). Furthermore, no pathogenic

325 bacteria were found in wash water after each H_2O_2 treatment. For this reason, 5 % of H_2O_2 was

326 selected for subsequent experiments. Similarly, Ramos et al. (2013) found that lower

327 concentrations of H_2O_2 (1–2 %) were not efficient in reducing the bacterial load of the fruit matrix.

328 For organic acids, reductions of $\geq$ 2.7 log CFU/g were reported for both bacterial strains in all

329 concentrations tested (Table 2). All lactic acid (LA) treatments were more effective than their

330 control with NaClO (100 ppm) for both microorganisms studied ($P < 0.05$). Instead, no significant

331 differences among the applied doses (1, 2.5 and 5 %) and the pathogenic bacteria were found.

332 Regarding citric acid (CA) treatments, the effect of the acid was greater than their control with

333 chlorine (100 ppm) only for *S. enterica*. Similarly, the effectiveness of the acetic acid (AA) dose

334 was significant different ($P < 0.05$) for *S. enterica* strains, whereas no differences were reported

335 for *L. monocytogenes* reduction. In fact, the use of AA as a sanitizer was better for *S. enterica*

336 than for *L. monocytogenes*, being the *S. enterica* reduction of 5 % AA statistically higher than any

337 reduction of *L. monocytogenes* for all AA doses applied. As for the *S. enterica* strains, 3.9 ± 0.6

338 log CFU/g of the pathogen were removed from the surface of fresh strawberries with 5 % of AA,

339 but no significant differences were observed with 2.5 % AA (3.2 ± 0.0 log CFU/g).

340 Regarding wash water, microorganisms were found in the concentration 1 % LA and AA, while

341 for the treatments with CA, all doses tested presented presence of foodborne pathogens after

342   washing. Some works have demonstrated that exceed levels of organic acids would not prevent

343   adverse effects on the sensory quality of produce (Koutsoumanis et al., 2013) and it is

344   demonstrated that 2 % is generally considered as the appropriate concentration of organic acids

345   for reducing *Escherichia coli* O157:H7, *Salmonella* Typhimurium, and *L. monocytogenes* in fruits

346   (Neal et al., 2012; Ramos-Villarroel et al., 2015; Sagong et al., 2011; Salinas-Roca et al., 2016;

347   Wang et al., 2013). Moreover, high concentrations would imply higher costs for their application

348   and therefore, 2.5 % was the concentration selected for organic acids tested in the present study.

349   **3.1.2. Effect of selected doses on pathogenic bacteria**

350   All selected treatments (80 ppm of PA, 5 % H_2O_2 and 2.5 % of organic acids) were tested again

351   together, using the same batch of strawberries, storage conditions and inoculum. The inoculum

352   level of fresh strawberries with tested bacterial strains was ca. 4.40 and 4.70 log CFU/g for *L.*

353   *monocytogenes* and *S. enterica*, respectively (data not shown). After all washing treatments,

354   bacteria populations were statistically lower than the initial population (Fig. 1). For both

355   *S. enterica* and *L. monocytogenes* strains, ca. 2.0 log of the pathogens were removed from the

356   surface of fresh strawberries by 100 ppm chlorine washing (control group). Similarly, > 2.0 log

357   reductions could be achieved for both pathogenic bacteria tested with the other alternative

358   sanitizers studied. Therefore, there were no statistical differences among alternative treatments

359   and NaClO (100 ppm) effect ($P > 0.05$), being potential alternatives for the disinfection of

360   strawberries destined to processing and frozen purposes. Likewise, for both microorganisms, it

361   has been seen that there were no significant differences between *L. monocytogenes* and

362   *Salmonella* spp. among the different sanitizers and doses tested.

363   In strawberries, pathogenic microorganisms have been reported in other investigations to pose a

364   health concern, namely tested microorganisms studied in the present study and *Escherichia coli*

365   O157:H7 (European Food Safety Authority, 2014) and several disinfectants have been tested for

366   their reduction. For instance, Guo et al. (2018) studied the effect of PA at 90 ppm for 2 min and

367   found a reduction of *Salmonella* spp. and *E. coli* O157:H7 of > 1.2 log CFU/g after the washing

368   treatments. Similar results with the present investigation were described by Zhou et al. (2017),

which used 0.5 % levulinic acid (LVA; organic acid) plus 0.5 % sodium dodecyl sulphate (SDS), achieving ≥ 2.0 log CFU/g reductions of *L. monocytogenes* and *Salmonella* spp., and 1.8 log CFU/mL of *E.coli* O157:H7. Gómez-Aldapa et al. (2017) reported that washing with 0.5 % of acetic acid for 10-min caused a significant reduction *(P < 0.05)* in the level of concentration of pathogenic bacteria, including *S.* typhimurium and *L. monocytogenes,* on fresh strawberries compared to the control with water alone, achieving reductions between 0.8 and 1.4 log CFU/g. However, it has to be under consideration that long processing times that have been studied are not feasible for practical application.

Other environmentally friendly and safe approaches have been developed for the disinfection of fruit and vegetables in the food industry as a possible alternative to chlorine disinfection. These comprise the use of novel chemical strategies, such as electrolyzed oxidizing water (EOW) (Udompijitkul et al., 2007), ozone (Brodowska et al., 2017), or chlorine dioxide (Aday et al., 2014) on strawberries. Physical strategies include high pressure processing (HPP) (Huang et al., 2014), UV-C light alone (Butot et al., 2018), or combined with a photocatalysis technology (TiO_2) (Lee et al., 2018), or intense pulsed light (IPL) processing (Duarte-Molina et al., 2016). Some of these strategies have suggested that their use in the food industry has potential applications in microbial decontamination (Lafarga et al., 2018).

In wash water counts after the treatments (2 min), pathogenic bacteria were present in water with organic acids (LA, AA and CA). For LA, 1.91 log CFU/mL of *S. enterica* was found and *L. monocytogenes* was not detected. In case of AA, 2.56 log CFU/g was found for *S. enterica* and 1.20 log CFU/g for *L. monocytogenes*. Similar results were reported for CA, with a total of 2.21 log CFU/g and 1.91 log CFU/g of *S. enterica* and *L. monocytogenes,* respectively. Concerning PA and H_2O_2 effect, no population of both microorganisms were found, as in the chlorine treatment (100 ppm). In short, PA and H_2O_2 were more effective to reduce cross-contamination levels in wash water and maintain the water quality during processing compared to organic acids. Furthermore, in comparison with chlorine, PA has less potential of producing undesirable by-products, which are easily dissolved in water, thus making these sanitizers a good alternative to

396   chlorine and for maintain the water washing safe and quality (Banach et al., 2015). The reported

397   ability of PA to reduce biofilm formation would make this product a suitable sanitizer to add in

398   the washing step (Barbosa et al., 2016). On the other hand, H_2O_2 had relatively high cost and is

399   not recommended for use in berry produce (bleaching of anthocyanins in berries) (Sapers, 2003).

400   Ramos et al. (2013) found that high concentrations (> 5 %) of H_2O_2 could interfere with the overall

401   quality of the fruit (Beltrán et al., 2005; Ölmez & Kretzschmar, 2009; Rico et al., 2007). Maintain

402   the 5 % solution of H_2O_2 in dump tanks and flumes with a few thousand fruit capacity would

403   require continuous supply of substantial quantities of concentrated H_2O_2 (Pietrysiak et al., 2019).

404   **3.2. Evaluation of chemical sanitizers on the infectivity of MNV-1**

405   The inoculum of MNV-1, as a human norovirus surrogate, on strawberries reached to 3.8 log

406   $TCID_{50}$/mL (data not shown). Reductions of viral strain were > 1.7 $logTCID_{50}$/mL for all the

407   sanitizers tested (Fig. 2). Viral population removal of different treatments was not statistically

408   different among them, so the efficiency of these sanitizers were equivalent to NaClO (100 ppm)

409   ($P > 0.05$). Previous publications reported that tap water alone and chlorine solution (100-200

410   ppm) gave < 1.2-log reductions in virus titer on fresh strawberries (Predmore et al., 2011). Baert

411   et al. (2009) found that tap water washing only gave an average reduction of 0.94 logs of MNV-

412   1 in shredded lettuce, while the addition of 200 ppm of NaClO only led to an additional 0.48 logs,

413   and the addition of 80 ppm of PA acid brought about a reduction of only 0.77 log. Huang et al.

414   (2015) shown the effectiveness of water pulsed-light (WPL) combined with H_2O_2 in reducing

415   MNV-1, on berries. For strawberries, WPL – H_2O_2 treatment achieved a significantly higher ($P <$

416   0.05) reduction of MNV-1 than control washing (H_2O), by reducing 2.2 log PFU/g of MNV-1.

417   Other studies combined 0.5 % levulinic acid (LVA) plus 0.5 % sodium dodecyl sulfate (SDS)

418   wash and obtained 1.40 log reduction for MNV-1, which were comparable with the reductions

419   induced by chlorine (1.5 log reduction) ($P > 0.05$) (Zhou et al., 2017). Other treatments, including

420   short-wave ultraviolet light (UV-C) and gaseous ozone achieved reductions about < 2 log $TCID_{50}$

421   of human norovirus in fresh strawberries (Butot et al., 2018; Zhou et al., 2018). The results

422   highlight an urgent need to develop a more effective sanitizer for removal of norovirus from berry

industry, specially taking account the huge increasing number of people that striving to eat healthier by increasing their ingesting that are at high risk for norovirus contamination. The modest practice of washing raw fruits and vegetables using cold or warm water has been shown to remove some of the bacteria on produce, but studies showing the efficacy of these treatments on enteric viruses are limited (Butot et al., 2018). Natural extracts, such as green tea and grape seed extracts, have also been evaluated as natural sanitizers on fresh vegetables (Randazzo et al., 2017).

3.3. Evaluation of chemical sanitizers on non-inoculated strawberries

3.3.1. Quality changes

Physicochemical changes in strawberries, pH, TSS contents, TA, firmness and colour are shown in Table 3. Values of these parameters of non-washed strawberries were in concordance with the literature (Ayala-Zavala et al., 2004). Values of TSS and TA contents showed barely statistically significant differences among treatments. Although existing differences between treatments, there was not a general predisposition that explains changes in TSS and TA contents. TA values were higher when strawberries were washed with 100 ppm NaClO and CA, achieving a maximum of 9.16 ± 0.04 and 8.90 ± 0.29 mg citric acid/L juice, respectively when treatment time was 2 min.

Strawberry colour before any sanitization washing, expressed as CIELab coordinates, was L* 40.76 ± 3.39, a* 31.96 ± 1.62 and b* 23.26 ± 4.80. These values were comparable to those found in previous researches (Van de Velde et al., 2014). Statistical differences among treatments regarding each CIE-Lab coordinates were observed, and PA-washed samples seem to have more luminosity (L*) and H_2O_2 5 % have more a* value, that indicates the red intensity. Colour is an important visual parameter that influence the consumers' acceptance and buying intention (Barrett et al., 2010). Alexandre et al. (2012) found that microbial loads of strawberries washed with H_2O_2 resulted in lower microbial loads but caused significant changes in key attributes such as colour and total anthocyanins content.

448 On the other hand, texture was evaluated by firmness test. The obtained results for firmness

449 showed no statistical differences among treatments and initial value. Firmness values were in the

450 range of those reported by previous studies (Duvetter et al., 2005).

451 ### 3.3.2. Native microbiota counts

452 Regarding epiphytic microbiota, the initial concentration was ca. 3.0 - 3.5 log CFU/g on untreated

453 strawberries (Fig. 3). Remaining total aerobic mesophyll (TAM) population after NaClO (100

454 ppm) washing was 1.7 ± 0.3 log CFU/g. Only NaClO, LA and AA treatments were statistically

455 most effective than the other disinfectants compared with the initial population (untreated

456 strawberries). For all treatments, TAM reduction range was between 0.0 – 1.4 log CFU/g. The

457 alternative sanitizers effect was comparable to that of NaClO (100 ppm), as there were no

458 significant differences between populations, except for PA 80 ppm. The population reported in

459 the PA treatment was statistically the same as the initial population (untreated). Native microbiota

460 of fruits and vegetables is a complex and heterogenic community. However, dissimilar

461 proportions of each genre of microorganisms and different loads can be found between cultivars,

462 batches or years and even among fruits (Baugher and Jaykus, 2016; Jensen et al., 2013). Hereto,

463 a higher sensitivity to washing procedures depending on the main genres existing in the

464 population may occur, as it has been proved that there are inter-specific differences on how

465 microorganisms are inhibited by this product. PA disrupts the chemiosmotic function of the

466 lipoprotein cytoplasmic membrane and rupture the cell walls promoting catalase inactivation, so

467 variances in membrane composition and bacteria heterogenicity of TAM could be a reason for

468 comparative sensitivity among PA (Banach et al., 2015).

469 For fungi, significant reductions were found on mould and yeast (M&Y) populations among some

470 treatments compared with the initial population (untreated strawberries), except for PA, LA and

471 CA sanitizers (Fig. 3). The most effective treatments for M&Y reduction were the NaClO, H_2O_2

472 and AA treatments. M&Y reduction range was between 0.3 - 1.8 log CFU/g. Previous

473 publications reported maximum TAM reductions of 1.5 log CFU/g in berries when using citric or

474 malic acid, whereas M&Y reductions below 1 log CFU/g were achieved (Wei et al., 2017).

475   Microbial contamination of washing solutions after disinfection changing between 1.7 and 3.2

476   log CFU/mL (Fig. 3), except for NaClO and H_2O_2 5 %, in which both TAM and M&Y were

477   reduced completely.

478   **4.   Conclusion**

479   For each treatment, different concentrations were studied in order to optimize the decontamination

480   washing step with chemical sanitizers. The best concentrations were 80 ppm for PA, 5 % for H_2O_2

481   and 2.5 % for organic acids after 2 min treatment. Results indicate that the sanitizers selected may

482   be a feasible alternative to chlorine (100 ppm) for removing pathogenic microorganisms from

483   fresh strawberries destined to frozen and processing purposes, reducing the number of produce-

484   related food-borne outbreaks. Despite good results were also obtained with H_2O_2, we opt for PA

485   as a good alternative to chlorine disinfection against microbial pathogens. PA at 80 ppm gave

486   reductions of 3.5 log-reduction for *L. monocytogenes*, 2.6 log-reduction for *S. enterica*, and 1.9

487   log-reduction for MNV-1. Additionally, no remaining population of pathogenic bacteria was

488   detected after PA sanitization in wash water, thus preventing possible subsequent cross-

489   contamination. This sanitizer does not form undesirable by-products derived from chlorine and

490   did not affect the physicochemical quality of strawberries. Furthermore, PA is used in less

491   quantity compared with the other sanitizers. To improve PA implementation, its combination with

492   other chemical/physical technologies, such as the combination with water-assisted ultraviolet

493   (UV-C) light, should be investigated.

494

501 **Conflict of interest**

502 The authors declare no conflict of interest.

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738 **Table 1.** Wash water parameters (pH, oxidation reduction potential (ORP) and

739 concentration of sanitizer). Values are the mean of the 6 repetitions ± standard deviation.

740 NaClO, sodium hypochlorite; PA, peracetic acid; LA, lactic acid; AA, acetic acid; CA,

741 citric acid.

Sanitizer	Concentration	Concentration of free chlorine NaClO or PA (ppm)	pH	ORP (mV)
NaClO	100 ppm	112 ± 5.7	6.7 ± 0.1	873 ± 0.0
PA	40 ppm	56.6 ± 3.6	5.6 ± 0.2	563 ± 13.3
PA	80 ppm	81.3 ± 3.2	4.6 ± 0.1	602 ± 16.5
PA	120 ppm	114 ± 3.1	4.4 ± 0.0	595 ± 5.6
NaClO	100 ppm	113 ± 0.0	6.7 ± 0.1	891 ± 9.9
H_2O_2	1%	-	7.6 ± 0.1	239 ± 10.1
H_2O_2	2.5%	-	7.6 ± 0.1	239 ± 0.0
H_2O_2	5%	-	7.4 ± 0.1	247 ± 7.0
NaClO	100 ppm	115.5 ± 4.2	6.8 ± 0.1	895 ± 7.1
LA	1%	-	2.3 ± 0.0	755 ± 49.5
LA	2.5%	-	2.5 ± 0.0	711 ± 10.6
LA	5%	-	1.8 ± 0.0	703 ± 3.2
NaClO	100 ppm	133.8 ± 11.7	6.6 ± 0.0	898 ± 12.7
AA	1%	-	2.9 ± 0.0	755 ± 33.1
AA	2.5%	-	2.6 ± 0.0	738 ± 8.0
AA	5%	-	2.4 ± 0.00	740 ± 6.7
NaClO	100 ppm	124 ± 19.1	6.8 ± 0.0	876 ± 5.7
CA	1%	-	2.2 ± 0.0	480 ± 38.9
CA	2.5%	-	1.9 ± 0.0	504 ± 2.5
CA	5%	-	1.8 ± 0.0	516 ± 3.8

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Treatment	Dose	Reduction (log CFU/g)			
		L. monocytogenes		*Salmonella* spp.	
		Strawberry	Water	Strawberry	Water
PA	40 ppm	3.8 ± 0.0^a	≤ 1.7	4.1 ± 0.0^a	≤ 1.7
	80 ppm	3.8 ± 0.0^a	0.0	4.1 ± 0.0^a	0.0
	120 ppm	3.8 ± 0.0^a	0.0	4.1 ± 0.0^a	0.0
NaClO	100 ppm	2.4 ± 0.1^b	0.0	2.3 ± 0.1^b	0.0
H$_2$O$_2$	1%	2.4 ± 0.9^c	**2.7**	2.3 ± 0.4^c	**3.1**
	2.5%	3.8 ± 0.7^b	0.0	3.8 ± 0.9^b	0.0
	5%	5.4 ± 0.8^a	0.0	4.9 ± 0.0^a	0.0
NaClO	100 ppm	2.9 ± 0.4^{bc}	0.0	2.7 ± 0.4^{bc}	0.0
LA	1%	2.7 ± 0.6^a	≤ 1.7	2.7 ± 0.6^a	**2.0**
	2.5%	3.0 ± 0.0^a	0.0	2.9 ± 0.6^a	0.0
	5%	2.7 ± 0.9^a	0.0	2.8 ± 0.8^a	0.0
NaClO	100 ppm	1.3 ± 0.4^b	0.0	1.2 ± 0.4^b	0.0
AA	1%	2.4 ± 0.6^a	0.0	3.1 ± 0.6^b	≤ 1.7
	2.5%	2.5 ± 0.6^a	0.0	3.2 ± 0.0^{ab}	0.0
	5%	2.8 ± 0.6^a	0.0	3.9 ± 0.6^a	0.0
NaClO	100 ppm	3.2 ± 0.3^a	0.0	2.9 ± 0.3^b	0.0
CA	1%	3.2 ± 0.2^a	≤ 1.7	3.3 ± 0.4^{ab}	**2.0**
	2.5%	4.0 ± 0.6^a	**2.5**	3.8 ± 0.6^a	**2.8**
	5%	4.2 ± 0.6^a	≤ 1.7	3.8 ± 0.0^a	≤ 1.7

		NaClO	100 ppm	2.7 ± 0.4^a	0.0	2.5 ± 0.4^b	0.0

745 **Table 2.** Average reductions (log Colony-forming unit (CFU)/g) of *L. monocytogenes*

746 and *S. enterica* after 2 minutes on strawberries. Means ± standard deviation followed by

747 the same small letter indicate no significant differences among the different concentration

748 tested for each treatment ($p \le 0.05$; n=6). For washing solutions, values represent the

	pH	Firmness (N)	TA (g citric acid/L juice)	TSS (ºB)	Colour		
					L*	a*	b*
Initial	3.49 ± 0.19^a	3.61 ± 1.57^a	9.84 ± 0.91^a	7.80 ± 0.00^e	40.76 ± 3.39^{ab}	31.96 ± 1.62^{ab}	23.26 ± 4.80^a
NaClO 100	3.46 ± 0.02^a	4.27 ± 1.88^a	9.16 ± 0.04^{ab}	8.07 ± 0.06^d	43.61 ± 5.40^{ab}	31.31 ± 2.19^{ab}	28.99 ± 7.01^a
PA 80	3.61 ± 0.22^a	4.33 ± 1.56^a	8.10 ± 0.06^{bc}	7.93 ± 0.05^{de}	45.48 ± 2.7^a	31.16 ± 1.22^{ab}	30.17 ± 4.25^a
H$_2$O$_2$ 5%	3.42 ± 0.04^a	3.38 ± 0.97^a	8.19 ± 0.18^{bc}	8.53 ± 0.05^c	43.16 ± 0.79^{ab}	33.53 ± 0.74^a	27.46 ± 1.8^a
LA 2.5%	3.49 ± 0.13^a	3.45 ± 1.26^a	7.86 ± 0.36^c	9.26 ± 0.05^a	41.86 ± 4.09^{ab}	31.53 ± 0.88^{ab}	24.43 ± 4.49^a
AA 2.5%	3.56 ± 0.06^a	3.11 ± 0.47^a	8.04 ± 0.56^{bc}	9.00 ± 0.00^b	38.35 ± 2.64^b	30.11 ± 1.25^b	21.25 ± 3.64^a
CA 2.5%	3.43 ± 0.09^a	3.65 ± 1.21^a	8.90 ± 0.29^{abc}	9.46 ± 0.23^a	42.65 ± 4.08^{ab}	31.91 ± 2.5^{ab}	27.3 ± 4.51^a

749 population of pathogenic bacteria, and were obtained from one sample and repeated

750 twice. NaClO, sodium hypochlorite; PA, peracetic acid; LA, lactic acid; AA, acetic acid;

751 CA, citric acid.

752 **Table 3.** Values of pH, firmness, titratable acidity (TA), total soluble solids (TSS) and

753 colour (L*, a* and b*) of strawberries for each washing treatment. Values are expressed

754 as the mean of 6 reps ± standard deviation. Different letters indicate statistically

755 significant differences ($p < 0.05$) among treatments. Sodium hypochlorite (NaClO),

756 peracetic acid (PA), lactic acid (LA), acetic acid (AA) and citric acid (CA).

757 *Different letters indicate statistically significant differences ($p < 0.05$) among doses.*
758

Figure 1. Reduction of *L. monocytogenes* in strawberries (grey bars, log Colony-forming

unit (CFU)/g) and *Salmonella enterica* (white bars, log CFU/g). Population of *L.*

monocytogenes in water (●, log CFU/mL) and *Salmonella enterica* (▲, log CFU/mL).

Bacterial reduction 9999values in strawberries are the mean of 6 reps ± standard

deviation. Remaining bacterial population values in water were obtained from one sample

for each microorganism and were repeated twice. The straight line indicates the detection

limit (dl) of the bacterial population on strawberries. NaClO, sodium hypochlorite; PA,

peracetic acid; LA, lactic acid; AA, acetic acid; CA, citric acid.
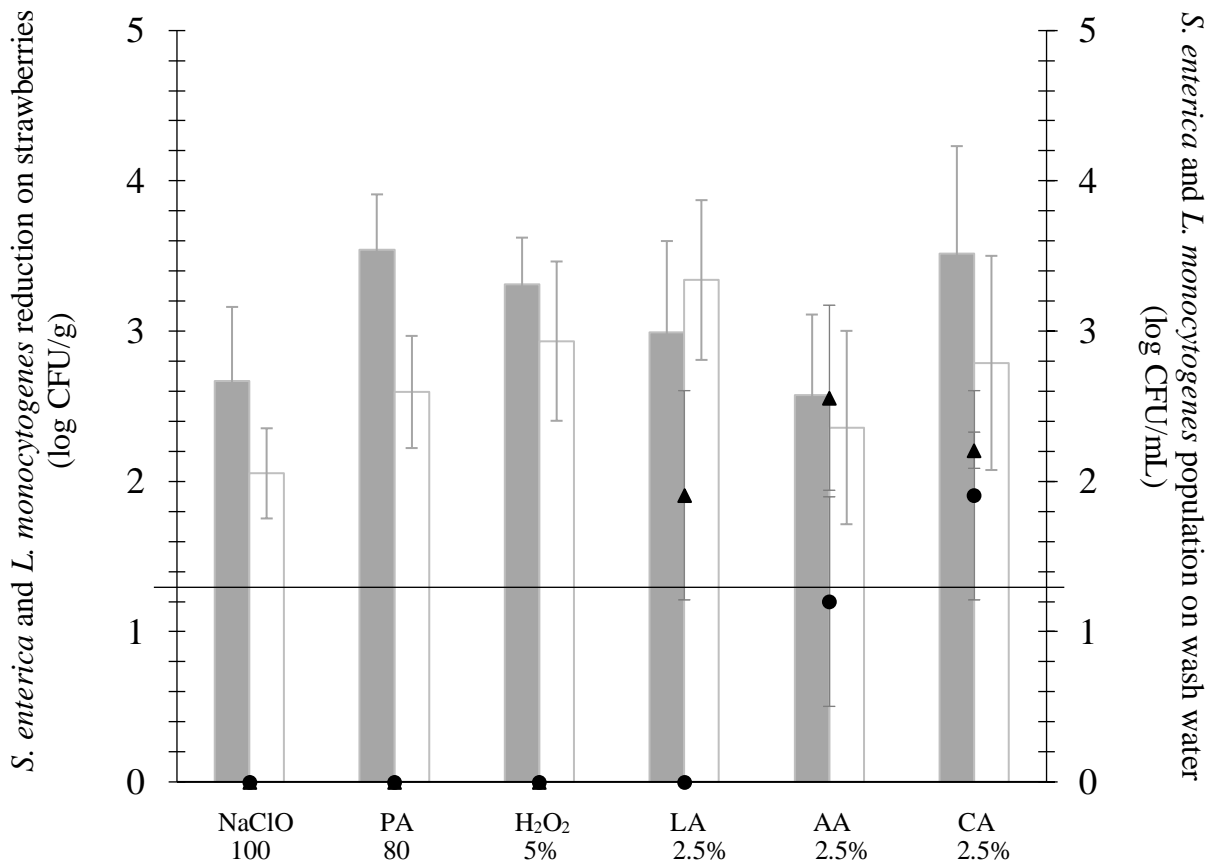
774 **Figure 2.** Reduction of the infectivity of murine norovirus (MNV-1) in fresh strawberries

775 (log 50 % Tissue Culture Infectious Dose ($TCID_{50}$)/mL) after disinfection treatments at

776 2 min. Detection limit was 0.8 log $TCID_{50}$/mL. Results are the mean of 6 repetitions ±

777 standard deviation. NaClO, sodium hypochlorite; PA, peracetic acid; LA, lactic acid; AA,
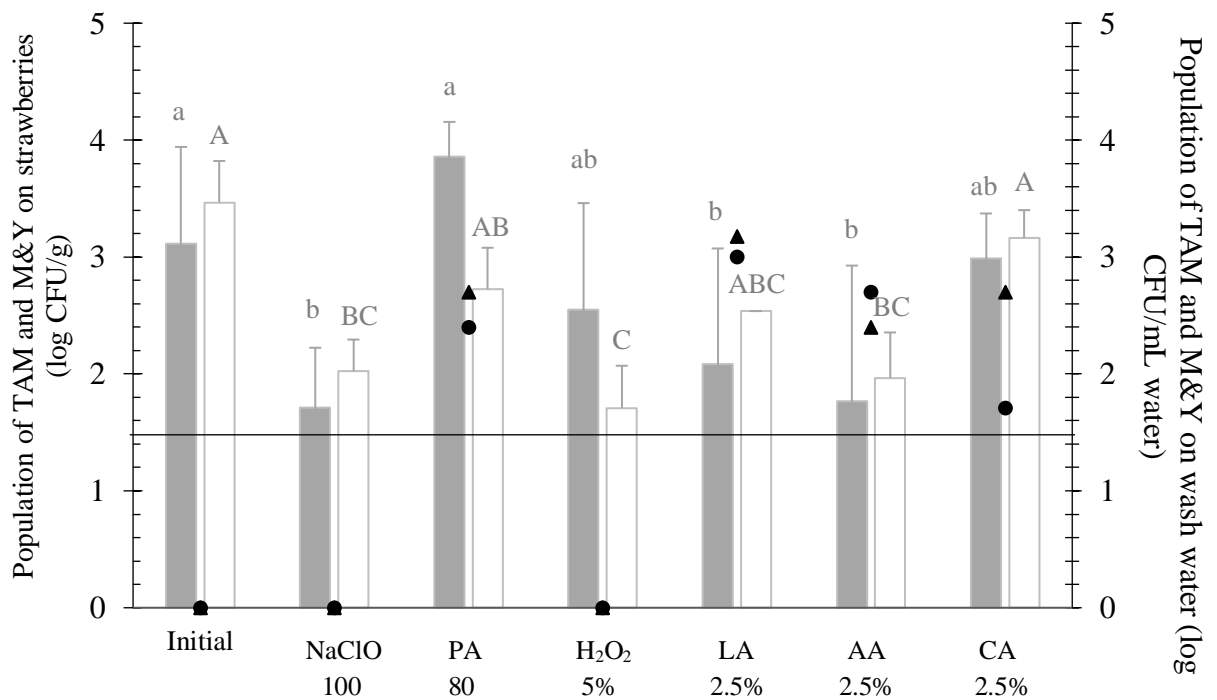
778 acetic acid; CA, citric acid.

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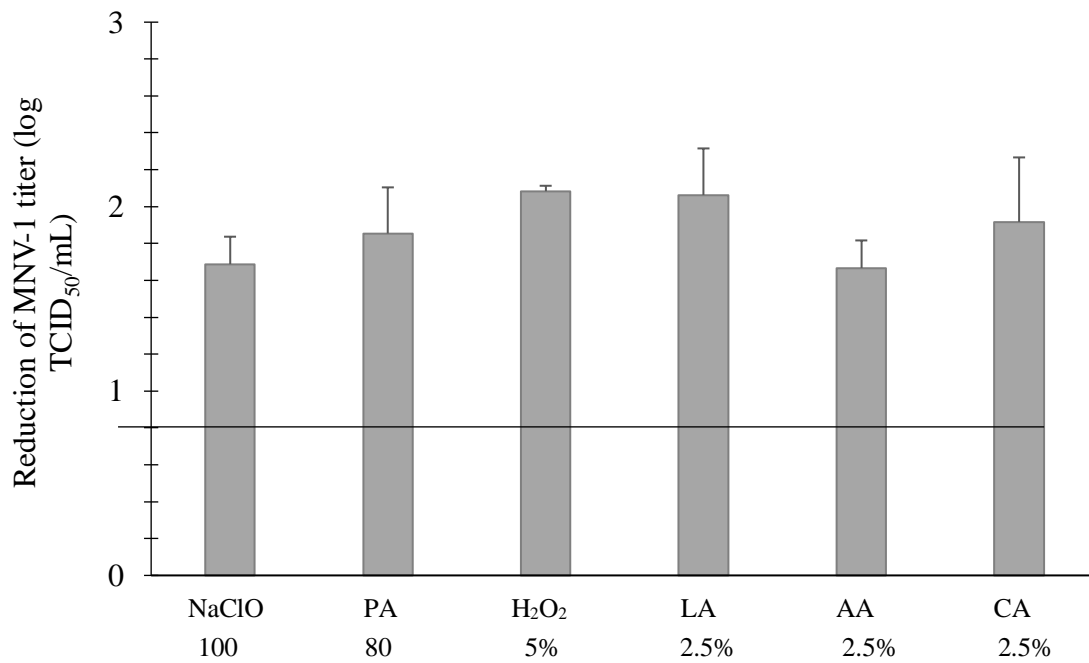
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784 **Figure 3.** Population (log Colony-forming unit (CFU)/g strawberry) of total aerobic
785 mesophylls (TAM) (grey), or molds and yeasts (M&Y) (white) on strawberries. Values
786 are the mean of 3 reps ± standard deviation. Different letters indicate significant
787 statistically differences ($P < 0.05$) among treatments. Remaining counts (log CFU/mL)
788 of TAM (●), or M&Y (▲) in washing solutions. Values were obtained from one sample.
789 The straight line indicates the detection limit (dl). NaClO, sodium hypochlorite; PA,
790 peracetic acid; LA, lactic acid; AA, acetic acid; CA, citric acid.

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