LWT - Food Science and Technology 98 (2018) 524–532

Contents lists available at ScienceDirect

LWT - Food Science and Technology

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

# Physicochemical and antibacterial effects of sodium bicarbonate and brine water on the electrolysed water generated by a portable sanitising unit

Jufang Zhang[a,b], Shaojuan Lai[c,d], Hongshun Yang[a,b,*]

[a] Food Science and Technology Programme, c/o Department of Chemistry, National University of Singapore, Singapore, 117543, Singapore
[b] National University of Singapore (Suzhou) Research Institute, 377 Lin Quan Street, Suzhou Industrial Park, Suzhou, Jiangsu, 215123, PR China
[c] College of Food Science and Technology, Henan University of Technology, Zhengzhou, Henan, 450001, PR China
[d] Guangzhou Pulu Medical Technology Co., Ltd, Guangzhou, Guangdong, 510800, PR China

ARTICLE INFO

*Keywords:*
Chlorine
Electrolyzed water
Sanitization
Sodium bicarbonate
Free radical

ABSTRACT

A novel method was used to produce near neutral pH electrolysed water (NEW) by developing a portable electrochemical sanitising unit that uses diluted sodium chloride and sodium bicarbonate solution (6 mM) as electrolytes. The unit produced NEW at pH 5.70 to 7.1, an oxidation-reduction potential of 802.2–933.8 mV, and free available chlorine (FAC) of 3.3–70 mg/L. NEW produced by the unit with NaCl 10 g/L showed stronger bactericidal effects than NEW produced by mixing the anode and cathode product from commercial unit's ($P < 0.05$): 2.03 log colony forming units (CFU)/mL reductions compared with 1.66 log CFU/mL reductions. To further understand the sanitising result, electron spin resonance and flow cytometry were performed. The results showed NEW produced by the developed unit induced 82.2% injured cells compared with 54.4% by NEW without detected free radicals. Overall, the current efficiency and power consumption were increased during NEW generation for NEW using sodium bicarbonate compared with sole NEW. The developed NEW generator is a promising sanitising unit for consumers and food industry to control foodborne pathogens.

## 1. Introduction

It is essential to provide adequate sanitising treatments during the processing and preparation of fruit and vegetables in family kitchens and in the food industry, especially because of the increasing consumption of organic foods worldwide (Yu & Yang, 2017). Electrolysed water (EW), also referred to as acidic EW, which is produced by electrolysing a diluted NaCl solution with direct current (DC) (Liu, Tan, Yang, & Wang, 2017a; Liu et al., 2017b; Sow, Tirtawinata, Yang, Shao, & Wang, 2017), has been approved as a food sanitiser by the Food and Drug Administration (Zhang & Yang, 2017). However, at low pH, EW is corrosive, unstable, might be toxic to operators, and causes corrosion of different types of metals. A feasible approach is the application of a nearly neutral EW (NEW; pH 6.0–7.5) (Ayebah & Hung, 2005).

A number of studies demonstrate that NEW has a strong antibacterial ability against a variety of foodborne pathogens, such as *Salmonella enteritidis*, *Listeria monocytogenes*, *Bacillus cereus*, and *Escherichia coli* O157:H7 in fruit and vegetables, and food processing equipment (Gil, Gómez-López, Hung, & Allende, 2015; Len, Hung, Erickson, & Kim, 2000; Liu et al., 2018; Luo, Kim, Wang, & Oh, 2016; Park, Guo, Rahman, Ahn, & Oh, 2009; Xuan et al., 2016). However, the NEW used was mainly produced by current commercial NEW-producing units with NaCl or NaCl mixed with HCl as electrolytes. These units are quite large so that they are not convenient for households and small food industries (Zhang, Zhou, Chen, & Yang, 2017; Yang, Feirtag, & Diez-Gonzalez, 2013).

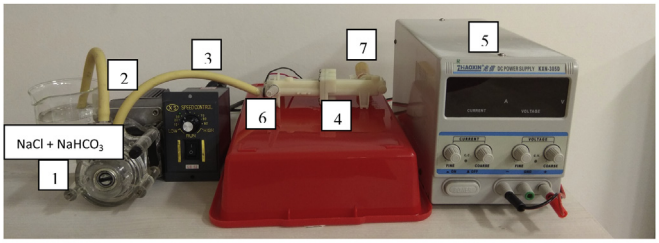
$NaHCO_3$ is a food additive that is allowed for application on various commodities. In addition, $NaHCO_3$ is one of the most effective electrolytes because of its high ionic mobility between cations and anions (NaCl: 0.767, $NaHCO_3$: 0.887) (Hanaoka, Sun, Lawrence, Kamitani, & Fernandes, 2004). During electrolysis, the carbonate radical ($CO_3^{\cdot-}$) may be produced, which is a potent strong one-electron oxidant (1.78 V *versus* the standard hydrogen electrode (SHE) at pH 7) (Augusto et al., 2002; Behar, Czapski, & Duchovny, 1970; Saha, Furuta, & Nishiki, 2004). Thus, the electrolysis of $NaHCO_3$ with an NaCl solution might enhance the sanitising effect of NEW.

In our previous study, we developed a portable flow-through electrochemical sanitising unit that can produce NEW (Zhang, Yang, & Chan, 2018; Zhang et al., 2017); however, the current efficiency (CE) is low and the energy consumption is high because the two electrolytic cells need to be used at the same time.

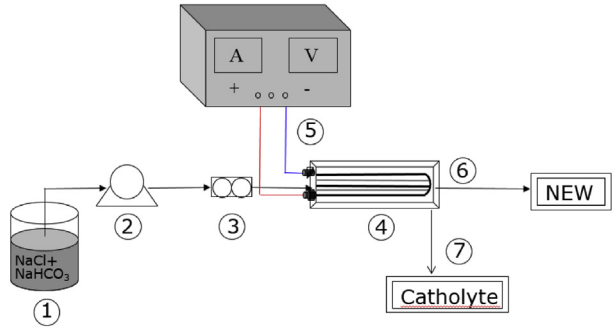
The objective of this research was to investigate the addition of

* Corresponding author. Food Science and Technology Programme, c/o Department of Chemistry, National University of Singapore, Singapore, 117543, Singapore.
*E-mail address:* chmynghs@nus.edu.sg (H. Yang).

https://doi.org/10.1016/j.lwt.2018.08.048
Received 17 January 2018; Received in revised form 26 June 2018; Accepted 23 August 2018
Available online 30 August 2018
0023-6438/ 




(A)




(B)

**Fig. 1.** (A) Overview of the portable neutral electrolysed water (NEW) producing unit. (B) Schematic illustration of the portable NEW producing unit: 1, electrolyte; 2, pump; 3, controller; 4, electrolytic cell; 5, power supply; 6, NEW outlet; 7, catholyte outlet.

$NaHCO_3$ on the properties (free available chlorine (FAC), oxidation-reduction potential (ORP), and pH) of electrolysed water based on an NaCl solution. The effect on current efficiency and power consumption during EW generation was also evaluated. The sanitising effect of NEW yielded by the developed unit, and NEW produced by a commercial EW generator on *E. coli* O157:H7 C7927 and *L. monocytogenes* BAA-839 were compared. Flow cytometry (FCM) analysis and electron spin resonance (ESR) were used to further understand the sanitising mechanism.

## 2. Materials and methods

### 2.1. Portable NEW producing unit

Fig. 1A shows an overview of the developed portable NEW producing unit, which consists of two kinds of electrolyte (NaCl solution and $NaHCO_3$ solution), a peristaltic pump, a controller (Nanjing Runzefluid control equipment CO., Ltd, Nanjing, Jiangsu, China), an electrolytic cell (Dongguan Sunrise Environmental Technology Co., Ltd, Guangzhou, Guangdong, China) (L × W × H, 100 mm × 50 mm × 10 mm), and a DC power supply (KXN-305D, Shenzhen Zhaoxin Electronic Instrument Equipment Co., Ltd, Shenzhen, Guangdong, China). Fig. 1B shows a schematic illustration of the portable NEW producing unit with the addition of $NaHCO_3$. NEW was generated from the outlet of the anode chamber of the electrolytic cell.

### 2.2. Analytical measurements of NEW

All chemicals used in this study were of analytical grade. Deionised water (DI) was used for cleaning and dissolving solutes. The FAC concentration was determined by the iodometric method (Tang, Li, Li, Chen, & Zeng, 2016). Briefly, potassium iodide was mixed with EW. Chlorine was reduced by potassium iodide, forming an equivalent amount of iodine, which was titrated with sodium thiosulfate ($Na_2S_2O_3$). ORP was measured using a Mettler Toledo Seven compact

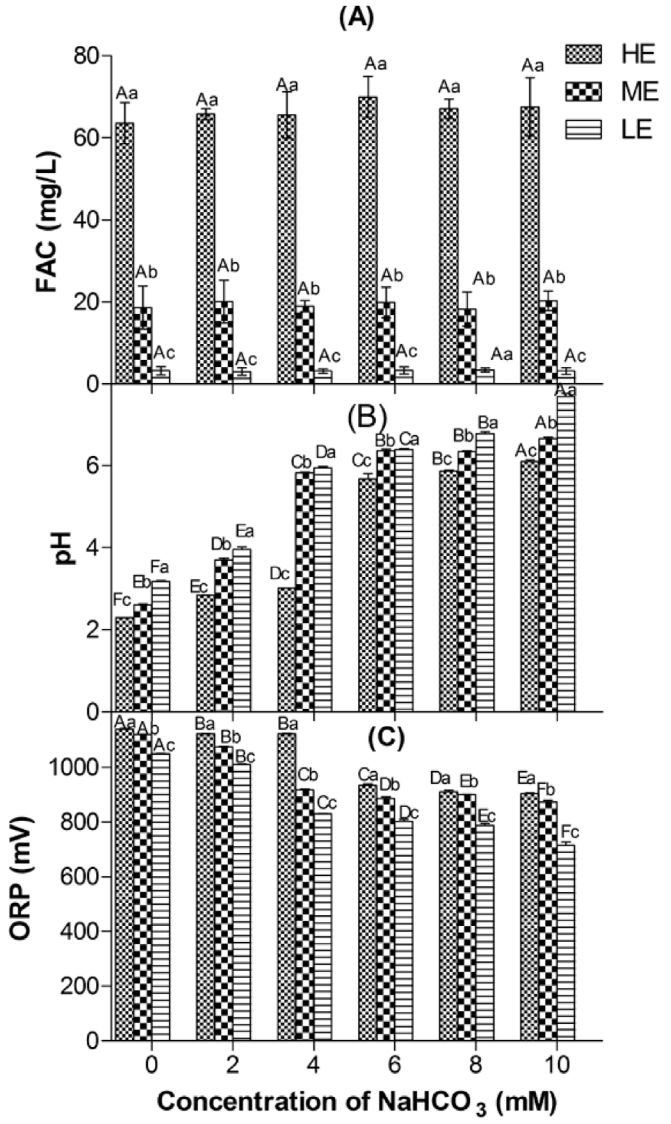



**Fig. 2.** Influence of $NaHCO_3$ concentration on free available chlorine (FAC), pH and oxidation-reduction potential (ORP) of electrolysed water* with supply of dilute NaCl solution**.

*HE: high electrolysis conditions, 10 g/L NaCl concentration, 400 mL/min, 30 mA/cm$^2$; ME: middle electrolysis condition, 6 g/L NaCl concentration, 600 mL/min, 22.5 mA/cm$^2$; LE: low electrolysis condition: 2 g/L NaCl concentration, 900 mL/min, 7.5 mA/cm$^2$.

**Means within each $NaHCO_3$ concentration with different lowercase letters are significantly different among the different electrolysis conditions ($P < 0.05$); means within each group with different capital letters are significantly different among different $NaHCO_3$ concentrations ($P < 0.05$).

ORP meter (Metrohm Singapore Pte, Ltd, Singapore), and the pH was measured with a Thermo Orion 410 pH meter (Thermo Scientific, Waltham, MA, USA).

### 2.3. Current efficiency and energy consumption

The CE is defined as the ratio of actual active chlorine productivity to theoretical chlorine productivity. Producing chlorine through electrochlorination is a two-electron transfer process; therefore, the CE was calculated according to Faraday's law, as follows (Choi, Shim, & Yoon, 2013):

$$\text{Current Efficiency (\%)} = \frac{\text{vol}\times\Delta C \times n \times F}{i_{app} \times t} \times 100\%$$

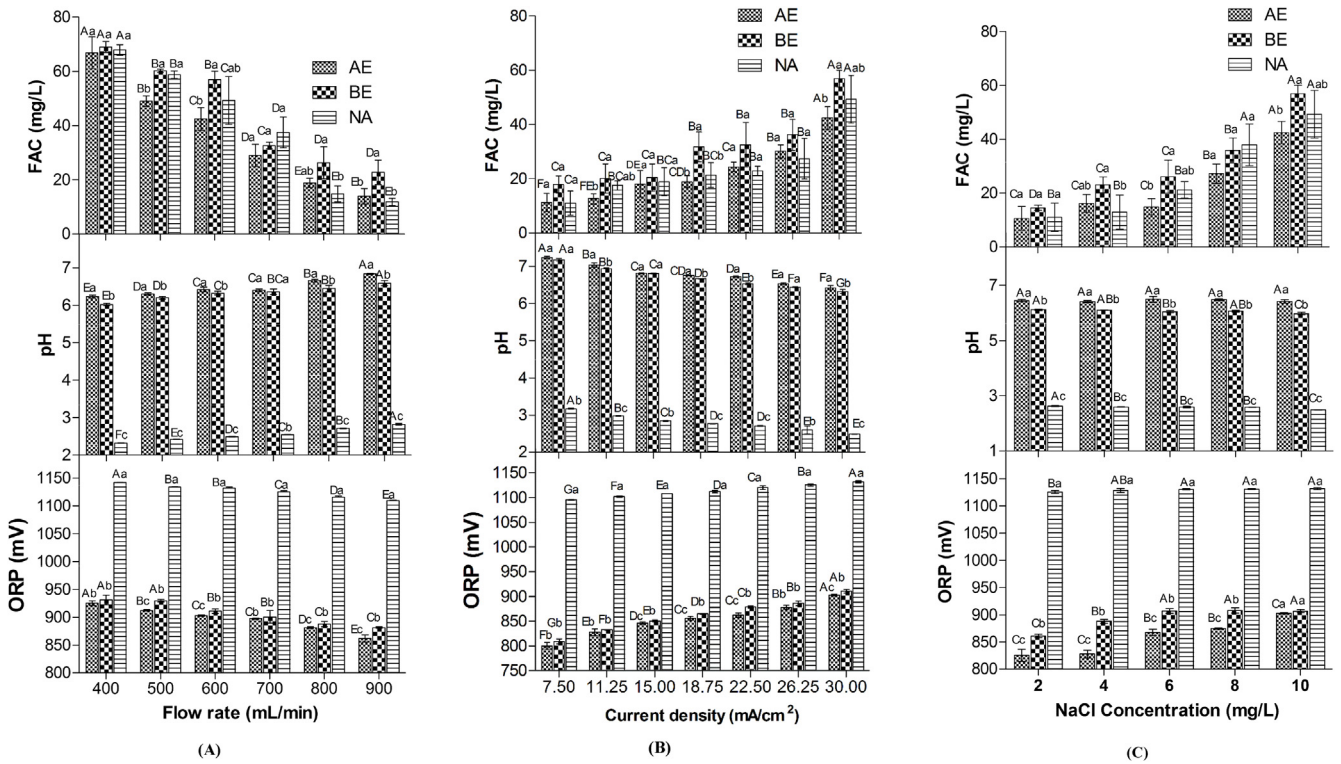



**Fig. 3.** Effect of flow rate (A), current density (B) and NaCl concentration (C) on the free available chlorine (FAC) concentration, pH, and oxidation-reduction potential (ORP) of neutral electrolysed water (NEW)* electrolysis process **.

*AE: add $NaHCO_3$ after electrolysis; BE: add $NaHCO_3$ before electrolysis; NA: without adding $NaHCO_3$.

** Means within each flow rate with different lowercase letters are significantly different among different electrolysis processes ($P < 0.05$); means within each group with different capital letters are significantly different among different flow rates ($P < 0.05$).

where $i_{app}$ is the applied current (A), $t$ is the elapsed time (s), Vol is the solution volume (L), $\Delta C$ is the concentration difference in chlorine (M), n is the number of electrons required, and F is the Faraday constant (96,485 C mol$^{-1}$).

Energy consumption was calculated as reported by Zaviska, Drogui and Pablo (2012), with a slight modification:

$$E = \frac{U*I}{v}$$

where $E$ is the energy consumption (kWh × m$^{-3}$), $I$ is the current intensity (A), $U$ is the electrical potential (V), and $v$ is the flow rate of NaCl solution (m$^3$/h).

### 2.4. Efficacy of sanitising effect

The sanitising effects of NEW samples (20 mL) obtained from the developed portable electrolytic unit were compared to those of NEW samples prepared from a commercial electrolytic unit (Hoshizaki ROX-10WB3-EW, Smitech (Asia) Pte Ltd, Singapore). *E. coli* O157:H7 (strain C7927), and *L. monocytogenes* (strain ATCC BAA-839) were used in this study. The bactericidal activity of the NEW samples was determined as previously reported, with slight modifications (Yang et al., 2013; Zhao, Zhang, & Yang, 2017). Briefly, 24-h bacterial suspensions (10 mL each) were centrifuged (3000 × *g*, 4 °C) for 10 min, and the resulting pellets were rinsed with 10 mL of sterile 0.1% peptone water (PW), centrifuged, and re-suspended in 10 mL of PW. Subsequently, 1 mL of each bacterial suspension was mixed with 9 mL of each of the NEW samples. Aliquots (1 mL) were added to 9 mL of neutralising buffer solutions (5.2 g/L; Becton, Dickinson and Company, Sparks, MD, USA). The neutralised mixture was serially diluted for plating on petri dishes containing nutrient agar. Following incubation at 37 °C for 24 h, bacterial colonies were counted. For each bacterial strain, two separate experiments were performed independently. For each experiment, parallel groups were carried out in duplicate resulting in four observations for each strain.

### 2.5. Identification of free radical species by electron spin resonance (ESR)

The free radicals were examined according to the previous studies, with slight modifications (Mokudai, Nakamura, Kanno, & Niwano, 2012; Xiong, Liu, & Liu, 2010). An aliquot (180 μL) of NEW produced by the portable electrolytic sanitising unit and CNEW (produced by mixing the anode and cathode product) was mixed with 40 μL of 1 M 5, 5-dimethyl-1-pyrroline-*N*-oxide (DMPO) for 10 s. Immediately after mixing, the mixture was transferred to an ESR spectrometry cell, and the ESR measurements were started after 30 s on an X-band of JEOL (FA200) spectrometer. The measurement conditions were as follows: field modulation frequency, 100 kHz; field modulation width, 0.1 mT; amplitude, 2; sweep time, 30 s; time constant, 0.03 s; microwave frequency, 9.192 GHz; and microwave power, 1 mW.

### 2.6. Staining procedure and flow cytometry analysis

Membrane damage in bacteria following treatment by NEW produced from the developed sanitising unit was evaluated using SYTO9 (a green fluorescent nucleic acid stain that stains live and dead gram-positive and gram-negative bacteria) and propidium iodide (PI) dye from the LIVE/DEAD BacLight kit (Molecular Probes, Thermo Fisher Scientific, Eugene, OR, USA). The stock solutions of the dyes were prepared according to the LIVE/DEAD BacLight kit instruction and diluted 2× in 0.22 μm-filtered sterilised DI water. Flow cytometry (FCM) analysis with fluorescence staining was performed according to the method described previously, with slight modification (Berney, Hammes, Bosshard, Weilenmann, & Egli, 2007). Briefly, after

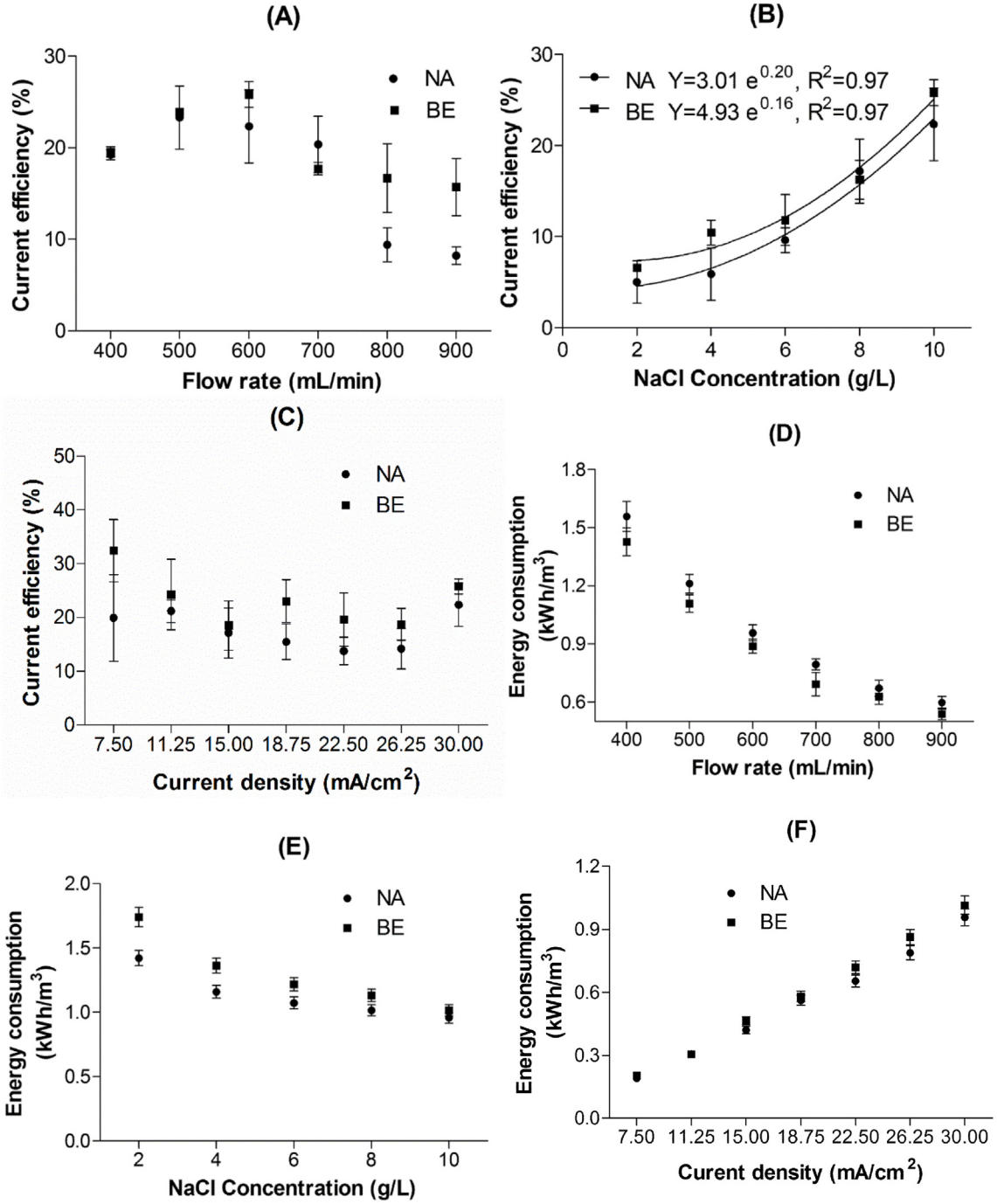



**Fig. 4.** Current efficiency and energy consumption as a function of flow rate, NaCl concentration and current density (A, B: current density = 30 mA/cm$^2$, [NaCl] = 10 g/L; C, D, current density = 30 mA/cm$^2$, flow rate = 600 mL/min; E, F: [NaCl] = 10 g/L, flow rate = 600 mL/min). BE: add $NaHCO_3$ before electrolysis; NA: without adding $NaHCO_3$.

treatment, the neutralised *E. coli* (by 0.22 μm-filtered neutralising buffer) samples (600 μL) were immediately stained with two mixed probes (SYTO9/PI). The mixtures were incubated in the dark for 15 min, then the stained samples were kept in the dark on ice and used within 1 h for FCM analysis (Berney et al., 2007).

FCM was performed on a CyAn$^{BC}$ ADP flow cytometer, with excitation/emission of 480/500 nm for SYTO9 and 490/635 nm for PI, respectively. The Summit 4.3 software package was used to analyse the FCM data. The signal/background ratio was calculated as fluorescence with sanitiser/fluorescence without sanitiser.

### 2.7. Statistical analysis

Data were reported as the mean ± the standard deviation. ANOVA and Duncan's test were performed using SAS software (SAS Institute Inc., Cary, NC, USA). Statistical significance was set at $P < 0.05$.

## 3. Results and discussion

### 3.1. Development of the NEW generating unit

#### 3.1.1. The effect of the $NaHCO_3$ concentration on properties of EW

The influence of different concentrations of $NaHCO_3$ (0, 2, 4, 6, 8,

**Table 1**
Physicochemical properties of near neutral electrolysed water (NEW) solutions*.

| NEW group | FAC (mg/L) | ORP (mV)** | pH |
|---|---|---|---|
| DI*** | 0.0 ± 0.0$^{f}$ | 330.1 ± 8.0$^{g}$ | 7.02 ± 0.17$^{a}$ |
| CNEW**** | 4.2 ± 0.2$^{e}$ | 830.3 ± 14.0$^{f}$ | 6.33 ± 0.11$^{bc}$ |
| | 8.3 ± 0.4$^{d}$ | 832.0 ± 7.7$^{f}$ | 6.29 ± 0.12$^{bc}$ |
| | 10.4 ± 0.5$^{c}$ | 839.8 ± 5.4$^{f}$ | 6.18 ± 0.15$^{bc}$ |
| | 20.2 ± 2.0$^{b}$ | 844.8 ± 9.3$^{f}$ | 6.09 ± 0.17$^{bcd}$ |
| | 41.7 ± 2.1$^{a}$ | 868.6 ± 6.8$^{de}$ | 6.04 ± 0.14$^{cd}$ |
| NEWC | 4.3 ± 0.1$^{e}$ | 835.7 ± 18.8$^{f}$ | 6.48 ± 0.22$^{b}$ |
| | 8.6 ± 0.2$^{d}$ | 872.5 ± 4.5 $^{cde}$ | 6.2 ± 0.15$^{bc}$ |
| | 11.2 ± 0.8$^{c}$ | 876.8 ± 3.1$^{bcde}$ | 6.14 ± 0.06$^{bcd}$ |
| | 19.7 ± 1.5$^{b}$ | 887.9 ± 3.5$^{bc}$ | 5.99 ± 0.12$^{cd}$ |
| | 40.3 ± 1.5$^{a}$ | 910.8 ± 3.8$^{a}$ | 5.91 ± 0.11$^{d}$ |
| NEWCD | 4.0 ± 0.2$^{e}$ | 862.9 ± 12.6$^{e}$ | 6.18 ± 0.13$^{bc}$ |
| | 8.1 ± 0.3$^{d}$ | 868.2 ± 3.9 $^{de}$ | 6.13 ± 0.06$^{bcd}$ |
| | 10.1 ± 0.4$^{c}$ | 881.4 ± 10.3$^{bcd}$ | 6.09 ± 0.05$^{bcd}$ |
| | 20.2 ± 0.8$^{b}$ | 891.4 ± 9.7$^{b}$ | 5.99 ± 0.11$^{cd}$ |
| | 40.3 ± 1.5$^{a}$ | 908.8 ± 6.7$^{a}$ | 5.92 ± 0.10$^{d}$ |

* Different lowercase letters within a column represent significant differences ($P < 0.05$).
** FAC: Free available chlorine concentration, ORP: oxidation-reduction potential.
***DI: Deionised water.
**** CNEW: produced by a commercial sanitiser.
NEWC: produced by electrolysis of NaCl + $NaCHO_3$.
NEWCD: produced by diluting NEWC.

and 10 mM) on the properties (FAC, ORP and pH) of EW produced under three electrolysis conditions (HE, high electrolysis condition: 10 g/L NaCl, 400 mL/min, 30 mA/cm$^2$; ME, middle electrolysis condition: 6 g/L NaCl, 600 mL/min, 22.5 mA/cm$^2$; and LE, low electrolysis condition: 1 g/L NaCl, 900 mL/min, 7.5 mA/cm$^2$) is shown in Fig. 2.

Generally, with increasing $NaHCO_3$ concentration, ORP was significantly decreased and pH was significantly increased from acidic (2.7) to nearly neutral (6.0–7.5) and then to weak alkaline (greater than 7.5 (Gil et al., 2015)); however, there was no significant change in the FAC level for each electrolysis condition. According to these results, it was obvious that an $NaHCO_3$ concentration of 6 mM was the ideal concentration, at which NEW (pH: 5.70–7.1, ORP: 802.2–933.8 mV, FAC: 3.3–70 mg/L) could be produced under all three electrolysis conditions. Although an $NaHCO_3$ concentration of 8 mM could also produce NEW under the three electrolysis conditions, the ORP was significantly decreased compared with that of 6 mM and the highest pH was much greater than 7 (7.3). Thus, 6 mM of $NaHCO_3$ was chosen to produce NEW in the following sections.

#### 3.1.2. The influence of flow rate, current density, and the NaCl concentration on the FAC, ORP, and pH of the NEW

The electrochemical production of NEW in this study was performed by adding the selected $NaHCO_3$ concentration (6 mM) into the electrolyte before electrolysis (BE) under different conditions. This process was evaluated by comparing with two other conditions: the EW producing process using NaCl as the electrolyte without the addition of $NaHCO_3$ (NA), and the NEW produced by adding $NaHCO_3$ (6 mM) to the EW after electrolysis (AE). The influence of flow rate, current density, and NaCl concentration on the FAC, ORP, and pH of the three types of EW is shown in Fig. 3A–C, respectively. With a decrease in flow rate, and an increase in current density and NaCl concentration, FAC levels increased, which agreed with previous studies (Zhang et al., 2017). Interestingly, BE NEW showed significantly higher FAC, ORP, and a lower pH than AE NEW under all conditions. This will be discussed further in terms of the relationship between current and potential.

### 3.2. The influence of flow rate, current density, and NaCl concentration on the CE and energy consumption of the NEW generator

The CE and energy consumption are important for the characterisation of the electrolysis process (Choi et al., 2013; Parsons, 1992).

For better comparison, the electrolysis process using the electrolyte without adding $NaHCO_3$ (NA) was also evaluated with the electrolyte containing $NaHCO_3$ (BE) under the same conditions. The total molar of $Na^+$ for the two situations was the same. The influence of flow rate, current density, and NaCl concentration on the CE and energy consumption of the NEW generator were examined. As expected, the result showed that after the addition of $NaHCO_3$, the CE improved together with slightly increased energy consumption under all electrolysis conditions (Fig. 4A–F). In Fig. 4A, with the increase of the flow rate, the CE increased initially, followed by slight decrease. However, it was slightly decreased with increasing current density (Fig. 4C), but significantly increased from 6% to 25% with increasing NaCl concentration (Fig. 4B). Energy consumption showed the opposite trend to CE (Fig. 4D–F).

Increasing the flow rate could reduce the residence time and thus decrease the electrolysis efficiency (Grinberg, Skundin, & Tuseeva, 2001). However, the anodic CE of chlorine production can be reduced by cathodic reduction, which will be partly diminished at low flow rates (Bergmann & Koparal, 2005; Grinberg et al., 2001). This could explain the trend of CE *vs*. flow rate in our study.

A higher current density can enhance electrochemical chlorine production, but not necessarily the CE. With the increase in current density, the limited concentration of chloride could not meet the requirement of a sufficient chloride diffusion rate, leading to a decrease in the CE (Bergmann & Koparal, 2005; Shao, Yan, Cao, Li, & Xu, 2014). At the same time, a higher current density will promote the side reaction of oxygen evolution, decreasing the CE of chlorine evolution (Choi et al., 2013). In our study, the CE decreased as the current density increased, which was consistent with these previous reports.

Several studies have demonstrated that the CE increased initially and the energy consumption decreased with increasing NaCl concentrations. These factors then plateaued when the level of NaCl became too high (higher than 0.5 mol/L) (Choi et al., 2013; Nath, Wang, Torrens, & Langdon, 2011). The maximum NaCl concentration used in our study was 10 g/L, that was why the CE kept increasing in our study. In Nath et al. (2011)'s study, the CE responses were linear at low NaCl concentrations (< 29 g/L). However, in our study, the CE showed an exponential-law dependence on NaCl concentration ($R^2 = 0.97$ for both producing conditions). This could be caused by the vertical electrolytic cell structure used in our study, which is different from the perforated one used in Nath et al.'s study.

Although the CE was not markedly enhanced by adding $NaHCO_3$, the properties of the NEW finally produced were improved compared with NEW of AE.

### 3.3. Efficacy of sanitising effects and FCM analysis

In this section, the antibacterial abilities of three groups of NEW (NEWC: produced directly from the developed equipment; NEWCD: produced by diluting NEWC with the highest FAC concentration; and CNEW: produced by a commercial EW generator) at different concentrations (4, 8, 10, 20, and 40 mg/L) against *E. coli* O157:H7 C7927 and *L. monocytogenes* BAA-839 were compared (Table 1). The pH values of the NEWs produced from different generators and principles ranged from 5.92 to 6.48. The ORP values ranged from 830.3 to 908.8 mV. FAC wasn't detected in deionised water.

The surviving populations of *E. coli* and *L. monocytogenes* after treatment with the NEW solutions are shown in Fig. 5A–B. Generally, the sanitising effect was enhanced with increasing FAC concentration in different NEWs. At FAC concentrations of 20 and 40 mg/L and 30 s of exposure to NEW, the populations of *E. coli* and *L. monocytogenes* in the

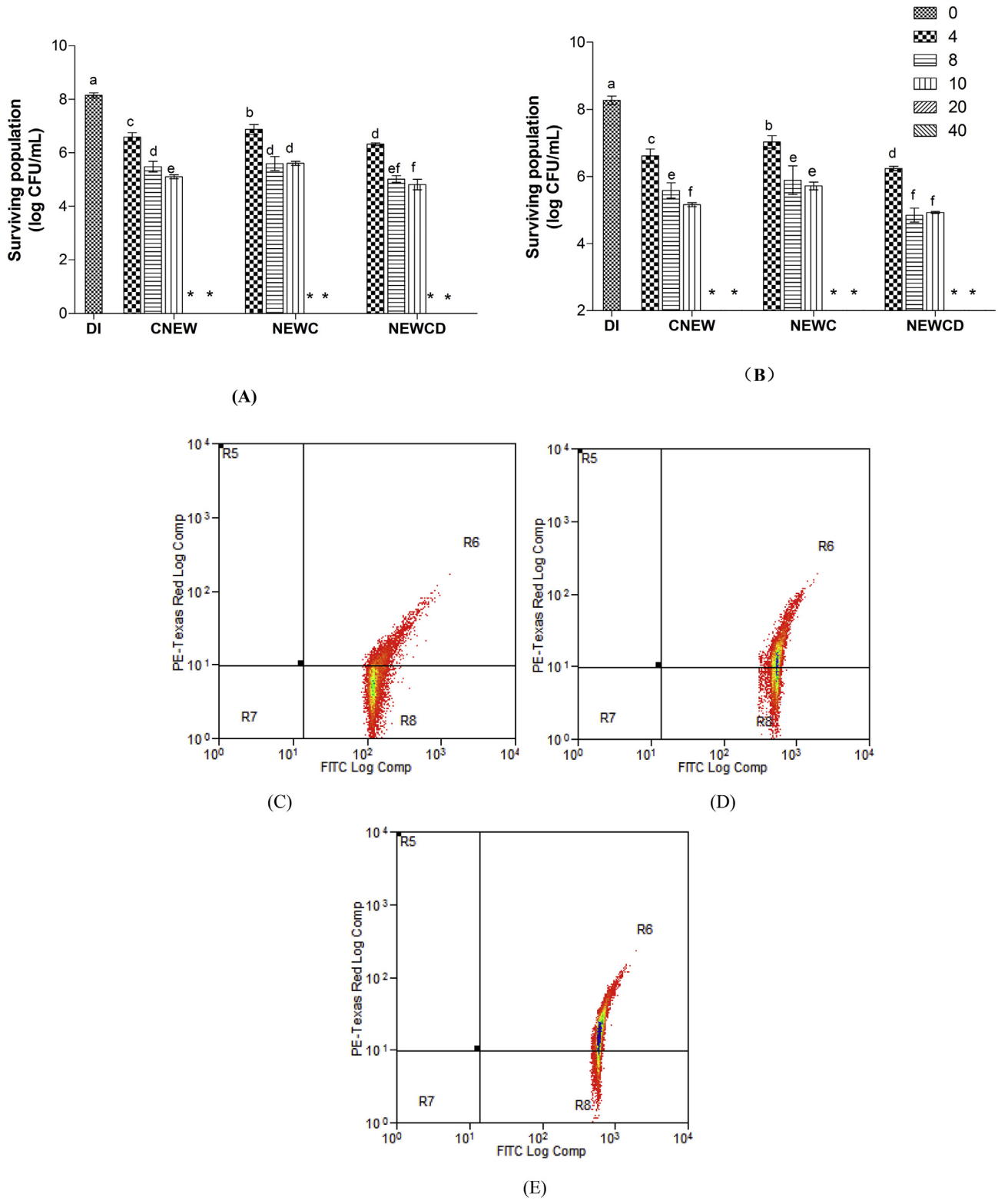



**Fig. 5.** Efficacy of sanitising effects and FCM analysis after treatment with neutral electrolysed water (NEW) solutions.**

(A) The surviving population of *Escherichia coli* O157:H7 (C7927).

(B) The surviving population of *Listeria monocytogenes* (BAA-839).

(C–E) Flow cytometry (FCM) density plots of *E. coli* stained with SYTO9/propidium iodide (PI) after different treatments: C, DI; D, 4 mg/L NEWC, E, 4 mg/L NEWCD.

*Not detectable by direct plate count or negative on enrichment media.

CNEW: produced by a commercial sanitiser, with free available chlorine (FAC) levels of 4, 8, 10, 20, and 40 mg/L; NEWC: produced by electrolysis of NaCl + NaCHO3, with FAC levels of 4, 8, 10; 20 and 40 mg/L; NEWCD: produced by dilution of NEWC, to 4, 8, 10 20, and 40 mg/L.

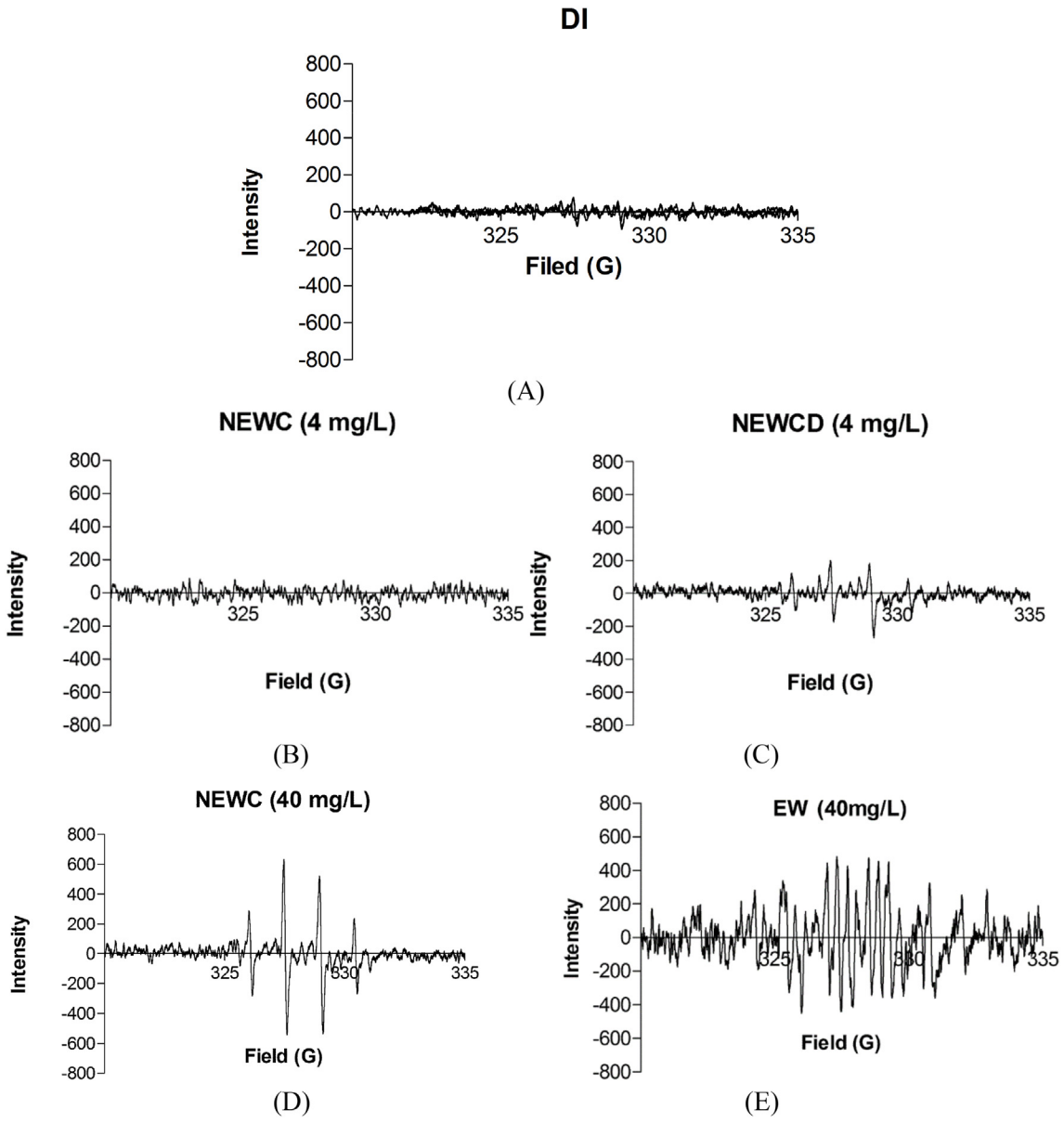



**Fig. 6.** Electron spin resonance (ESR) spectra of neutral electrolysed water (NEW) produced by the portable NEW producing unit.
(A) Deionised water (DI), (B) NEWC (produced by electrolysis of NaCl + $NaCHO_3$, with free available chlorine of 4 mg/L), (C) NEWCD (produced by diluting NEWC, with free available chlorine of 4 mg/L), (D) NEWC (produced by electrolysis of NaCl + $NaCHO_3$, with free available chlorine of 40 mg/L), (E) EW (produced by electrolysis of NaCl, with free available chlorine of 40 mg/L).

treated samples decreased to undetectable levels as determined by both plating and enrichment procedures. It is worth noting that, with a FAC of 4 mg/L, NEWCD reduced the *E. coli* by 2.03 log CFU/mL, which was significantly ($P < 0.05$) higher than the 1.25 and 1.66 log CFU/mL achieved by NEWC and CNEW, respectively. A similar trend was observed for *L. monocytogenes*.

EW produced using $NaHCO_3$ alone as an electrolyte proved to be an effective method to control postharvest rots of citrus fruit (Fallanaj et al., 2016); however, it was ineffective in the control of viable cells because of the low concentration of oxidant (sodium percarbonate) produced by dimensionally stable anodes (around 1:5000 of that produced by using NaCl alone as electrolyte) (Pinto, Ippolito, & Baruzzi, 2015; Velazquez-Pena et al., 2013). However, in our study, the NEWCD produced by electrolysing sodium bicarbonate and sodium chloride showed strong bactericidal effects. Interestingly, NEWC (4 mg/L FAC) produced by same method and same electrolyser, and with similar FAC and pH with NEWCD, had a significantly ($P < 0.05$) weaker sanitising effect. Thus, FCM was used to further evaluate the difference in cytoplasmic membrane damage in cells caused by these two kinds of NEW.

Fig. 5C–E shows the FCM density plots of two *E. coli* samples stained with SYTO9/PI after treatment with 4 mg/L NEW produced directly (NEWC) and that produced by dilution of NEWC with a high concentration of FAC (NEWCD). The control was treated with DI. PI-stained (membrane damaged) cells were 55.4 ± 7.6 and 82.2 ± 3.5% (Fig. 5D–E) of total two-colour stained samples for NEWC and NEWCD treatment, respectively, demonstrating a sharp increase of damaged cells by NEWCD treatment. This finding is in agreement with the results obtained in the above sanitising experiment. We assumed that more free radicals existed in NEWCD than NEWC, which resulted in more damaged cellular structures and further disrupted the normal cellular function of the microorganism (Xiong et al., 2010). Subsequently, electron spin resonance (ESR) was applied to analyse the free radicals in

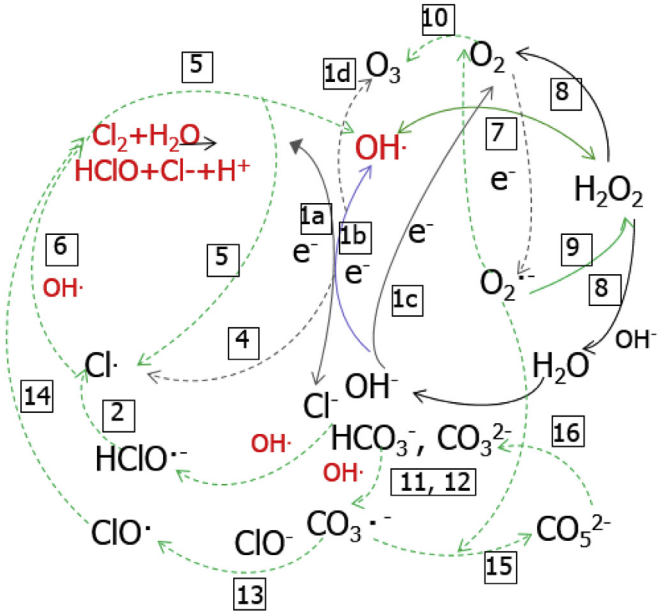



**Fig. 7.** Proposed reaction pathways of the hypochlorous acid in neutral electrolysed water (NEW) produced with the addition of $NaHCO_3$ in electrolyte.*
(1a) $2Cl^- \rightarrow Cl_2 + 2e$ (1b) $OH^- \rightarrow OH\cdot + e$ (1c) $2H_2O \rightarrow O_2 + 4H^+ + 4e$
(1d) $3H_2O \rightarrow O_3 + 6H^+ + 6e$ (2) $Cl^- + OH\cdot \rightarrow HClO^{\cdot-}$ (3) $HClO^{\cdot-} + H^+ \rightarrow Cl\cdot + H_2O$
(4) $2Cl^- \rightarrow 2Cl\cdot + 2e$ (5) $HClO \rightarrow OH\cdot + Cl\cdot$ (6) $OH\cdot + Cl\cdot \rightarrow HClO$
(7) $OH\cdot + OH\cdot \leftrightarrow H_2O_2$ (8) $2H_2O_2 \rightarrow 2H_2O + O_2$ (9) $2O_2^{\cdot-} + 2H^+ \rightarrow H_2O_2 + O_2$
(10) $O_2 + O\cdot \rightarrow O_3$ (11) $OH\cdot + HCO_3^- \rightarrow H_2O + CO_3^{\cdot-}$ (12) $OH\cdot + CO_3^{2-} \rightarrow OH^- + CO_3^{\cdot-}$
(13) $CO_3^{\cdot-} + ClO^- \rightarrow CO_3^{2-} + ClO\cdot$ (14) $ClO\cdot + ClO\cdot \rightarrow Cl_2 + O_2$
(15) $CO_3^{\cdot-} + O_2^{\cdot-} \rightarrow CO_5^{2-}$ (16) $CO_5^{2-} \rightarrow CO_3^{2-} + O_2$
*The red colour represents the detected reaction product and its intermediates. The dotted arrows represent the reaction steps without definite verification. Green and blue arrows represent processes with distinguishing radical chain reaction mechanisms and catalytic reaction mechanisms, respectively; black arrows represent the common chemical reactions. Diagram with steps 1–10 was reported previously (Zhang et al., 2018).

these two kinds of NEW.

### 3.4. Identification of free radicals in neutral electrolysed water

Free radicals were detected in NEW (4 mg/L) produced by electrolysis of NaCl and $NaHCO_3$ solution, including that produced directly (NEWC) and that produced by dilution of NEWC with a high concentration of FAC (NEWCD). Fig. 6 shows the representative ESR spectra of DI (A), NEWC: 4 mg/L (B), NEWCD: 4 mg/L (C), NEWC: 40 mg/L (D), and EW: 40 mg/L (E). The presence of a DMPO-OH-like signal in NEWC (40 mg/L) was confirmed by hyper fine coupling constants (HFCC) of $|A_N| = |A_H| = 1.5$ mT (Saito, Kohno, Yoshizaki, & Niwano, 2008). Comparing Fig. 6A with 6B and 6C, no clear signal from DMPO-OH was observed in the NEWC (4 mg/L) sample; however, a DMPO-OH-like signal appeared in the NEWCD (4 mg/L) sample. This indicated that a higher FAC could enhance the ·OH production in NEW; and at the same FAC level, by comparing Fig. 6D with 6E the addition of $NaHCO_3$ could promote the ·OH content in NEWC.

The reaction pathways was proposed in Fig. 7 based on our previous study, in which hydroxide ions could promote the production of ·OH in NEW (Reaction 1–10) (Zhang et al., 2018). With the existence of $CO_3^{2-}$ and $HCO_3^-$, ·OH is captured and produces carbonate radicals ($CO_3^{\cdot-}$), as described in equation (11), (12) (Buxton & Elliot, 1986; Czapski, Lymar, & Schwarz, 1999; Saha et al., 2004). In the presence of $ClO^-$, the precursor of $Cl_2$ might be produced through equation (13) and (14), which again could yield ·OH through equation (15) and (16) (Clyne & Coxon, 1966; Huie, Clifton, & Neta, 1991; Saran, Beck-Speier, Fellerhoff, & Bauer, 1999; Swanson & Fu, 2017). However, $CO_3^{\cdot-}$ decays in the reaction (17, 18) with the extinction of $O_2^{\cdot-}$ and produces $CO_3^{2-}$ (Behar et al., 1970; Czapski et al., 1999; Jamieson, Mebel, & Kaiser, 2007).

## 4. Conclusion

A portable electrolytic sanitising unit was developed to produce NEW via a novel method: using diluted sodium chloride and sodium bicarbonate solution (6 mM) as electrolytes. It provided NEW with a FAC ranging from 3.3 to 70.0 mg/L, near neutral pH between 5.7 and 7.1, and an ORP between 802.2 and 933.8 mV. The produced NEW (generated by dilution of NEW with high FAC) had strong bactericidal activity: a FAC concentration of 40 mg/L achieved > 6 log CFU/mL reductions, and a FAC concentration of 4 mg/L achieved > 2 log CFU/mL reductions. Moreover, the NEW produced by this method had stronger bactericidal effects on both *E. coli* O157:H7 and *L. monocytogenes* than NEW produced using a commercial EW generator. In addition, ·OH, a strong antimicrobial agent, was present in the NEW produced by the portable electrochemical sanitising unit. The results suggested that the developed portable electrolytic unit is promising to produce NEW that effectively control foodborne pathogens.

## Acknowledgements

We thank projects 31371851 and 31471605 supported by NSFC, Singapore Ministry of Education Academic Research Fund Tier 1 (R-143-000-A40-114), Natural Science Foundation of Jiangsu Province (BK20141220), Applied Basic Research Project (Agricultural) Suzhou Science and Technology Planning Programme (SYN201522), and a project from Guangzhou Kaijie Power Supply Industrial Co., Ltd (R-143-000-576-597).

## Abbreviations

| | |
|---|---|
| CE | current efficiency |
| FAC | free available chlorine |
| ORP | oxidation-reduction potential |
| NEW | near neutral pH electrolysed water |
| EW | Electrolysed water |

## References

Augusto, O., Bonini, M. G., Amanso, A. M., Linares, E., Santos, C. C., & De Menezes, S. I. L. (2002). Nitrogen dioxide and carbonate radical anion: Two emerging radicals in biology. *Free Radical Biology and Medicine, 32*(9), 841–859.
Ayebah, B., & Hung, Y.-C. (2005). Electrolyzed water and its corrosiveness on various surface materials commonly found in food processing facilities. *Journal of Food Process Engineering, 28*(3), 247–264.
Behar, D., Czapski, G., & Duchovny, I. (1970). Carbonate radical in flash photolysis and pulse radiolysis of aqueous carbonate solutions. *Journal of Physical Chemistry, 74*(10), 2206–2210.
Bergmann, M., & Koparal, A. (2005). Studies on electrochemical disinfectant production using anodes containing $RuO_2$. *Journal of Applied Electrochemistry, 35*(12), 1321–1329.
Berney, M., Hammes, F., Bosshard, F., Weilenmann, H. U., & Egli, T. (2007). Assessment and interpretation of bacterial viability by using the LIVE/DEAD BacLight kit in combination with flow cytometry. *Applied and Environmental Microbiology, 73*(10), 3283–3290.
Buxton, G. V., & Elliot, A. J. (1986). Rate constant for reaction of hydroxyl radicals with bicarbonate ions. *International Journal of Radiation Applications and Instrumentation - Part C: Radiation Physics and Chemistry, 27*(3), 241–243.
Choi, J., Shim, S., & Yoon, J. (2013). Design and operating parameters affecting an electrochlorination system. *Journal of Industrial and Engineering Chemistry, 19*(1), 215–219.
Clyne, M., & Coxon, J. (1966). Reactions of chlorine oxide radicals. Part 1.—reaction kinetics of the ClO radical. *Transactions of the Faraday Society, 62*, 1175–1189.
Czapski, G., Lymar, S. V., & Schwarz, H. A. (1999). Acidity of the carbonate radical. *The Journal of Physical Chemistry A, 103*(18), 3447–3450.
Fallanaj, F., Ippolito, A., Ligorio, A., Garganese, F., Zavanella, C., & Sanzani, S. M. (2016).

Electrolyzed sodium bicarbonate inhibits Penicillium digitatum and induces defence responses against green mould in citrus fruit. *Postharvest Biology and Technology, 115*, 18–29.
Gil, M. I., Gómez-López, V. M., Hung, Y.-C., & Allende, A. (2015). Potential of electrolyzed water as an alternative disinfectant agent in the fresh-cut industry. *Food and Bioprocess Technology, 8*(6), 1336–1348.
Grinberg, V., Skundin, A., & Tuseeva, E. (2001). Electrochemical production of medicinal sodium hypochlorite solutions in a flow-through electrolyzing cell. *Russian Journal of Electrochemistry, 37*(4), 431–434.
Hanaoka, K., Sun, D., Lawrence, R., Kamitani, Y., & Fernandes, G. (2004). The mechanism of the enhanced antioxidant effects against superoxide anion radicals of reduced water produced by electrolysis. *Biophysical Chemistry, 107*(1), 71–82.
Huie, R. E., Clifton, C. L., & Neta, P. (1991). Electron transfer reaction rates and equilibria of the carbonate and sulfate radical anions. *International Journal of Radiation Applications and Instrumentation - Part C: Radiation Physics and Chemistry, 38*(5), 477–481.
Jamieson, C. S., Mebel, A. M., & Kaiser, R. I. (2007). First detection of the C2 symmetric isomer of carbon pentaoxide ($CO_5$) at 10 K. *Chemical Physics Letters, 443*(1–3), 49–54.
Len, S.-V., Hung, Y.-C., Erickson, M., & Kim, C. (2000). Ultraviolet spectrophotometric characterization and bactericidal properties of electrolyzed oxidizing water as influenced by amperage and pH. *Journal of Food Protection, 63*(11), 1534–1537.
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., 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., 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.
Luo, K., Kim, S. Y., Wang, J., & Oh, D.-H. (2016). A combined hurdle approach of slightly acidic electrolyzed water simultaneous with ultrasound to inactivate *Bacillus cereus* on potato. *LWT-food Science and Technology, 73*, 615–621.
Mokudai, T., Nakamura, K., Kanno, T., & Niwano, Y. (2012). Presence of hydrogen peroxide, a source of hydroxyl radicals, in acid electrolyzed water. *PLoS One, 7*(9), 46392.
Nath, H., Wang, X., Torrens, R., & Langdon, A. (2011). A novel perforated electrode flow through cell design for chlorine generation. *Journal of Applied Electrochemistry, 41*(4), 389–395.
Park, Y. B., Guo, J., Rahman, S., Ahn, J., & Oh, D. H. (2009). Synergistic effect of electrolyzed water and citric acid against *Bacillus cereus* cells and spores on cereal grains. *Journal of Food Science, 74*(4), M185–M189.
Parsons, R. (1992). In H. Gerischer, & C. W. Tobias (Vol. Eds.), *Advances in electrochemical science and engineering. 2. Advances in electrochemical science and engineering* (pp. 269–). Weinheim: VCH Publishers, Elsevier DM 168.
Pinto, L., Ippolito, A., & Baruzzi, F. (2015). Control of spoiler Pseudomonas spp. on fresh cut vegetables by neutral electrolyzed water. *Food Microbiology, 50*, 102–108.
Saha, M. S., Furuta, T., & Nishiki, Y. (2004). Conversion of carbon dioxide to peroxycarbonate at boron-doped diamond electrode. *Electrochemistry Communications, 6*(2), 201–204.
Saito, K., Kohno, M., Yoshizaki, F., & Niwano, Y. (2008). Antioxidant properties of herbal extracts selected from screening for potent scavenging activity against superoxide anions. *Journal of the Science of Food and Agriculture, 88*(15), 2707–2712.
Saran, M., Beck-Speier, I., Fellerhoff, B., & Bauer, G. (1999). Phagocytic killing of microorganisms by radical processes: Consequences of the reaction of hydroxyl radicals with chloride yielding chlorine atoms. *Free Radical Biology and Medicine, 26*(3), 482–490.
Shao, D., Yan, W., Cao, L., Li, X., & Xu, H. (2014). High-performance Ti/Sb–SnO 2/Pb 3 O 4 electrodes for chlorine evolution: Preparation and characteristics. *Journal of Hazardous Materials, 267*, 238–244.
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.
Swanson, S., & Fu, T. J. (2017). Effect of water hardness on efficacy of sodium hypochlorite inactivation of *Escherichia coli* O157:H7 in water. *Journal of Food Protection, 80*(3), 497–501.
Tang, W., Li, Y., Li, W., Chen, X., & Zeng, X. (2016). Preparation of a coated Ti anode for producing acidic electrolyzed oxidizing water. *LWT-food Science and Technology, 66*, 606–614.
Velazquez-Pena, S., Saez, C., Canizares, P., Linares-Hernandez, I., Martinez-Miranda, V., Barrera-Diaz, C., et al. (2013). Production of oxidants via electrolysis of carbonate solutions with conductive-diamond anodes. *Chemical Engineering Journal, 230*, 272–278.
Xiong, K., Liu, H.-j., & Liu, R. (2010). Differences in fungicidal efficiency against *Aspergillus flavus* for neutralized and acidic electrolyzed oxidizing waters. *International Journal of Food Microbiology, 137*(1), 67–75.
Xuan, X., Wang, M., Ahn, J., Ma, Y., Chen, S., Ye, X., et al. (2016). Storage stability of slightly acidic electrolyzed water and circulating electrolyzed water and their property changes after application. *Journal of Food Science, 81*(3), 610–617.
Yang, H., Feirtag, J. M., & Diez-Gonzalez, F. (2013). Sanitizing effectiveness of commercial "active water" technologies on *Escherichia coli* O157:H7, *Salmonella enterica* and Listeria monocytogenes. *Food Control, 33*(1), 232–238.
Yu, X., & Yang, H. (2017). Pyrethroid residue determination in organic and conventional vegetables using liquid-solid extraction coupled with magnetic solid phase extraction based on polystyrene-coated magnetic nanoparticles. *Food Chemistry, 217*, 303–310.
Zaviska, F., Drogui, P., & Pablo, G. (2012). Statistical optimization of active chlorine production from a synthetic saline effluent by electrolysis. *Desalination, 296*, 16–23.
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.
Zhang, J., Yang, H., & Chan, J. Z. Y. (2018). Development of portable flow-through electrochemical sanitizing unit to generate near neutral electrolyzed water. *Journal of Food Science, 83*, 780–790.
Zhang, J., Zhou, S., Chen, R., & Yang, H. (2017). Development of a portable electrolytic sanitising unit for the production of neutral electrolysed water. *LWT-food Science and Technology, 82*, 207–215.
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.