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Inactivation kinetics of *Escherichia coli* O157:H7 and *Salmonella* Typhimurium on organic carrot (*Daucus carota* L.) treated with low concentration electrolyzed water combined with short-time heat treatment

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ABSTRACT

In the present study, the synergistic disinfection efficacy of low concentration electrolyzed water (LcEW) (free available chlorine, 4 mg/L) combined with brief heat enhancement was evaluated and the bactericidal mechanism was investigated by atomic force microscopy (AFM). The inactivation kinetics of *Escherichia coli* O157:H7 and *Salmonella* Typhimurium on organic carrot were fitted with Weibull model to evaluate the synergistic effects. LcEW is effective at inactivating *E. coli* O157:H7 and *S.* Typhimurium on organic carrots, and the efficacy is dependent on the temperature. The combined treatment with LcEW at 80 °C resulted in decimal reduction time (T_R) of 7.42 and 3.27 s for *E. coli* O157:H7 and *S.* Typhimurium, respectively. The reactive oxygen species generated from LcEW were responsible for the microbial inactivation. In addition, AFM observation of *E. coli* O157:H7 and *S.* Typhimurium revealed morphological alterations in the bacterial cell structure, which illustrated the damage of cell membrane injury and intracellular component leakage. Quality attributes of carrot treated with LcEW and short-time heating (70 °C, 1 min) were not significantly different from controls. Compared to the control group, the combined treatment exhibited significantly ($P < 0.05$) greater inhibition of naturally occurring microbiota on organic carrots during storage at 4 °C. Consequently, the application of LcEW combined with short-time heat improved safety of organic carrot, without negatively affecting the sensory properties, which can be explored by the organic industry.

1. Introduction

Organic product sales have increased at a healthy rate over the last decade worldwide and will continue to grow in the coming years (Chen et al., 2019; Willer & Lernoud, 2016; Yu & Yang, 2017). Organic farming policies restrict the usage of pesticides and chemosynthetic fertilizers, but recommend regular organic fertility input into the soil. However, the use of animal manure as fertiliser input on vegetable and fruit crops has raised safety concerns regarding an increased risk of pathogenic bacteria entering the food supply chain (Leifert, Ball, Volakakis, & Cooper, 2008; Liu, Tan, Yang, & Wang, 2017a; Zhang & Yang, 2017). In addition, outbreaks of *Escherichia coli* O157:H7 and *Salmonella* infection linked to organic produce contribute safety concerns (CDC, 2012; 2016), thereby it is urgent to develop more sanitizing strategies for organic produce.

Many chemical sanitizing agents are prohibited and the concentrations of sanitizers are also fairly restricted due to the strict regulations in the organic system plan. Electrolyzed water (EW) as a disinfectant has gained highly interest for organic produce due to its promising sanitizing efficacy and environmentally-friendly nature (Liu et al., 2017b, 2018; Zhang, Zhou, Chen, & Yang, 2017; Zhao, Zhang, & Yang, 2017; Zhao, Zhao, Phey, & Yang, 2019). EW with high free available chlorine (FAC) of 30–90 mg/L was usually used for reducing the population of many pathogens (Hao, Wu, Li, & Liu, 2017; Waters & Hung,

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2014). However, the potential application of strong acid EW in organic food is limited due to strict rules regarding chlorine residue. Referring to US Department of Agriculture's guidance on organic produce, chlorine containing sanitizers can be used, but the residual chlorine levels in the water after washing must not exceed limit level of 4 mg/L FAC, which is the maximum residual disinfectant limit under the Safe Drinking Water Act of the US Environmental Protection Agency (Sow et al., 2017; Zhang & Yang, 2017).

Consequently, low concentration EW (LcEW) could be one ideal potential alternative sanitizer for organic fresh produce sanitization because of its high bactericidal efficacy and compatibility with organic processing regulations. EW is an effective antimicrobial agent that is environmentally-friendly, relatively inexpensive, and easy to use (Han, Hung, & Wang, 2018). Several previous studies reported that LcEW (FAC: 2–6 mg/L) was a satisfactory antimicrobial agent against native microbiota and different pathogenic microorganisms including *S.* Enteritidis, *E. coli* O157:H7 and *Listeria monocytogenes* (Cao, Zhu, Shi, Wang, & Li, 2009; Park, Hung, & Chung, 2004; Rahman, Ding, & Oh, 2010) and demonstrated greater antimicrobial effects than NaClO (FAC, 100 mg/L) with efficacy similar to strong acid EW (FAC, 50 mg/L) (Ding, Rahman, & Oh, 2011). Combination of LcEW with other measures is also possible. The combination of a heat treatment with many sanitizers, such as acidic and alkaline EW, organic acid and hydrogen peroxide, achieves higher efficiency at reducing microbial load than treatment only (Liu et al., 2017a; Xuan et al., 2017). Carrot is a rich source of essential nutritional components and suitable for being used as a fresh-cut vegetable or salad (Vandekinderen et al., 2008). However, a number of outbreaks have been traced to fresh-cut vegetables (Ackers et al., 1998; Greene et al., 2008). In particular, several foodborne illness outbreaks have been associated with carrots (Gaynor et al., 2009; Kangas et al., 2008). To date, there is no formal report on inactivation kinetics of pathogens to assess the combined bactericidal efficacy of LcEW and short-time heat treatment on organic carrot.

The objectives of this study were to quantitatively describe the inactivation kinetics of *E. coli* O157:H7 and *S.* Typhimurium from inoculated shredded carrots and explore the ultrastructure changes of *E. coli* O157:H7 and *S.* Typhimurium at nanoscale; and investigate the growth of indigenous microflora throughout shelf life at 4 °C of treated samples and examine the effects color and texture to reveal any quality deterioration after treatment.

2. Materials and methods

2.1. Sample preparation

Certified organic carrots from Australia were bought from a local supermarket in Singapore and kept at 4 °C. Firstly, the carrot samples were washed for 1 min with cold tap water to remove undesired organic residues and cut into 2.5 g pieces (approximately 0.5 cm in height, 2.5 cm in diameter) with a sterile knife. These pieces were randomly assigned into untreated or experimental groups. Each sample consisted of four pieces (totally 10 g) and was in sterile bags before the experiment.

2.2. Bacterial strains

E. coli O157:H7 (EDL 933) and *Salmonella enterica* serovar Typhimurium ATCC 14028 were obtained from Food Science and Technology Programme, National University of Singapore. Frozen stock cultures (0.1 mL) from −80 °C were activated in 10 mL of sterile tryptic soy broth (TBS, Oxoid, Basingstoke, UK) at 37 °C for 18 h. Working cultures at stationary phase were used for experiment after two consecutive transfers.

2.3. Inoculation procedure

Dip inoculation was chosen to simulate such a process because immersion could be a suspected point that caused contamination in the food industry (Ruiz-Cruz, Acedo-Félix, Díaz-Cinco, Islas-Osuna, & González-Aguilar, 2007). All inocula were prepared by centrifugation (5000 *g*, 10 min), washed twice with saline and resuspended in peptone water to give bacterial suspensions with a cell density of about 10^7 CFU/mL. Shredded carrots were immersed in the inoculum suspension (sample: inoculum = 1:6 w/v) for 30 min. The samples were then drained and air-dried in a laminar flow biosafety cabinet for 1 h until treatment with the sanitizer solutions.

2.4. Sanitizing treatments

EW was collected from an EW unit (ROX-10WB3, Hoshizaki Electric Company, Aichi, Japan), in which diluted sodium chloride solution underwent electrolysis. FAC concentration was measured using a colorimetric test kit (model CN-66, Hash Co. Ames, IA, USA), and properly diluted with deionized water (DI) to achieve a final concentration of 4 mg/L (pH = 4.02 ± 0.6, ORP = 956.8 ± 55 mV). Solutions of LcEW and DI were store in glass containers and the temperature immediately controlled at 60, 70, 80 °C in a water bath. Inoculated and uninoculated sliced carrots (10 g each) were immersed into 200 mL of each solution for 10–180 s. Each experiment consists of two independent trials with triplicate samples. For determination of shelf life, control and uninoculated samples after treatment were packaged using low-density polyethylene Ziploc bags and stored at 4 °C for up to 7 days and subsamples were analyzed at 2-day intervals.

2.5. Microbiological analysis

Carrot samples from treatment procedures were aseptically collected in a sterile stomacher bag containing 90 mL of 0.1% peptone water and homogenized for 180 s using a stomacher (Masticator Stomacher, IUL Instruments, Germany). The aliquot was serially diluted and plated onto Sorbitol-MacConkey agar (SMAC, Oxoid, UK), Xylose lysine deoxycholate (XLD, Oxoid, UK) agar, plate count agar (PCA, Oxoid, UK), violet red bile glucose agar (VRBGA, Oxoid, UK), and potato dextrose agar (PDA, Oxoid, UK) to enumerate *E. coli* O157:H7, *S.* Typhimurium, aerobic mesophilic bacteria (AMB), *Enterobacteriaceae*, and yeast and mould counts (YMC), respectively. All other agar plates were incubated at 37 °C for 24 h. The plates for YMC were incubated at 25 °C for 4–5 days.

2.6. Inactivation kinetics

Log surviving fractions were fitted to the Weibull model using equation Eq. (1) (Vaid, Linton, & Morgan, 2010)

$$\log\left(\frac{N}{N_0}\right) = -\frac{1}{2.303}\left(\frac{t}{\alpha}\right)^{\beta} \quad (1)$$

where N and N_0 are the survival population at time t (CFU/g) and the initial population (CFU/g), respectively. t is the treatment time (s), α is a coefficient in the Weibull distribution, called scale factor, and β is the shape parameter. The value of β relates to the concavity of the survival curve, while $\beta < 1$ and $\beta > 1$ reveals concave-upward and concave-downward survival curves, respectively. OriginPro 2015 was applied for computing Weibull parameters, with which T_d (time required for d log cycle reduction) (Luo and Oh, 2016) was calculated (Eq. (2)):

$$T_d = \alpha\left(-\ln(10^{-d})^{\frac{1}{\beta}}\right) \quad (2)$$

Thus, reliable life T_R could be used for assessing the time required for microorganism reduction by a factor of 10 (similar as D value),

which indicated 90% reliability of a population (Eq. (3)):

$$T_R = \alpha\left((2.303)^{\frac{1}{\beta}}\right) \tag{3}$$

where T_R is the time needed to reduce 90% population of the pathogen, α is the scale factor, and β is the shape parameter.

The fitting wellness of the Weibull model was judged by root mean squared error (RMSE) and adjusted R-Square (Adj-R^2). Adj-R^2 was calculated by OriginPro 2015 (OriginLab, Northampton, MA, USA), while RMSE was calculated from the equation (Luo and Oh 2016):

$$\text{RMSE} = \sqrt{\sum_{i=1}^{n} \frac{(y_{obs} - y_{pre})^2}{N_t} - N_p} \tag{4}$$

where y_{obs} represented the experimental value, y_{pre} represents the model predicted data, N_t is the number of data, N_p is the number of parameters, and n is the number of observations (Luo & Oh, 2016).

2.7. Detection of intracellular reactive oxidative species (ROS)

Intracellular ROS generation levels in *E. coli* O157:H7 and *S.* Typhimurium were assayed by the oxidant-sensitive probe 2',7-dichlorodihydrofluorescein diacetate (H_2DCFDA). Briefly, bacteria were sedimented, washed and resuspended in 50 mM sodium phosphate buffer pH 7.4. One millilitre containing 10^8 CFU *E. coli* O157:H7 or *S.* Typhimurium was pre-incubated with 20 μM H_2DCFDA in a 1.5 mL tube at 37 °C. Following 1 h incubation, the bacteria were washed twice and then resuspended in PBS. Electrolyzed water (final FAC 4 mg/L) was then added to the bacteria suspension and treated for 180 s at 60, 70 and 80 °C. Then neutralize solution of 0.5% sodium thiosulfate was applied to quench the excess FAC. For all sanitation treatments, fluorescence intensity of 100 μL E. *coli* O157:H7 and *S.* Typhimurium suspension was measured using a Spectrafluor Plus fluorescence plate reader (Tecan, Durham, NC, USA) of 488/520 nm at excitation/emission wavelengths.

2.8. Morphology observation from atomic force microscopy (AFM)

E. coli O157:H7 and *S.* Typhimurium suspensions were washed twice with PBS and then re-disperzed in deionized water. The bacteria were then stressed in 70 °C DI water or EW for 1 min with approximately of 10^7 CFU/mL. Then, 50 μL of each sample was pipetted on a freshly cut mica surface and aired dried in a biosafety cabinet for 1 h. Morphology of bacteria cells was assessed by AFM workshop (Signal Hill, CA, USA) with a Sensaprobe TM190-A-15 tip from Applied Nanostructures (Mountain View, CA, USA). AFM images were obtained in noncontact mode, scan rate of 512 pixels/line and 1 Hz scan rate (Liu et al., 2017b) and processed by Gwyddion software to quantitatively depict the topography of the bacterial surface. RMS was computed from two separate areas ($0.4 \times 0.4\ \mu m^2$) of the height images, and roughness' results were obtained from the central zones of at least 15 cells.

2.9. Color and texture analyses

The physicochemical properties determined were the color and hardness of the sliced carrots before and after treatment. Shredded carrot samples of 5 g were used for physical quality evaluation. Color changes of the sliced carrots were measured using a Minolta Colorimeter CM-3500d (Konica Minolta, Inc., Japan) at 3 locations on each sample and revealed by L^*, a^*, and b^* values. The overall color difference (ΔE^*) was calculated by applying the following formula (Zhang & Yang, 2017):

$$\Delta E^* = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2} \tag{5}$$

where ΔL^*, Δa^* and Δb^* indicate the differences between the color parameters of the sample and the control. The "browning index (BI)" was calculated using Eqs (6) and (7). The relative change between the BI corresponding to a treatment and untreated control (BI/BIo) was analyzed (Pathare, Opara, & Al-Said, 2013).

$$\text{BI} = \frac{[100 \times (x - 0.31)]}{0.17} \tag{6}$$

$$x = \frac{(a^* + 1.75 \times L^*)}{(5.645 \times L^* + a^* - 3.012 \times b^*} \tag{7}$$

All analyses were conducted triplicated with independently prepared samples.

The texture of the samples was determined by a TA-XT2i Texture analyser (Stable Micro Systems Ltd, Godalming, UK). A cylinder probe with diameter of 6 mm was applied with test speed of 1 mm/s, penetrating distance of 12 mm. At least six independently prepared samples were analyzed by the texture analyser.

2.10. Statistical analysis

Data were analyzed with one-way ANOVA using SPSS statistical software (IBM, Armonk, NY, USA). Differences analysis between treatments were performed by Duncan's test. Comparisons with *P*-value lower than 0.05 were considered statistically significant.

3. Results and discussion

3.1. Effect of treatments on survival of E. coli O157:H7 and S. Typhimurium on organic carrots

Surviving populations of *E. coli* O157:H7 and *S.* Typhimurium on organic carrots are shown in Figs. 1 and 2, respectively. The graphs show the population of surviving cells (log CFU/g) with respect to treatment time (s) for different microorganisms and temperatures. The Weibull model was applied to fit the results obtained from surviving population vs. treatment time. In general, the survival patterns in the two microorganisms were similar and reflected a greater log-decrease for the LcEW treatment. The figures show that at any temperature studied, LcEW was much more effective than DI treatment. Treatments with LcEW at 60 °C for 10–180 s were more effective than washing with DI at 60 °C for 10–180s, with an observed bacterial population reduction of 1.14 and 0.9 log CFU/g for *E. coli* O157:H7 and *S.* Typhimurium, respectively. Treatment with LcEW at 70 °C for 180 s resulted in 2.20 and 2.70 log CFU/g reductions for both pathogens. Further significant ($P < 0.05$) reductions of 3.5 and 3.0 log CFU/g for *E. coli* O157:H7 and *S.* Typhimurium were achieved by 180 s treatment at 80 °C with LcEW. The greatest difference in log counts produced by LcEW and DI treatments was at 70 °C and *E. coli* O157:H7 was slightly more resistant than *S.* Typhimurium to the LcEW.

Using a higher temperature of treatment is a conventional technique used to improve the efficacy of a sanitizer. Selma, Ibáñez, Allende, Cantwell and Suslow (2008) studied the effect of gaseous ozone and hot water (75 °C) for 1 min, alone or in combination, on microbiological quality of cantaloupe melon. The combination of the two treatment was the most effective for controlling microbial growth. There was no evidence of quality compromised to melons, and the melon samples maintained their initial texture and aroma. EW at lower temperatures achieved less bactericidal effect. Acidic EW (40 mg/L FAC) used at 50 °C reduced *E. coli* O157: H7 by 2.9 log CFU/g, compared to a reduction of 0.78 log CFU/g treated at 4 °C (Koseki, Yoshida, Kamitani, Isobe, & Itoh, 2004).

The current results indicated LcEW was pretty effective while at low concentrations as Kiura et al. (2002) demonstrated the germicidal effect on *Mycobacteria* and spores of *Bacillus subtilis.* The effect was only slightly weaker than that of 50 mg/L FAC EW. The mechanism of the bactericidal effect could be attributed to the inactivation of cytoplasmic enzymes, caused blebs and breaks in the outer membrane. However, for

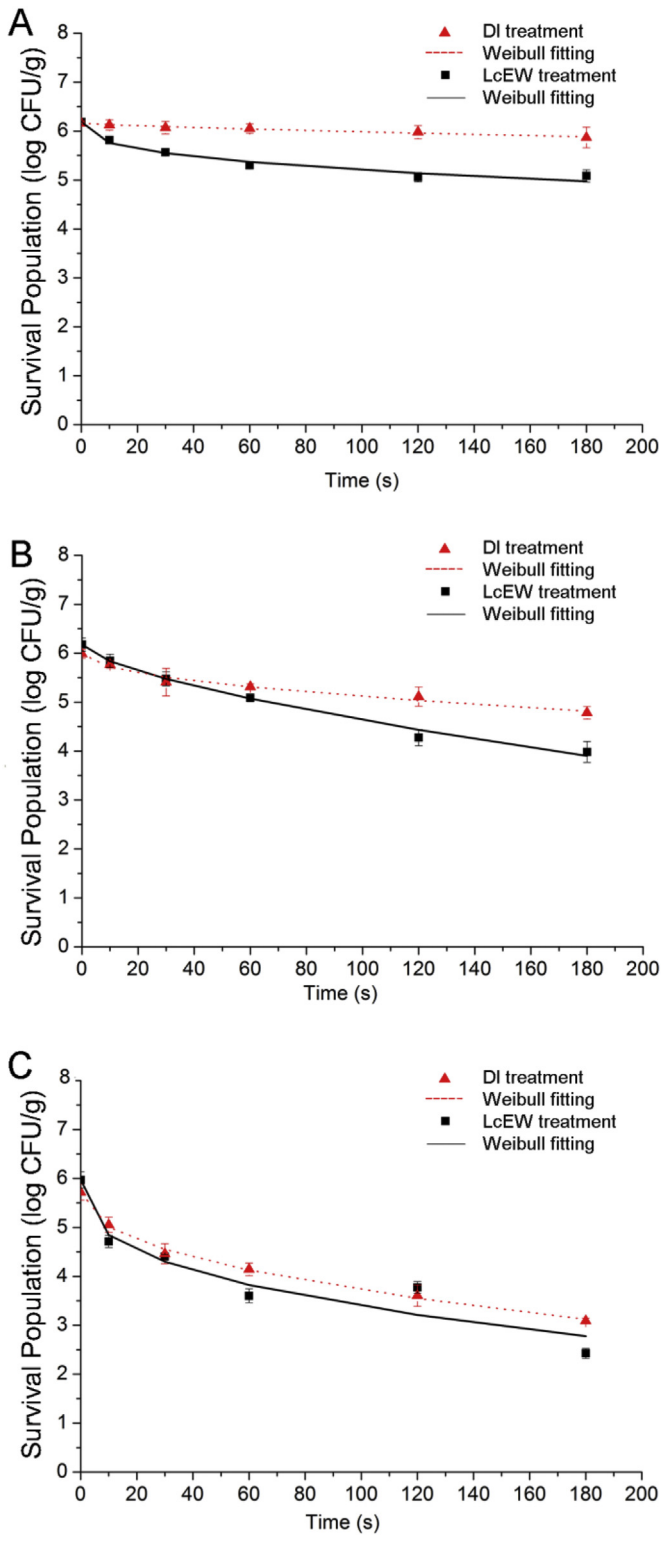

Fig. 1. Survival curves of *Escherichia coli* O157:H7 inoculated on organic carrots after DI and low concentration electrolyzed water (FAC: 4 mg/L) treatments at 60 °C (A), 70 °C (B) and 80 °C (C). The Weibull model was used for curve fitting.

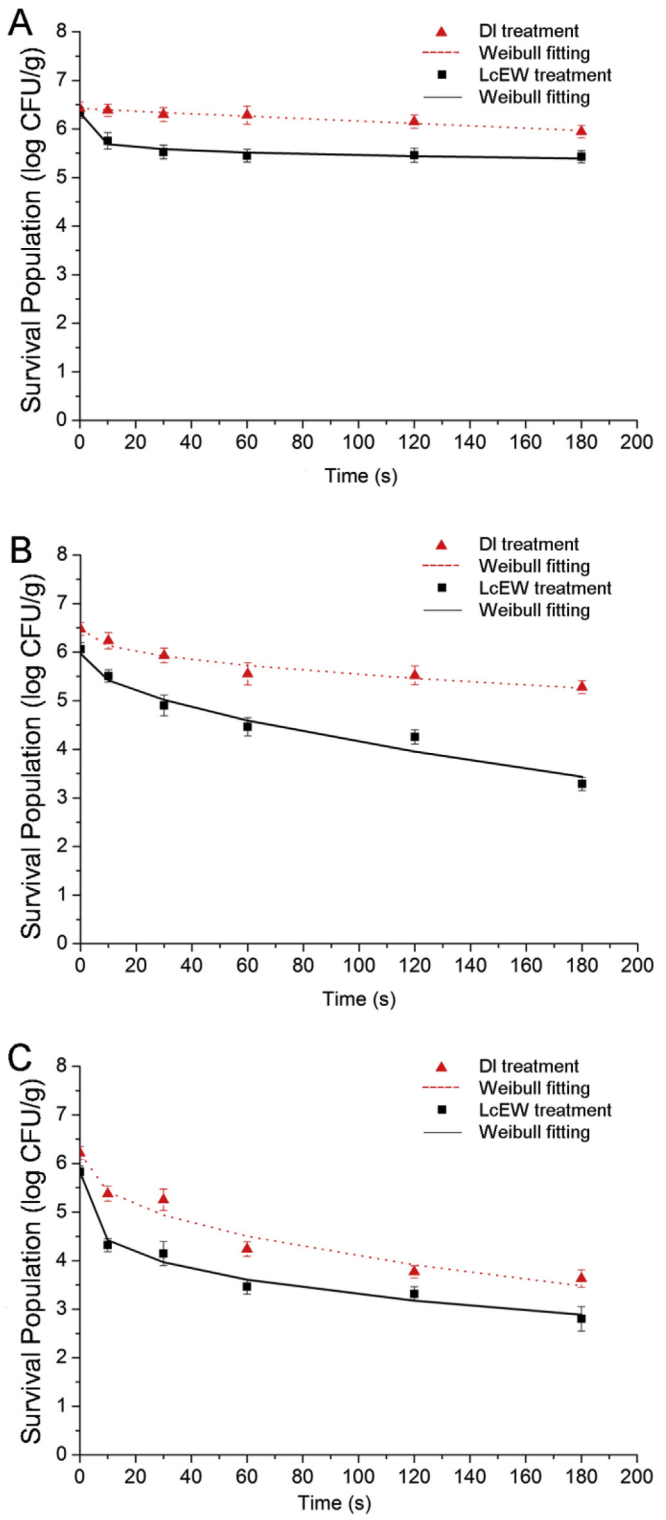

Fig. 2. Survival curves of *Salmonella* Typhimurium inoculated on organic carrots after DI and low concentration electrolyzed water (FAC: 4 mg/L) treatments at 60 °C (A), 70 °C (B) and 80 °C (C). The Weibull model was used for curve fitting.

food system, this sanitizing efficacy might be limited. Keskinen, Burke and Annous (2009) reported the efficacy of acidic EW (50 mg/L FAC, pH 2.6) at reducing populations of *E. coli* O157:H7 on artificially inoculated iceberg lettuce, and only 0.68 log CFU/g reduction was observed after 2 min treatment at 22 °C.

Our results presented a remarkable reduction of LcEW used in combination with short-time heat compared to the untreated control regardless of dipping time or temperature. The bacterial reductions of LcEW showed a significant difference ($P < 0.05$). Within 30 s of dipping time, the difference was not significant ($P > 0.05$) between 60 and 70 °C. There was a significant difference ($P < 0.05$) between 60 s and 0, 10, or 30 s dipping time at 60 and 70 °C. As with the results

Table 1
Kinetic parameters of the Weibull model for reduction of *E. coli* O157:H7 and *S.* Typhimurium on organic carrots in different sanitizing treatments.

	Temp (°C)	Treatment	T_R (s)	α	β	R^2	RMSE
E. coli O157:H7	60	EW	103.31 ± 5.61^{C}	10.61 ± 5.61^{B}	0.36 ± 0.08^{B}	0.96	0.14
		DI	2519.35 ± 40.00^{A}	381.17 ± 28.47^{A}	0.47 ± 0.08^{B}	0.95	0.05
	70	EW	51.69 ± 2.95^{D}	14.57 ± 1.39^{B}	0.66 ± 0.02^{A}	0.98	0.13
		DI	138.61 ± 14.35^{B}	23.48 ± 5.99^{B}	0.49 ± 0.12^{B}	0.96	0.11
	80	EW	7.42 ± 2.05^{E}	0.78 ± 0.41^{B}	0.36 ± 0.04^{B}	0.95	0.26
		DI	22.86 ± 6.79^{DE}	3.90 ± 2.30^{B}	0.45 ± 0.06^{B}	0.99	0.11
S. Typhimurium	60	EW	302.18 ± 30.32^{b}	0.46 ± 0.24^{d}	0.12 ± 0.01^{d}	0.96	0.09
		DI	412.71 ± 43.05^{a}	170.72 ± 6.96^{a}	0.98 ± 0.19^{a}	0.96	0.04
	70	EW	39.48 ± 2.14^{d}	9.73 ± 1.90^{c}	0.60 ± 0.06^{b}	0.97	0.21
		DI	113.74 ± 17.32^{c}	16.34 ± 2.07^{b}	0.43 ± 0.04^{c}	0.99	0.05
	80	EW	3.27 ± 0.53^{d}	0.17 ± 0.15^{d}	0.26 ± 0.05^{d}	0.97	0.18
		DI	17.01 ± 1.13^{d}	2.45 ± 0.41^{d}	0.43 ± 0.02^{c}	0.98	0.23

Note: T_R, time required for 1 log reduction of bacteria population; α, scale factor; β, shape parameter; RMSE, root mean squared error; R^2, R-Square; DI, deionized water; EW, electrolyzed water. Mean values of *E. coli* O157:H7 with different capital letters are significantly different ($P < 0.05$); means of *S.* Typhimurium with different small case letters are significantly different ($P < 0.05$).

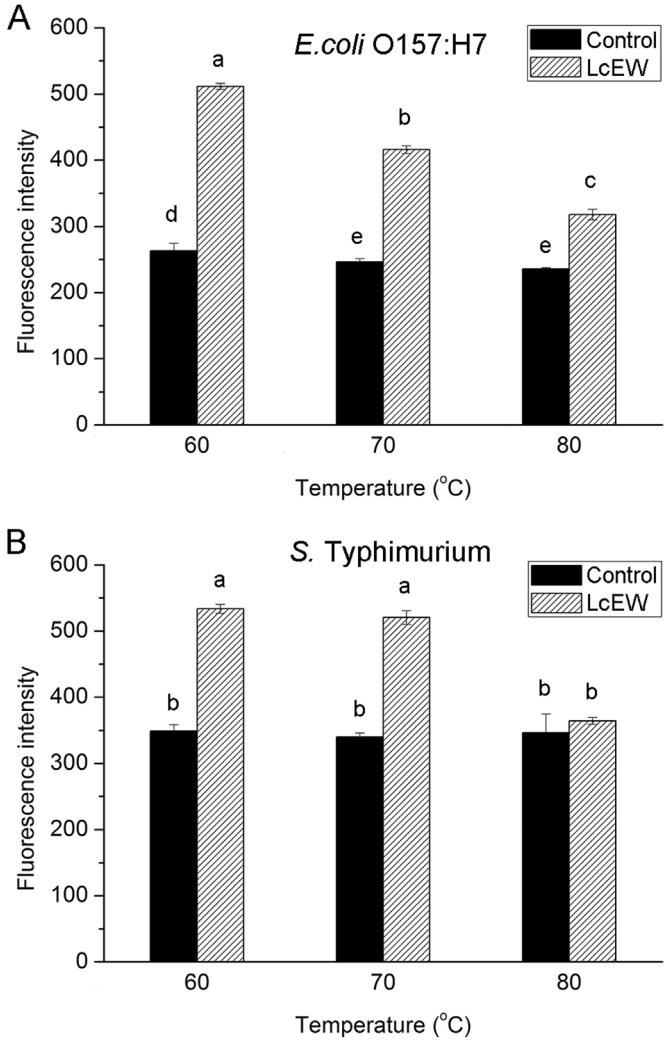

Fig. 3. Fluorescence intensity as a response of H_2DCFDA probe to different temperature of *E. coli* O157:H7 (A) and *S.* Typhimurium (B) in the absence and presence of low concentration electrolyzed water (FAC: 4 mg/L). Bars with the same letters are not significantly different using Duncan Multiple Range Test (n = 3, $P < 0.05$).

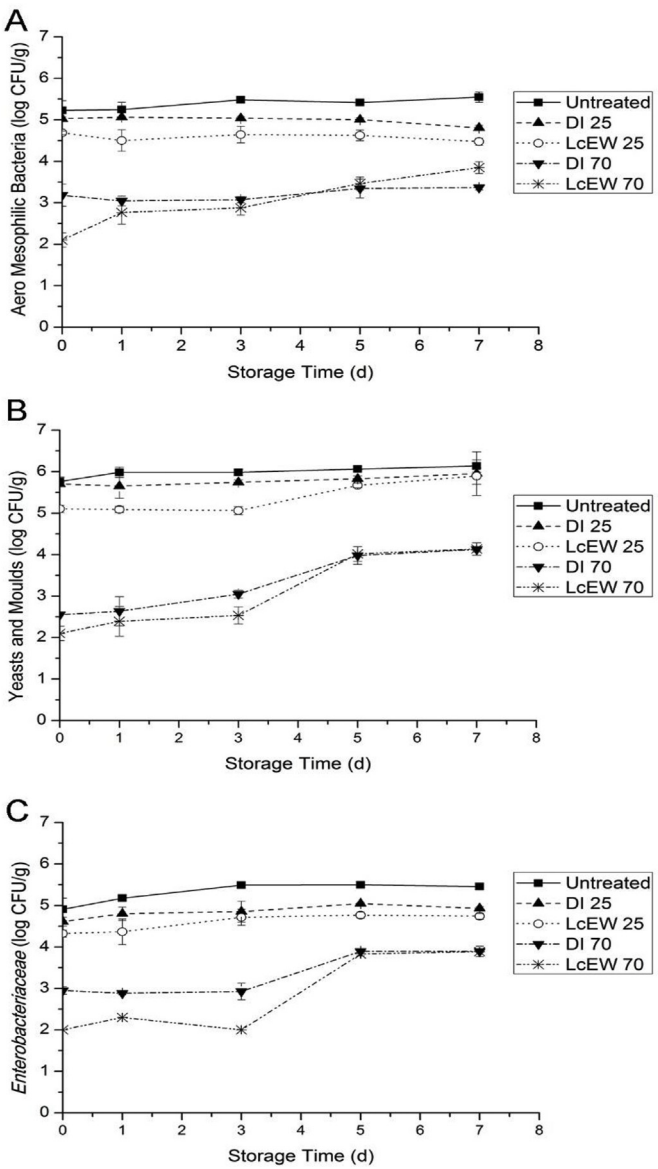

Fig. 4. Effect of different treatments on aerobic mesophilic bacteria (A), yeasts and moulds (B), and *Enterobacteriaceae* (C) on organic carrot stored for 7 days at 4 °C. DI 25: deionized water at 25 °C; LcEW 25: electrolyzed water (FAC, 4 mg/L) at 25 °C; DI 70: deionized water at 70 °C; LcEW 70: electrolyzed water (FAC, 4 mg/L) at 70 °C.

Table 2
Effect of treatments on the dimension and surface roughness of *E. coli* O157:H7 and *S.* Typhimurium.

		Length (μm)	Width (μm)	Height (nm)	RMS roughness (nm)
E. coli O157:H7	Control	2.64 ± 0.45 A	1.77 ± 0.43 A	255.40 ± 17.11 B	22.48 ± 3.24 B
	DI 70	2.85 ± 0.25 A	1.76 ± 0.45 A	129.71 ± 3.45 C	22.12 ± 4.35 B
	EW 25	2.37 ± 0.67 A	1.11 ± 0.56 AB	295.53 ± 6.78 A	31.72 ± 2.15 AB
	EW 70	1.33 ± 0.32 B	0.82 ± 0.25 B	107.32 ± 2.13 D	34.92 ± 8.98 A
S. Typhimurium	Control	2.22 ± 0.38 a	1.34 ± 0.45 a	191.16 ± 9.87 bc	7.19 ± 1.89 c
	DI 70	2.91 ± 0.41 a	1.58 ± 0.29 a	171.20 ± 7.62 c	9.49 ± 2.05 bc
	EW 25	3.12 ± 0.78 a	1.19 ± 0.24 a	216.93 ± 14.23 a	14.92 ± 2.23 b
	EW 70	2.45 ± 0.29 a	1.02 ± 0.17 a	202.55 ± 12.67 ab	25.55 ± 4.67 a

Note: The same uppercase or lowercase letters within the same column for each parameter show that the results are not statistically significantly different ($P > 0.05$).
DI 70: deionized water at 70 °C; EW 25: electrolyzed water (FAC, 4 mg/L) at 25 °C; EW 70: electrolyzed water (FAC, 4 mg/L) at 70 °C.

reported here, sanitizing solutions of LcEW at 70 °C for 60 s achieved 1.01 and 1.41 log CFU/g reduction of *E. coli* O157:H7 and *S.* Typhimurium, respectively. Furthermore, heat treatment has been showed effective for improving postharvest fresh produce's quality. Gonçalves, Pinheiro, Abreu, Brandão and Silva (2010) reported that treatment at 70 °C could better retain the color and texture quality attributes for a process compared to higher temperature (75–90 °C), and peroxidase enzyme (POD) became monophasic and demonstrated 100% inactivation at 65–75 °C. For the subsequent analyses of shelf life and physical qualities, the condition of LcEW treatment at 70 °C for 1 min was chosen as a hurdle approach to determine the effect of this approach on the microbial loads of organic carrots during storage.

3.2. Pathogen inactivation kinetics in different sanitizing treatments

The inactivation kinetics of *E. coli* O157:H7 and *S.* Typhimurium were analyzed by 5 model types. The Weibull model provided the best fit for the data among the tested models. The corresponding and other additional parameters are shown in Table 1, and they all demonstrate a very reasonable fit. The low RMSE values demonstrated the good applicability of the Weibull model. The regression coefficients (R^2) in all treatments were over 0.95. All survival curves displayed upward concavity without shoulders. According to the previous report by Peleg and Penchina (2000), a survival curve's concavity was not only served as an indicator of lethal inactivation, but also can be translated into a physiological effect such as a cumulative manifestation. An upwardly concave curve most likely indicates that on the time scale of the experiment, as the sensitive bacteria cells of the population are destroyed, it will become increasingly more difficult to further inactivate the remaining sturdy survivors (Miller, Gil, Brandão, Teixeira, & Silva, 2009).

The pattern of survival curves in this study was similar to that reported by Luo and Oh (2016), which showed an upwardly concave curve. However, the shape parameter is also a reflection of and affected by the status of the tested strains and conditions such as ionic strength, pH, etc. Table 1 represents that the shape parameter β was smaller than 1, and both the scale and shape parameters did not depend on the temperature in a systematic way.

The parameter T_R from modelling to estimate the time needed for 90% reduction. The T_R was obviously dependent on temperature, and decreased T_R values were obtained by increasing the temperature. At each temperature, the T_R values of LcEW treatment were significantly lower than those of DI groups. The minimal T_R for both *E. coli* O157:H7 (7.42 s) and *S.* Typhimurium (3.27 s) was obtained from LcEW treatment at 80 °C. Our results indicated that simultaneous treatment with LcEW and short-time heat efficiently enhanced the bactericidal efficacy against *E. coli* O157:H7 and *S.* Typhimurium on organic carrots.

3.3. Intracellular level of reactive oxidative species of E. coli O157:H7 and S. Typhimurium

The generation of free radicals has been shown to contribute to EW-triggered cytotoxicity. In this study, ROS accumulation in *E. coli* O157:H7 *and S.* Typhimurium due to LcEW treatment exposure was investigated in terms of fluorescence emission. H_2DCFDA was used as an intracellular ROS-indicator for LcEW treated cells to measure the burst of free radicals and reactive oxygen species (ROS). An exposure time of 180 s was used to better discriminate differences among treatments.

Fig. 3 shows that exposure to LcEW resulted in a significant increase in fluorescence signal, indicating generation of intracellular ROS in both *E. coli* O157:H7 and *S.* Typhimurium. Higher fluorescence intensity was observed for the H_2DCFDA labelled cells treated with LcEW as compared to the DI treated control except *S.* Typhimurium at 80 °C. In addition, for the LcEW treated groups, the fluorescence intensity showed a declining trend with an increase in temperature. ROS such as $\cdot OH^-$ and O_3 have been reported as bactericidal agents in EW, which may inactivate bacteria by reducing activities of dehydrogenase and nitrate reductase (Kiura et al., 2002). In the present study, accounting for the results of fluorescence intensity for LcEW at different temperatures and the disinfection efficacy of the treatments, considering the extreme low chlorine concentration of EW, the accumulated ROS rather than the chlorine compounds might play a key role in the disinfection by LcEW.

3.4. Effect of LcEW treatment on the microbial load of organic carrots during storage

The evolution of native flora (AMB, YMC, and *Enterobacteriaceae*) after different treatments at room temperature (25 °C) and 70 °C for 60 s during 7 days of storage (4 °C) is shown in Fig. 4. The initial AMB, YMC and *Enterobacteriaceae* count of the untreated control sample was 5.22 ± 0.24, 5.76 ± 0.1 and 4.91 ± 0.27 log CFU/g, respectively. The initial microbial loads of the organic carrots were higher than that of conventional ones as reported previously (Gómez-López, Devlieghere, Ragaert, & Debevere, 2007; Vandekinderen, Devlieghere, De Meulenaer, Ragaert, & Van Camp, 2009).

The current results support the statement that organic produce poses a greater risk of microbial contamination than does conventional produce. DI treatment at 25 °C had very little effect on the initial population of AMB whereas LcEW produced reasonable (0.53 log reduction) population reduction at the same temperature. After treatment at 70 °C, the initial populations of AMB on carrots after being treated by DI and LcEW were reduced by 2.04 and 3.12 log CFU/g, respectively. AMB counts gradually increased in the untreated group during 7 days of storage and showed the highest count (5.54 log CFU/g) on day 7 compared to treated samples (3.36–4.80 log CFU/g).

Postharvest rotting and spoilage occurs mainly due to yeasts and

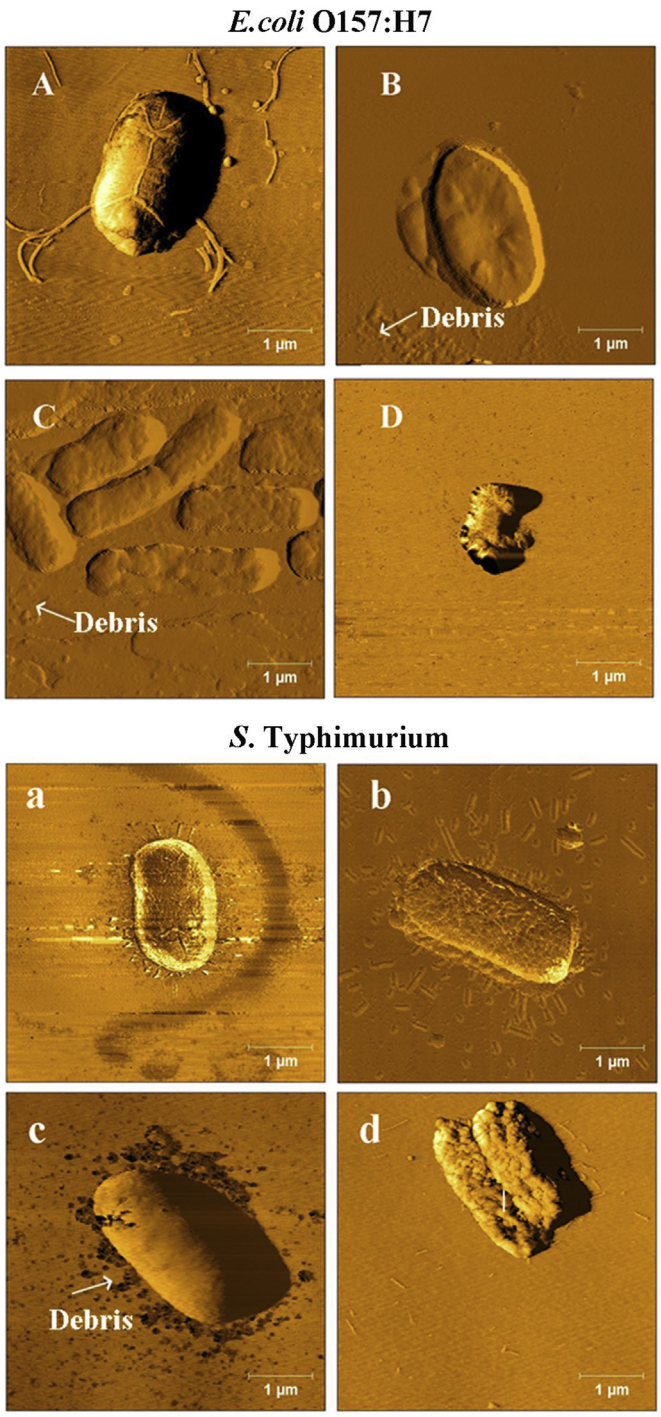

Fig. 5. AFM images of *E. coli* O157:H7 (A–D) and *S.* Typhimurium (a–d), untreated (A, a) and after treatment with DI at 70 °C (B, b), EW at 25 °C (C, c), and EW at 70 °C (D, d).

moulds. Organic produce is more susceptible to faecal contamination due to the use of organic fertiliser often consisting of manure. The *Enterobacteriaceae* family is a common indicator of faecal contamination (Miranda et al., 2008). As shown in Fig. 4 (B, C), YMC and *Enterobacteriaceae* showed quite similar patterns to that of the AMC. The initial populations of YMC and *Enterobacteriacea* were 5.76 and 4.90 log CFU/g, respectively. The maximum YMC and *Enterobacteriacea* reductions of 3.66 and 2.93 log CFU/g on organic carrots were observed by LcEW at 70 °C. On the contrary, immersing in DI reduced the YMC and *Enterobacteriacea* by 3.22 and 1.96 log CFU/g, respectively.

In addition to the fact that LcEW was able to reduce the indigenous microflora counts immediately after treatment, it better prevented the recovery of microbial load for 5 days than DI treatment at the same temperature. The delay in the native flora counts could be attributed to an additive inhibitory effect of LcEW. By this day, untreated AMB, YMC and *Enterobacteriaceae* counts increased to 5.42, 6.06 and 5.50 log CFU/g, respectively. Control of background microorganisms can play a key role in the improvement of quality of produce like carrots. In the present work, the background microbial loads for untreated carrots increased gradually over 7 days of storage (5.45–6.13 log CFU/g). It is worth noting that for treated carrots, the initial native flora (AMB, YMC and *Enterobacteriaceae*) population of organic carrots was significantly reduced ($P < 0.05$) to 3.84–4.13 log CFU/g due to the LcEW treatment at 70 °C. This is in agreement with a report (Forghani et al., 2013); LcEW demonstrates as a good potential sanitizer to improve microbiological safety.

3.5. Effect of EW and heat treatments on cell morphology and membrane integrity

AFM was used to observe topological changes of the cells of both bacteria when treated with EW (4 mg/L FAC) and short-time heat treatments. To understand the underlying mechanisms better, quantitative analysis was carried out further to determine the morphological changes of cells after being subject to the treatments (Table 2). As presented in Fig. 5(A) and (a), the surface membrane was integrated, smooth, and structured for fresh *E. coli* O157:H7 and *S.* Typhimurium, and there were no obvious grooves and indentations on its surface.

E. coli O157:H7 and *S.* Typhimurium cells showed different degree of membrane and structure damage by EW and heat treatments. The bacterial cell membrane collapsed and leaking of intracellular component happened when treated with LcEW. Morphology of *E. coli* O157:H7 and *S.* Typhimurium stressed with 70 °C LcEW exhibited greatest cell shrinkage with the highest R_{rms} of 34.92 ± 8.98 and 25.55 ± 4.67, respectively. Untreated *S.* Typhimurium had smooth surfaces (R_{rms} = 7.1 ± 0.58 nm, n = 15) with intact flagella, which is typically found in *Salmonella* species. *S.* Typhimurium cells treated with 70 °C DI water could still maintain intact shape, while breakage of flagella and some indentations were observed on the surface. Fig. 5 (D) and (d) show that EW combined with 70 °C caused most severe damage. Consistent with a previous report (Liu et al., 2017b; Liu & Yang, 2019), AFM results of morphological changes suggested that EW induced visible lesions and impaired membrane structure of *E. coli* O157:H7 and *S.* Typhimurium with the leakage of cytosolic components.

3.6. Changes in physical qualities

Changes in the surface color and hardness of carrot samples before and after the treatments are shown in Table 3. Because untreated samples that have not been immersed in either DI water or LcEW tend to change color or texture slightly after immersion, it is reasonable that DI immersed samples were the best to use for the comparison of physical properties among treated samples. As shown in Table 3, there was no significant difference ($P < 0.05$) in L^*, a^* or b^*, which correlated with visual appearance of lightness, redness-greenness and yellowness-blueness, respectively. Moreover, there was no significance ($P < 0.05$) in total color difference or BI/BI_0 values among samples treated with DI, heated DI, LcEW and heated LcEW. The ΔE of samples treated with DI and heated LcEW was quite low at 0.39, and it was considered an unremarkable change.

Browning is one of the important visual symptoms of fresh-cut produce deterioration due to the activity of enzymes involved in the browning process. In this study, the technological parameter BI was used to measure brownness of sliced carrots after different treatments. The results demonstrated that carrot samples treated with DI and LcEW had slightly higher BI/BI_0 value than untreated slices, indicating

Table 3
Effects of treatments on surface color and hardness of organic carrot samples.

	L*	a*	b*	ΔE	BI/BI0	Hardness
Control	57.36 ± 1.96^{A}	31.63 ± 4.08^{A}	45.51 ± 1.72^{A}	–	1.00 ± 0.03^{A}	242.15 ± 30.02^{A}
DI 25	57.48 ± 1.79^{A}	34.52 ± 1.52^{A}	45.75 ± 0.65^{A}	3.42 ± 1.00^{A}	1.02 ± 0.06^{A}	215.80 ± 7.34^{A}
EW 25	55.92 ± 2.79^{A}	32.94 ± 2.55^{A}	44.52 ± 0.53^{A}	3.65 ± 1.43^{A}	1.02 ± 0.07^{A}	221.71 ± 15.58^{A}
DI 70	57.10 ± 2.93^{A}	34.45 ± 0.50^{A}	45.30 ± 1.59^{A}	3.89 ± 0.64^{A}	1.02 ± 0.08^{A}	223.82 ± 16.26^{A}
EW 70	54.19 ± 2.96^{A}	33.59 ± 0.72^{A}	45.24 ± 1.48^{A}	3.81 ± 1.40^{A}	1.08 ± 0.02^{A}	217.74 ± 18.23^{A}

Note: The same uppercase letters within the same column for each parameter show that the results are not statistically significantly different ($P > 0.05$).
DI 25: deionized water at 25 °C; DI 70: deionized water at 70 °C; EW 25: electrolyzed water (FAC, 4 mg/L) at 25 °C; EW 70: electrolyzed water (FAC, 4 mg/L) at 70 °C.

slightly more brown color development.

The firmness of fresh-cut produce reveals the integrity of tissue and is considered a crucial feature influencing the purchase decision of customers. No significant differences in hardness were found between untreated and treated samples. All carrot samples subject to disinfection treatments showed slight losses in firmness, which could be explained by the shredding and immersion operations that induced the exudation of proteolytic and pectinolytic enzymes from bruised cells into inner tissues (Soliva-Fortuny & Martín-Belloso, 2003).

The obtained physical quality values in this study were similar to that recorded by Koide, Shitanda, Note and Cao (2011), who showed that surface color was not affected by mildly heated EW (FAC 23 mg/L, pH 5.5), and there were insignificant differences in the hardness of sliced carrots. The physical data obtained in this study suggested that dipping in heated LcEW is suitable and practical for organic produce compared with DI or other types of electrolyzed water. From a practical point of view, LcEW treatment is an inexpensive and environment-friendly method of processing that leaves no residues behind; thus, this intervention method can be adopted for use post-harvest for microbiological enhancement and to maintain quality of organic carrots. However, further studies should be conducted to simulate commercial and practical conditions in other types of microbial pathogens and organic vegetables.

4. Conclusions

The synergistic effects of low concentration EW and heat treatment on the inactivation efficacy of *E. coli* O157:H7 and *S.* Typhimurium on organic carrot were fitted by Weibull model. The T_R values of LcEW treatment were significantly lower than those of DI groups. Morphology of bacteria surface provided the direct evidence for revealing the bactericidal mechanism. The AFM images demonstrated that bacteria cells were severely damaged and intracellular components were leaked. With respect to shelf life evaluation, LcEW at 70 °C for 1 min was capable of significantly reducing the indigenous microbiota immediately after treatment, moreover, better prevented the recovery of AMB, YMC, and *Enterobacteriaceae* for 5 days than control groups. The results indicate the technology is promising for fresh-cut produce like carrot.

Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.foodcont.2019.06.028.

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