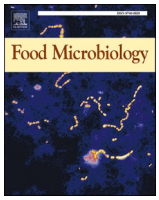
Food Microbiology 93 (2021) 103610

Contents lists available at ScienceDirect

Food Microbiology

journal homepage: www.elsevier.com/locate/fm



Neutral electrolyzed oxidizing water is effective for pre-harvest decontamination of fresh produce

Abiodun D. Ogunniyi [a,*,1], Catherine E. Dandie [a,1], Gianluca Brunetti [a], Barbara Drigo [a], Samuel Aleer [a], Barbara Hall [b], Sergio Ferro [c], Permal Deo [d], Henrietta Venter [d], Baden Myers [e], Erica Donner [a], Enzo Lombi [a]

[a] Future Industries Institute, University of South Australia, Mawson Lakes, South Australia, Australia
[b] Plant Health and Biosecurity, SARDI, Adelaide, South Australia, Australia
[c] Ecas4 Australia Pty Ltd, 8/1 London Road, Mile End South, South Australia, Australia
[d] Health and Biomedical Innovation, Clinical and Health Sciences, University of South Australia, Adelaide, South Australia, Australia
[e] Australian Flow Management Group & UniSA STEM, University of South Australia, Mawson Lakes, South Australia, Australia

ARTICLE INFO

Keywords:
Electrolyzed oxidizing water
Sodium hypochlorite
Pre-harvest sanitization
Lettuce
Baby spinach
Foodborne pathogens

ABSTRACT

Pre-harvest sanitization of irrigation water has potential for reducing pathogen contamination of fresh produce. We compared the sanitizing effects of irrigation water containing neutral electrolyzed oxidizing water (EOW) or sodium hypochlorite (NaClO) on pre-harvest lettuce and baby spinach leaves artificially contaminated with a mixture of *Escherichia coli*, *Salmonella* Enteritidis and *Listeria innocua* (~1×10^8 colony-forming units/mL each resuspended in water containing 100 mg/L dissolved organic carbon, simulating a splash-back scenario from contaminated soil/manure). The microbial load and leaf quality were assessed over 7 days, and post-harvest shelf life evaluated for 10 days. Irrigation with water containing EOW or NaClO at 50 mg/L free chlorine significantly reduced the inoculated bacterial load by $\geq$ 1.5 $\log_{10}$, whereas tap water irrigation reduced the inoculated bacterial load by an average of 0.5 $\log_{10}$, when compared with untreated leaves. There were no visual effects of EOW or tap water irrigation on baby spinach or lettuce leaf surfaces pre- or post-harvest, whereas there were obvious negative effects of NaClO irrigation on leaf appearance for both plants, including severe necrotic zones and yellowing/browning of leaves. Therefore, EOW could serve as a viable alternative to chemical-based sanitizers for pre-harvest disinfection of minimally processed vegetables.

1. Introduction

Microbial contamination of fresh, minimally processed foods such as lettuce, spinach, parsley and other leafy greens by opportunistic and human pathogens is of serious health and economic concern worldwide (Food and Agriculture Organization of the United Nations, 2018; World Health Organization, 2018). Microbiologically impacted irrigation water or splash-back from contaminated soil during irrigation can function as a conduit for pathogen transfer to fresh produce (Jongman and Korsten, 2018; Lee et al., 2019; Markland et al., 2017). Leafy greens are particularly vulnerable to irrigation-mediated contamination with opportunistic human pathogens because they have large surface areas, are often grown in close proximity to soil, are irrigated intensively, and are mostly consumed raw (De Keuckelaere et al., 2015). In their investigation of splash transfer of *Salmonella* to a range of field-grown produce, Lee and colleagues (Lee et al., 2019) demonstrated the potential for splash transfer as a route of pre-harvest contamination. For fresh produce, pre-harvest (i.e. irrigation water) and post-harvest (i.e. washing water) water sources have been identified as the main sources of contamination associated with illnesses (FSANZ, 2011). Indeed, investigations of recent outbreaks have focused on the quality of water used in produce processing, for instance in outbreaks associated with *Salmonella* spp. and *Listeria monocytogenes* in cantaloupes, pre-packed lettuce and baby spinach leaves (FSANZ, 2016; Zhu et al., 2017).

Abbreviations: FAC, free available chlorine; EOW, electrolyzed oxidizing water; SEM, standard error of the mean; DOC, dissolved organic carbon; OM, organic matter.

* Corresponding author. Future Industries Institute, Building X, Mawson Lakes Campus, Mawson Lakes Boulevard, Mawson Lakes, 5095, South Australia, Australia.
E-mail address: david.ogunniyi@unisa.edu.au (A.D. Ogunniyi).

[1] Co-first author.

https://doi.org/10.1016/j.fm.2020.103610
Received 26 March 2020; Received in revised form 28 July 2020; Accepted 29 July 2020
Available online 3 August 2020

Contaminated irrigation water has been implicated in outbreaks of verotoxigenic *E. coli* in lettuce in Sweden and Denmark (Ethelberg et al., 2010; Söderström et al., 2008) and in outbreaks of *Salmonella* in tomatoes and serrano pepper in the US (Greene et al., 2008; Hanning et al., 2009). Thus, the quality of irrigation water is paramount in ensuring the safety of edible produce and it is important to select an appropriate water source and/or disinfection method to reduce the potential for fresh produce contamination (De Keuckelaere et al., 2015; Mogren et al., 2018; Uyttendaele et al., 2015).

Disinfection processes for on-site treatment of microbiologically-impaired irrigation water commonly involve application of chemicals, such as chlorine, ozone, peroxyacetic acid or hydrogen peroxide (Dandie et al., 2019; Premier, 2013). While there is a substantial industry around post-harvest washing/processing of fresh produce (Premier, 2013), there are few studies on the application of such processes for pre-harvest sanitization of fresh produce. However, it is becoming increasingly evident that the best strategy to reduce fresh produce contamination is to prevent contamination occurring in the first instance, for various reasons. This is a significant part of the hurdle approach to reducing food safety risks (Mogren et al., 2018; Sigge et al., 2016), which emphasizes pre-harvest treatments and using clean irrigation water. This approach is further supported by the fact that removing/eliminating bacteria from leaf surfaces through post-harvest washing is not always possible once the bacteria are irreversibly attached (Yaron and Romling, 2014). For example, it has been demonstrated that even with several washes of a chlorine-based sanitizer, pathogens such as *E. coli* and *S.* Typhimurium were difficult to remove from produce surfaces once firmly attached (Banach et al., 2017). There is further evidence that bacteria can be internalized through various routes and thus are not generally susceptible to removal by post-harvest washing procedures (Alegbeleye et al., 2018).

Given the paucity of data on the pre-harvest sanitization of fresh produce, there is a great need to evaluate the efficacy of on-farm irrigation of fresh produce using a common chlorine based sanitizer, sodium hypochlorite (NaClO), and examine its effects on overall leaf quality and post-harvest shelf life. However, currently used chemical-based sanitizers such as NaClO have a number of drawbacks including efficacy, limited range of application and safety concerns (Dandie et al., 2019). It has recently been highlighted that growers need more alternatives for the treatment of irrigation water (Allende et al., 2018), therefore global efforts are focused on the development and testing of alternative sanitization methods that address these shortcomings, without compromising efficacy. Electrolyzed oxidizing water (EOW) is an alternative sanitization technology (Rahman et al., 2016; Veasey and Muriana, 2016) mainly used in the healthcare industry to control *Legionella* in water supplies (Ferro, 2015; Migliarina and Ferro, 2014). EOW has also gained attention in the food industry (Hricova et al., 2008; Khazandi et al., 2017; Rahman et al., 2016; Veasey and Muriana, 2016) and has been used successfully for sanitizing household utensils such as plastic and wooden kitchen cutting boards (Deza et al., 2007). Of the various types of EOW, the pH-neutral EOW is considered the most promising as it contains predominantly HOCl, which is more effective than ClO^- in NaClO for microbial cell wall penetration and oxidative attack while not presenting the corrosiveness of the acidic or slightly acidic forms (Rahman et al., 2016; Veasey and Muriana, 2016). In a recent study, we demonstrated that EOW was as effective or better than two other chemical based sanitizers (NaClO and ClO_2) for pathogen reduction in contaminated water, and that its efficacy was not affected under a range of pH or buffer conditions (Ogunniyi et al., 2019). In that study, the efficacy of EOW was superior to that of NaClO and ClO_2, being bactericidal at 20 mg/L of free available chlorine (FAC) in the presence of high organic matter content (100 mg/L), while NaClO was only bactericidal at 50 mg/L of FAC, and ClO_2 showed no effectiveness at the highest concentration used (equivalent to 50 mg/L of FAC).

In this study, we compared the sanitizing effects of irrigation water containing either EOW or NaClO on pre-harvest lettuce and baby spinach leaves artificially contaminated with a mixture of *E. coli*, *S.* Enteritidis and *Listeria innocua*. *L. innocua* is routinely used as a proxy of *L. monocytogenes*, a pathogen of fresh produce, since it displays similar behavior; the main advantage is that it does not require Biosafety level 2 containment (Rasch, 2004). We created a worst-case scenario of splash-back of contaminated soil/manure by preparing a high-concentration bacterial inoculant in a manure suspension and then manually spraying this onto the plants. We hypothesized that the EOW would be at least as effective, if not more effective, than NaClO in reducing concentrations of the target microorganisms, whilst also being less harmful to the crop at the equivalent concentration of free chlorine. Changes in the abundance of inoculated bacteria, total bacterial and fungal populations were monitored for 7 days after irrigation treatment. The overall quality and post-harvest shelf life of the vegetables were evaluated.

2. Materials and methods

2.1. Bacterial inocula

The bacterial strains used in this study were *Escherichia coli* (ATCC 25922), *Listeria innocua* 6a (ATCC 33090) and *Salmonella enterica* serovar Enteritidis 11RX (Ogunniyi et al., 1994; Ushiba et al., 1959). Glycerol stock cultures were maintained at −80 °C until use and were streaked onto Luria Bertani (LB) agar (Oxoid; Thermo Fisher Scientific Australia Pty Ltd., Scoresby, Australia) to obtain isolated colonies. Single colonies were streaked onto the following selective agar plates (Thermo Fisher Scientific) to confirm purity: eosin methylene blue (EMB) agar (PP2169) for *E. coli*; *Listeria* selective Oxford (OXF) agar (PP2141) for *L. innocua* 6a, and xylose lysine deoxycholate (XLD) agar (PP2004) for *S.* Enteritidis 11RX.

For experiments, single colonies from selective agar plates were suspended in LB broth and grown overnight at 37 °C with aeration at 150 rpm on a digital platform mixer (Ratek Instruments Pty Ltd., Boronia, Australia). Thereafter, bacteria were subcultured at a 1:10 dilution into fresh LB broth and incubated further at 180 rpm for 2–3 h until optical densities of $A_{600} = 1.0$ (for *E. coli* and *S.* Enteritidis 11RX) and $A_{600} = 0.5$ (for *L. innocua* 6a) were reached, equivalent to approx. 1×10^9 colony-forming units (CFU)/mL for *E. coli* and *S.* Enteritidis 11RX and to approx. 5×10^8 CFU/mL for *L. innocua* 6a. Bacteria were then harvested and washed in autoclave-sterilized Milli-Q water (PURELAB Classic, ELGA; Thermo Fisher Scientific) to remove residual culture medium and resuspended in the filtered manure mix as described below.

2.2. Manure mixture preparation

Blended cow manure (Fine Farm Organics, Charlton, Australia) was obtained from a local distributor and was γ-sterilized at Steritech (Melbourne, Australia). The sterilized manure was subsequently dried in an oven at 37 °C and ground to a fine powder using an analytical mill (IKA, Selangor, Malaysia), resuspended to the equivalent of 10 g/L in sterile Milli-Q water (pH 7.0) and then passed through a 0.45 μm filter to remove particulate matter (Bolan et al., 2011). The dissolved organic carbon (DOC) content in the manure suspension was measured on a Shimadzu TOC-L total organic carbon analyzer (Shimadzu Australasia, Rydalmere, Australia). A mixed suspension of *E. coli*, *L. innocua* 6a and *S.* Enteritidis 11RX organisms pre-washed in sterile Milli-Q water was added to the manure suspension to a final concentration of approx. 1×10^8 CFU/mL of each strain.

2.3. Plant growth conditions

Cos lettuce (*Lactuca sativa* L. var. *longifolia*) and baby spinach (*Spinacia oleracea* L.) seedlings were purchased from commercial plant suppliers in the local area (Virginia Nursery, Virginia, SA, Australia; Bunnings Pty Ltd., Parafield, SA, Australia). Rockwool was purchased

from Complete Hydroponics (Salisbury East, SA, Australia). Plants were grown in a hydroponic system of troughs, with 20 L of nutrient solution supplied to each set of three troughs (containing 15 plants). The nutrient solution was a dilute Hoagland's solution (1/2 or 1/4 strength for lettuce and spinach, respectively) prepared from stock solutions of concentrated nutrients (Hoagland and Arnon, 1950). Individual seedlings were re-potted into pre-wetted rockwool cubes in plastic mesh pots. The pots were then placed into the trough system and a pump (Aqua One Maxi 104, Kong's Australia Pty Ltd., Ingleburn, Australia) submerged in a storage tank was used to circulate the nutrient solution through the troughs, wetting the rockwool to allow access to the nutrients for growth. The nutrient solution was continually recirculated and replaced weekly. The plants were grown in a greenhouse with temperature settings of 22 °C/15 °C day/night 12 h:12 h for lettuce and 24 °C/19 °C day/night 12 h:12 h for spinach. No supplementary lighting was supplied.

2.4. Reagents, solutions and instruments

Electrolyzed oxidizing water (EOW) was kindly provided by Ecas4 Australia at 300–350 mg/L of FAC. Sodium hypochlorite (NaClO) was obtained as a 12.5% solution (CAS no. 7681-52-9) from Chemwell Pty. Ltd., Melbourne, Australia. The amount of FAC in EOW and NaClO was measured using a free chlorine and chlorine ultra-high range portable photometer (HI 96771C; Hanna Instruments Australia, Keysborough, Australia) according to the manufacturer's instructions. The pH and oxidation-reduction potential (ORP) of EOW, NaClO and tap water were measured using a Eutech PC700 m (John Morris Scientific, Wayville, Australia) with separate pH and ORP probes, respectively.

2.5. Plant inoculation experiments and bacterial recovery from plant leaves

2.5.1. Plant inoculation

Plant seedlings were grown for 7 days (for cos lettuce) or 14 days (for baby spinach) prior to application of the bacterial inocula. The bacteria/manure mix was applied manually using a 500-mL spray bottle. Approximately 10 mL (measured) of the mixed bacterial suspension (equivalent to approx. 1×10^9 CFU total for each strain) was applied to each plant. Plants were allowed to air-dry for 2–3 h after application of the bacterial inocula in a manner similar to that described by others (Jacob and Melotto, 2020; Van der Linden et al., 2014).

2.5.2. Plant washing

Solutions for the washing procedure were freshly prepared at the time of application. EOW (~350 mg/L FAC) and NaClO (12,500 mg/L FAC) were diluted to the appropriate concentration with tap water (0.16 mg/L FAC) and the FAC content of the diluted solutions was tested as described above; pH and ORP were also recorded for each trial. The pH and ORP of the test solutions were as follows: EOW pH 6.8, ORP 855 mV; NaClO pH 10.3, ORP 616 mV. Sixty-liter drums were filled with the diluted solutions and tap water was used as the control. A sprinkler system was flushed with the test solutions prior to use on the plants. Plants were sprinkler irrigated from an overhead sprinkler system with approx. 25 L of solution over a period of 10 min (equivalent to a 6 mm irrigation event). Plants were left to dry for 1–2 h after the washing procedure and prior to sampling.

2.5.3. Leaf harvesting and bacterial enumeration

Leaves were harvested from growing plants at days 0, 3 and 7 after application of the inocula and the washing procedure ($n = 5$ per treatment per time point). Leaves were cut at the base of the stem and placed into sterile stomacher bags (Thermo Fisher Scientific); the wet weight of plant material was recorded. Sterile peptone water (0.1%; Thermo Fisher Scientific) was added (50 or 100 mL) and the leaves were processed in a Seward BA6021 Stomacher (Seward Limited, Worthing, UK) for 1 min to extract and wash intact microbes into solution. The bacterial suspension was then serially diluted in sterile peptone water and surviving bacterial colonies were enumerated on selective media as described above; total bacterial counts were also enumerated on plate count agar (PCA; PP2145, Thermo Fisher Scientific). Agar plates were incubated at 37 °C for 24–36 h. Bacterial counts were reported as CFU/g wet weight plant material.

2.5.4. Sensory evaluation of lettuce and spinach leaves

A post-harvest quality rating scheme for cos lettuce and baby spinach leaves was used for post-harvest quality assessment. For each treatment, five individual leaves were packed in separate sealable plastic bags. All bags with leaves were stored in a container with ice or ice packs at 4 °C for 10 days. Photographs of the leaves were taken on days 0 and 10 post sampling and the samples were independently assessed for post-harvest quality by five trained sensory panelists. For sensory evaluation, a previously optimized shelf-life quality rating scheme was used (Table 1).

2.5.5. DNA extraction, quantitative PCR and microbial ecology analysis

In order to determine the overall microbial composition of leaves, 15 mL aliquots from the processed (homogenized) samples from Section 2.5.3 ($n = 5$) of each treatment at days 0 and 3 were centrifuged at 4,000×*g* for 7 min, the supernatant was decanted and DNA was extracted from the pellet using the DNeasy PowerSoil® kit (QIAGEN Cat No 12888-100). DNA was eluted in 100 μL of RNAse and DNAse free water and the amount of DNA extracted from each sample was determined on a DS-11 Series spectrophotometer (DeNovix Inc, Wilmington, DE, USA). Quantitative PCR (qPCR) was performed in a LightCycler®480 II instrument (Roche Diagnostics GmbH) using gene-specific primers and associated cycling parameters (*cdsA* for *L. innocua*, 16S rRNA gene for total bacteria and internal transcribed spacer (ITS) region for fungi). The numbers of copies of the qPCR standards were calculated by assuming average molecular masses of 340 Da for 1 nucleotide of single-stranded DNA according to the following equation: copies per nanogram = (amount × NL)/(n × 10^9 x *mw*), where amount is the concentration of template in ng, NL is the Avogadro constant (6.022 × 10^{23} molecules per mol), n is the length of the strain in base pairs or nucleotides and *mw* is the average molecular weight per bp or nucleotide. The sample copy numbers were determined from the standard curve and subsequently standardized to copy numbers per gram of material. In all runs, standard curves and the amplification efficiency were calculated using the software manufactured by Roche. The efficiency of the different real-time PCRs ranged from 95 to 100%. The threshold of each single run was placed above any baseline activity and within the exponential increase phase. The cycle thresholds (C_T) were determined by a mathematical analysis of the resulting curve using the software manufactured by Roche. The C_T values of the no-template controls were always around 40, indicating no amplification and

Table 1
Quality rating scheme for cos lettuce and baby spinach leaves used in this study.

Criteria	Rating				
Yellowing	No yellowing	slight yellowing	just acceptable	unacceptable yellowing	very severe yellowing
Bruising	No bruising	slight bruising	just acceptable	unacceptable bruising	very severe bruising
Wilting	No wilting	slight wilting	just acceptable	unacceptable wilting	very severe wilting
Sliming	No Sliming	No rating	sliming evident	bad sliming	very severe sliming
Browning	No browning,	slight browning	just acceptable	unacceptable browning	very severe browning

internal positive control strains were around 25. Melting curves were determined for qPCR products to confirm product integrity and assess the presence of inhibitors, including the presence of primer-dimers. Among the different qPCR coefficients, attention was given to the R^2 coefficient which was used to analyze the standard curves obtained by linear regression analysis. For each run, the R^2 was ≥0.99 (values between zero and −1 a negative correlation and between zero and +1 for a positive correlation). Most of the samples, and all standards, were assessed in at least two different runs to confirm the reproducibility of the quantification and all the samples were free of PCR inhibitors. The primers used are listed in Table 2.

2.6. Statistical analyses

All figures were drawn and statistical analyses performed using Prism v8 (GraphPad Software, San Diego, CA, USA). A two-way analysis of variance (Tukey's multiple comparisons) was performed to evaluate statistical differences between mean bacterial counts, chlorophyll content or gene copy numbers between groups for each time point. A *p*-value of <0.05 was considered statistically significant.

3. Results

3.1. Determination of the effective concentrations of EOW and NaClO for contaminated lettuce leaves

In a preliminary experiment, we investigated the ability of EOW and NaClO solutions at an average value of 5.8 mg/L of FAC in the presence of 100 mg/L of DOC to significantly reduce the microbial load on contaminated cos lettuce leaves relative to tap water-treated plants. There were no statistically significant differences among the treatments at this concentration of FAC (Fig. 1). We attributed the overall lack of efficacy of EOW and NaClO at this concentration to the consumption/quenching of the FAC by the DOC in the organic matter (Ogunniyi et al., 2019). The leaf visual quality during the 7-days experimentation and shelf life post-harvest were not affected by the use of EOW or NaClO at the above FAC concentration (Figure S1A, B).

3.2. EOW at 20 mg/L and 50 mg/L FAC is effective in reducing microbial load on contaminated cos lettuce leaves in the presence of 100 mg/L DOC

Because of the quenching of EOW and NaClO at high DOC content, we examined EOW efficacy in reducing bacterial contamination on plant leaves at increased FAC concentrations of 20 and 50 mg/L. At both of these concentrations, EOW substantially reduced the microbial contamination of the lettuce leaves, with EOW at 50 mg/L of FAC showing the most pronounced and statistically significant difference in reducing *L. innocua* populations from the lettuce leaves by day 3 post-inoculation (>2 $\log_{10}$ reduction; Fig. 2).

Again, the overall leaf appearance during the 7-days evaluation and the shelf life of the leaves post-harvest were not affected by the use of EOW at either 20 mg/L or 50 mg/L of FAC (Figure S2A, B), leading to the choice of EOW at 50 mg/L of FAC for further experimentation.

Table 2
Primers used for quantification of surrogate bacterial pathogens.

Primer name	Primer sequence (5' → 3')	Product length	Source/reference
cdsA F	GTGGTTAGTTGTCGTGCCAGATAG	163	This work
cdsA R	AGCAGCAACCATACAAATTCCAAC		
16S F	TCCTACGGGAGGCAGCAGT	195	Modified from (
16S R	ATTACCGCGGCTGCTGG		Muyzer et al., 1993)
ITS F	AGAGCACTGTGCACTTAAG	208	Chiang et al. (2011)
ITS R	CATTATCACGGTAATTAGTG		

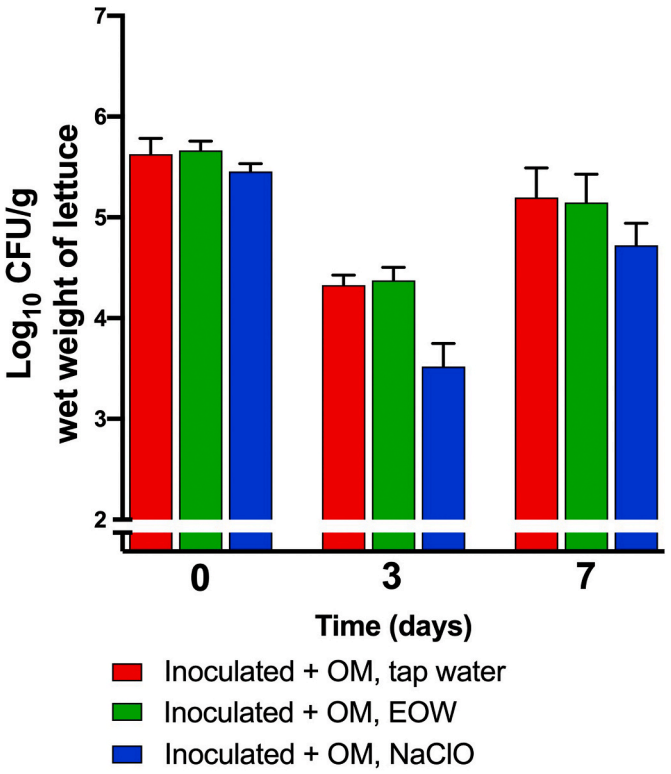


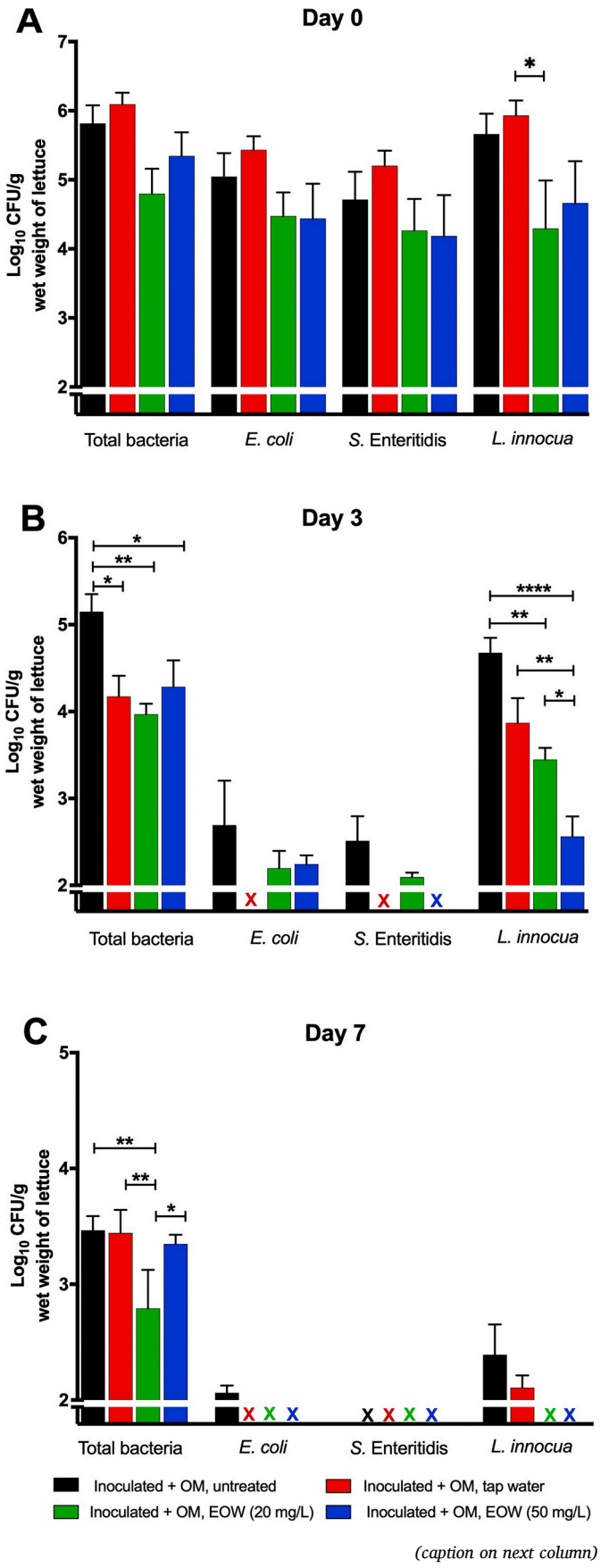
Fig. 1. Preliminary assessment of the efficacy of ~5.8 mg/L of free available chlorine for either electrolyzed oxidizing water (EOW) or sodium hypochlorite (NaClO) in comparison with tap water (control) in reducing total microbial load (on plate count agar) on contaminated cos lettuce leaves in the presence of organic matter (OM: 100 mg/L of dissolved organic carbon). CFU: colony forming units. Values presented are mean ± SEM ($n = 3$); horizontal segment shows the limit of detection (100 CFU).

3.3. EOW is more effective than NaClO at 50 mg/L FAC in reducing microbial load on contaminated cos lettuce leaves

We carried out a further assessment of 50 mg/L of FAC for either EOW or NaClO in reducing microbial load on contaminated cos lettuce leaves. In this experiment, both EOW and NaClO treatment led to statistically significant reductions in abundance of all microbial populations tested when compared with untreated plants (mean reductions of 1.2, 1.2, 1.0 and 1.3 $\log_{10}$ for total bacteria, *E. coli*, *S.* Enteritidis 11RX and *L. innocua*, respectively; $p < 0.001$). In addition, EOW treatment resulted in statistically significant reductions in *E. coli* (0.7 $\log_{10}$ reduction; $p < 0.05$) and *L. innocua* (0.8 $\log_{10}$ reduction; $p < 0.05$) abundance while NaClO treatment resulted in a statistically significant reduction in *L. innocua* (0.8 $\log_{10}$ reduction; $p < 0.05$) abundance when compared with tap water treatment at day 0 (Fig. 3). EOW treatment did not affect leaf quality during the lettuce growth period whereas the overall leaf quality after NaClO treatment deteriorated from day 3 onwards with severe necrotic zones, yellowing and browning of leaves (Figure S3A, B).

3.4. EOW and NaClO at 50 mg/L of FAC are both effective in reducing microbial load on contaminated baby spinach leaves

The experiment performed on lettuce leaves using 50 mg/L of FAC for either EOW or NaClO was repeated for baby spinach. In this experiment, EOW treatment resulted in statistically significant reductions in *E. coli* (1.5 $\log_{10}$ reduction; $p < 0.05$), *S.* Enteritidis 11RX (1.8 $\log_{10}$ reduction; $p < 0.01$) and *L. innocua* (1.5 $\log_{10}$ reduction; $p < 0.05$) abundance, while NaClO treatment resulted in statistically significant reductions in *E. coli* (1.5 $\log_{10}$ reduction; $p < 0.05$) and *S.* Enteritidis



(caption on next column)

Fig. 2. Assessment of the efficacy of 20 and 50 mg/L of free available chlorine for electrolyzed oxidizing water (EOW) in reducing microbial load on contaminated cos lettuce leaves in the presence of 100 mg/L of dissolved organic carbon. A: day 0; B: day 3; C: day 7 post-irrigation treatment. *E. coli*: *Escherichia coli*; *S.* Enteritidis: *Salmonella* Enteritidis 11RX; *L. innocua*: *Listeria innocua* 6a. CFU: colony-forming units; OM: 100 mg/L of dissolved organic carbon. Values presented are mean ± SEM ($n = 3$); horizontal segment shows the limit of detection (100 CFU); X in colour denotes no colonies detected. *$p < 0.05$; **$p < 0.01$; ****$p < 0.0001$; two-way analysis of variance (Tukey's multiple comparisons). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

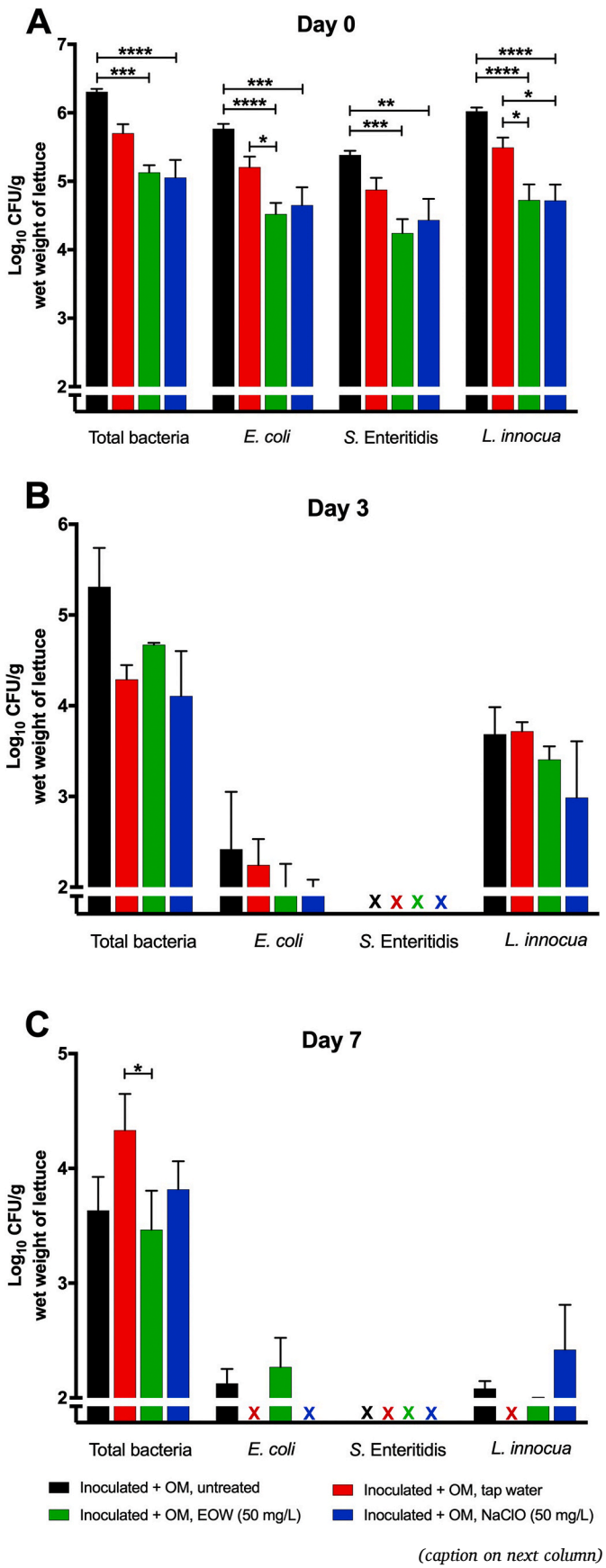
11RX (1.5 $\log_{10}$ reduction; $p < 0.05$) when compared with untreated plants at Day 0 (Fig. 4). EOW treatment also showed statistically significant reductions in *S.* Enteritidis 11RX (1.4 $\log_{10}$ reduction; $p < 0.05$) and *L. innocua* (1.4 $\log_{10}$ reduction; $p < 0.05$) abundance when compared with tap water treatment at day 0. The *L. innocua* numbers were higher for EOW treatment compared to NaClO treatment at day 3, but there was no statistically significant difference in numbers by day 7 between the treatment groups (Fig. 4). As observed for lettuce leaves, EOW treatment did not affect the quality of baby spinach leaves whereas the overall quality of the leaves treated with NaClO deteriorated from day 3 onwards (Figure S4A, B).

3.5. EOW treatment does not affect the post-harvest quality of lettuce and spinach leaves

To assess any post-harvest effects of irrigating lettuce and spinach plants with EOW or NaClO, photographs of five individual leaves packed in separate sealable bags were taken at days 0 and 10 post-harvest, and were independently assessed for post-harvest quality by five trained sensory panelists using a previously optimized shelf-life quality rating scheme. We found that there were no visual effects of EOW or tap water irrigation on baby spinach or lettuce leaf surfaces pre- or post-harvest, whereas there were obvious negative effects of NaClO irrigation on leaf appearance for both plants, including severe necrotic zones and yellowing/browning of leaves (Fig. 5).

3.6. EOW and NaClO significantly reduce bacterial contamination, but not the overall microbial load of leaves

In order to complement the results obtained on the effectiveness of EOW or NaClO at decontaminating or reducing the microbial contamination of lettuce and spinach leaves, the *cdsA* gene was used to determine the abundance of *L. innocua* populations, 16S rRNA was used for total bacterial load and ITS was used for total fungal load by quantitative PCR. There was a statistically significant reduction in gene copy numbers for the *L. innocua cdsA* gene from lettuce leaves treated with EOW (1.1 $\log_{10}$ reduction; $p < 0.05$) or NaClO (1.4 $\log_{10}$ reduction; $p < 0.05$) when compared with untreated leaves at day 0 (Fig. 6). There were statistically significant reductions in *cdsA* gene copy number for baby spinach leaves treated with EOW or NaClO when compared with tap water (0.7 $\log_{10}$ reduction, $p < 0.01$ and 1.2 $\log_{10}$ reduction, $p < 0.001$, respectively) or untreated leaves (0.7 $\log_{10}$ reduction, $p < 0.001$ and 1.2 $\log_{10}$ reduction, $p < 0.0001$, respectively) at this time point; no statistically significant differences were seen at day 3 for both leaves. (Fig. 6). Quantitative PCR was not carried out for day 7 as viable counts were very low across all the samples at this time point. Together, these results corroborate the reduction in the *L. innocua* viable counts obtained from both types of fresh produce. However, apart from a statistically significant reduction in the ITS gene abundance in the EOW-treated spinach leaves at day 0 (0.9 $\log_{10}$ reduction, $p < 0.05$), there were no statistically significant differences in the copy numbers for 16S rRNA and ITS genes. The lack of decrease in total abundance of bacteria and fungi on the leaf surface indicated that the EOW treatment did not result in substantial disruption of the resident leaf microbiota, but was effective at reducing



(caption on next column)

Fig. 3. Comparative assessment of 50 mg/L of free available chlorine for either electrolyzed oxidizing water (EOW) or sodium hypochlorite (NaClO) in reducing microbial load on contaminated cos lettuce leaves. A: day 0; B: day 3; C: day 7 post-irrigation treatment. *E. coli*: *Escherichia coli*; *S.* Enteritidis: *Salmonella* Enteritidis 11RX; *L. innocua*: *Listeria innocua* 6a. CFU: colony-forming units; OM: 100 mg/L of dissolved organic carbon. Values presented are mean ± SEM ($n = 5$); horizontal segment shows the limit of detection (100 CFU); X in colour denotes no colonies detected. $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$; $^{****}p < 0.0001$; two-way analysis of variance (Tukey's multiple comparisons). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

the abundance of newly-inoculated bacteria in the scenario tested here.

4. Discussion

Safe, effective and environmentally-friendly strategies for sanitization of fresh produce are being evaluated and promoted worldwide. Sanitization strategies should ideally target spoilage and foodborne pathogens without affecting the indigenous microbiome present on fresh produce, which acts as a "natural biological barrier" against colonization by spoilage organisms and pathogens (Andrews and Harris, 2000; Barth et al., 2009; Janisiewicz and Korsten, 2002). As such, it is crucial to avoid contamination of leaf surfaces in the first instance as post-harvest washing to remove bacteria from leaf surfaces is not always effective once the bacteria are firmly attached (Sigge et al., 2016; Yaron and Romling, 2014). Therefore, to maximize the quality of fresh produce, it is critical that good agricultural practices are implemented before, during and after harvest to maintain good soil and water quality and promote a balanced and functioning microbial ecosystem, as defined in the Codex General Principles of Food Hygiene (Codex Alimentarius Commission, 2003).

In this work, we compared the efficacy of EOW and NaClO in reducing artificial microbial contamination of pre-harvest cos lettuce and baby spinach plants grown under hydroponic conditions in a greenhouse. Lettuce and spinach are minimally processed ready-to-eat vegetables known to be susceptible to colonization by foodborne pathogens (Hackl et al., 2013). The plants were spray-inoculated in a manure suspension with a mixture of three model organisms (*E. coli*, *S.* Enteritidis and *L. innocua*) that are representatives or surrogates of important pathogenic bacteria of fresh produce. This simulated a worst-case scenario of soil/manure splash-back onto plant leaves with high contamination levels. The plants were then irrigated with tap water, EOW at 5.8, 20 and 50 mg/L FAC or NaClO at 5.8 and 50 mg/L FAC. The controlled greenhouse environment allowed us to assess the effect of the irrigation treatment without introducing any further confounding factors. The concentrations of these sanitizers were chosen based on our recent work, which showed that concentrations of EOW and NaClO at 20–50 mg/L FAC were effective in eliminating microbial contamination from contaminated water in the presence of very high DOC (Ogunniyi et al., 2019). Post-irrigation evaluation of the sanitizer efficacy at the highest examined rate of 50 mg/L FAC showed that treatment with EOW and NaClO resulted in significant reductions in inoculum survival at day 0 for both lettuce and spinach leaves. Furthermore, there were no visual effects of irrigation with EOW or tap water on the lettuce and spinach leaf surfaces; however, there were obvious negative effects of NaClO at that concentration on leaf appearance, with severe necrotic zones, yellowing and browning of the leaves appearing from day 3 post-irrigation. These findings clearly indicate the potential for EOW pre-harvest sanitization of fresh produce, even at free chlorine concentrations that would otherwise be detrimental to produce quality.

Although significant reductions in viable counts were observed for the irrigation water treatments applied, these did not reduce the concentrations of applied bacteria to below acceptable guideline levels for consumption [set at less than 3 cfu for *E. coli* per 25 g and no cfu for *Listeria* spp or *Salmonella* spp per 25 g of ready-to eat foods] (FSANZ,

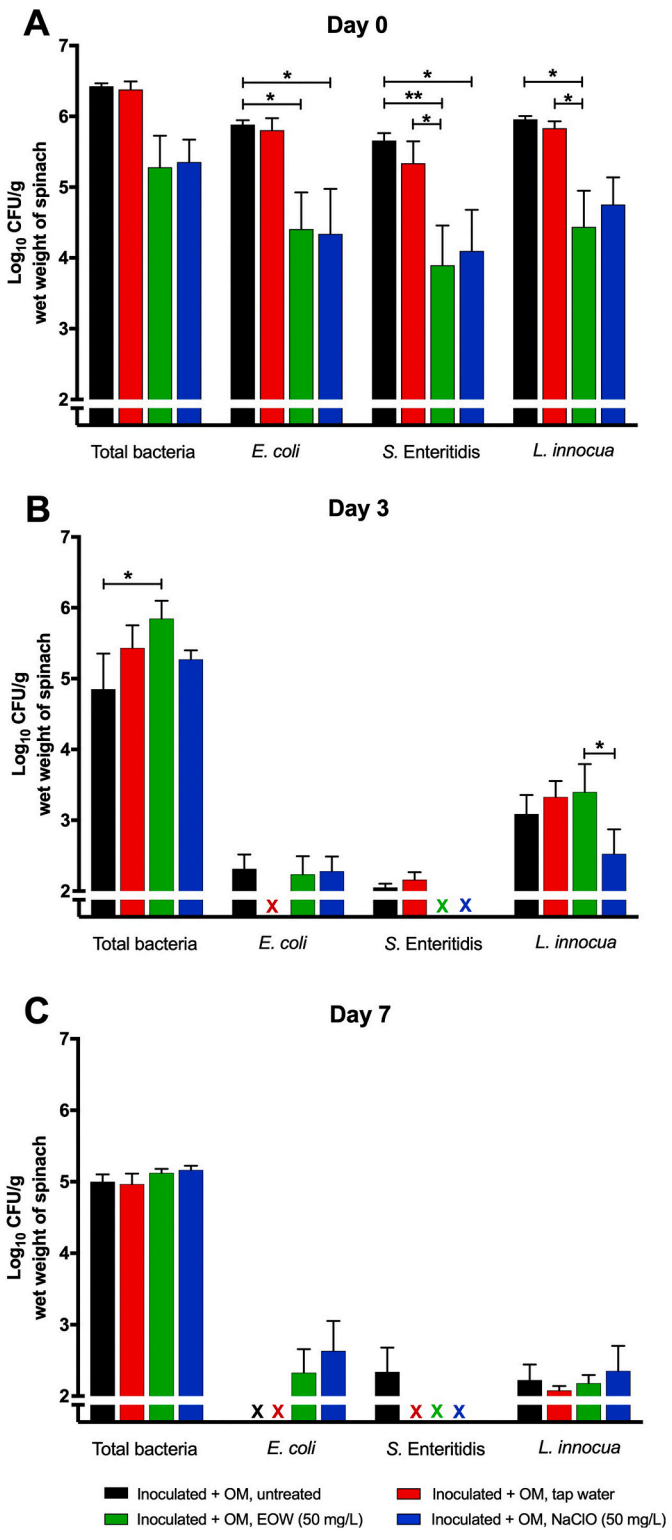


Fig. 4. Comparative assessment of 50 mg/L of free available chlorine for either electrolyzed oxidizing water (EOW) or sodium hypochlorite (NaClO) in reducing microbial load on contaminated baby spinach leaves. A: day 0; B: day 3; C: day 7 post-irrigation treatment. *E. coli*: *Escherichia coli*; *S.* Enteritidis: *Salmonella* Enteritidis 11RX; *L. innocua*: *Listeria innocua* 6a. CFU: colony-forming units; OM: 100 mg/L of dissolved organic carbon. Values presented are mean ± SEM ($n = 5$); horizontal segment shows the limit of detection (100 CFU); X in colour denotes no colonies detected. *$p < 0.05$; **$p < 0.01$; two-way analysis of variance (Tukey's multiple comparisons). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

2018). However, the very high concentrations of bacteria applied in this scenario (10^6 cfu/g wet weight leaf material) means that it would be extremely unlikely for any treatment to reduce counts by 4–6 $\log_{10}$ to comply with the food guideline requirements. This experiment was a worst case scenario to demonstrate the potential efficacy of the irrigation water treatment in reducing microbial counts on contaminated leaves, as part of a multiple hurdle approach to reducing the risk of pathogen contamination of leafy greens. Other components of this hurdle approach might include the use of withholding periods prior to harvest, post-harvest washing and other treatments, each of which might not on their own be sufficient to control foodborne pathogens, but which together significantly reduce the risk (Mogren et al., 2018).

The microbiome on fresh produce can act as a natural biological barrier against spoilage organisms and invading pathogens (Andrews and Harris, 2000; Barth et al., 2009; Janisiewicz and Korsten, 2002), and treatments should preferably not affect this barrier. The main contributing factors to changes in the microbial ecology in soil, vegetables and fruits after treatment include irrigation water quality, soil type, harvest season, harvest techniques, pre- and post-harvest sanitization practices, nature and relative abundance of resident microbiota in the rhizosphere, and treatment processes (Allende and Monaghan, 2015; Berg and Smalla, 2009; Cluff et al., 2014; Frenk et al., 2014). There are few published articles on changes to the microbial ecology of soil and foliar tissues after irrigation with treated irrigation water. Yin et al. (2019) showed that the transfer of indicator organisms from irrigation waters to spinach leaves was dependent on the type of water and growing season.

Chlorine-based sanitizers have been shown to reduce beneficial inhibitory microbes in lettuce and spinach (Johnston et al., 2009). However, a study using low residual ClO_2 concentrations (approx. 0.25 mg/L) to treat irrigation water throughout the growing period of baby spinach concluded that the phyllosphere bacterial community of the leaves was mainly influenced by the soil bacterial community (Truchado et al., 2018). The study also demonstrated that the ClO_2 treatment only caused changes in two bacterial families of the baby spinach and soil microbiota, without affecting the major phyla and classes, and our preliminary investigations support this finding. While treatment of lettuce and baby spinach leaves with EOW and NaClO significantly reduced the abundance of the bacteria inoculated onto the leaves, neither EOW nor NaClO significantly changed the overall abundance of microbial (bacterial and fungal) communities present on the leaves, except for a significant reduction in the ITS gene in the EOW-treated spinach leaves. Although the abundance did not generally change, it is possible that irrigation with treated water could change the microbial community composition, with potential effects on disease resistance, growth rate, post-harvest losses or other properties. The effect of EOW on the microbial ecology of ready-to-eat leafy vegetables requires further evaluation.

There are several other considerations in the application of EOW in the field, including potential effects on soil, cost and feasibility of large-scale application. The use of NaCl-based EOW has potential negative effects because of the addition of Na ions to soil, which could result in problems with sodicity and dispersive soils. The use of KCl could overcome some of these issues, given that K-based EOW is just as effective as Na-based EOW (Ogunniyi et al., 2019) and that K can be used as a plant nutrient. The cost and feasibility of application are issues that would have to be addressed at a local site scale.

5. Conclusions

We tested the efficacy of EOW and NaClO at 50 mg/L FAC as pre-harvest sanitizers of artificially-contaminated lettuce and spinach grown under hydroponic greenhouse conditions. While both sanitizers were effective in reducing microbial counts on contaminated leaves, NaClO had severe negative effects on leaf quality both pre- and post-harvest, whereas EOW showed no discernible negative effects on leaf quality throughout the experiment. This study provides essential

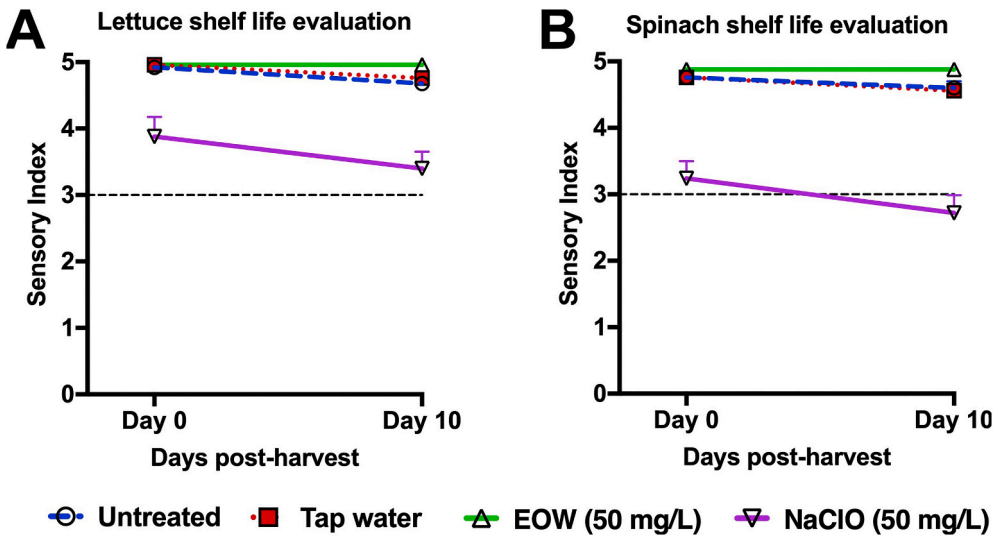


Fig. 5. Shelf life assessment of lettuce (A) and spinach (B) leaf quality after treatment with electrolyzed oxidizing water (EOW) or sodium hypochlorite (NaClO) at 50 mg/L of free available chlorine, compared with tap water-treated or untreated leaves. The results presented are mean (±SEM) scores of 5 sensory panelists based on the following sensory attributes: yellowing, bruising, wilting, sliming and browning. The sensory cut-off score was fixed at a SI of 3.

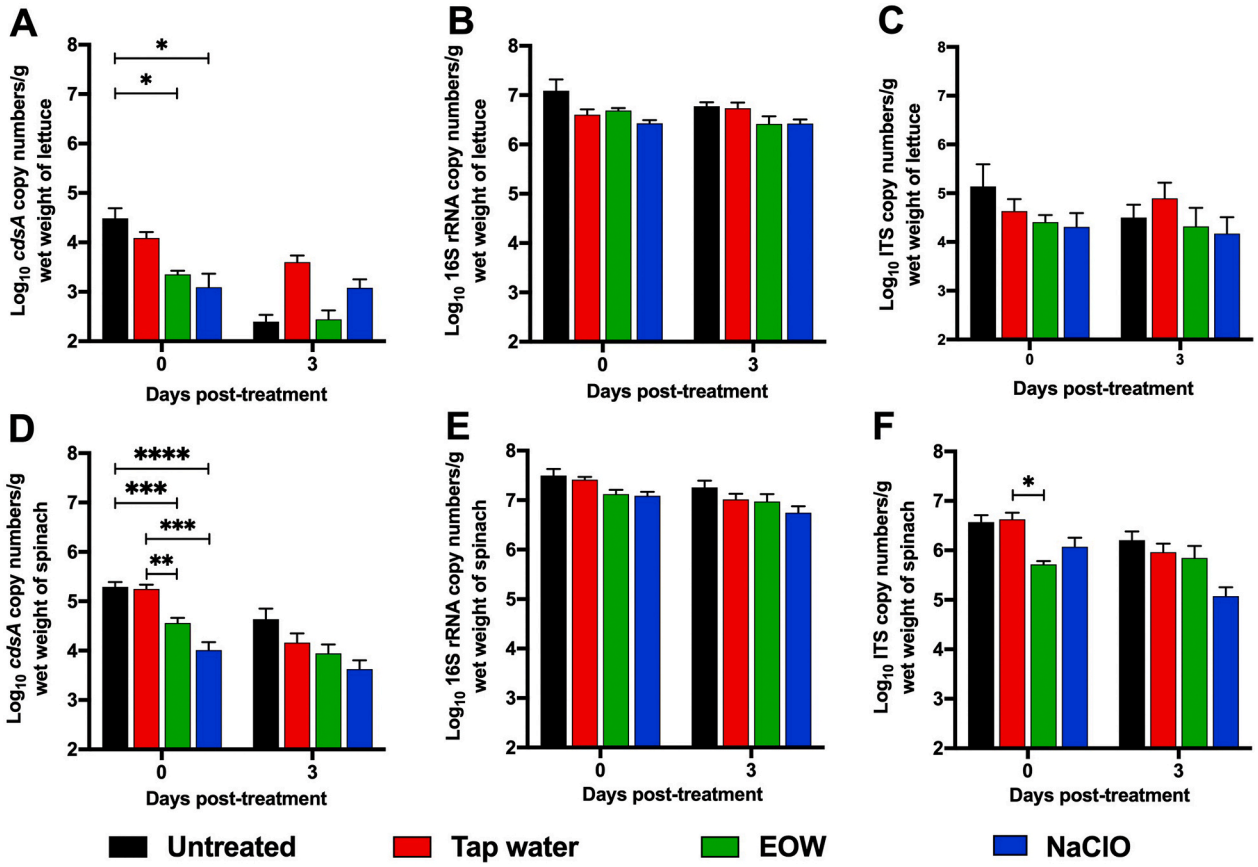


Fig. 6. Quantitative PCR analysis of the *Listeria innocua cdsA* (A, D), 16S rRNA (B, E) and internal transcribed spacer (ITS; C, F) genes from lettuce (A–C) and baby spinach (D–F) leaves treated with electrolyzed oxidizing water (EOW) or sodium hypochlorite (NaClO) at 50 mg/L of free available chlorine, compared with tap water-treated or untreated leaves. Values presented are mean ± SEM ($n = 5$) gene copy numbers at days 0 and 3 post treatment; *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$; ****$p < 0.0001$ two-way analysis of variance (Tukey's multiple comparisons).

information on the conditions required for efficient use of EOW for pre-harvest sanitization of fresh produce such as lettuce and spinach. However, the preliminary results obtained here on hydroponically-grown immature plants should be verified with large-scale field trials in agricultural soils. For a single application, FAC concentrations in EOW of up to 50 mg/L were not harmful to lettuce or spinach leaves under the conditions tested.

Funding information

This work was funded by Horticulture Innovation Grant VG15068 to University of South Australia, Australia.

Declaration of competing interest

Sergio Ferro is the technical manager of Ecas4 Australia.

Acknowledgements

The Authors would like to thank Dr Euan Smith (University of South Australia) for advice and discussions around this work. We also wish to acknowledge Judith Lukas, Amanda Kenyon and Lora Bowes (University of South Australia) for their participation in the shelf life assessments of the lettuce and spinach leaves.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fm.2020.103610.

References

Alegbeleye, O.O., Singleton, I., Sant'Ana, A.S., 2018. Sources and contamination routes of microbial pathogens to fresh produce during field cultivation: a review. Food Microbiol. 73, 177–208.

Allende, A., Datta, A.R., Smith, W.A., Adonis, R., MacKay, A., Adell, A.D., 2018. Implications of new legislation (US FSMA) and guidelines (EC) on the establishment of management systems for agricultural water. Food Microbiol. 75, 119–125.

Allende, A., Monaghan, J., 2015. Irrigation water quality for leafy crops: a perspective of risks and potential solutions. Int. J. Environ. Res. Publ. Health 12, 7457–7477.

Andrews, J.H., Harris, R.F., 2000. The ecology and biogeography of microorganisms on plant surfaces. Annu. Rev. Phytopathol. 38, 145–180.

Banach, J.L., van Bokhorst-van de Veen, H., van Overbeek, L.S., van der Zouwen, P.S., van der Fels-Klerx, H.J., Groot, M.N.N., 2017. The efficacy of chemical sanitizers on the reduction of *Salmonella* Typhimurium and *Escherichia coli* affected by bacterial cell history and water quality. Food Contr. 81, 137–146.

Barth, M., Hankinson, T.R., Zhuang, H., Breidt, F., 2009. Microbiological spoilage of fruits and vegetables. In: Sperber, W.H., Doyle, M.P. (Eds.), Compendium of the Microbiological Spoilage of Foods and Beverages, Food Microbiology and Food Safety. Springer Science+Business Media, LLC 2009.

Berg, G., Smalla, K., 2009. Plant species and soil type cooperatively shape the structure and function of microbial communities in the rhizosphere. FEMS Microbiol. Ecol. 68, 1–13.

Bolan, N.S., Adriano, D.C., Kunhikrishnan, A., James, T., McDowell, R., Senesi, N., 2011. Chapter One - dissolved organic matter: biogeochemistry, dynamics, and environmental significance in soils. In: Sparks, D.L. (Ed.), Advances in Agronomy. Academic Press 1–75.

Chiang, M.-C., Kuo, S.-C., Chen, Y.-C., Lee, Y.-T., Chen, T.-L., Fung, C.-P., 2011. Polymerase chain reaction assay for the detection of *Acinetobacter baumannii* in endotracheal aspirates from patients in the intensive care unit. J. Microbiol. Immunol. Infect. 44, 106–110.

Cluff, M.A., Hartsock, A., MacRae, J.D., Carter, K., Mouser, P.J., 2014. Temporal changes in microbial ecology and geochemistry in produced water from hydraulically fractured Marcellus shale gas wells. Environ. Sci. Technol. 48, 6508–6517.

Codex Alimentarius Commission, 2003. Codex General Principles of Food Hygiene; CAC/RCP 1-1969, pp. 1–31.

Dandie, C.E., Ogunniyi, A.D., Ferro, S., Hall, B., Drigo, B., Chow, C.W.K., Venter, H., Myers, B., Deo, P., Donner, E., Lombi, E., 2019. Disinfection options for irrigation water: reducing the risk of fresh produce contamination with human pathogens. Crit. Rev. Environ. Sci. Technol. 1–31.

De Keuckelaere, A., Jacxsens, L., Amoah, P., Medema, G., McClure, P., Jaykus, L.-A., Uyttendaele, M., 2015. Zero risk does not exist: lessons learned from microbial risk assessment related to use of water and safety of fresh produce. Compr. Rev. Food Sci. Food Saf. 14, 387–410.

Deza, M.A., Araujo, M., Garrido, M.J., 2007. Efficacy of neutral electrolyzed water to inactivate Escherichia coli, Listeria monocytogenes, Pseudomonas aeruginosa, and Staphylococcus aureus on plastic and wooden kitchen cutting boards. J. Food Protect. 70, 102–108.

Ethelberg, S., Lisby, M., Bottiger, B., Schultz, A.C., Villif, A., Jensen, T., Olsen, K.E., Scheutz, F., Kjelso, C., Muller, L., 2010. Outbreaks of gastroenteritis linked to lettuce, Denmark, January 2010. Euro Surveill. 15.

Food and Agriculture Organization of the United Nations, 2018. Food Loss and Food Waste.

Ferro, S., 2015. Electrochemical activated solutions. The Australian Hospital Engineer 38, 50–53.

Frenk, S., Hadar, Y., Minz, D., 2014. Resilience of soil bacterial community to irrigation with water of different qualities under Mediterranean climate. Environ. Microbiol. 16, 559–569.

FSANZ, 2011. Supporting Document 2: Review of Foodborne Illness Associated with Selected Ready-To-Eat Fresh Produce (December 2011). Proposal P1015. Primary Production & Processing Requirements for Horticulture. Food Standards Australia New Zealand. ACT, Australia.

FSANZ, 2016. Woolworths Loose Leaf Lettuce Product Recall. Food Standards Australia New Zealand. ACT, Australia.

FSANZ, 2018. Compendium of Microbiological Criteria for Food. Food Standards Australia New Zealand. ACT, Australia, Wellington, New Zealand.

Greene, S.K., Daly, E.R., Talbot, E.A., Demma, L.J., Holzbauer, S., Patel, N.J., Hill, T.A., Walderhaug, M.O., Hoekstra, R.M., Lynch, M.F., Painter, J.A., 2008. Recurrent multistate outbreak of *Salmonella* Newport associated with tomatoes from contaminated fields, 2005. Epidemiol. Infect. 136, 157–165.

Hackl, E., Hölzl, C., Konlechner, C., Sessitsch, A., 2013. Food of plant origin: production methods and microbiological hazards linked to food-borne disease. Reference: CFT/EFSA/BIOHAZ/2012/01 Lot 1 (Food of plant origin with high water content such as fruits, vegetables, juices and herbs). EFSA Supporting Publications 10, 402E.

Hanning, I.B., Nutt, J.D., Ricke, S.C., 2009. Salmonellosis outbreaks in the United States due to fresh produce: sources and potential intervention measures. Foodb. Pathog. Dis. 6, 635–648.

Hoagland, D.R., Arnon, D.I., 1950. The water-culture method for growing plants without soil. Circular. California Agricultural Experiment Station 347, 32.

Hricova, D., Stephan, R., Zweifel, C., 2008. Electrolyzed water and its application in the food industry. J. Food Protect. 71, 1934–1947.

Jacob, C., Melotto, M., 2020. Human pathogen colonization of lettuce dependent upon plant genotype and defense response activation. Front. Plant Sci. 10.

Janisiewicz, W.J., Korsten, L., 2002. Biological control of postharvest diseases of fruits. Annu. Rev. Phytopathol. 40, 411–441.

Johnston, M.A., Harrison, M.A., Morrow, R.A., 2009. Microbial antagonists of *Escherichia coli* O157:H7 on fresh-cut lettuce and spinach. J. Food Protect. 72, 1569–1575.

Jongman, M., Korsten, L., 2018. Irrigation water quality and microbial safety of leafy greens in different vegetable production systems: a review. Food Rev. Int. 34, 308–328.

Khazandi, M., Deo, P., Ferro, S., Venter, H., Pi, H., Crabb, S., Amorico, T., Ogunniyi, A.D., Trott, D.J., 2017. Efficacy evaluation of a new water sanitizer for increasing the shelf life of Southern Australian King George Whiting and Tasmanian Atlantic Salmon fillets. Food Microbiol. 68, 51–60.

Lee, D., Tertuliano, M., Harris, C., Vellidis, G., Levy, K., Coolong, T., 2019. *Salmonella* survival in soil and transfer onto produce via splash events. J. Food Protect. 82, 2023–2037.

Markland, S.M., Ingram, D., Kniel, K.E., Sharma, M., 2017. Water for Agriculture: the convergence of sustainability and safety. Microbiol. Spectr. 5.

Migliarina, F., Ferro, S., 2014. A modern approach to disinfection, as old as the evolution of vertebrates. Healthcare 2, 516–526.

Mogren, L., Windstam, S., Boqvist, S., Vågsholm, I., Söderqvist, K., Rosberg, A.K., Lindén, J., Mulaosmanovic, E., Karlsson, M., Uhlig, E., Håkansson, Å., Alsanius, B., 2018. The hurdle approach-a holistic concept for controlling food safety risks associated with pathogenic bacterial contamination of leafy green vegetables. A review. Front. Microbiol. 9.

Muyzer, G., de Waal, E.C., Uitterlinden, A.G., 1993. Profiling of complex microbial populations by denaturing gradient gel electrophoresis analysis of polymerase chain reaction-amplified genes coding for 16S rRNA. Appl. Environ. Microbiol. 59, 695.

Ogunniyi, A.D., Dandie, C.E., Ferro, S., Hall, B., Drigo, B., Brunetti, G., Venter, H., Myers, B., Deo, P., Donner, E., Lombi, E., 2019. Comparative antibacterial activities of neutral electrolyzed oxidizing water and other chlorine-based sanitizers. Sci. Rep. 9, 19955.

Ogunniyi, A.D., Manning, P.A., Kotlarski, I., 1994. A *Salmonella enteritidis* 11RX pilin induces strong T-lymphocyte responses. Infect. Immun. 62, 5376–5383.

Premier, R., 2013. Evaluation of vegetable washing chemicals, final report for horticulture Australia Ltd Project VG09086, pp. 1–57. ISBN 0 7341 3028 7.

Rahman, S.M.E., Khan, I., Oh, D.-H., 2016. Electrolyzed water as a novel sanitizer in the food industry: current trends and future perspectives. Compr. Rev. Food Sci. Food Saf. 15, 471–490.

Rasch, M., 2004. Experimental design and data collection. In: McKellar, R.C., Lu, X. (Eds.), Modeling Microbial Responses in Foods. CRC Press, Boca Raton.

Sigge, G.O., Olivier, F., Bester, C., Giddey, K.F., van rooyen, B., Kotze, M., Blom, N., Bredenhann, L., Britz, T.J., Lamprecht, C., 2016. Scoping study on different on-farm treatment options to reduce the high microbial contaminant loads of irrigation water to reduce the related food safety risk. In: Sigge, G.O., Lamprecht, C. (Eds.), Stellenbosch University, Department of Food Science.

Söderström, A., Österberg, P., Lindqvist, A., Jönsson, B., Lindberg, A., Blide Ulander, S., Welinder-Olsson, C., Löfdahl, S., Kaijser, B., De Jong, B., Kühlmann-Berenzon, S., Boqvist, S., Eriksson, E., Szanto, E., Andersson, S., Allestam, G., Hedenström, I., Ledet Muller, L., Andersson, Y., 2008. A large *Escherichia coli* O157 Outbreak in Sweden associated with locally produced lettuce. Foodborne Pathogens and Disease 5, 339–349.

Truchado, P., Gil, M.I., Suslow, T., Allende, A., 2018. Impact of chlorine dioxide disinfection of irrigation water on the epiphytic bacterial community of baby spinach and underlying soil. PloS One 13, e0199291.

Ushiba, D., Saito, K., Akiyama, T., Nakano, M., Sugiyama, T., Shirono, S., 1959. Studies on experimental typhoid: bacterial multiplication and host cell response after infection with *Salmonella enteritidis* in mice immunized with live and killed vaccines. Jpn. J. Microbiol. 3, 231–242.

Uyttendaele, M., Jaykus, L.-A., Amoah, P., Chiodini, A., Cunliffe, D., Jacxsens, L., Holvoet, K., Korsten, L., Lau, M., McClure, P., Medema, G., Sampers, I., Rao Jasti, P., 2015. Microbial hazards in irrigation water: standards, norms, and testing to manage use of water in fresh produce primary production. Compr. Rev. Food Sci. Food Saf. 14, 336–356.

Van der Linden, I., Cottyn, B., Uyttendaele, M., Vlaemynck, G., Maes, M., Heyndrickx, M., 2014. Evaluation of an attachment assay on lettuce leaves with temperature- and starvation-stressed Escherichia coli O157:H7 MB3885. J. Food Protect. 77, 549–557.

Veasey, S., Muriana, P.M., 2016. Evaluation of electrolytically-generated hypochlorous acid ('electrolyzed water') for sanitation of meat and meat-contact surfaces. Foods 5.

World Health Organization, 2018. Food Safety.

Yaron, S., Romling, U., 2014. Biofilm formation by enteric pathogens and its role in plant colonization and persistence. Microb Biotechnol 7, 496–516.

Yin, H.B., Gu, G., Nou, X., Patel, J., 2019. Comparative evaluation of irrigation waters on microbiological safety of spinach in field. J. Appl. Microbiol. 127, 1889–1900.

Zhu, Q., Gooneratne, R., Hussain, M.A., 2017. *Listeria monocytogenes* in fresh produce: outbreaks, prevalence and contamination levels. Foods 6.