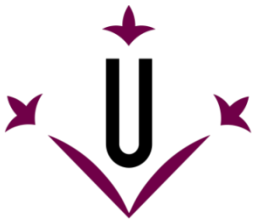

Strawberry sanitization by peracetic acid washing and its effect on fruit quality.

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Highlights

- Washing time was irrelevant to reduce epiphytic microbiota and *L. innocua* populations.
- Aerobic mesophylls were reduced similarly by peracetic acid (PA) and NaClO washes.
- All PA washing treatments reduced the *L. innocua* populations by 4 log units.
- *L. innocua* counts in PA washing solutions were 4-log units lower than they were in control water.
- Sanitization had no relevant impact on quality nor on biochemical characterization.

Abstract

The risk posed by outbreaks associated with strawberries together with the safety issues of by-products from chlorine disinfection in the fruit industry has led to a search for alternative sanitizers. The disinfection capacity of peracetic acid (PA) at three concentrations (20, 40 and 80 ppm) and washing times (1 and 2 min) was compared to sodium hypochlorite (200 ppm) (NaClO) treatments and a water control, and its influence on the physico-chemical, biochemical and nutritional quality of strawberries was also studied. Counts on total aerobic mesophilic microorganisms were comparable between NaClO and PA. For yeasts and molds, only NaClO and 80 ppm PA reduced contamination in washing water, but no differences wereobserved in strawberries. Artificially inoculated *L. innocua* was reduced by at least 4 log cfu/g in strawberry by all the PA treatments, except at 20 ppm PA for 1 min. Total soluble solids, pH, titratable acidity, antioxidant activity and total phenolic content values were maintained after all treatments. Only anthocyanin content was affected. Treatments of 20 and 40 ppm PA did not significantly affect fruit color, and there were no losses on strawberry firmness. PA, as a GRAS substance that has shown potential to reduce microorganisms present in strawberries without any major physicochemical or sensorial alteration, could be a suitable alternative to chlorine disinfection.

Keywords:
Listeria innocua, native microbiota, nutritional, biochemical, disinfection

Abbreviations:

DPPH, 2,2-diphenyl-1-picrylhydrazyl; FRAP, ferric reducing antioxidant power; FW, fresh weight; PA, peracetic acid; T, temperature; TAM, total aerobic mesophyll; M&Y; moulds and yeasts; TPTZ, 2,4,6-tris(2-pyridyl)-s-triazine

1. Introduction

Strawberries are rich in vitamins (i.e. ascorbic acid) and other antioxidants (i.e. phenolic acids, anthocyanins), and other bioactive molecules. There is increasing evidence to suggest that these active phytochemicals have anti-inflammatory, antimicrobial, anti-carcinogenic, anti-mutagenic and neuroprotective effects. Thus, berry consumption seems to be beneficial for human health (Mortas and Sanlier, 2017).

Strawberry production exceeds 740,000 tones in Europe, and it is widely consumed in both fresh and frozen forms (Fruit Logistica, 2018). Fresh strawberries have a short life of 13 days on average if correctly stored at 5 °C (Leithner, 2017) and losses due to shelf-life issues can range up to 53%, as reported in Meyer et al. (2017). Even though no bacterial pathogenic microorganisms have been found on strawberries (Delbeke et al., 2015), the EFSA (European Food Safety Authority, 2014) emitted a scientific opinion on the risk posed by *Salmonella* spp. and norovirus in berries. Hadjilouka et al. (2014) reported presence of *Listeria monocytogenes* in 3.8% of strawberry samples.

Strawberry contamination can occur at the pre-harvest or post-harvest stage by numerous sources including insects, soil, water, equipment or human handling (Zhu et al., 2017). Disinfection is a critical step in the inactivation of pathogenic and spoilage microorganisms. In fruits, a first approach for this purpose consists of a washing step in which fruits are immersed in a sanitizer solution. Among available sanitizers, chlorine is the first choice due to its low price, simplicity of use and effectiveness against vegetative bacteria. But since its action is highly pH dependent and it reacts with organic matter, producing unhealthy by-products including carcinogenic and mutagenic chlorinated compounds, it has already been banned in some European countries (Fallik, 2014; Meireles et al., 2016). It has also been included in the indicative list of the

71 Directive on Industrial Emissions (IPPC, 2007/0286(COD), to reduce harmful industrial
72 emissions across the EU (European Commission, 2007).

73 Subsequently, effective disinfection alternatives to chlorine have been studied,
74 including other sanitizers like organic acids or essential oils, or physical methods such
75 as ultrasound or ultraviolet processing (Ramos et al., 2013). As the washing water may
76 also increase the bacterial counts by cross-contamination, it is important that the
77 washing step not only removes bacteria from the strawberry surface but also maintains
78 water quality (Pablos et al., 2018). Peracetic acid (PA) is an unspecific, persistent
79 oxidizer of C-C double bonds and reduced atoms. This mode of action would imply a
80 poor chance for the development of resistance in microorganisms, as borne out by the
81 absence of such reports in the literature (Wessels and Ingmer, 2013). It has revealed to
82 be effective on decontamination procedures, making it a good choice as a sanitizing
83 agent (Singh et al., 2018). Its use up to 80 ppm is permitted in USA for the washing of
84 fruits and vegetables (FDA CFR 173.315).

85 Alternative disinfection methods to chlorine must be found in order to provide
86 consumers with safe fresh-cut fruits and vegetables. Hence, the objectives of this study
87 were to assess the adequacy of peracetic acid as a sanitizer in strawberry washing
88 processes to decrease native microbiota and artificially inoculated *L. innocua* and to
89 study its effect on the nutritional and commercial quality of the fruits.

2. Materials and methods

2.1. Materials

Strawberries (*Fragaria* x *ananassa*) were purchased from local distributors. Calix and leaves were carefully removed before the treatment.

Peracetic acid 15% was purchased from PanReac AppliChem (Barcelona, Spain). Triptone soy broth (TSB), triptone soy agar (TSA), Palcam base agar, yeast extract, plate count agar (PCA), dichloran rose bengale chloramphenicol agar (DRBC), potassium bisulfate, sodium chloride and peptone were purchased from Biokar Diagnostics (Allonne, France).

Ascorbic acid, gallic acid, 2,4,6-tris(2-pyridyl)-s-triazine (TPTZ), 2,2-diphenyl-1-picrylhydrazyl (DPPH), sodium carbonate, were purchased from Sigma-Aldrich (Steinheim, Germany). Methanol, acetone, chlorhidric acid (37%), sodium acetate, sodium hydroxide, potassium chloride, ferric chloride hexahydrate and Folin Ciocalteau's reagent were purchased from Panreac (Llinars del Vallès, Spain).

2.2. Bacterial strains and culture conditions

L. innocua strain CECT-940 (*Colección Española de Cultivos Tipo*, Burjassot, Spain) was used in this study. It was grown for 24 h in 50 mL of TSB supplemented with 6 g/L of yeast extract, 2.5 g/L glucose and 2.5 g/L K_2HPO_4 (TSBYE) at 37±1°C in a rotatory shaker set at 150 rpm. Afterwards, the culture was centrifuged at 9800 × *g*, at 10°C, for 10 min, and the pellet was suspended in an adequate volume of saline peptone, 8.5 g NaCl and 1 g peptone (PS) to obtain a concentrated suspension, which was approximately 10^{10} cfu/mL. Concentration in the suspension was checked by plating in TSAYE and Palcam followed by incubation at 37±1ºC for 48 h.

2.3. Strawberry inoculation with *Listeria innocua*

The day before the experiment, strawberries were inoculated with 50 µL of the prepared suspension of *L. innocua* at 10^{10} cfu/mL, to reach a theoretical initial concentration of 2 × 10^7 cfu/g. Inoculation was done by pipetting small droplets on the surface of each strawberry and allowing them to dry for approximately 3 h at room temperature (22ºC). Inoculated strawberries were stored at 4±1°C for 20 h until the assay. Prior to the experiments, the initial concentration of *L. innocua* was checked as explained below.

2.4. Experimental design

Two types of experiments were carried out. On one hand, an experiment was conducted in artificially inoculated strawberries to determine *L. innocua* populations after the treatments (Figure 1). This experiment was done once, with 3 determinations (repetitions). On the other hand, the experiment in non-inoculated strawberries was replicated three times, two to ascertain the effect of washing treatments on epiphytic microbiota and one to perform the quality and nutritional determinations. Treatment solutions were prepared: tap water with sodium hypochlorite at 200 ppm pH 6.6 (NaClO) adjusted using 3 M citric acid, and tap water with peracetic acid at concentrations of 20 ppm (PA20), 40 ppm (PA40) or 80 ppm (PA80). In microbiological assays, tap water (W) was added as a control in order to verify whether reductions could be due to the physical removal of water itself or if further reductions could be achieved by the use of a germicidal effect of PA. For washing treatments, 20 fruits were submerged for 1 or 2 min in 2 L of each solution. After the hypochlorite treatment, fruits were rinsed in 2 L of tap water. Fruits were kept to dry at room temperature. Free chlorine concentration was checked with an ion specific meter Hanna Instruments HI 95734-11 (Rhode Island, USA) and peracetic acid concentration was determined by titration.

Moreover, in the experiments with non-inoculated strawberries, microbiological and quality analysis were performed. For biochemical determinations, an aliquot of each replication was frozen with liquid nitrogen, milled using a MINIMOKA GR-020 grinder (Taurus Group, Barcelona, Spain) and stored at -80°C until analysis.

2.5. Microbiological analysis

In the artificially inoculated experiments, one strawberry per repetition was weighted, placed in a sterile filter bag (80 mL BagPage®, Interscience BagSystem, Saint Nom, France) and diluted with buffered peptone water 1:4 (w:v). It was mashed in a paddle blender (MiniMix, Interscience, France) for 2 min at 9 strokes/s. Aliquots of the mixture were serially diluted in saline peptone (SP), plated in duplicate on Palcam agar and plates were incubated at 37 ± 1°C for 48 h.

In experiments with epiphytic microbiota, two strawberries per repetition were weighed, placed in a sterile filter bag, diluted and homogenized as explained above. A 10-fold serial dilutions were made in SP and plated in duplicate on PCA for total aerobic mesophilic counts (TAM) and in DRBC for molds and yeasts (M&Y). Plates were incubated at 30±1 ºC for 3 days for TAM and at 25±1 ºC for 3 to 5 days for M&Y. Results were expressed as log cfu/g and the detection limit was 20 cfu/g. This experiment was repeated twice.

Moreover, after each washing treatment, the population of *L. innocua* and TAM and M&Y was determined in the wash water. One milliliter of water was added to neutralizing Dey-Engley medium and plated as described before. Results were expressed as log cfu/mL, and the detection limit was 50 cfu/mL. When quantification was below the detection limit, its presence was confirmed by Dey-Engley change in color followed by streaking onto PCA, DRBC or Palcam.

2.6. Quality analysis

Quality analyses were only determined in non-inoculated strawberries

2.6.1. pH, titratable acidity and total soluble solids

For pH, titratable acidity (TA) and total soluble solids (TSS) determination, strawberries were smashed in a blender to obtain their juice. For each replication, 25 mL of strawberry juice were prepared, and determined twice. pH was determined using an electrode in a pH-meter model GLP22 (Crison Instruments SA, Barcelona, Spain). TA was measured by diluting 10 mL of strawberry juice with 10 mL of distilled water and titrated with 0.1 M NaOH until pH 8.2 was reached. Results were expressed as mg of citric acid per L. TSS was measured at 20 °C with a refractometer (Atago Co. Ltd., Tokyo, Japan), and the results expressed as °Brix.

2.6.2. Color

Color of 20 strawberries was measured on 3 sides of each sample by using a CR-200 Minolta Chroma Meter (Minolta, INC., Tokyo, Japan). Color was expressed as CIE L* a* b* coordinates, using a D65 illuminant and 10° observer angle. These values were used to calculate the total color difference (TCD) (Eq. 1),

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

where f = final (strawberries after each treatment) and i = initial (strawberries before any treatment).

2.6.3. Texture

To assess changes in texture, compression and firmness measured by the maximum penetration force were determined using the TA.XT Plus Connect texture analyzer (Stable Micro systems Ltd., Surrey, England).

185 **Compression** force readings were taken by recording the maximum force required to
186 compress a strawberry half 6 mm using 2 horizontal parallel plates. The compression
187 pre-test and test were both run at 5 mm/s speed with a trigger force of 0.1 N.

188 **The firmness** test was performed using a cylindrical probe (4 mm). Pre-test and test
189 were both run at 5 mm/s speed and using a trigger force of 0.1 N, allowing the probe to
190 enter 8.0 mm deep into the tissue, measuring the maximum force encountered.

191 2.7. Biochemical analysis

192 2.7.1. Antioxidant activity

193 Antioxidant activity was assessed in the frozen strawberries using two methodologies:
194 ferric reducing antioxidant power (FRAP) and DPPH scavenging activity assays. For
195 the extraction, 6.0 ± 0.1 g were mixed with 20 ml of methanol 70% (v/v) and
196 homogenized in a vortex for 20 s. Samples were immediately placed in a stirrer at 4 °C
197 working at 195 rpm for 5 min and centrifuged using a Sigma-3-18 KS centrifuge
198 (Sigma Laborzentrifugen GmbH, Osterode am Harz, Germany) at 13 500 x *g* for 20 min
199 at 4 °C. Supernatant was then filtered and marked to 25 mL with methanol 70%.
200 Extracts were stored at -80 °C for further determinations.

201 The **FRAP** reagent was prepared with a mixture of acetate buffer 0.3 M pH 2.6, TPTZ
202 40 mM in HCl and $FeCl_3 \cdot 6H_2O$ 20 mM in distilled water in 10:1:1 (v:v:v) proportion.
203 The determination was performed by adding 0.1 mL of the extract to 1.4 mL of FRAP
204 reagent and incubating in a thermostatic bath at 37 °C for 20 min in the dark.
205 Absorbance was read at 593 nm using GENESYS^{TM} 10S UV-Vis spectrophotometer
206 (Thermo Fisher Scientific, MA, USA).

207 **DPPH**· radical was prepared daily by diluting a stock solution of DPPH· 1mM in
208 methanol 100%, until an absorbance at 515 nm of 0.750 ± 0.50 was reached. Then, the

determination was performed by adding 0.1 mL of the extract to 1.4 mL of DPPH· reagent and incubating at RT for 1 h in the dark. Absorbance was read at 515 nm using GENESYS^{TM} 10S UV-Vis spectrophotometer (Thermo Fisher Scientific, MA, USA).

Standard curves with ascorbic acid for both methods were prepared daily by using the same procedure as with the samples. Results were expressed as mg of ascorbic acid equivalents / 100 g of fresh weight (FW).

2.7.2. Anthocyanin content

Anthocyanin extraction for further determination was performed as following. Briefly, 5.0 ± 0.1 g of frozen sample were mixed with 10 mL of methanol 80% (v/v) and vortexed for 20 s. After stirring at 200 rpm for 10 min at 4 °C, the mixture was centrifuged using a Sigma-3-18 KS centrifuge (Sigma Laborzentrifugen GmbH, Osterode am Harz, Germany) at 12,000 rpm for 15 min at 4 °C. Supernatant was then filtered and stored at -80°C until needed.

Determination was accomplished by adding a 0.5 mL aliquot of the extract to potassium chloride buffer 0.025 M, pH 1.0 and also to sodium acetate buffer 0.400 M, pH 4.5 to a final volume of 5 mL. Absorbance of both solutions was read at 510 and 700 nm. For quantification, Eq. 2 was used:

$$\Delta A = (A_{510} - A_{700})_{pH\ 1.0} - (A_{510} - A_{700})_{pH\ 4.5} \qquad \text{Eq. 2}$$

Where A is absorbance at a certain wavelength. Anthocyanin content was expressed as mg of cianidine-3-glucosyde / 100 g FW following the calculations described by (Meyers et al., 2003).

2.7.3. Total phenolic content (TPC)

The TPC was determined by the Folin-Ciocalteau method. The test was performed on the same extract used for antioxidant activity determination.

The assay was performed by adding 4.3 mL of distilled water and 0.5 mL of Folin-Ciocalteu's reagent to 0.7 mL of extract. After shaking and incubation for 5 min at RT in the dark, 2 mL of saturated sodium carbonate were added. The mixture was again shaken and incubated for 1 h in the dark. Absorbance was read at 760 nm using GENESYS^{TM} 10S UV-Vis spectrophotometer (Thermo Fisher Scientific, MA, USA). Standard curve with gallic acid was prepared daily using the same procedure as with the samples. Results were expressed as mg of gallic acid equivalents per 100 g FW.

2.8. Statistical analysis

Results are expressed by mean ± standard deviation (SD) of 3 repetitions. All data were checked for significant differences by applying analysis of variance test (ANOVA). The criterion for statistical significance was $p < 0.05$. When significant differences were observed, Tukey's Honest Significant Difference (HSD) of the means was applied. All statistical analysis was carried out using JMP 13 (SAS Institute Inc., Cary, USA).

3. Results and discussion

3.1. Effect of PA on microorganisms

Concentrations of sanitizers, pH and ORP values are detailed in Table 1. In the PA washing solutions, pH and POR values were lower than those observed in NaClO treatment, which ranged from 6.5 to 6.65 and 881 to 894 mV, respectively.

3.1.2. *L. innocua* experiments

The initial population of *L. innocua* on strawberries was 5.70 ± 0.50 log cfu/g (Figure 2) After all washing treatments, *L. innocua* populations were statistically lower than the initial population. When washing with 200 ppm hypochlorite (NaClO) for 2 min, *L. innocua* population in strawberries was 0.50 ± 0.50 log cfu/g fruit. This 5.50 log cfu/g reduction was higher than those reported in other studies on fresh-cut produce such as avocados disinfected with hypochlorite 75 ppm for 15 s (Rodríguez-García et al., 2011), or romaine lettuce and cantaloupe, immersed in a 200 ppm NaClO solution for 10 min (Guzel et al., 2017). *L. innocua* populations achieved after PA treatments in all combinations were equivalent to those observed after hypochlorite washing, ranging from 1.69 ± 0.74 to 0.40 ± 0.52 log cfu/g, when washing with PA40 for 2 min or PA80 for 2 min, respectively. Reductions of about 4 log units observed in this study were in accordance with other authors, who 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 (Singh et al., 2018). Contrarily, other authors found lower reductions at similar PA concentrations (25, 50 and 75 ppm) on sprouts (Neo et al., 2013). These differences could be attributed to variations in the inoculation step (method or pathogen concentration), the strain used or on the characteristics of the fruit and vegetable surface, as this parameter affects the adherence of the microorganism (São José et al., 2014). As *L. monocytogenes* is a pathogen that can grow in the

conditions in which strawberries are stored, other studies have used different sanitizers to reduce its populations. For instance, Zhou et al. (2017) used 0.5 % levulinic acid plus 0.5 % sodium dodecyl sulphate, achieving 2 log cfu/g reductions. In strawberries, other pathogenic microorganisms have been reported to pose a health concern, namely *Salmonella* spp., *E. coli* O157:H7 and norovirus (EFSA, 2014). Guo et al. (2018) have studied the effect of PA at 90 ppm for 2 min and found a reduction of *Salmonella* and *E. coli* O157:H7 of 1.2 log cfu/g after the washing treatments. In other vegetable products, Silveira et al. (2018) found a decrease of *S. enterica* Typhimurium of 2.4 log cfu/g when using PA 50 ppm for 5 min.Wang and Riser (2014) also found that the decrease of Salmonella Typhimurium after washing tomatoes with PA 40 ppm for 2 min was 2.5 log cfu/g. *L. innocua* has demonstrated to be a good surrogate for *L. monocytogenes* (Francis and O' Beirne, 1997). However, the lower reductions of other pathogens compared to ones found in our study with *L. innocua* should be considered. Further investigations should be done targeting common pathogenic microorganisms of strawberries, so as to confirm the effect of PA on them. Removal of microorganisms from the produce surface as a result of washing is critical, as it is the quality of water used. In this study, *L. innocua* on strawberries after W washing was not statistically different from other treatments, demonstrating that there was a physical removal of microorganism during washing. However, the remaining population in wash water after treatments was higher (more than 5 log cfu/mL) than it was when a sanitizer was used. Except for PA20 for 1 min, other PA and NaClO treatments achieved a final population of less than 1.5 log cfu/mL in water, thus preventing subsequent cross contamination of *L. innocua*. However, as can be seen below, the population of natural microbiota found in washing solutions was higher than it was for the pathogenic strain. The 2-4 log cfu/mL of TAM and Y&M found after treatments in washing solutions could be a

296 drawback when recommending PA for water reprocessing. On the other hand, the
297 reported ability of PA to reduce biofilm formation would make this product a suitable
298 sanitizer to add in the washing step (Barbosa et al., 2016). Furthermore, compared to
299 other wash water disinfectants, PA has less potential of producing degradation by-
300 products, which are easily dissolved in water and non-toxic, thus making this sanitizer a
301 good alternative to chlorine (Banach et al., 2015a).

302 3.1.2. Native microbiota

303 Regarding epiphytic microbiota, remaining TAM population after NaClO washing was
304 3.32 ± 0.68 log cfu/g (Figure 3). The PA and NaClO effect were comparable, as there
305 were no significant differences between populations. Washing time, 1 or 2 min, did not
306 significantly affect the results. Remaining TAM in strawberries after treatments with
307 PA ranged from 3.42 ± 0.38 to 3.93 ± 0.29 log cfu/g when using PA80 or PA20 for 2
308 min, respectively. These counts were significantly lower than those observed after the
309 washing with water for 2 min (W, control) with populations of 4.74 ± 0.58 log cfu/g,
310 thus implying a sanitizing effect attributed to PA. Nevertheless, no significant
311 differences were found on M&Y populations between the treatments and the control, so
312 the cell decrease could be attributed to a physical removal due to water forces on the
313 surface (Castro-Ibáñez et al., 2017). Microbial contamination of washing solutions after
314 washing was between 2.5 and 4.2 log cfu/mL, except for sodium hypochlorite, in which
315 both TAM and M&Y were reduced below 2 log cfu/mL. The experiment was repeated
316 using a different batch of strawberries. Results showed that even if the initial population
317 on strawberries was similar (3.96 ± 0.14 and 3.88 ± 0.14 log cfu/ g strawberry), the
318 effectiveness of some of the treatments was statistically different. Overall, reductions
319 observed were lower in the second repetition than they were in the first assay. However,
320 PA80 results were comparable to those obtained with NaClO being final populations of

TAM after NaClO and PA80 for 1 min treatments 3.32 ± 0.68 and 3.51 ± 0.14 log cfu/g strawberry, respectively. These differences could be partially explained by the fact that native microbiota of fruits and vegetables is a complex and heterogenic community. Bacteria belonging to *Serratia*, *Pseudomonas*, *Enterobacter* and *Rahnella* genera, yeasts like *Candida*, *Cryptococcus* and *Rhodotorula* and molds such as *Cladosporium*, *Penicillium* and *Botrytis cinerea* are most likely to be found in strawberries (Baugher and Jaykus, 2016). However, dissimilar proportions of each genre and different loads can be found between cultivars, batches or years and even among fruits (Baugher and Jaykus, 2016; Jensen et al., 2013). Hereto, a higher sensitivity to washing procedures depending on the main genres existing in the population may occur, as it has been proved that there are inter-specific differences on how microorganisms are inhibited by this product (K. Banach et al., 2015a). It is suggested that PA disrupts the chemiosmotic function of the lipoprotein cytoplasmic membrane and transport by dislocation or rupture of cell walls and promotes catalase inactivation. Variances in membrane composition could be a reason for comparative sensitivity (Banach et al., 2015c).

Other sanitizers have been used in order to reduce natural microbiota of strawberries. For instance, organic acids such as citric acid (20 g/L, pH 2.1), lactic acid (20 mL/L, pH 2.1), and malic acid (20 g/L, pH 3.3) were used for strawberry washing by Wei et al. (2017). They reported maximum TAM reductions of 1.5 log cfu/g when using citric or malic acid, whereas M&Y reductions below 1 log cfu/g were achieved. This was attributed to the observed results in non-washed strawberries regarding the TAM, M&Y counts being less than those obtained after the different treatments.

3.2. Quality changes

3.2.1. pH, TSS, TA

Physicochemical changes in strawberries, pH, TSS contents and TA are shown in Table 2. Values of these parameters of non-washed strawberries were 3.39 ± 0.01, 5.9 ± 0.1 and 6.37 ± 0.30 mg citric acid/L juice, respectively, which were in concordance with the literature (Ayala-Zavala, et al., 2004). Values of pH and TSS contents indicated barely detectable statistically significant differences among treatments. Although existing differences between treatments, there was not a general tendency that explains changes in pH and TSS contents. TA values were higher when strawberries were washed with PA80, achieving a maximum of 8.54 ± 0.17 mg citric acid/ L juice when treatment time was 2 min.

3.2.2. Color

Strawberry color before any sanitization washing, expressed as CIE-Lab coordinates, was L* 40.04 ± 3.20, a* 32.69 ± 2.57 and b* 26.14 ± 5.40 (Table 3). These values were comparable to those found in the literature (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 and to be less yellowish and reddish, as L* values are higher and a* and b* lower in these samples. However, TCD was not statistically influenced by treatments. It has been stablished that when TCD is higher than 3.5, a clear difference in color is noticed by the inexperienced viewer (Mokrzycki and Tatol, 2011). A general trend was found in TCD, markedly observed when using PA at 80 ppm, with values of 4.76 ± 1.69 and 4.85 ± 3.88 for 1 and 2 min, respectively. When washed with hypochlorite, TCD was 0.84 ± 1.13, indicating that there was no visible alteration in color. Color is one of the sensory parameters that may affect consumers' acceptance and buying intention (Barrett et al., 2010).

3.2.3. Texture

Texture was evaluated by compression and firmness tests (Table 3). The obtained results for firmness showed no statistical differences among treatments and initial value. Firmness values were in the range of those reported by other authors (Duvetter et al., 2005). However, compression values showed a statistical difference between non-washed and PA80 2 min washed strawberries. After washing with PA 80 ppm for 2 min, maximum force at compression was 48.57 ± 12.28 N, higher than the 30.67 ± 7.30 N obtained in non-washed strawberries (initial). This increase in texture may be considered to be an undesirable impact of this washing treatment on strawberry quality, as consumers search for 'moderate hardness' against firm or smooth strawberries (Bhat et al., 2015).

3.3. Biochemical characterization

3.3.1. Antioxidant activity

Antioxidant activity of samples washed with NaClO or PA was assessed by FRAP and DPPH· free radical scavenging ability assays (Table 4).

FRAP results indicated that control strawberries had an antioxidant capacity equivalent to 145.93 ± 8.09 mg ascorbic acid/100 g FW. DPPH· results showed values of 138.04 ± 12.21 mg ascorbic acid equivalents/100g FW. Nevertheless, antioxidant activity was maintained in strawberries washed with hypochlorite or PA at different concentration and time combinations, as no statistical differences were observed between samples.

3.3.2. Anthocyanin content

Initial anthocyanin content of strawberries was 1.90 ± 0.18 mg/100 g FW (Table 4). Significant increases of anthocyanin values were found after the treatments PA20 2 min and PA 80 1 min, but a general tendency was not observed. To date, no studies have

393 been found on how PA can affect anthocyanin content of strawberries. Anthocyanin
394 values obtained with strawberries used in this study were lower than those found by
395 Nowicka et al. (2019) and Van de Velde et al. (2014). This could be attributed to the use
396 of different strawberry varieties or maturity stage, or by differences in the anthocyanin
397 extraction method, as ultrasound was used to assist extraction in those studies, which
398 makes anthocyanins more accessible as it helps to break cell walls and remove
399 boundaries (Meyers et al., 2003).

400 3.3.3. Total phenolic content

401 Values of TPC are shown in Table 4. Initial phenolic content of strawberries was 83.01
402 ± 1.58 mg/100 g FW, which was in similar amounts to those reported in the literature
403 (Perin et al., 2019; Yeoh and Ali, 2017). Even so, Avalos-Llano et al., 2018 found
404 greater values of TPC in strawberry (550 mg/100 g FW). These dissimilarities could be
405 attributed to fruit differences in maturity stage (Ban et al., 2018) or cultivar (Šamec et
406 al., 2016), for instance. Also, different extraction methods and interferences by other
407 compounds could mark a difference on the values obtained (Azmir et al., 2013). TPC in
408 washed strawberries did not statistically change either with NaClO solution (83.56 ±
409 5.01 mg/ 100 g FW) or PA solutions at different concentrations or times. Similarly, no
410 significant differences were observed by Vandekinderen and Devlieghere (2017), in
411 carrots washed with 80 or 250 ppm PA. Contrarily, Ling et al. (2018) found a
412 significant increase in TPC when washing loquat fruit with a higher dose of PA (4000)
413 ppm for a longer time (6 min) with respect to the control.

414

4. Conclusions

The results of this study demonstrated the effectivity of peracetic acid treatments in reducing artificially inoculated *L. innocua* in both, strawberries and wash water, which would reduce cross-contamination in washing steps. Concerning native microbiota, mesophilic bacteria and molds and yeasts reduction values were lower than those observed with *L. innocua* but similar to those obtained with a standard treatment using sodium hypochlorite. PA in general did not affect the physicochemical and nutritional quality of strawberries.

Future experiments should be carried on in order to validate the efficacy of PA against the strawberry pathogens of concern, namely Salmonella, STEC, norovirus, or hepatitis A virus. Further investigations should be focused on the effect of PA during shelf life and subsequent processing of strawberries.

In this paper, the effect of PA has been studied against a pathogen surrogate and epiphytic microbiota, and the results have shown that PA washing seems to be a good alternative to chlorine disinfection for pathogens. However, results demonstrated that its efficacy against natural microbiota was lower than hypochlorite treatment, especially for the number of these microorganisms that remained in the wash water. To overcome this weakness, more studies should be carried on, including combination of PA with other physical technologies, such as ultrasounds or ultraviolet light, in order to promote a synergistic effect and increase shelf-life of strawberries washed with these procedures.

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Conflict of interests

The authors declare no conflict of interests.

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Figure 1. Experimental design.

See *Experimental design* attached as a PowerPoint document.

Here there is a preview:

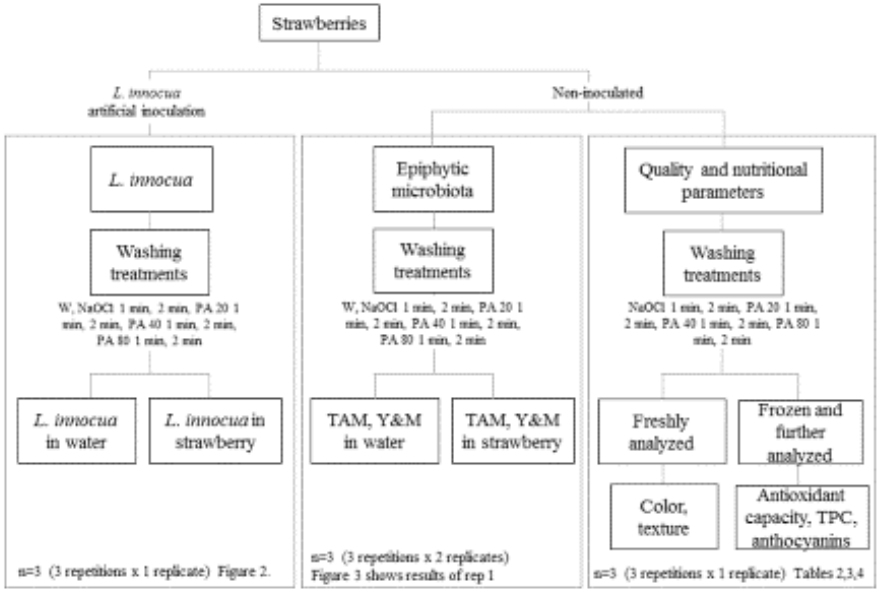

Figure 2. Population of *L. innocua* in strawberries (bars, log cfu/g) and in water (●, log cfu/mL). *L. innocua* values in strawberries are the mean of 3 reps ± standard deviation. *L. innocua* values in water were obtained from one sample.

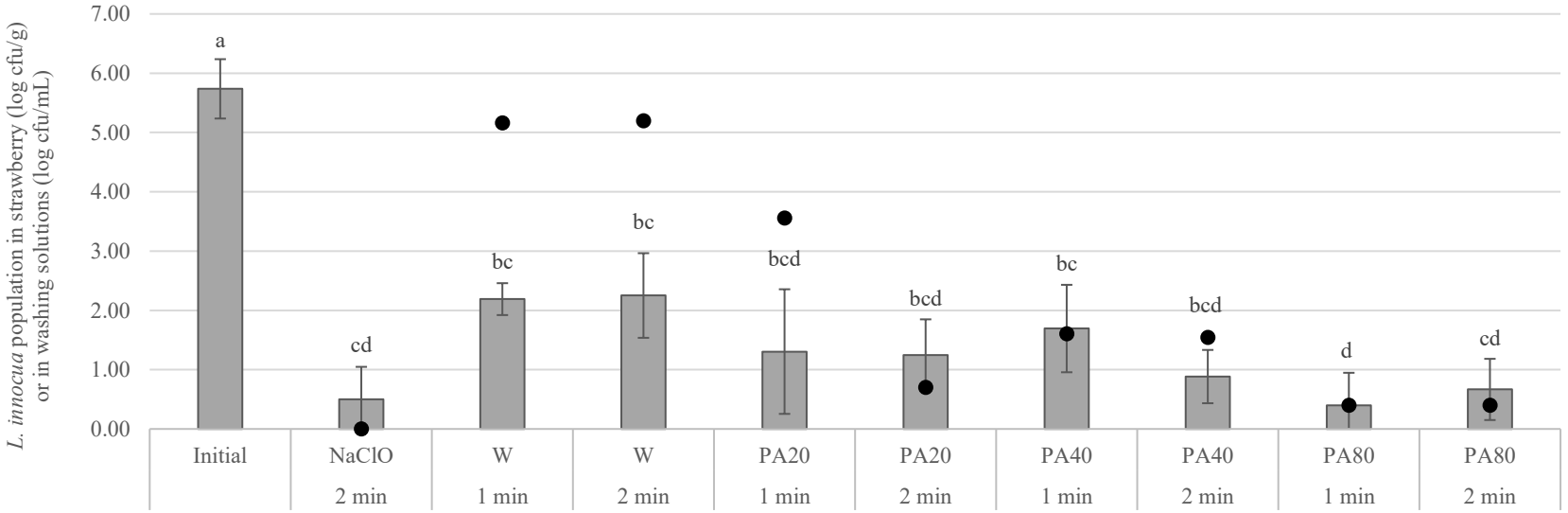

Figure 3. Population (log cfu/g strawberry) of total aerobic mesophylls (grey), or molds and yeasts (white) on strawberries. Values are the mean of 3 reps ± standard deviation. Different letters indicate significant statistically differences ($p < 0.05$) between treatments. Counts (log cfu/mL) of total aerobic mesophylls (●), or molds and yeasts (▲) in washing solutions. Values were obtained from one sample.

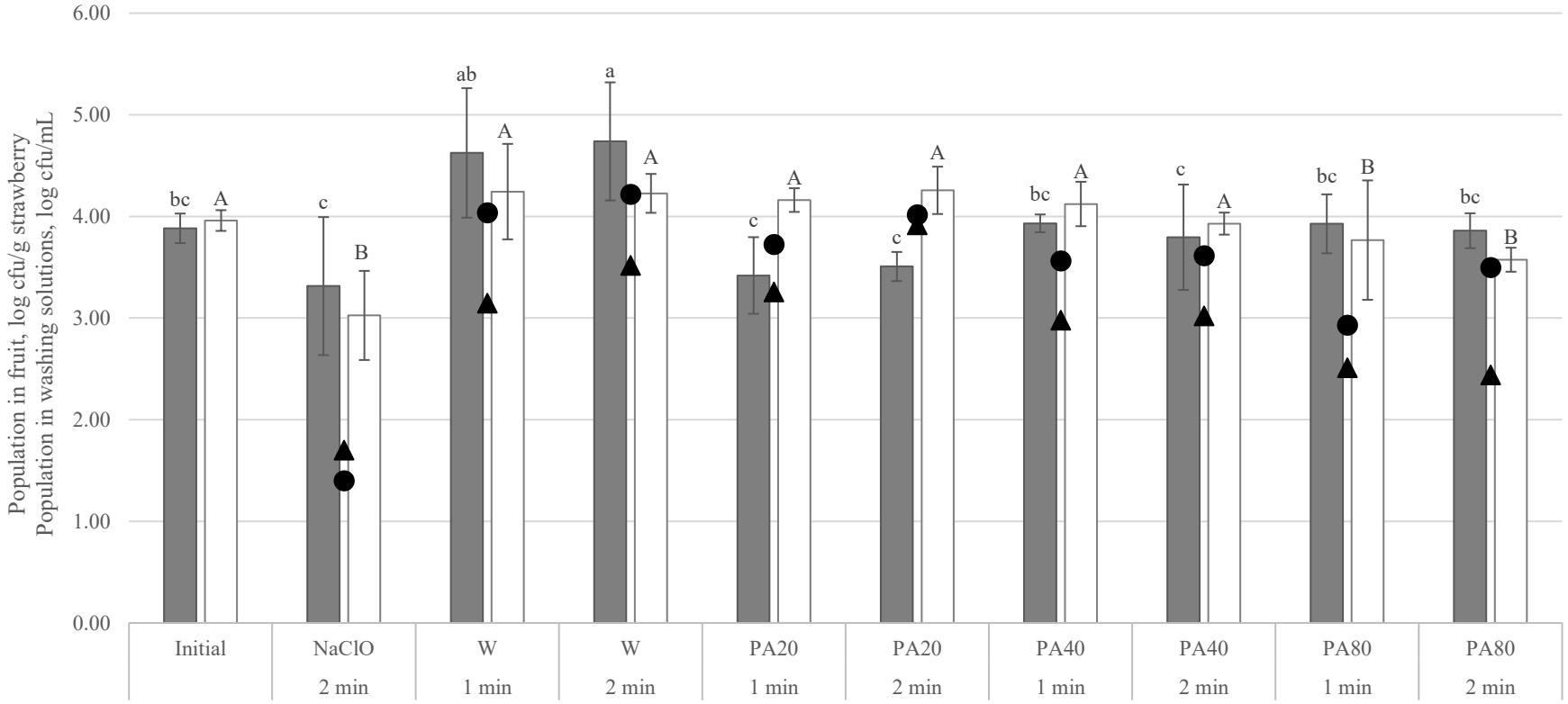

Table 1. Water parameters: pH, ORP, concentration of sanitizer. Values are the mean of the 3 repetitions ± standard deviation.

	Native microbiota experiment			***L. innocua* experiment**		
Treatment	**pH**	**ORP (mV)**	**Concentration of free chlorine or PA (mg/L)**	**pH**	**ORP (mV)**	**Concentration of free chlorine or PA (mg/L)**
Water	7.84 ± 0.15	279 ± 5	<0.01	7.84 ± 0.15	279 ± 5	<0,01
NaClO	6.5±0.0	894±14	138±6	6.65±0.07	881±3	173±4
PA20	5.5±0.1	460±3	26±2	6.54±0.09	464±5	23±1
PA40	4.5±0.0	493±5	46±2	4.83±0.01	506±7	46±6
PA80	4.11±0.02	515±3	76±1	4.17±0.03	523±6	87±8

650 **Table 2**. Values of pH, TSS and TA of strawberries for each washing treatment. Values are expressed as the mean of 3 reps ± standard deviation.
651 Different letters indicate statistically significant differences ($p < 0.05$) between treatments.

Treatment	Treatment time	pH	TSS (°B)	TA (g citric acid/ L juice)
Initial	-	3.39 ± 0.01 ^{bc}	5.9 ± 0.1 ^{a}	6.37 ± 0.30 ^{cd}
NaClO	2 min	3.36 ± 0.02 ^{bcd}	5.8 ± 0.1 ^{ab}	6.56 ± 0.22 ^{c}
PA20	1 min	3.40 ± 0.01 ^{b}	5.5 ± 0.1 ^{bc}	5.96 ± 0.02 ^{de}
PA20	2 min	3.47 ± 0.03 ^{a}	5.2 ± 0.1 ^{d}	6.19 ± 0.07 ^{e}
PA40	1 min	3.33 ± 0.01 ^{d}	5.1 ± 0.1 ^{d}	6.32 ± 0.06 ^{cd}
PA40	2 min	3.45 ± 0.03 ^{a}	5.5 ± 0.1 ^{bc}	5.5 ± 0.16 ^{e}
PA80	1 min	3.34 ± 0.01 ^{cd}	5.5 ± 0.0 ^{c}	7.06 ± 0.15 ^{b}
PA80	2 min	3.26±0.01 ^{e}	5.2 ± 0.1 ^{d}	8.54 ± 0.17 ^{a}

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653

654 **Table 3**. Values of CIE Lab coordinates, total color difference (TCD) and firmness measured by compression and pricking tests. Values are the
655 mean of 20 samples by 3 reps ± standard deviation. Different letters indicate statistically significant differences ($p < 0.05$) between treatments.

Treatment	Treatment time	Color				Firmness	
		L*	a*	b*	TCD	Compression (N)	Firmness (N)
Initial	-	40.04 ± 3.20 ^{ab}	32.69 ± 2.57 ^{ab}	26.14 ± 5.40 ^{abc}	-	30.67 ± 7.30 ^{a}	2.67 ± 1.06 ^{a}
NaClO	2 min	39.28 ± 3.66 ^{ab}	32.66 ± 1.56 ^{ab}	26.48 ± 5.62 ^{abc}	0.84 ± 1.13 ^{a}	36.51 ± 12.01 ^{ab}	2.23 ± 0.88 ^{a}
PA20	1 min	39.83 ± 3.10 ^{ab}	33.18 ± 1.80 ^{a}	28.49 ± 4.74 ^{ab}	2.41 ± 1.02 ^{a}	34.06 ± 10.02 ^{a}	2.46 ± 1.22 ^{a}
PA20	2 min	41.61 ± 3.63 ^{ab}	32.84 ± 1.78 ^{ab}	28.27 ± 5.34 ^{ab}	2.65 ± 0.90 ^{a}	31.37 ± 8.90 ^{a}	2.30 ± 0.65 ^{a}
PA40	1 min	41.17 ± 3.05 ^{ab}	32.66 ± 1.88 ^{abc}	27.31 ± 5.03 ^{abc}	1.61 ± 0.80 ^{a}	34.32 ± 9.92 ^{a}	2.82 ± 0.90 ^{a}
PA40	2 min	38.82 ± 2.80 ^{b}	31.73 ± 1.81 ^{abc}	22.86 ± 5.12 ^{bc}	3.63 ± 0.90 ^{a}	32.23 ± 6.90 ^{a}	2.31 ± 0.63 ^{a}
PA80	1 min	38.70 ± 3.83 ^{b}	30.55 ± 3.28 ^{bc}	22.11 ± 6.81 ^{c}	4.76 ± 1.70 ^{a}	38.09 ± 12.50 ^{ab}	2.98 ± 0.91 ^{a}
PA80	2 min	42.92 ± 6.66 ^{a}	29.74 ± 4.17 ^{c}	28.09 ± 6.13 ^{a}	4.85 ± 3.90 ^{a}	48.57 ± 12.28 ^{b}	3.59 ± 1.67 ^{a}

656

Table 4. Anthocyanin content, total phenolic content (TPC), and antioxidant activity (FRAP and DPPH· methods) of strawberries for each washing treatment. Values as expressed as a mean of 3 reps ± standard deviation. Different letters indicate significant statistically differences ($p < 0.05$) between treatments.

Treatment	Treatment time	Anthocyanin content (mg / 100 g FW)	TPC (mg / 100 g WF)	FRAP (mg AA / 100 g FW)	DPPH· (mg AA / 100 g FW)
Initial	-	1.90 ± 0.18 ^{cd}	83.01 ± 1.58 ^{a}	145.92 ± 8.09 ^{a}	138.04 ± 12.21 ^{a}
NaClO	2 min	1.91 ± 0.13 ^{cd}	83.56 ± 5.01 ^{a}	136.05 ± 21.75 ^{a}	133.35 ± 6.51 ^{a}
PA20	1 min	1.96 ± 0.06 ^{cd}	75.78 ± 2.79 ^{a}	123.90 ± 5.44 ^{a}	119.77 ± 1.16 ^{a}
PA20	2 min	2.42 ± 0.05 ^{ab}	75.84 ± 4.26 ^{a}	216.35 ± 6.63 ^{a}	118.26 ± 5.95 ^{a}
PA40	1 min	1.82 ± 0.11 ^{cd}	72.09 ± 0.25 ^{a}	133.81 ± 4.32 ^{a}	124.98 ± 8.99 ^{a}
PA40	2 min	2.08 ± 0.02 ^{bc}	77.89 ± 8.47 ^{a}	125.80 ± 6.70 ^{a}	112.86 ± 4.83 ^{a}
PA80	1 min	2.56 ± 0.01 ^{a}	82.89 ± 3.5 ^{a}	140.94 ± 1.75 ^{a}	129.6 ± 1.58 ^{a}
PA80	2 min	1.66 ± 0.05 ^{d}	77.77 ± 2.92 ^{a}	59.07 ± 6.19 ^{a}	116.88 ± 11.3 ^{a}