Food Control 101 (2019) 241–250

Contents lists available at ScienceDirect

Food Control

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

Efficacy of low concentration acidic electrolysed water and levulinic acid combination on fresh organic lettuce (*Lactuca sativa* Var. *Crispa* L.) and its antimicrobial mechanism

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ARTICLE INFO

Keywords:
Electrolyzed water
Organic acid
Modeling
Epifluorescence microscopy
Atomic force microscopy
Nanostructure
Listeria
Escherichia coli
Protein
Organic produce
Sanitization
Mechanism
Bacterium
Microbial safety
SDS-PAGE
Physicochemical property

ABSTRACT

The sanitising effect of low concentration acidic electrolysed water (AEW, free available chlorine (FAC): 4 mg/L) combined with levulinic acid (LA, 3% v/v) on fresh organic lettuce during 7-day storage was evaluated. The combined sanitising method showed additional bactericidal efficacy against naturally existing microbiota, while LA alone and combined with AEW could reduce survival population of *Escherichia coli* ATCC 25922 and *Listeria innocua* Seeliger ATCC 33090 inoculated on lettuce surface effectively, with 3.5–4.0 log CFU/g reduction for both during storage. Moreover, the modified Gompertz model provided a good fitness to the sanitising results during storage, with highest R^2 in AEW group for *E. coli* (0.99) and in combination group for *L. innocua* (1.00), respectively. In addition, the physicochemical properties of organic lettuce treated by each sanitising treatment were not changed significantly during storage. Epifluorescence microscopy and atomic force microscopy (AFM) revealed that the cell permeability and morphology of *E. coli* and *L. innocua* were changed after sanitising treatments, with damaged cell membrane and disordered cellular structure in different degrees. Besides, the size of cells became smaller after combined sanitising treatment, with 2.12 and 1.24 μm^2 for *E. coli* and *L. innocua*, respectively, indicating some cytoplasm leakage. Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) showed the number and intensities of the protein bands of *E. coli* were reduced, while those of *L. innocua* remained similar after sanitising treatments. The results suggest that low concentration AEW combined with LA is a potential effective approach to sanitise organic produce.

1. Introduction

The purchases of organic food have been dramatically increased over the past decades. For instance, organic sales in the U.S. have increased rapidly from $\$3.6 \times 10^9$ in 1997 to $\$49.4 \times 10^9$ in 2017 (OTA, 2018; Sow, Tirtawinata, Yang, Shao, & Wang, 2017). However, as a primary part of organic food, organic produce is vulnerable to be contaminated by pathogens due to using organic fertilisers, bringing microbiological safety concerns to consumers (Ilic et al., 2012). Among them, organic lettuce (*Lactuca sativa*) is most likely to become a major source of spreading pathogens, considering it is often consumed as ready-to-eat salad and responsible for many outbreaks of foodborne illnesses (Zhang & Yang, 2017). Therefore, appropriate sanitisation before consumption is required to enhance microbiological safety of organic lettuce.

When only limited numbers of synthetic sanitisers have been allowed for organic food processing due to strict regulations (Chen et al., 2019), acidic electrolysed water (AEW), which is generated by electrolysing the dilute sodium chloride solution, can act as an efficient sanitiser against various kinds of pathogenic bacteria, like *Escherichia coli* O157:H7, *Salmonella* spp. and *Listeria monocytogenes*. The antimicrobial mechanisms of AEW could be mainly attributed to three factors, free available chlorine (FAC), oxidation reduction potential (ORP) and pH (Hati et al., 2012). There exist interaction effects among them, for example, the FAC and ORP values were found to decrease significantly when the pH increased (Rahman, Ding, & Oh, 2010). Besides, high ORP played an important role in the bactericidal effects of AEW, as it could damage cell membrane and increase membrane penetrability to make intracellular components released, leading to necrosis of bacteria (Liao, Chen, & Xiao, 2007). In addition to strong antimicrobial activity, environmentally-friendly nature, safe characteristic, and economical running expense also account for the

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https://doi.org/10.1016/j.foodcont.2019.02.039
Received 28 December 2018; Received in revised form 26 February 2019; Accepted 28 February 2019
Available online 05 March 2019
0956-7135/

popularity of AEW in food industry. However, for chlorine-based sanitisers, the National Organic Program (NOP) of US Department of Agriculture (USDA) regulates that the FAC concentration in organic-compatible sanitisers after processing cannot exceed 4 mg/L (NOP 5026, 2011). Therefore, in order to achieve a desirable sanitising result, low concentration AEW should be combined with another method for organic food application.

Levulinic acid, a 5-carbon organic acid, has been designated as Generally Recognised as Safe (GRAS) by FDA. It could be derived from degradation of cellulose or produced by heating hexose (Smith, 2011). Like other organic acids, levulinic acid also possesses a certain antimicrobial effect. By combining 0.5% levulinic acid with 0.05% sodium dodecyl sulphate as a sanitising method, the population of Shiga toxin–producing *E. coli* in a pure culture was reduced to an undetectable level (Zhao et al., 2014). Besides, the salt of levulinic acid like sodium levulinate also exhibited an effective sanitising ability for spoilage bacteria in fresh sausage (Vasavada, Carpenter, Cornforth, & Ghorpade, 2003). However, studies related to the sanitising effect of levulinic acid on microorganisms during the preservation of organic produce are limited.

Therefore, the main objective of this study was to evaluate the sanitising effect of low concentration AEW (4 mg/L FAC) combined with levulinic acid (LA) on *E. coli* ATCC 25922 and *L. innocua* Seeliger ATCC 33090 of organic lettuce during 7-day storage by model fitting test, and its influence on physicochemical qualities of lettuce. Furthermore, the antimicrobial mechanisms of this combined sanitising treatment were also elucidated, by determining the damage to membrane permeability, morphological changes and protein profile changes, respectively. The results would contribute to the development of a potential sanitising approach to enhance food safety of organic produce as a novel food preservation technology.

2. Materials and methods

2.1. Materials

Certified organic lettuces were purchased from an organic farm in Singapore. After being transported to laboratory, the lettuces were stored at 4.0 °C and used within 24 h. The injured leaves of lettuces were removed and the remaining intact leaves similar in size and colour were gently rinsed by sterilised water to remove the soil (Zhang & Yang, 2017). Lettuces were cut into pieces of 3 cm × 4 cm. Each single set contained around 10 g of lettuces and triplicate samples from each treatment were analysed.

2.2. Bacterial strains and inoculation

E. coli ATCC 25922 and *L. innocua* Seeliger ATCC 33090 were obtained from ATCC. After grown to the stationary phase, they were adapted to the media supplemented with 200 μg/mL nalidixic acid (Sigma-Aldrich, USA), which was added by stepwise increments after each transfer. This was to rule out any effects that naturally existing microbiota on lettuce surface might exert for later enumeration. Adapted *E. coli* and *L. innocua* were diluted in 0.1% peptone water to achieve final cell concentration of 10^7 colony forming unit (CFU)/mL for the inoculums. Submerged in *E. coli* and *L. innocua* suspensions for 5 min respectively, the inoculated lettuces were air-dried for 30 min in a laminar flow biosafety cabinet.

2.3. Sanitising treatments

Uninoculated (for natural microbiota analysis) and inoculated lettuces (10 g) were dipped into 200 mL of following sanitiser solutions for 7 min respectively: (i) AEW (with 4 mg/L FAC, generated by electrolysing 0.9% NaCl solution in an electrolysis device (ROX-10WB3, Hoshizaki Electric Company, Japan)); (ii) LA (3% v/v, prepared from a solution of 97% v/v, Sigma-Aldrich, USA); (iii) the combination of AEW and LA. Besides, the deionised water (DW) was served as control. The properties of each sanitising treatment are shown in Table 1. The FAC value was measured by using a chlorine test kit (Merck, Darmstadt, Germany), followed by measuring pH value using a pH meter (Thermo Orion pH meter, Waltham, USA) and the ORP value using an ORP meter (HM Digital ORP-200, Culver City, USA). After the dipping, all lettuce samples were air-dried again. Then all the treated lettuces were stored in zipper bags (16.8 cm × 8.2 cm) and refrigerated (7.0 ± 1.0 °C) up to a period of 7 days with different sampling time point (Day 0, 1, 3, 5 and 7) being analysed.

Table 1
The concentration, pH and ORP of different treatment solutions.

Solution	Concentration	pH	ORP* (mV)
DW	–	7.11 ± 0.13^{c}	300.0 ± 20.0^{A}
LA	3% (v/v)	2.66 ± 0.02^{a}	513.0 ± 10.5^{B}
AEW	4 mg/L (FAC*)	3.84 ± 0.12^{b}	920.5 ± 30.5^{C}
Combination	4 mg/L + 3%	2.66 ± 0.03^{a}	1007.5 ± 10.5^{D}

*FAC: Free available chlorine; ORP: oxidation reduction potential.
Within each column, mean values with different lowercase letters are significantly different pH among different treatment solutions ($P < 0.05$), and mean values with different capital letters are significantly different ORP among different treatment solutions ($P < 0.05$). DW, deionised water; LA, levulinic acid; AEW, acidic electrolysed water; Combination: AEW + LA.

2.4. Microbiological analysis

At each sampling time point, a 10 g of lettuce in each group were put in a stomacher bag containing sterile peptone water (0.1%, 90 mL) for 3-min homogenisation by using a Stomacher (Masticator Stomacher, IUL Instruments, Germany). Serial dilution was performed and the desired dilution (0.1 mL) of each group was used for spread plating. For aerobic mesophilic count (AMC), plate count agar (PCA, Oxoid, Britain) was utilised for inoculation, followed by incubation at 37 °C for 48 h. Yeasts and moulds were incubated on potato dextrose agar (PDA, Oxoid, Britain) at 25 °C for 72 h. For inoculated bacteria, *E. coli* was enumerated by counting the colonies grown on tryptic soy agar (TSA, Oxoid, Britain) containing 200 μg/mL nalidixic acid at 37 °C for 24 h, while *L. innocua* counts were determined from tryptic soy agar with 0.6 g/100 mL yeast extract (TSAYE) containing 200 μg/mL nalidixic acid at 37 °C for 48 h. The results were showed as log CFU/g sample (Liu, Tan, Yang, & Wang, 2017a).

2.5. Mathematical modeling of sanitising effect

A modified Gompertz model was used to describe the changes of microbial loads on organic lettuces during storage (Valdivia-Nájar, Martín-Belloso, Giner-Seguí, & Soliva-Fortuny, 2017):

$$y = y_1 + A\exp\{-\exp[-B(t-M)]\}$$

where y is the cell population surviving at representative day (log (CFU/g), y_1 is the initial cell population at the beginning of storage period (log (CFU/g), A is the different asymptotic values from the beginning of the storage to the end, B is the relative rate of change at time M, M is the time (day) with the maximum absolute rate of change, and t is the storage time (day).

The goodness of the model fitting was tested by R-square (R^2) and Residual Mean Square Error (RMSE) values using MATLAB R2018a (The Mathworks, Inc., Natick, USA). Besides, the Akaike Information Criterion (AIC) values were also calculated to analyse the overfitting using following formula:

$$AIC = n\ln(SSE) + 2p$$

where n is the number of data points used for modeling, SSE is the Sum

of Squares for Error and p is the number of parameters used in the model (Josewin, Ghate, Kim, & Yuk, 2018).

2.6. Physicochemical property analyses

After each sanitising treatment, firmness, electrolyte leakage and colour changes of lettuces were measured during storage. The firmness of samples was determined by using a TA-XT2i Texture Analyser (Stable Micro Systems Ltd., Goldaming, UK) following a reported approach (Salgado, Pearlstein, Luo, & Feng, 2014). Moving at a speed of 5 mm/s, the blade plunger was stopped once it was 1 cm below the bottom of the press holder. Twenty independent replicates were performed for each group and the results were exhibited as the maximum cut force.

The electrolyte leakage changes of treated lettuces were measured according to a previous method with some modifications (Kim, Luo, Tao, Saftner, & Gross, 2005). The lettuces (2 g) were cut into small pieces and transferred into a centrifuge tube containing 20 mL deionised water for 0.5 h interaction. A conductivity meter Horiba ES-14 (Horiba. Ltd., Kyoto, Japan) was used to measure the electrical conductivity, which was noted as conductivity of sample at 0.5 h. Then the sample was boiled for 15 min, cooled to room temperature and measured its conductivity again. The electrolyte leakage rate was calculated based on the following formula:

$$\text{Electrolyte leakage (\%)} = \frac{\text{conductivity of sample at 0.5 h} - \text{conductivity of deionised water}}{\text{conductivity of sample after 15 min boiling}} \times 100\%$$

The colour of lettuce samples was analysed using a Minolta Colorimeter CM-3500d (Konica Minolta, Inc., Tokyo, Japan) after calibration. Hunter's colour parameters (L, a, b) were tested at 2 locations of each lettuce piece and a total of 20 readings for each group were obtained. Overall colour difference was calculated according to the following formula (Zhang & Yang, 2017):

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

where ΔE* represents the overall colour difference, ΔL*, Δa* and Δb* were generated by the colourimeter as colour changes compared to the corresponding values of lettuce untreated initially.

2.7. Antimicrobial mechanism analysis

2.7.1. Determination of cell membrane permeability

Cell membrane permeability after each sanitising treatment was observed using epifluorescence microscopy (Olympus BX51, Melville, USA) after the cells were stained by two dyes in the LIVE/DEAD® *Bac*Light™ Viability Kit L-7007 (Molecular Probes™, Eugene, USA). The preparation of samples was conducted according to the previous methods with some modifications (Kim, Mikš-Krajnik, Kumar, & Yuk, 2016). The two dyes were SYTO®9 and propidium iodide (PI) and after they were mixed thoroughly by equal volumes, 3 μL of the mixture was added into 1 mL of treated *E. coli* and *L. innocua* suspension for a 15-min incubation in the dark at room temperature. Afterwards, 10 μL of the suspension was placed onto glass slides covered with square coverslips and examined immediately by microscopy which was equipped with a set of fluorochrome filters.

2.7.2. AFM analysis of morphological changes

Atomic force microscopy (AFM) has recently been introduced to food safety as a powerful tool to study bacterial morphology. After the bacterial suspension was prepared, treated and neutralised according to the methods in our previous study (Zhao, Zhang, & Yang, 2017), about 20 μL of treated *E. coli* and *L. innocua* solutions were pipetted onto freshly cleaved mica sheets which were stuck to AFM specimen discs. After drying, characterisation of bacterial morphology was carried out by AFM (TT-AFM workshop, Signal Hill, USA) using a Sensaprobe

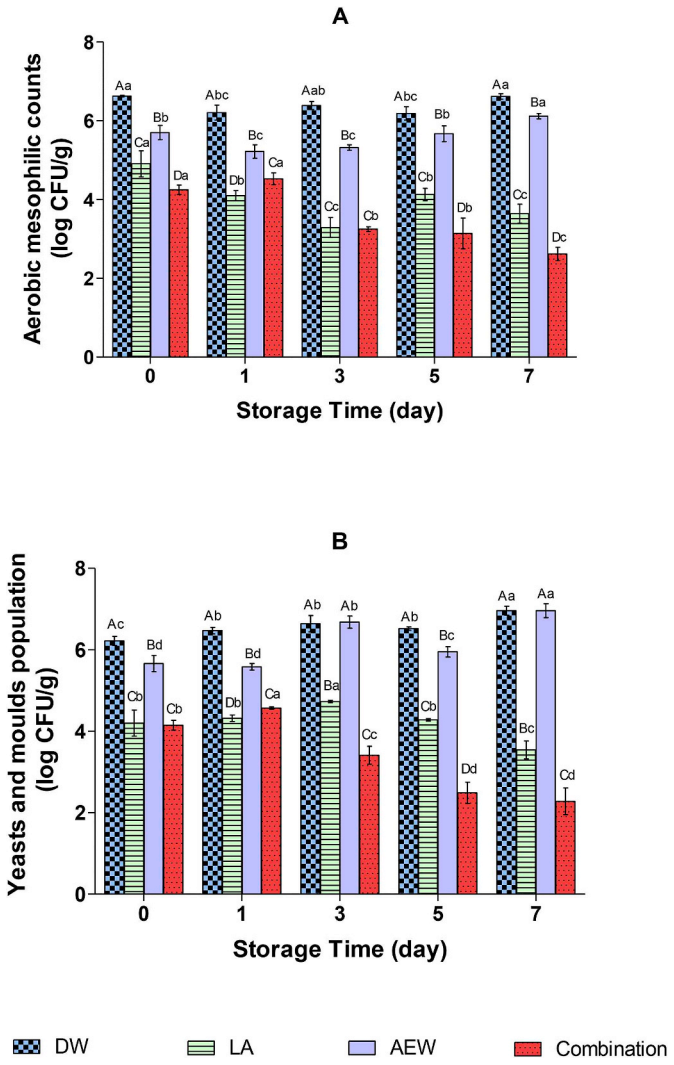

Fig. 1. Sanitising effect of different treatments on aerobic mesophilic counts (A) and yeasts and moulds (B) of organic lettuce during storage. Data are presented as mean values ± standard deviation. Mean values within the same storage time by different treatments with different capital letters are significantly different; mean values for the same treatments at different storage times with different lowercase letters are significantly different ($P < 0.05$). DW: deionised water; LA: levulinic acid; AEW: acidic electrolysed water; Combination: AEW + LA.

TM190-A-15 tip (Applied Nanostructures, Mountain View, USA) in the mode of 512 pixels/line and 0.5 Hz scan rate. The AFM images were processed and analysed offline using Gwyddion software (Liu & Yang, 2018).

2.7.3. SDS-PAGE protein analysis

Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) has become an ideal technique to investigate the mechanism of antimicrobial action (Walker, 2002, pp. 11–14). For whole protein extraction, cell pellets (~1.0 g) were resuspended in 10 mL of chilled lysis buffer and cooled on ice for 10 min, followed by sonication (22.5 kHz, 100 W) on wet ice with 10 cycles of 10 s pulse and 30 s stops. Lysis buffer contained 50 mM Tris-HCl (pH 7.5), 100 mM NaCl, 5 mM 1,4-dithiothreitol (DTT) and 1 mM phenylmethylsulfonyl fluoride (PMSF). The protein supernatant from lysed cells were obtained by centrifugation at 5000 × *g* at 4 °C for 10 min. 100 μL of 20% SDS mixed with 900 μL of STB (sample treatment buffer) was prepared as SDS-STB for use with proteins. STB was the mixture of 950 μL of 2x Laemmli sample buffer (Bio-Rad, Singapore) and 50 μL of 2-mercaptoethanol (Sigma-

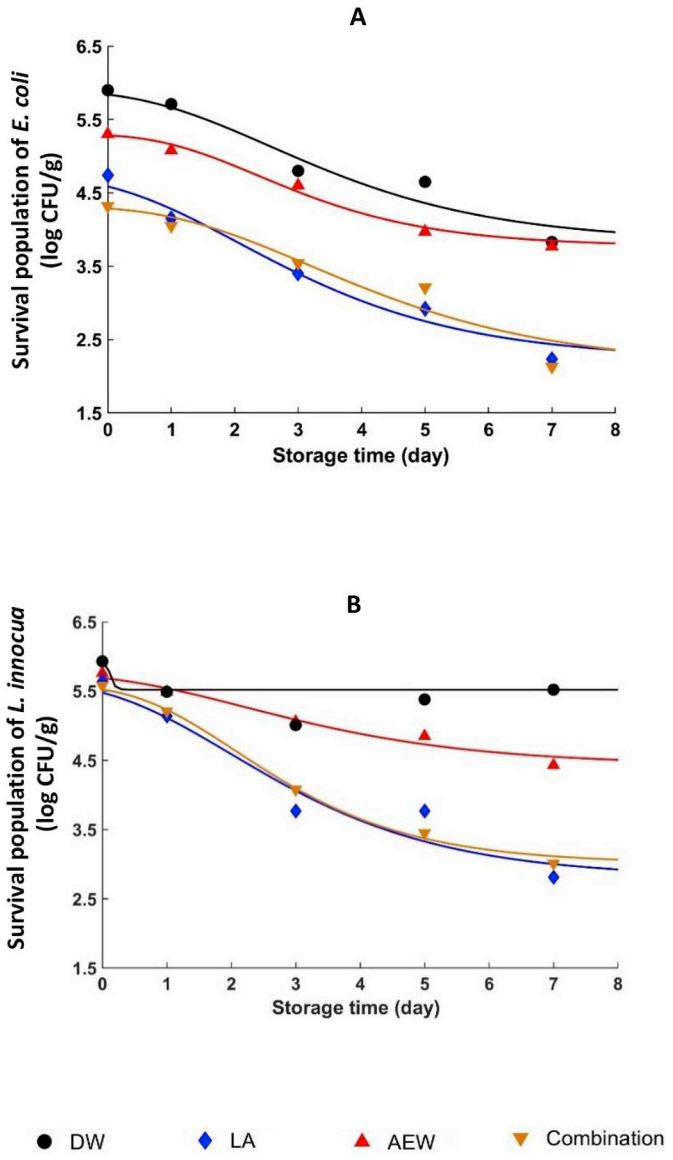

Fig. 2. *E. coli* (A) and *L. innocua* (B) counts on treated organic lettuce through storage. Points are the mean of three determinations. Lines represent the fit of a modified Gompertz model to the experimental data. DW: deionised water (black circle); LA: levulinic acid (blue diamond); AEW: acidic electrolysed water (red triangle); Combination: AEW + LA (yellow inverted triangle). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

Aldrich, USA). Then equal volumes of SDS-STB and protein supernatant were mixed, followed by heating for 3 min at 96 °C. Subsequent steps of performing SDS-PAGE, staining and destaining of proteins were based on the methods of Cloete, Thantsha, Maluleke, and Kirkpatrick (2009).

2.8. Statistical analysis

All experiments were repeated thrice. Means with standard deviation were compared using ANOVA ($P < 0.05$) and Duncan's multiple-range test to assess the difference among different treatment groups with an IBM SPSS statistical software (version 24; IBM Corp., Armonk, USA). For AFM and epifluorescence microscopy, parallel images were compared to get representative results.

3. Results and discussion

3.1. Sanitising effect on natural microbiota of lettuce

Fig. 1 shows the sanitising effect of different treatments on natural microbiota of organic lettuce. The concentration of LA (3% v/v) and the treatment time (7 min) used in current study were based on our pervious study in *vitro*, which were the optimal conditions. The initial population of aerobic mesophilic counts (AMC) and yeasts and moulds on lettuce before treatments were approximate 6.72 and 6.68 log CFU/g, respectively (data not shown). As shown in Fig. 1A, decreased population of AMC was observed immediately (day 0) after AEW, LA and combination treatments, with around 1.02, 1.80 and 2.47 log CFU/g reduction, respectively, compared to the untreated lettuces. For yeasts and moulds, all treatment groups resulted in a significant reduction immediately (day 0) compared to the untreatment, with around 0.46, 1.02, 2.48 and 2.53 log CFU/g reduction in DW, AEW, LA and combination groups, respectively (Fig. 1B). Besides, during 7 days of storage at 7 °C, the populations of AMC and yeasts and moulds increased in both DW and AEW groups, while the most dramatic reduction happened in the combination groups, with more than 4.0 log CFU/g reduction for AMC and 4.5 log CFU/g reduction for yeasts and moulds, respectively, compared to the control group (Fig. 1A and B).

The antimicrobial mechanism of LA could be attributed to its undissociated form, which can passively diffuse through the bacterial cell membrane. Once in the cell, the higher intracellular pH will lead to proton dissociation, making cellular pH drop and then setting off a series of metabolic reactions, and finally the cell death occurs (Guo & Olsson, 2014). Continuous microbial reduction was observed in LA treated groups during 7-day storage in current study, mainly because the decline of internal pH can persist over the storage period. Therefore, LA is able to impart residual inhibition of microorganisms for some days after treatment (Chhetri, Janes, King, Doerrler, & Adhikari, 2019). AEW was found to be less effective against bacteria on lettuce than in *vitro*, which could be attributed to the biofilm formation, a complex structure

Table 2
Fitting parameters of each treatment by modified Gompertz model.

Inoculum	Treatment	y_1*	A*	B*	M*	R^2	RMSE	SSE	AIC
E. coli	DW	5.90^{a}	-2.07^{b}	0.50^{b}	2.55^{b}	0.94^{c}	0.23^{b}	0.16^{b}	-5.16^{b}
	LA	4.74^{c}	-2.51^{d}	0.50^{b}	2.07^{d}	0.97^{b}	0.19^{c}	0.11^{c}	-7.04^{c}
	AEW	5.30^{b}	-1.53^{a}	0.64^{a}	2.36^{c}	0.99^{a}	0.09^{d}	0.02^{d}	-15.56^{d}
	Combination	4.32^{d}	-2.19^{c}	0.45^{c}	3.17^{a}	0.92^{d}	0.28^{a}	0.23^{a}	-3.35^{a}
L. innocua	DW	5.93^{a}	-0.41^{a}	21.49^{a}	0.10^{d}	0.35^{d}	0.30^{b}	0.28^{b}	-2.36^{b}
	LA	5.64^{c}	-2.83^{d}	0.53^{c}	1.98^{c}	0.93^{c}	0.34^{a}	0.34^{a}	-1.39^{a}
	AEW	5.76^{b}	-1.33^{b}	0.49^{d}	2.21^{a}	0.97^{b}	0.11^{c}	0.04^{c}	-12.09^{c}
	Combination	5.58^{d}	-2.57^{c}	0.64^{b}	2.10^{b}	1.00^{a}	0.08^{d}	0.02^{d}	-15.56^{d}

*The parameters of modified Gompertz model. $y = y_1 + A\exp\{-\exp[-B(t-M)]\}$.
Note: y_1, decimal logarithm of initial load (log (CFU/g)); A, difference in value of the upper and the lower asymptotes (log (CFU/g)); B, relative growth or death rate at time equal to M(log (CFU/g)/day); M, time at which growth or death rates (B) are maxima (day); Regression coefficient (R^2); Root Mean Squared Error (RMSE); Sum of Squares for Error (SSE); Akaike Information Criterion (AIC). Different letters within the column indicate that the mean values are significantly different in each strain ($P < 0.05$). DW, deionised water; LA, levulinic acid; AEW, acidic electrolysed water; Combination: AEW + LA.

surrounding the cells to protect them from adverse environmental stresses (Flemming & Wingender, 2010). Besides, the chemically reactive molecules of AEW could interact with the organic materials on lettuce surfaces, such as dusts, soil particles and organic fertiliser residues, which could decrease chlorine availability of AEW and reduce its bactericidal effect (Hao et al., 2013). In addition, the microbial reduction in combination group was more than LA and AEW each used alone, indicating that additional sanitising action might occur when combining LA and AEW. The possible reason for additional sanitising effect is that LA with low pH and its undissociated form can act as a permeabiliser of the bacterial cell membrane as well as act as a potentiator to enhance the bactericidal effect of AEW (Tango, Mansur, Kim, & Oh, 2014).

3.2. Model fitting of sanitising effect on E. coli and L. innocua of lettuce

The model fitting curves of each treatment during storage are presented in Fig. 2. The initial inoculums of *E. coli* and *L. innocua* on lettuces were 6.28 and 6.77 log CFU/g, respectively (data not shown). A decreasing trend of all treatment groups during storage was observed for both bacteria, except the population of *L. innocua* on DW-treated lettuces, remaining almost unchanged (Fig. 2A and B). The combined-treated lettuces provided the most adverse environment for the two bacteria to grow, causing 3.5–4.0 log CFU/g reduction for them during storage, a similar result to that in LA group.

The modified Gompertz model provided a good fitness to the experimental data. As can be seen in Table 2, high R^2 values (0.92–1.00) were shown in each treatment group (control group excluded) for both bacteria, with highest in AEW group for *E. coli* (0.99) and in combination group for *L. innocua* (1.00), respectively. On the contrary, lowest RMSE and AIC values were also observed in that two groups. Moreover, to validate the model's fitness, additional data of Day 4 were conducted to determine the error percentage between the predicted value from the model and the actual experimental value. All error percentages of Day 4 were less than 10% in all treatment groups for both bacteria (data not shown), indicating good predictability. Considering RMSE can act as the most simple and informative indicator of goodness-of-fit for inactivation curves and AIC can check the overfitting of the model by taking into account the sample size and parameter number, the overall results demonstrated good appropriateness of the modified Gompertz model in current study (Pla, Oltra, Esteban, Andreu, & Palop, 2015).

To further analyse the sanitising effect during storage in detail, the model parameters in each treatment group are also shown in Table 2. For *E. coli*, the time at which death rates were maxima (M) was extended in combination group as compared to the control. However, decreasing trend of relative death rate (B) was recorded in most of the treated groups, which might be attributed to the lower initial microbial loads on lettuces that the sanitising treatments had caused at the beginning (Valdivia-Nájar et al., 2017). For *L. innocua*, the lower value of B and the higher value of M were observed in all treated groups compared to the control. However, the microbial loads at the beginning of storage after being treated immediately (y_1 values in Table 2) were higher than those for *E. coli*, showing *L. innocua* exhibited more resistance to sanitising solutions (Ahn & Balasubramaniam, 2007).

Mathematical models can predict microbial safety and provide practical guidance for food industry. While most of the models were applied to describe the inactivation behaviour during sanitising process, modeling the changes of microbial growth or death on food matrix during storage period is limited. For example, Gil, Miller, Brandao, and Silva (2011) used Gompertz model to predict microbial thermal inactivation under static and dynamic temperature during 200 min, showing that Gompertz model was effective if re-parameterised forms were applied. Besides, other models like Weibull and Baranyi models were used to describe the inactivation effect of light emitting diode illumination on *Salmonella* spp. on fresh-cut pineapples during a certain sanitising time, demonstrating good fitness (Ghate et al., 2017). Not

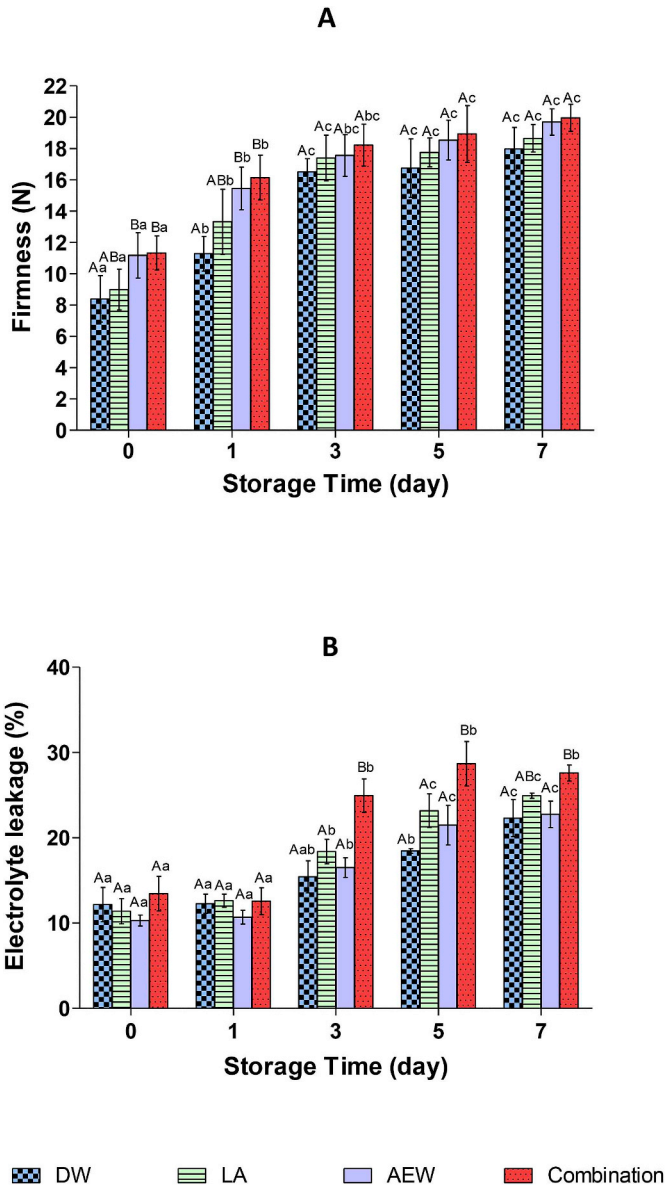

Fig. 3. Effect of different sanitising treatments on the firmness (A) and electrolyte leakage (B) of organic lettuce during storage. Data are presented as mean values ± standard deviation. Mean values within the same storage time by different treatments with different capital letters are significantly different; mean values for the same treatments at different storage times with different lowercase letters are significantly different ($P < 0.05$). DW: deionised water; LA: levulinic acid; AEW: acidic electrolysed water; Combination: AEW + LA.

similar to the inactivation process, which generally presents a shape containing a shoulder, linear phase and tail residual for microbial survival under model description (Koyama, Hokunan, Hasegawa, Kawamura, & Koseki, 2017), describing microbial growth or death on food matrix during storage through models needs to be investigated further, as it involves more complicated factors, like the storage temperature, food matrix, biofilm formation and organic materials on food surfaces, which could all have a cross-impact on microbial behaviour (Adhikari, Syamaladevi, Killinger, & Sablani, 2015).

3.3. Physicochemical property of lettuce

The physicochemical properties of lettuce can directly affect its shelf life, therefore, postharvest treatments on reducing microorganisms and

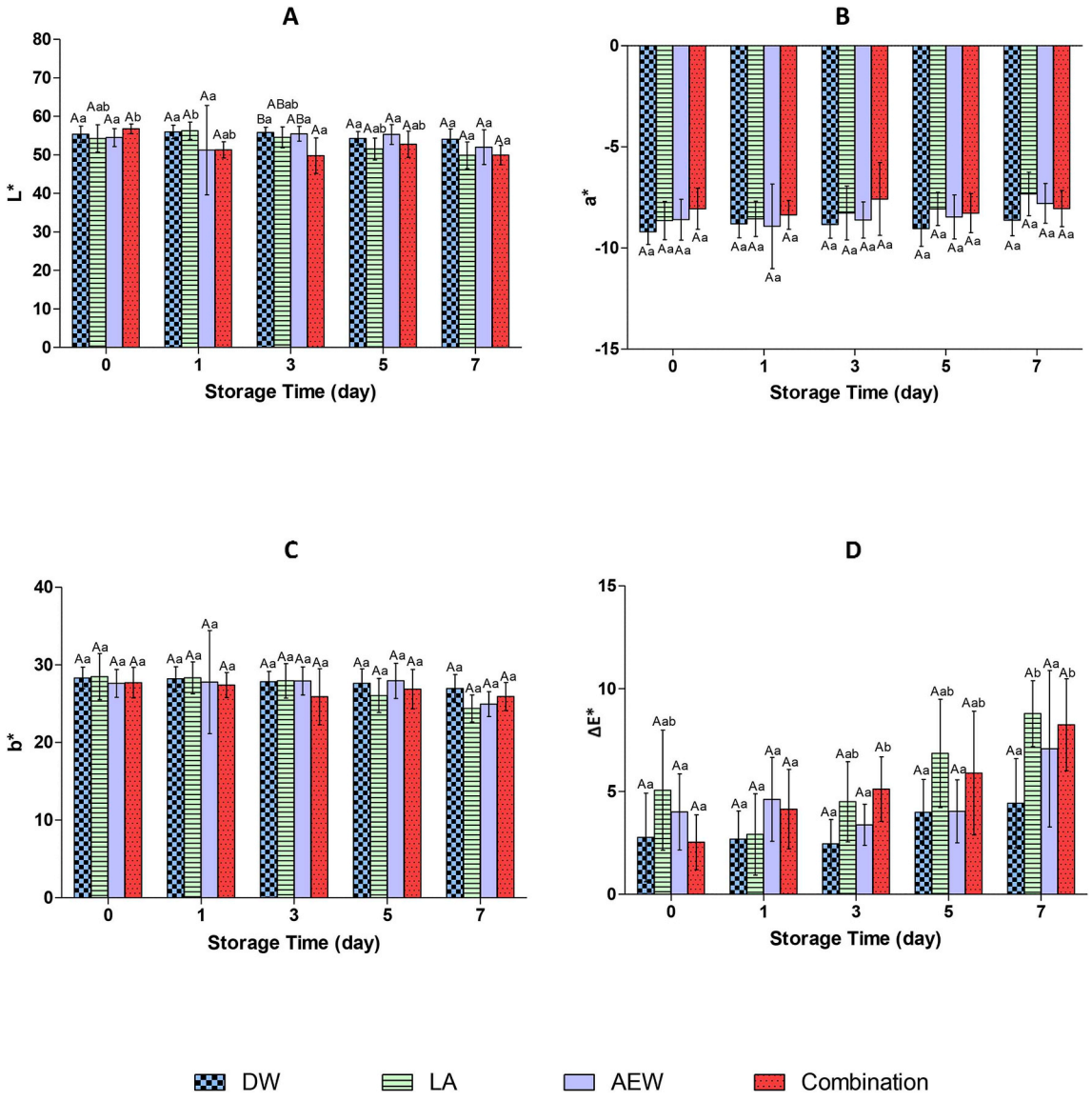

Fig. 4. Effect of different sanitising treatments on colour changes of organic lettuce during storage. (A) L*; (B) a*; (C) b*; (D) overall colour difference of organic lettuce (ΔE*). Data are presented as mean values ± standard deviation. Mean values within the same storage time by different treatments with different capital letters are significantly different; mean values for the same treatments at different storage times with different lowercase letters are significantly different ($P < 0.05$). DW: deionised water; LA: levulinic acid; AEW: acidic electrolysed water; Combination: AEW + LA. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

maintaining produce's quality are of equal importance (Qi, Hu, Jiang, Tian, & Li, 2011).

Fig. 3 shows the effects of different treatments on firmness and electrolyte leakage of organic lettuce during storage. As shown in Fig. 3A, firmness of each group's samples increased significantly as the storage time increased. Considering the firmness was represented by maximum cut force, which can be higher if produce's tissues become softer (Ali, Chin, & Lazan, 2004), the increased firmness results indicated some loss of freshness of lettuce in our current study. It is generally believed that changes of cell wall structures and components can result in increased firmness of produce, and the reactions that happened between the sanitising components and related macromolecules within lettuce could also be a reason (Saftner, Bai, Abbott, & Lee, 2003). Although some slightly different values were observed for different treatments, there was no significant difference in firmness among different treatment groups after the same storage period.

For electrolyte leakage shown in Fig. 3B, an increasing trend appeared in all treatment groups over storage time, with the highest electrolyte leakage happening in combination group from the third day of storage (~25%). Considering electrolyte leakage is connected with cell membrane integrity (Demidchik et al., 2014), higher electrolyte leakage means more tissue damage of produce. The oxidised unsaturated fatty acids located in cell membranes could be one of the reasons that caused ion leakage in some fruits and vegetables (Wang, Feng, & Luo, 2004). When AEW was combined with LA, its oxidation property might be improved due to lower pH and higher ORP values, which could promote unsaturated fatty acid oxidation and decrease membrane fluidity, causing increased electrolyte leakage in produce (Salgado et al., 2014).

The results of colour changes of organic lettuce treated by different sanitisers during storage are shown in Fig. 4. No significant difference was observed among different treatment groups on the same day for L*, a* and b*, individually. However, the overall colour difference, ΔE*, showed to be with more fluctuations and more obvious increasing trends as storage time going on (Fig. 4D). Considering colour is an indicator of the degree of freshness (Zhang & Yang, 2017), our results

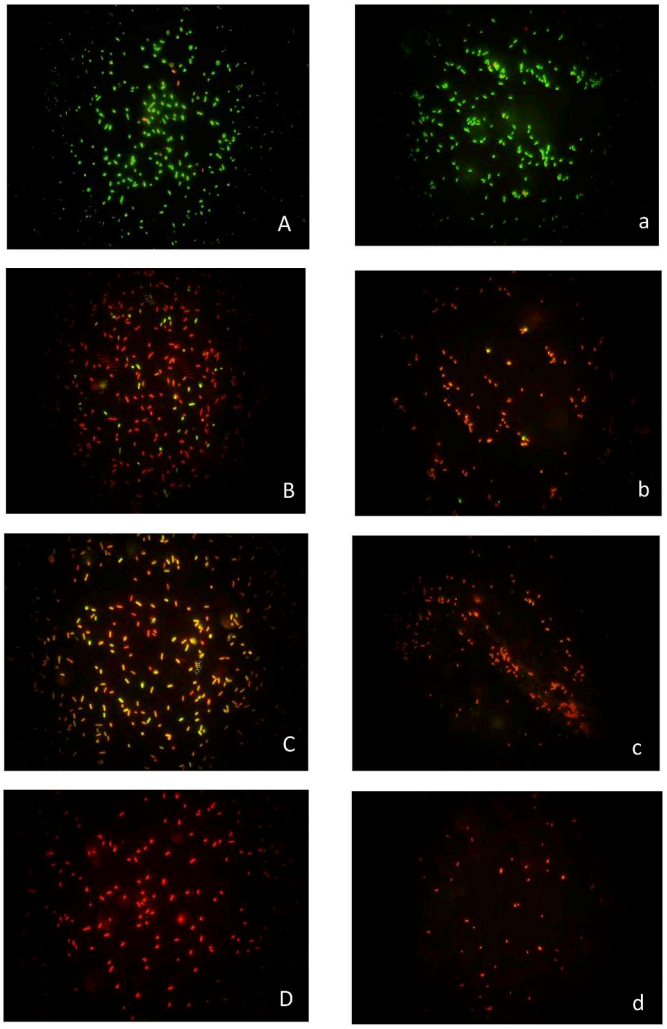

Fig. 5. Fluorescence microscopy images of *E. coli* (first column) and *L. innocua* (second column) cells stained with LIVE/DEAD® *Bac*Light™ after different sanitising treatments. (A and a) DW; (B and b) LA; (C and c) AEW; (D and d) Combination. DW: deionised water; LA: levulinic acid; AEW: acidic electrolysed water; Combination: AEW + LA.

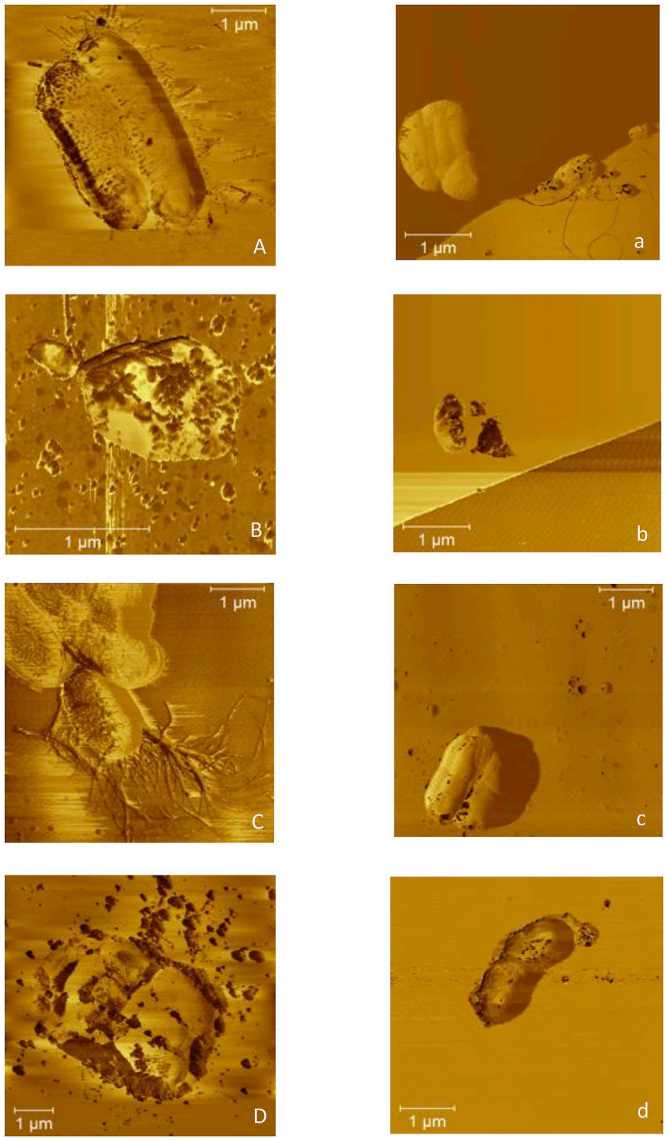

Fig. 6. AFM images of *E. coli* (first column) and *L. innocua* (second column) cells after different sanitising treatments. (A and a) DW; (B and b) LA; (C and c) AEW; (D and d) Combination. DW: deionised water; LA: levulinic acid; AEW: acidic electrolysed water; Combination: AEW + LA. AFM: Atomic force microscopy.

demonstrated that different sanitisers did not accelerate the loss of freshness of organic lettuce, compared to DW treatment. Colourimetric data is frequently used to show the degree of browning, which is also a sign of vegetable aging in fresh-cut products (Cho & Moon, 2014). The higher a* values and the lower L* and b* values of lettuces after 7-day storage indicated the loss of lightness and fresh green colour due to vegetable aging.

3.4. Loss of cell membrane permeability after sanitising treatments

To further analyse the antimicrobial mechanism of each sanitising treatment, LIVE/DEAD® *Bac*Light™ assay kit was used to examine the cell membrane permeability. The kit comprises of two stains, SYTO®9 and propidium iodide (PI). SYTO®9 showing green fluorescence could penetrate both intact and damaged cells' membranes as its molecular weight is lower (~400 Da), while PI of higher molecular weight (668 Da) could only penetrate the damaged cell membrane, showing red fluorescence (Bleichert, Santo, Hanczaruk, Meyer, & Grass, 2014).

In this study, the control group after 7-min DW treatment revealed green fluorescent signal of SYTO®9, whereas other three treatment groups all showed red fluorescence (Fig. 5). The results showed that the cells under LA, AEW and their combination treatments all lost their membrane integrity, which was quantified and shown in Table S1. The percentages of membrane integrity loss were significantly increased after all sanitising treatments, with more than 95% in combination group for both bacteria. The permeability change could be attributed to many factors, such as membrane potential, enzyme activity and pump activity (Joux & Lebaron, 2000). However, in current study, the membrane permeability change caused by the loss of a permeability barrier might be the primary cause, considering only PI is able to enter the cells when permeability barrier is absent.

However, as the epifluorescence microscopy could only determine the cell membrane permeability in a macroscopic scale, the specific damage level of cell membranes caused by each sanitising treatment remained unclear. Therefore, it is necessary to further understand the disinfection mechanism on a nanoscale basis using AFM.

3.5. AFM analyses of cell morphological changes

Applications of AFM involve many areas, such as analysing food components and investigating the structural properties of microbial surfaces related to food safety issues (Liu & Yang, 2018). The

Table 3
Effects of different treatments on the quantification of *E. coli* and *L. innocua* dimension.

Treatment	*E. coli*			*L. innocua*		
	Length (μm)	Width (μm)	Area (μm^2)	Length (μm)	Width (μm)	Area (μm^2)
DW	2.31 ± 0.08^a	0.88 ± 0.04^a	2.83 ± 0.09^a	1.32 ± 0.14^a	0.75 ± 0.08^a	1.53 ± 0.11^a
LA	1.86 ± 0.05^c	0.52 ± 0.04^c	2.15 ± 0.13^c	1.10 ± 0.09^c	0.49 ± 0.08^c	1.28 ± 0.10^c
AEW	2.16 ± 0.11^b	0.62 ± 0.05^b	2.44 ± 0.09^b	1.24 ± 0.13^b	0.64 ± 0.06^b	1.42 ± 0.08^b
Combination	1.89 ± 0.09^c	0.52 ± 0.06^c	2.12 ± 0.10^c	1.14 ± 0.10^c	0.50 ± 0.08^c	1.24 ± 0.10^c

Within each column, means with different letters are significantly different among different treatments ($P < 0.05$). DW, deionised water; LA, levulinic acid; AEW, acidic electrolysed water; Combination: AEW + LA.

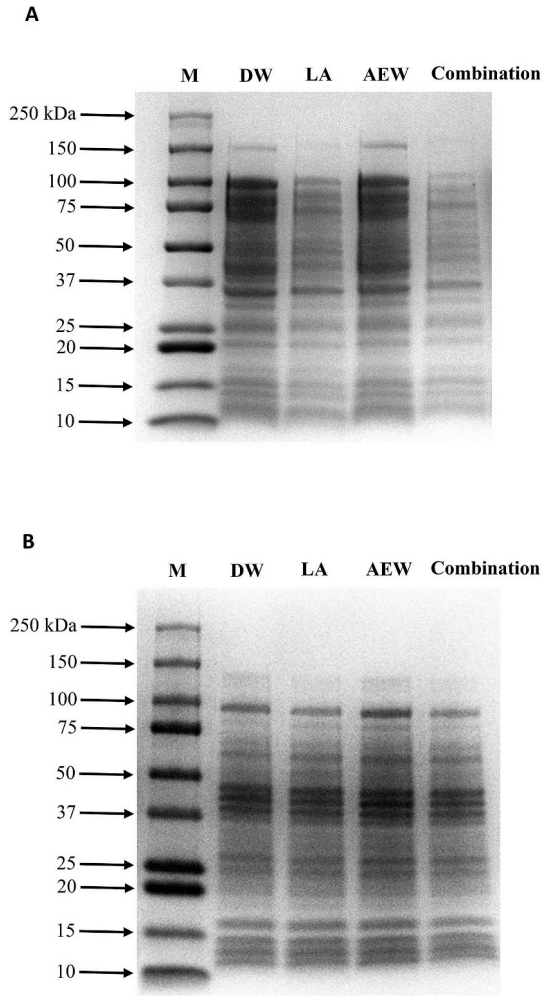

Fig. 7. SDS-PAGE whole protein profiles from *E. coli* (A) and *L. innocua* (B) after different sanitising treatments. M: molecular weight marker; DW: deionised water; LA: levulinic acid; AEW: acidic electrolysed water; Combination: AEW + LA.

nanostructures of *E. coli* and *L. innocua* after each treatment are shown in Fig. 6. There were no visible damages for both cells after 7-min DW treatment, showing typical rod-shaped morphology with smooth surfaces and intact membranes (Fig. 6Aa). After being treated with AEW, there were some slight wrinkles in the surface, but the profiles were still normal with well-defined outer walls (Fig. 6Cc). However, in the groups of LA alone and LA combined with AEW, both *E. coli* and *L. innocua* cells were observed with more disordered cellular structure, irregular and wrinkled surfaces and damaged cell wall (Fig. 6Bb, Dd). In addition to profile changes, some small substances could also be observed, which might be the leakage of intracellular contents of the cells or the aggregation of the cytoplasmic components caused by the change of cell membrane permeability. The results were in good accordance with the epifluorescent micrographs shown in Fig. 5. However, *L. innocua* cells were observed with smaller damage and fewer substances surrounded, compared to *E. coli* cells treated by the same sanitiser (Fig. 6bcd), showing *L. innocua* was more resistant to sanitisers as mentioned in 3.2.

Dimensions of two strains after each sanitising treatment were quantified (Table 3). For each strain in each group, the parallel number was ca. 100 to make results more representative. The cell bodies of both bacteria became smaller after being treated by LA, AEW and their combination, compared to the control group, which might be due to the leakage of cytoplasm as mentioned above (Osafune, Ehara, & Ito, 2006).

In other studies, the structural changes to bacterial cells treated with organic acids and AEW were also examined by TEM. For example, Feliciano, Lee, and Pascall (2012) found that when an organic acidic formulation was used to treat *E. coli* and *L. innocua* cells respectively, the former was more susceptible with a phase separation of the cytoplasm, while the latter could keep its structure intact better, although some losses of definition appeared in the cell wall. The results shown in Feliciano's study supported the current results.

3.6. Analysis on protein SDS-PAGE

After each treatment, the whole cell protein bands of *E. coli* and *L. innocua* in SDS-PAGE are shown in Fig. 7. For *E. coli*, more and clearer protein bands were observed for DW and AEW treated cells, while fewer and fainter bands were observed for the cells treated by LA alone or combined with AEW (Fig. 7A). Besides, the coefficients of similarity of whole cell protein patterns between each two treatments were calculated, which are shown in Table S2. For *E. coli*, the whole cell protein patterns after DW and AEW treatments were very similar with a coefficient of 100%, whereas much lower coefficients of similarity were observed after LA and combined treatments, with 68.7% between DW and LA and 62.5% between DW and combination group. The results obtained for *L. innocua* showed some differences. The number and intensities of the protein bands for all treated cells were similar, with higher coefficients of similarity of whole cell protein patterns between each two treatments, suggesting that current sanitising methods might cause the bacteria death of *L. innocua* not through the destruction of cellular proteins (Fig. 7B, Table S2).

The protein profiles of bacteria can be altered during exposure to stressed environment. Previous studies found that anolyte solution could break down the covalent bonds in proteins and cause the destruction, which might be attributed to the oxidising compounds existed in anolyte (Zinkevich, Beech, Tapper, & Bogdarina, 2000). Moreover, bacteria can synthesise and replace damaged proteins under stress, by activating related encoding genes (Kochhar & Kochhar, 2005). These results are in agreement with our current results, although our change might not be so evident due to our low chlorine concentration in AEW. In addition to protein profile change, some metabolic pathways of bacteria could also be altered under AEW treatment, such as aminoacyl-tRNA biosynthesis, arginine and proline metabolism, which need further investigations (Liu et al., 2017b, 2018).

4. Conclusion

The combination of low concentration AEW (4 mg/L FAC) and LA (3% v/v) showed additional microbiocidal efficacy on fresh organic lettuce in a broad-spectrum range (aerobic bacteria, yeasts and moulds, *E. coli* and *L. innocua*), and did not change the lettuce qualities significantly during storage. Through epifluorescence microscopy and AFM studies, the membrane permeability and morphologies of *E. coli* and *L. innocua* showed visible changes after being treated by LA alone or LA + AEW combination, observed from a macro and micro perspective, respectively. Further analysis of protein profile revealed that *E. coli* and *L. innocua* had different levels of protein degradation due to oxidative stress. Therefore, AEW in combination with LA could be developed as a potential sanitising approach to enhance food safety of fresh organic produce.

Acknowledgements

This study was funded by the Singapore Ministry of Education Academic Research Fund Tier 1 (R-143-000-A40-114), projects 31371851 supported by NSFC, Natural Science Foundation of Jiangsu Province (BK20181184) and an industry grant supported by Shenzhen Zhiyun Optoelectronics Co., Ltd. (R-143-000-A24-597).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodcont.2019.02.039.

References

Adhikari, A., Syamaladevi, R. M., Killinger, K., & Sablani, S. S. (2015). Ultraviolet-C light inactivation of *Escherichia coli* O157: H7 and *Listeria monocytogenes* on organic fruit surfaces. *International Journal of Food Microbiology, 210*, 136–142.

Ahn, J., & Balasubramaniam, V. (2007). Inactivation kinetics of *Listeria innocua* ATCC 33090 at various temperature heating-up and pressure building-up rates. *Food Science and Biotechnology, 16*(2), 85–89.

Ali, Z. M., Chin, L.-H., & Lazan, H. (2004). A comparative study on wall degrading enzymes, pectin modifications and softening during ripening of selected tropical fruits. *Plant Science, 167*(2), 317–327.

Bleichert, P., Santo, C. E., Hanczaruk, M., Meyer, H., & Grass, G. (2014). Inactivation of bacterial and viral biothreat agents on metallic copper surfaces. *Biometals, 27*(6), 1179–1189.

Chen, L., Zhang, H., Liu, Q., Pang, X., Zhao, X., & Yang, H. (2019). Sanitising efficacy of lactic acid combined with low-concentration sodium hypochlorite on *Listeria innocua* in organic broccoli sprouts. *International Journal of Food Microbiology, 295*, 41–48.

Chhetri, V. S., Janes, M. E., King, J. M., Doerrler, W., & Adhikari, A. (2019). Effect of residual chlorine and organic acids on survival and attachment of *Escherichia coli* O157:H7 and *Listeria monocytogenes* on spinach leaves during storage. *LWT-Food Science and Technology, 105*, 298–305.

Cho, J.-S., & Moon, K.-D. (2014). Comparison of image analysis methods to evaluate the degree of browning of fresh-cut lettuce. *Food Science and Biotechnology, 23*(4), 1043–1048.

Cloete, T. E., Thantsha, M., Maluleke, M., & Kirkpatrick, R. (2009). The antimicrobial mechanism of electrochemically activated water against *Pseudomonas aeruginosa* and *Escherichia coli* as determined by SDS-PAGE analysis. *Journal of Applied Microbiology, 107*(2), 379–384.

Demidchik, V., Straltsova, D., Medvedev, S. S., Pozhvanov, G. A., Sokolik, A., & Yurin, V. (2014). Stress-induced electrolyte leakage: The role of K^+-permeable channels and involvement in programmed cell death and metabolic adjustment. *Journal of Experimental Botany, 65*(5), 1259–1270.

Feliciano, L., Lee, J., & Pascall, M. A. (2012). Transmission electron microscopic analysis showing structural changes to bacterial cells treated with electrolyzed water and an acidic sanitizer. *Journal of Food Science, 77*(4), M182–M187.

Flemming, H.-C., & Wingender, J. (2010). The biofilm matrix. *Nature Reviews Microbiology, 8*(9), 623.

Ghate, V., Kumar, A., Kim, M.-J., Bang, W.-S., Zhou, W., & Yuk, H.-G. (2017). Effect of 460 nm light emitting diode illumination on survival of *Salmonella* spp. on fresh-cut pineapples at different irradiances and temperatures. *Journal of Food Engineering, 196*, 130–138.

Gil, M. M., Miller, F. A., Brandao, T. R., & Silva, C. L. (2011). On the use of the Gompertz model to predict microbial thermal inactivation under isothermal and non-isothermal conditions. *Food Engineering Reviews, 3*(1), 17–25.

Guo, Z., & Olsson, L. (2014). Physiological response of *Saccharomyces cerevisiae* to weak acids present in lignocellulosic hydrolysate. *FEMS Yeast Research, 14*(8), 1234–1248.

Hao, X., Li, B., Zhang, Q., Lin, B. Z., Ge, L., Wang, C., et al. (2013). Disinfection effectiveness of slightly acidic electrolysed water in swine barns. *Journal of Applied Microbiology, 115*(3), 703–710.

Hati, S., Mandal, S., Minz, P., Vij, S., Khetra, Y., Singh, B., et al. (2012). Electrolyzed oxidized water (EOW): Non-thermal approach for decontamination of food borne microorganisms in food industry. *Food and Nutrition Sciences, 3*(06), 760–768.

Ilic, S., Rajić, A., Britton, C. J., Grasso, E., Wilkins, W., Totton, S., et al. (2012). A scoping study characterizing prevalence, risk factor and intervention research, published between 1990 and 2010, for microbial hazards in leafy green vegetables. *Food Control, 23*(1), 7–19.

Josewin, S. W., Ghate, V., Kim, M.-J., & Yuk, H.-G. (2018). Antibacterial effect of 460 nm light-emitting diode in combination with riboflavin against *Listeria monocytogenes* on smoked salmon. *Food Control, 84*, 354–361.

Joux, F., & Lebaron, P. (2000). Use of fluorescent probes to assess physiological functions of bacteriaat single-cell level. *Microbes and Infection, 2*(12), 1523–1535.

Kim, J. G., Luo, Y., Tao, Y., Saftner, R. A., & Gross, K. C. (2005). Effect of initial oxygen concentration and film oxygen transmission rate on the quality of fresh-cut romaine lettuce. *Journal of the Science of Food and Agriculture, 85*(10), 1622–1630.

Kim, M.-J., Mikš-Krajnik, M., Kumar, A., & Yuk, H.-G. (2016). Inactivation by 405 ± 5 nm light emitting diode on *Escherichia coli* O157: H7, *Salmonella* typhimurium, and *Shigella sonnei* under refrigerated condition might be due to the loss of membrane integrity. *Food Control, 59*, 99–107.

Kochhar, S., & Kochhar, V. (2005). Expression of antioxidant enzymes and heat shock proteins in relation to combined stress of cadmium and heat in *Vigna mungo* seedlings. *Plant Science, 168*(4), 921–929.

Koyama, K., Hokunan, H., Hasegawa, M., Kawamura, S., & Koseki, S. (2017). Estimation of the probability of bacterial population survival: Development of a probability model to describe the variability in time to inactivation of *Salmonella enterica*. *Food Microbiology, 68*, 121–128.

Liao, L. B., Chen, W. M., & Xiao, X. M. (2007). The generation and inactivation mechanism of oxidation–reduction potential of electrolyzed oxidizing water. *Journal of Food Engineering, 78*(4), 1326–1332.

Liu, Q., Tan, C. S. C., Yang, H., & Wang, S. (2017a). Treatment with low-concentration acidic electrolysed water combined with mild heat to sanitise fresh organic broccoli (*Brassica oleracea*). *LWT-Food Science and Technology, 79*, 594–600.

Liu, Q., Wu, J. e., Lim, Z. Y., Aggarwal, A., Yang, H., & Wang, S. (2017b). Evaluation of the metabolic response of *Escherichia coli* to electrolysed water by ^{1}H NMR spectroscopy. *LWT-Food Science and Technology, 79*, 428–436.

Liu, Q., Wu, J. e., Lim, Z. Y., Lai, S., Lee, N., & Yang, H. (2018). Metabolite profiling of *Listeria innocua* for unravelling the inactivation mechanism of electrolysed water by nuclear magnetic resonance spectroscopy. *International Journal of Food Microbiology, 271*, 24–32.

Liu, Q., & Yang, H. (2018). Application of atomic force microscopy in food microorganisms. *Trends in Food Science & Technology*. https://doi.org/10.1016/j.tifs.2018.05.010.

[NOP]. National Organic Program (2011). *The use of chlorine materials in organic production & handling.* NOP 5026 https://www.ams.usda.gov/sites/default/files/media/5026.pdf (Accessed 7 Dec. 2018) .

Osafune, T., Ehara, T., & Ito, T. (2006). Electron microscopic studies on bactericidal effects of electrolyzed acidic water on bacteria derived from kendo protective equipment. *Environmental Health and Preventive Medicine, 11*(4), 206–214.

OTA (2018). *Organic trade association.* https://ota.com/resources/organic-industry-survey (Accessed 7 Dec. 2018).

Pla, M.-L., Oltra, S., Esteban, M.-D., Andreu, S., & Palop, A. (2015). Comparison of primary models to predict microbial growth by the plate count and absorbance methods. *BioMed Research International, 2015*, 365025 14 pages.

Qi, H., Hu, W., Jiang, A., Tian, M., & Li, Y. (2011). Extending shelf-life of fresh-cut 'Fuji'apples with chitosan-coatings. *Innovative Food Science & Emerging Technologies, 12*(1), 62–66.

Rahman, S., Ding, T., & Oh, D.-H. (2010). Effectiveness of low concentration electrolyzed water to inactivate foodborne pathogens under different environmental conditions. *International Journal of Food Microbiology, 139*(3), 147–153.

Saftner, R. A., Bai, J., Abbott, J. A., & Lee, Y. S. (2003). Sanitary dips with calcium propionate, calcium chloride, or a calcium amino acid chelate maintain quality and shelf stability of fresh-cut honeydew chunks. *Postharvest Biology and Technology, 29*(3), 257–269.

Salgado, S. P., Pearlstein, A. J., Luo, Y., & Feng, H. (2014). Quality of iceberg (*Lactuca sativa L.*) and romaine (*L. sativa L. var. longifolial*) lettuce treated by combinations of sanitizer, surfactant, and ultrasound. *LWT-Food Science and Technology, 56*(2), 261–268.

Smith, J. V. (2011). *Evaluation of levulinic acid for topical decontamination of meat surfaces* (Dissertation/Thesis). ProQuest Dissertations Publishing.

Sow, L. C., Tirtawinata, F., Yang, H., Shao, Q., & Wang, S. (2017). Carvacrol nanoemulsion combined with acid electrolysed water to inactivate bacteria, yeast in *vitro* and native microflora on shredded cabbages. *Food Control, 76*, 88–95.

Tango, C. N., Mansur, A. R., Kim, G. H., & Oh, D. H. (2014). Synergetic effect of combined fumaric acid and slightly acidic electrolysed water on the inactivation of food-borne pathogens and extending the shelf life of fresh beef. *Journal of Applied Microbiology, 117*(6), 1709–1720.

Valdivia-Nájar, C. G., Martín-Belloso, O., Giner-Seguí, J., & Soliva-Fortuny, R. (2017). Modeling the inactivation of *Listeria innocua* and *Escherichia coli* in fresh-cut tomato treated with pulsed light. *Food and Bioprocess Technology, 10*(2), 266–274.

Vasavada, M., Carpenter, C. E., Cornforth, D. P., & Ghorpade, V. (2003). Sodium levulinate and sodium lactate effects on microbial growth and stability of fresh pork and Turkey sausages. *Journal of Muscle Foods, 14*(2), 119–129.

Walker, J. M. (2002). *The bicinchoninic acid (BCA) assay for protein quantitation. The Protein Protocols Handbook.* Springer.

Wang, H., Feng, H., & Luo, Y. (2004). Microbial reduction and storage quality of fresh-cut cilantro washed with acidic electrolyzed water and aqueous ozone. *Food Research International, 37*(10), 949–956.

Zhang, J., & Yang, H. (2017). Effects of potential organic compatible sanitisers on organic and conventional fresh-cut lettuce (*Lactuca sativa* Var. *Crispa* L). *Food Control, 72*, 20–26.

Zhao, L., Zhang, Y., & Yang, H. (2017). Efficacy of low concentration neutralised electrolysed water and ultrasound combination for inactivating *Escherichia coli* ATCC 25922, *Pichia pastoris* GS115 and *Aureobasidium pullulans* 2012 on stainless steel coupons. *Food Control, 73*, 889–899.

Zhao, T., Zhao, P., Chen, D., Jadeja, R., Hung, Y.-C., & Doyle, M. P. (2014). Reductions of Shiga toxin–producing *Escherichia coli* and *Salmonella* Typhimurium on beef trim by lactic acid, levulinic acid, and sodium dodecyl sulfate treatments. *Journal of Food Protection, 77*(4), 528–537.

Zinkevich, V., Beech, I., Tapper, R., & Bogdarina, I. (2000). The effect of super-oxidized water on *Escherichia coli*. *Journal of Hospital Infection, 46*(2), 153–156.