

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



Iolanda Nicolau-Lapeña^a, Maribel Abadias^{b,*,**}, Gloria Bobo^b, Ingrid Aguiló-Aguayo^b, Tomás Lafarga^b, Inmaculada Viñas^{a,*}

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

^b IRTA, XaRTA-Postharvest, Parc Científic i Tecnològic Agroalimentari de Lleida, Parc de Gardery, Edifici Fruitcentre, 25003, Lleida, Catalonia, Spain

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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 were observed 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.

1. Introduction

Strawberries are rich in vitamins (i.e. ascorbic acid) 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, and 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 fruit, a first approach for this purpose consists of a washing step in which fruit 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 Directive on Industrial Emissions (IPPC, 2007/0286(COD)), to reduce harmful industrial emissions across the EU (European Commission, 2007).

Subsequently, effective disinfection alternatives to chlorine have

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, molds and yeasts; TPTZ, 2,4,6-tris(2-pyridyl)-s-triazine

* Corresponding author.

** Corresponding author.

E-mail addresses: isabel.abadias@irta.cat (M. Abadias), ivinas@tecal.udl.cat (I. Viñas).

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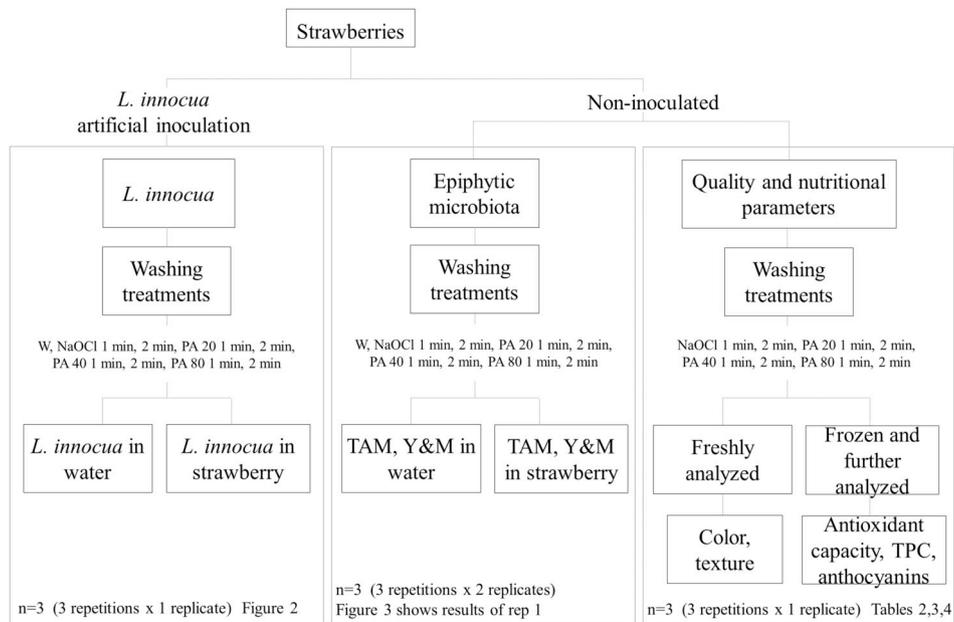


Fig. 1. Experimental design.

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

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

2. Materials and methods

2.1. Materials

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

Peracetic acid 15% was purchased from PanReac AppliChem (Barcelona, Spain). Tryptone 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 Ciocalteu'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 $9800 \times g$, 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 ± 1 °C for 48 h.

2.3. Strawberry inoculation with *Listeria innocua*

The day before the experiment, strawberries were inoculated with 50 μ L 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 (22 °C). 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 (Fig. 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 $-80\text{ }^{\circ}\text{C}$ 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 $37 \pm 1\text{ }^{\circ}\text{C}$ 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 $30 \pm 1\text{ }^{\circ}\text{C}$ for 3 days for TAM and at $25 \pm 1\text{ }^{\circ}\text{C}$ for 3–5 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 $20\text{ }^{\circ}\text{C}$ 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 points of each sample by using a CR-200 Minolta Chroma Meter (Minolta, INC., Tokyo, Japan). Color was expressed as CIE L^* , a^* , and b^* coordinates, using the D65 illuminant and a 10° observer angle. These values were used to calculate the total color difference (TCD) (Eq. (1)),

$$\text{TCD} = [(L_{*f} - L_{*i})^2 + (a_{*f} - a_{*i})^2 + (b_{*f} - b_{*i})^2]^{-\frac{1}{2}} \quad 1$$

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).

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

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

2.7. Biochemical analysis

2.7.1. Antioxidant activity

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

The **FRAP** reagent was prepared with a mixture of acetate buffer 0.3 M pH 2.6, TPTZ 40 mM in HCl and $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ 20 mM in distilled water in 10:1:1 (v:v:v) proportion. The determination was performed by adding 0.1 mL of the extract to 1.4 mL of FRAP reagent and incubating in a thermostatic bath at $37\text{ }^{\circ}\text{C}$ for 20 min in the dark. Absorbance was read at 593 nm using GENESYS[™] 10S UV-Vis spectrophotometer (Thermo Fisher Scientific, MA, USA).

DPPH radical was prepared daily by diluting a stock solution of DPPH 1 mM in 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[™] 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 follows. Briefly, $5.0 \pm 0.1\text{ g}$ 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 $4\text{ }^{\circ}\text{C}$, the mixture was centrifuged using a Sigma-3-18 KS centrifuge (Sigma Laborzentrifugen GmbH, Osterode am Harz, Germany) at $12,000 \times g$ for 15 min at $4\text{ }^{\circ}\text{C}$. Supernatant was then filtered and stored at $-80\text{ }^{\circ}\text{C}$ 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:

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

where A is absorbance at a certain wavelength. Anthocyanin content was expressed as mg of cyanidine-3-glucoside/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-Ciocalteu method. The test

was performed using 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™ 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 \pm 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 ORP values were lower than those observed in NaClO treatment, which ranged from 6.50 to 6.65 and 881–894 mV, respectively.

3.1.1. *L. innocua* experiments

The initial population of *L. innocua* on strawberries was 5.70 ± 0.50 log cfu/g (Fig. 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 melon, 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 (European Food Safety Authority, 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 Ryser (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 drawback when recommending PA for water reprocessing. On the other hand, the reported ability of PA to reduce biofilm formation would make this product a suitable sanitizer to add in the washing step (Barbosa et al., 2016). Furthermore, compared to other wash water disinfectants, PA has less potential of producing degradation by-products, which are easily dissolved in water and non-toxic, thus making this sanitizer a good alternative to chlorine (Banach et al., 2015a).

3.1.2. Native microbiota

Regarding epiphytic microbiota, remaining TAM population after NaClO washing was 3.32 ± 0.68 log cfu/g (Fig. 3). The PA effect and NaClO effect were comparable, as there were no significant differences between populations. Washing time, 1 or 2 min, did not significantly affect the results. Remaining TAM in strawberries after treatments with PA ranged from 3.42 ± 0.38 to 3.93 ± 0.29 log cfu/g when using PA80 or PA20 for 2 min, respectively. These counts were significantly lower than those observed after the washing with water for 2 min (W, control) with populations of 4.74 ± 0.58 log cfu/g, thus implying a sanitizing effect attributed to PA. Nevertheless, no significant differences were found on M&Y populations between the treatments and the control, so the cell decrease could be attributed to a physical removal

Table 1

Water parameters: pH, ORP, concentration of sanitizer. Values are the mean of the 3 repetitions \pm standard deviation.

| Treatment | Native microbiota experiment | | | <i>L. innocua</i> experiment | | |
|-----------|------------------------------|--------------|---|------------------------------|-------------|---|
| | 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 | 6.50 ± 0.0 | 894 ± 14 | 138 ± 6 | 6.65 ± 0.07 | 881 ± 3 | 173 ± 4 |
| PA20 | 5.50 ± 0.1 | 460 ± 3 | 26 ± 2 | 6.54 ± 0.09 | 464 ± 5 | 23 ± 1 |
| PA40 | 4.50 ± 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 |

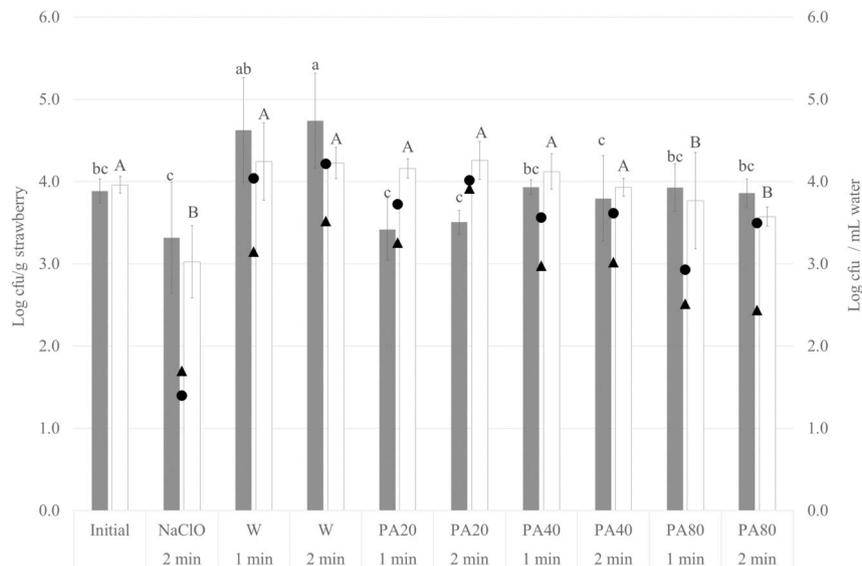


Fig. 2. Population of *L. innocua* in strawberries (bars, log cfu/g) and in water (●, log cfu/mL). *L. innocua* values in strawberries are the mean of 3 reps ± standard deviation. *L. innocua* values in water were obtained from one sample.

due to water forces on the surface (Castro-Ibáñez et al., 2017). Microbial contamination of washing solutions after washing changing between 2.5 and 4.2 log cfu/mL, except for NaOCl, in which both TAM and M&Y were reduced below 2 log cfu/mL. The experiment was repeated using a different batch of strawberries. Results showed that even if the initial population on strawberries was similar (3.96 ± 0.14 and 3.88 ± 0.14 log cfu/g strawberry), the effectiveness of some of the treatments was statistically different. Overall, reductions observed were lower in the second repetition than they were in the first assay. However, 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 (Baughner and Jaykus, 2016). However, dissimilar proportions of each genre and different loads can

be found between cultivars, batches or years and even among fruits (Baughner 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 (Banach et al., 2015a,b). 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.

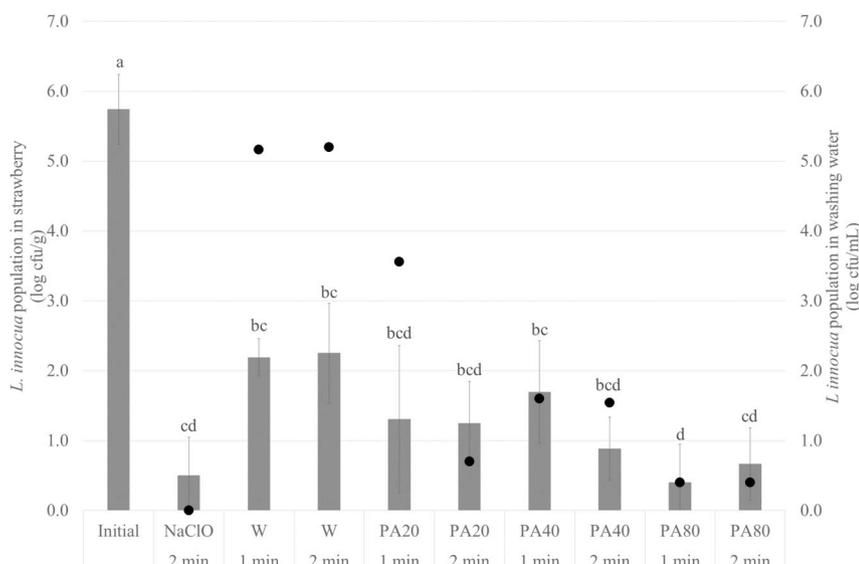


Fig. 3. Population (log cfu/g strawberry) of total aerobic mesophylls (grey), or molds and yeasts (white) on strawberries. Values are the mean of 3 reps ± standard deviation. Different letters indicate significant statistical differences ($p < 0.05$) between treatments. Counts (log cfu/mL) of total aerobic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

Table 2

Values of pH, TSS and TA of strawberries for each washing treatment. Values are expressed as the mean of 3 reps \pm standard deviation. 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 \pm 0.01 ^{bc} | 5.9 \pm 0.1 ^a | 6.37 \pm 0.30 ^{cd} |
| NaClO | 2 min | 3.36 \pm 0.02 ^{bcd} | 5.8 \pm 0.1 ^{ab} | 6.56 \pm 0.22 ^c |
| PA20 | 1 min | 3.40 \pm 0.01 ^b | 5.5 \pm 0.1 ^{bc} | 5.96 \pm 0.02 ^{de} |
| PA20 | 2 min | 3.47 \pm 0.03 ^a | 5.2 \pm 0.1 ^d | 6.19 \pm 0.07 ^e |
| PA40 | 1 min | 3.33 \pm 0.01 ^d | 5.1 \pm 0.1 ^d | 6.32 \pm 0.06 ^{cd} |
| PA40 | 2 min | 3.45 \pm 0.03 ^a | 5.5 \pm 0.1 ^{bc} | 5.5 \pm 0.16 ^e |
| PA80 | 1 min | 3.34 \pm 0.01 ^{cd} | 5.5 \pm 0.0 ^c | 7.06 \pm 0.15 ^b |
| PA80 | 2 min | 3.26 \pm 0.01 ^e | 5.2 \pm 0.1 ^d | 8.54 \pm 0.17 ^a |

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 $L^* 40.04 \pm 3.20$, $a^* 32.69 \pm 2.57$ and $b^* 26.14 \pm 5.40$ (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 established 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).

Table 3

Values of CIE Lab coordinates, total color difference (TCD) and firmness measured by compression and pricking tests. Values are the mean of 20 samples by 3 reps \pm 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 \pm 3.20 ^{ab} | 32.69 \pm 2.57 ^{ab} | 26.14 \pm 5.40 ^{abc} | – | 30.67 \pm 7.30 ^a | 2.67 \pm 1.06 ^a |
| NaClO | 2 min | 39.28 \pm 3.66 ^{ab} | 32.66 \pm 1.56 ^{ab} | 26.48 \pm 5.62 ^{abc} | 0.84 \pm 1.13 ^a | 36.51 \pm 12.01 ^{ab} | 2.23 \pm 0.88 ^a |
| PA20 | 1 min | 39.83 \pm 3.10 ^{ab} | 33.18 \pm 1.80 ^a | 28.49 \pm 4.74 ^{ab} | 2.41 \pm 1.02 ^a | 34.06 \pm 10.02 ^a | 2.46 \pm 1.22 ^a |
| PA20 | 2 min | 41.61 \pm 3.63 ^{ab} | 32.84 \pm 1.78 ^{ab} | 28.27 \pm 5.34 ^{ab} | 2.65 \pm 0.90 ^a | 31.37 \pm 8.90 ^a | 2.30 \pm 0.65 ^a |
| PA40 | 1 min | 41.17 \pm 3.05 ^{ab} | 32.66 \pm 1.88 ^{abc} | 27.31 \pm 5.03 ^{abc} | 1.61 \pm 0.80 ^a | 34.32 \pm 9.92 ^a | 2.82 \pm 0.90 ^a |
| PA40 | 2 min | 38.82 \pm 2.80 ^b | 31.73 \pm 1.81 ^{abc} | 22.86 \pm 5.12 ^{bc} | 3.63 \pm 0.90 ^a | 32.23 \pm 6.90 ^a | 2.31 \pm 0.63 ^a |
| PA80 | 1 min | 38.70 \pm 3.83 ^b | 30.55 \pm 3.28 ^{bc} | 22.11 \pm 6.81 ^c | 4.76 \pm 1.70 ^a | 38.09 \pm 12.50 ^{ab} | 2.98 \pm 0.91 ^a |
| PA80 | 2 min | 42.92 \pm 6.66 ^a | 29.74 \pm 4.17 ^c | 28.09 \pm 6.13 ^a | 4.85 \pm 3.90 ^a | 48.57 \pm 12.28 ^b | 3.59 \pm 1.67 ^a |

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/100 g 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 been found on how PA can affect anthocyanin content of strawberries. Anthocyanin values obtained with strawberries used in this study were lower than those found by Nowicka et al. (2019) and Van de Velde et al. (2014). This could be attributed to the use of different strawberry varieties or maturity stage, or by differences in the anthocyanin extraction method, as ultrasound was used to assist extraction in those studies, which makes anthocyanins more accessible as it helps to break cell walls and remove boundaries (Meyers et al., 2003).

3.3.3. Total phenolic content

Values of TPC are shown in Table 4. Initial phenolic content of strawberries was 83.01 ± 1.58 mg/100 g FW, which was in similar amounts to those reported in the literature (Perin et al., 2019; Yeoh and Ali, 2017). Even so, Avalos-Llano et al. (2018) found greater values of TPC in strawberry (550 mg/100 g FW). These dissimilarities could be attributed to fruit differences in maturity stage (Ban et al., 2018) or cultivar (Šamec et al., 2016), for instance. Also, different extraction methods and interferences by other compounds could mark a difference on the values obtained (Azmir et al., 2013). TPC in washed strawberries did not statistically change either with NaClO solution (83.56 ± 5.01

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 \pm 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 \pm 0.18 ^{cd} | 83.01 \pm 1.58 ^a | 145.92 \pm 8.09 ^a | 138.04 \pm 12.21 ^a |
| NaClO | 2 min | 1.91 \pm 0.13 ^{cd} | 83.56 \pm 5.01 ^a | 136.05 \pm 21.75 ^a | 133.35 \pm 6.51 ^a |
| PA20 | 1 min | 1.96 \pm 0.06 ^{cd} | 75.78 \pm 2.79 ^a | 123.90 \pm 5.44 ^a | 119.77 \pm 1.16 ^a |
| PA20 | 2 min | 2.42 \pm 0.05 ^{ab} | 75.84 \pm 4.26 ^a | 216.35 \pm 6.63 ^a | 118.26 \pm 5.95 ^a |
| PA40 | 1 min | 1.82 \pm 0.11 ^{cd} | 72.09 \pm 0.25 ^a | 133.81 \pm 4.32 ^a | 124.98 \pm 8.99 ^a |
| PA40 | 2 min | 2.08 \pm 0.02 ^{bc} | 77.89 \pm 8.47 ^a | 125.80 \pm 6.70 ^a | 112.86 \pm 4.83 ^a |
| PA80 | 1 min | 2.56 \pm 0.01 ^a | 82.89 \pm 3.5 ^a | 140.94 \pm 1.75 ^a | 129.6 \pm 1.58 ^a |
| PA80 | 2 min | 1.66 \pm 0.05 ^d | 77.77 \pm 2.92 ^a | 59.07 \pm 6.19 ^a | 116.88 \pm 11.3 ^a |

mg/100 g FW) or PA solutions at different concentrations or times. Similarly, no significant differences were observed by Vandekinderen and Devlieghere (2017), in carrots washed with 80 or 250 ppm PA. Contrarily, Ling et al. (2018) found a significant increase in TPC when washing loquat fruit with a higher dose of PA (4000) ppm for a longer time (6 min) with respect to the control.

4. Conclusions

Results demonstrated the effectivity of pPA 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 showed 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.

Conflicts of interest

The authors declare no conflict of interests.

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