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Comparative study on the stability of selected Neutral electrolyzed waters and their sanitizing effect on organic fresh-cut lettuce (*Lactuca sativa* Var. *crispa* L)

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Abstract

In this study, two near neutral pH electrolyzed water (NEW1 produced by redirecting of the catholyte solution back to the anode chamber and NEW2 produced by NaCl and $NaHCO_3$ as electrolyte) and control (NEW0, produced by commercial unit) were evaluated for their stability during 75 hr storage at 7°C. The physicochemical properties, bactericidal efficiencies, and sanitizing effects on organic fresh-cut lettuce of them were compared. The results showed that NEW2 was more stable than NEW1 and NEW0 during storage. The free available chlorine of it increased by approximately 35% after the storage. And, all three NEWs showed decreased bactericidal effects compared with that before the storage. In addition, all of them were effective against *Escherichia coli* and *Listeria innocua* Seeliger inoculated on organic fresh-cut lettuce, with 1.19–1.40 and 0.92–1.21 log CFU/g reductions, respectively. In terms of physicochemical parameters, there were no significant differences among different treatments.

Practical applications

Neutral electrolyzed waters (NEW) are a kind of potential organic compatible sanitizer and satisfy the urgent market need. But the stability of NEW has significant effects on their bactericidal activity. The results of the comparative study of different NEWs produced by developed NEW-producing unit could provide valuable reference data of NEWs regarding their stability during storage and the reduction of the risk from foodborne pathogens in a future application.

1 | INTRODUCTION

Consumer's consumption of organic fresh fruits and vegetables has increased dramatically because of their merits, such as, the low pesticide residues level (Yu & Yang, 2017). However, fresh organic produce might pose greater risk of microbial contamination because of the use of animal manure as fertilizer, especially the fresh-cut organic produce (Liu, Tan, et al., 2017; Zhang & Yang, 2017). Therefore, it is necessary to develop organic compatible sanitizers for fresh-cut organic produce. Electrolyzed water (EW) with free available chlorine (FAC) concentration less than 4 mg/L has been allowed to sanitize organic fresh fruits and vegetables, but its limitations include low pH, relatively low storage stability, and the corrosion caused on processing equipment. Newly developed near neutral pH electrolyzed water (NEW) could successfully overcome these limitations. At a near neutral pH (5.0 to 6.5), EW has highest proportion of hypochlorous acid (HClO), the bactericidal activity of which is 80 times greater than that of hypochlorite ion (ClO-) with equivalent concentration and same treatment time (Len et al., 2000; Zhao et al., 2017).

In general, NEW is produced by mixing catholyte with EW produced by a divided electrolytic cell, with diluted NaCl solution as the electrolyte. However, NEW produced by this way had lower oxidation–reduction potential (ORP) compared with EW with the same FAC (Yang et al., 2013; Zhao et al., 2017). There are also some

reports of producing NEW by electrolysis of hydrochloric acid (HCl) or diluted NaCl solution in a non-flow-through undivided electrolytic cell. However, higher acidity has a negative effect on the electrocatalytic activity of the electrode (Cao et al., 2009). In our previous studies (Zhang, Lai, & Yang, 2018; Zhang, Yang, & Chan, 2018; Zhang, Zhou, et al., 2017), two flow-through NEW generator systems were developed with RuO_2–IrO_2/TiO_2 electrodes. Both systems can produce NEW. The first produces NEW (NEW1) by redirecting of the catholyte solution back to the anode chamber. The second produces NEW (NEW2) using diluted sodium chloride and sodium bicarbonate solution as electrolyte. The produced NEW has a pH between 5.70 and 7.17, ORP between 805.5 and 933.8 mV, and FAC between 3.3 and 82.0 mg/L. At the same pH, and FAC, the ORP of NEW2 was a little higher than that of NEW1.

The stability of EW and NEW has significant effects on their bactericidal activity. Therefore, this study was aimed to evaluate the changes in the physicochemical properties (pH, ORP, and FAC) of NEW1, NEW2, and NEW0 (NEW produced by mixing of the catholyte and anolyte produced by commercial generator) during a 75 hr storage period. We also compared the bactericidal efficiency of all NEWs before and after the storage. In addition, to the best of our knowledge, few studies (Zhang & Yang, 2017; Zhao et al., 2019) were performed on the sanitizing of organic lettuce and most studies used conventional lettuce. Especially the studies using a low concentration of NEW produced directly as sanitizer were even more less. Thus, the application NEWs in organic fresh-cut lettuce was investigated in this study.

2 | MATERIALS AND METHODS

2.1 | Bacterial culture

Escherichia coli ATCC 25922 and *Listeria innocua* Seeliger ATCC 33090 were obtained from the Food Science and Technology Program, the National University of Singapore. The two strains were subcultured in 10 ml of sterilized tryptic soy broth (Oxoid, Hampshire, England) for three consecutive 24-hr transfers at 37°C. The cultures were centrifuged at 6,000× *g*, and 4°C for 5 min, washed twice with 1 × phosphate-buffered saline (PBS) (Vivantis Inc., Oceanside, CA, USA), and resuspended in PBS before use. The population of each working solution of *E. coli* and *Listeria* was approximately 9.2 to 9.6 log CFU/ml.

2.2 | Preparation of NEW and analytical measurements

NEW0 was prepared by mixing the anode and cathode products of a commercial EO water generator (model ROX-20TA, Hoshizaki Electric Inc., Aichi, Japan). NEW1 was produced by a developed flow-through NEW generator though redirecting cathode yields back to the anode chamber. NEW2 was produced by the developed flow-through NEW generator using NaCl and $NaHCO_3$ as the electrolyte. The physicochemical properties of these NEWs were measured immediately after being produced. The FAC was determined by a colorimetric method using a chlorine test kit and RQflex plus (Merck, Darmstadt, Germany). The pH was detected using a Thermo Orion 410 pH meter (Thermo Scientific, Waltham, MA, USA). The ORP was measured using a Mettler Toledo Seven compact ORP meter (Metrohm Singapore Pte, Ltd, Singapore). The FAC of the three NEWs before the storage was around 20 mg/L.

2.3 | Storage experiments

Three 100 ml autoclaved glass bottles (clear with cap) were used to collect the three NEW samples. The 100 ml clear glass bottles containing samples were stored in a refrigerator at 7°C for 75 hr in closed states. The pH, ORP, and FAC of the samples were measured at the 0th, 5th, 10th, 20th, 35th, 55th, and 75th hour. Two independent replicated experiments were conducted for each NEW. Each experiment had two parallel samples.

2.4 | Chlorine decay assays

FAC was determined using the colorimetric method described in Section 2.2. According to Henry's law, chlorine decay can be described mathematically as a function of time by the first-order kinetic equation as following (Len et al., 2002; Li et al., 2014):

$$\frac{dC}{dt} = -kc$$

where C is the FAC concentration (mg/L), k is the chlorine decay kinetic coefficient (mg/h). The analytical solution of the above first-order ordinary differential equation was obtained with the initial condition, $C = C_0$ at $t = 0$ hr, and used for describing chlorine decay:

$$C = C_0 \cdot \exp(-kt)$$

2.5 | Bactericidal efficiency of NEWs before and after storage

The bactericidal efficiencies of the three NEWs were investigated before and after the storage. Briefly, 1 ml of bacteria suspension of *E. coli* and *Listeria* (approximately 8.5 log CFU/ml) was individually mixed with 9 ml of NEW1, NEW2 or NEW0 at room temperature (20°C) for 5 min. Subsequently, 1 ml of the mixture was added to 9 ml of sterile neutralizing buffer solution (5.2 g/L; Becton, Dickinson and Company, Sparks, MD, USA) immediately. The neutralized mixture was serially diluted before plating on Tryptic Soya Agar (TSA). To obtain estimates of sublethal injured cells, treated samples were also plated on selective medium, TSA amended with 3% (w/w) sodium chloride, which assured the injured cells not able to recover whereas did not affect the growth of vital cells (Ghate et al., 2013; Ukuku

et al., 2008). Following incubation at 37°C for 24 hr, bacterial colonies were counted.

2.6 | Sanitizing effect of NEWs against *E. coli* and *Listeria* on organic lettuce

2.6.1 | Lettuce sample preparation

Organic lettuces were purchased from a local shop in Singapore. The vegetables were transported to the laboratory and stored at 4°C and used within 24 hr of purchase. The three outermost leaves and the inner part of each lettuce were removed, and the left clean ones were used. Then, a sterile kitchen knife was used to cut lettuce into pieces of 2.0 to 2.5 cm in a laminar flow hood and transferred to a sterile tray for treatments (Karaca & Velioglu, 2014).

2.6.2 | Inoculation and NEWs treatment

E. coli and *Listeria* were adapted to nalidixic acid (100 mg/L) by the stepwise increment method, and all cultures were grown at 37°C with 150 RPM agitation (Jadeja & Hung, 2013). Spot inoculation was used to inoculate the lettuce. To evenly inoculate the sample, each suspension (80 μl) was deposited on the surface with droplets at 16 minimum locations with a micropipettor and air-dried in a laminar flow hood for 30 min at room temperature (22 ± 2°C) to allow microbial attachment. Approximately 5 g lettuce were inoculated and used for each NEWs treatment. All media used in this part were supplemented with 100 mg/ml of nalidixic acid to differentiate from the background bacteria.

Inoculated samples were immersed immediately in the NEWs (diluted to 4 mg/L) for 5 min (Pinto et al., 2015). The ratio between the mass of the vegetable sample and the volume of solution was 50 g/L. In addition, the control experiment used sterile deionized water (DI) as the washing solution. The temperature of the solutions was 22 ± 2°C. Treated leaves were placed in a stomacher bag containing 45 ml of 0.1% peptone water. Samples were then homogenized in a stomacher (Stomacher 400 Circulator, Seward, London, UK) for 2 min. The samples were serially diluted with peptone water and plated on TSA.

2.6.3 | Firmness analysis and color measurement

Firmness of fresh-cut lettuce leaves was measured using a TA-XT2i Texture analyzer (Stable Micro Systems Ltd, Godalming, UK) according to Sindy Palma Salgado's (2014) method with a slight modification. The press holder and the blade plunger were moved down at a velocity of 5 mm/s to 1 cm below the bottom of the holder. The maximum cut force (MCF) was recorded using the Texture Expert Software (Nova-Tech International, Inc., Houston, TX, USA). These tests were conducted with six replicates for each group.

For color measurement, two pieces of cut lettuce leaves were withdrawn from each treatment and analyzed using a Minolta Colorimeter CM-3500d (Konica Minolta, Inc., Japan). Hunter's color values (*L*, *a*, *b*) were measured at three locations of each piece of lettuce and averaged for a total of six readings for each treatment. Each treatment was repeated at least twice. The overall color difference was calculated by applying formula as described by Bermudez-Aguirre and Barbosa-Canovas (2013), Pathare et al. (2013):

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

where $\triangle E^*$ represents the overall color difference. Standard white plate and black plate were used for instrument calibration.

2.7 | Statistical analysis

Data were reported as the mean ± standard deviation. Analysis of variance (ANOVA) and Duncan's test was performed using SAS software (SAS Institute Inc., Cary, NC, USA). Statistical significance was set at $p < .05$.

3 | RESULT AND DISCUSSION

3.1 | Comparisons of physicochemical property changes in NEWs during storage

To evaluate the stabilities of the three NEWs, two main factors were considered when conducting the experiment. The first factor was the initial concentration of the NEWs, which should be similar, because higher concentrations are more stable (Rossi-Fedele et al., 2011). Another important factor is temperature, as higher temperature can increase the chlorine decay constants (Hua et al., 1999; Robinson et al., 2012). Taking account of these two factors, in this study 7°C was chosen as the storage temperature and all the three NEWs had similar initial FAC (around 20 mg/L). Figure 1 shows changes in FAC, pH, and ORP of NEWs under closed storage conditions for 75 hr in 7°C fridge.

3.1.1 | Comparisons of FAC changes in NEWs during the storage

FAC of NEW0 and NEW1 decreased from 22.0 to 17.3 and 16.8 mg/L, respectively, after 35 hr storage, and then decreased slowly in the following 40 hr storage. In contrast, FAC of NEW2 increased from 26.7 to 34.0 mg/L after 10 hr storage, and then remained stable (Figure 1a).

Under the closed condition, the decomposition and evaporation of EW was the major reason causing the decay of chlorine (Len et al., 2002). Several studies demonstrated the FAC decay of EW during storage, and FAC of acid EW decreased much faster than

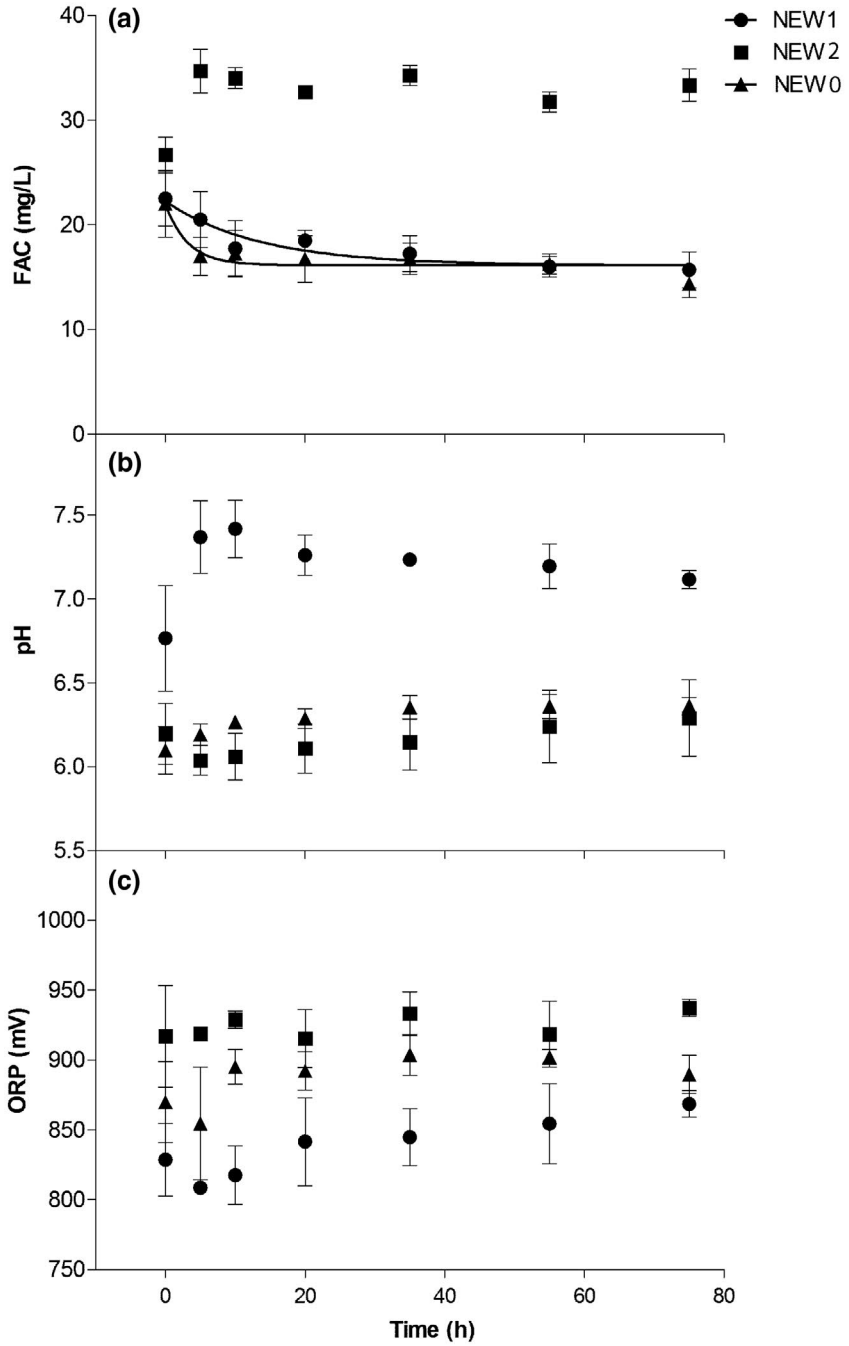

FIGURE 1 Changes in the properties of three different neutral pH electrolyzed waters (free available chlorine [FAC]: 20 mg/L) during storage. NEW0: neutral pH electrolyzed water produced by the commercial unit. NEW1:neutral pH electrolyzed water produced by redirecting of the catholyte solution back to the anode chamber. NEW2: neutral pH electrolyzed water produced by NaCl and $NaHCO_3$ as electrolyte

NEW, especially under open conditions (Len et al., 2002; Rahman et al., 2012; Xuan et al., 2016). Theoretically, at pH between 6.0 and 7.5, the dominant chlorine species in EW are HClO and OCl^- instead of dissolved chlorine gas, which has a weak release of chlorine gas (Cl_2). Therefore, the self-decomposition of HClO and OCl^- may be the main reason for the chlorine decay during storage.

3.1.2 | Comparisons of pH and ORP changes in NEWs during the storage

The pH and ORP values of NEW0 and NEW2 remained essentially stable during the 75 hr storage. However, the pH of NEW1 increased rapidly from 6.76 to 7.37 after 5 hr storage and then kept stable. The ORP dropped from 828.6 to 808.5 mV after 5 hr storage and then increased to approximately 868.6 mV (Figure 1b).

These differences might be due to the different properties of different NEW produced by various electrolytic systems. A similar result of NEW1 during storage has been reported by Cui et al. (2009) that ORP of NEW stored under closed condition increased dramatically during the initial 2 days of storage, followed by slower increase. One possible reason is the existence of dissolved oxygen (DO) in NEW, which would react with reducing components in NEW and increase the ORP (Cui et al., 2009; Hsu & Kao, 2004). Another reason might be the presence of hydroxyl free radicals. In Cui's study, NEWs (initial FAC around 20 mg/L) were obtained in the cell without

a membrane, by which the produced NEW had hydroxyl free radicals (Xiong et al., 2010), as well as NEW1 in our previous study. In addition, in our previous study (Zhang et al., 2018), NEW0 produced by commercial unit was checked without hydroxyl free radicals, and this maybe account for the different changes between NEW0 and NEW1, although their FAC content in NEW were the same (electrolysis of NaCl).

During the storage, with the increase in pH, the equilibrium in the solution will be shifted and lead to the formation of HClO and reduction of volatile chlorine gas in EW, thus leading to the increase of chlorine. However, this is applicable to EW, especially for acidic EW solution stored under open conditions (Len et al., 2000). For NEW, the pH increased while FAC decreased during the storage (Cui et al., 2009; Rahman et al., 2012).

In our study, only the NEW produced by $NaCHO_3$ and NaCl as electrolytes, was stable during storage, including FAC, pH, and ORP. It is probably because of the stronger ionic strength of NEW2 compared with NEW1 and NEW0, which can decrease the rate of NEW relaxation (or increase the stability) (Petrushanko & Lobyshev, 2001; Thorn et al., 2012).

3.2 | The decay kinetics of FAC of NEW1 and NEW0

Figure 1 shows the FAC decay kinetics of NEW1 and NEW0. FAC decay kinetics coefficients of NEWs are shown in Table 1. As can be seen from Table 1, the correlation coefficients R^2 were 0.91 and 0.86 for NEW1 and NEW0, respectively, which indicates that a first-order kinetic model can fit the experimental data of FAC of NEW1 and NEW0 decay during the 75 hr storage at 7°C. There was no significant difference in chlorine decay kinetic coefficients between the two NEWs.

FAC, which was consisted of dissolved chlorine gas (Cl_2), ClO^-, and HClO, is the main factor that accounts for the bactericidal capability of EW (Len et al., 2000; Li et al., 2014; Liu, Wu, et al., 2017; Sow et al., 2017; Xiong et al., 2010). But under some situations, EW was not freshly produced (i.e., electricity and EW generator are unavailable onsite to produce EW, no available salt or water supply, or economic reason) (Robinson et al., 2012). Thus, it is important to understand the decay of FAC of these NEWs during storage. In this study, the decay of FAC of NEW during the 75 hr storage can be described mathematically by a first-order kinetic model. Based on this model, FAC of NEW1 and NEW0 decreased significantly during the initial 35 and 10 hr storage, respectively. These results indicated that NEW1 has lower self-decomposition rate than NEW0 during the initial storage period, because, for NEW, the FAC decay is mainly due to the self-decomposition of chlorine species (Len et al., 2002; Li et al., 2014). To the best of our knowledge, there were no similar results in previous studies.

TABLE 1 The chlorine decay kinetic coefficients of free available chlorine of different neutral pH electrolyzed water during 75 hr storage*

NEWs**	K (mg/h) × 1,000	R^2
NEW1	4 ± 2^a	.91
NEW2	3 ± 1^a	.86

*Mean values with different small case letters are significantly different ($p < .05$) among different neutral pH electrolyzed water groups; **NEWs: neutral pH electrolyzed waters. NEW1: neutral pH electrolyzed water produced by reintroducing catholyte liquid to the anode part. NEW2: neutral pH electrolyzed water produced by NaCl and $NaHCO_3$ as electrolyte.

3.3 | Lethal and sublethal effect after treated by NEWs before and after the storage

The properties of different NEWs used to inactivate two bacterial strains in this study are presented in Table 2. The FAC of all NEWs was 4 mg/L. No FAC was detected in DI.

Lethal and sublethal effects after treatment by NEWs before and after the storage are shown in Table 3 (*E. coli*) and Table 4 (*Listeria*). Compared with NEWs before the storage, the bactericidal efficiency of *E. coli* decreased (before the 75 hr storage, with around 5.32, 5.14, and 4.97 log CFU/ml reductions for NEW1, NEW2, and NEW0, respectively; after the 75 hr storage, with around 3.88, 3.78, and 3.85 log CFU/ml reductions for NEW1, NEW2, and NEW0, respectively). Results

TABLE 2 Properties of 4 mg/L of neutral pH electrolyzed water before and after 75h storage*

	FAC		pH		ORP	
Water	Before storage	After storage	Before storage	After storage	Before storage	After storage
NEW1**	3.9 ± 0.3^{Aa}	4.2 ± 0.3^{Aa}	6.65 ± 0.32^{Aa}	6.74 ± 0.06^{Aa}	864.8 ± 3.9^{Aa}	852.9 ± 7.7^{Aa}
NEW2	4.3 ± 0.2^{Aa}	4.2 ± 0.1^{Aa}	6.19 ± 0.05^{Bb}	6.47 ± 0.01^{Ab}	862.5 ± 12.5^{Aa}	834.4 ± 9.6^{Ba}
NEW0	4.3 ± 0.4^{Aa}	4.1 ± 0.4^{Aa}	6.21 ± 0.10^{Bb}	6.41 ± 0.04^{Ab}	851.4 ± 9.9^{Aa}	853.6 ± 7.1^{Aa}

*Means with different capital letters are significantly different ($p < .05$) among different processes; Means with different small case letters are significantly different ($p < .05$) among different neutral pH electrolyzed water groups; **NEW0: neutral pH electrolyzed water produced by the commercial unit. NEW1: neutral pH electrolyzed water produced by reintroducing catholyte liquid to the anode part. NEW2: neutral pH electrolyzed water produced by NaCl and $NaHCO_3$ as electrolyte.

TABLE 4 Surviving and sublethal injured *Listeria innocua* Seeliger ATCC 33090 after treated by different 4 mg/L of neutral pH electrolyzed water before and after 75 hr storage*

Treatment	Before storage			After storage		
	Non-selective medium (log CFU/ml)	Selective medium (log CFU/ml)	Sublethally injured cells (log CFU/ml)	Non-selective medium (log CFU/ml)	Selective medium (log CFU/ml)	Sublethally injured cells (log CFU/ml)
DI**	8.43 ± 0.05^{a}	8.43 ± 0.06^{a}	0.01 ± 0.03^{a}	8.55 ± 0.10^{a}	8.48 ± 0.11^{a}	0.07 ± 0.04^{a}
NEW1	3.12 ± 0.15^{b}	3.06 ± 0.06^{c}	0.05 ± 0.16^{a}	4.58 ± 0.24^{b}	4.41 ± 0.16^{ab}	0.22 ± 0.32^{a}
NEW2	3.26 ± 0.22^{b}	3.25 ± 0.15^{bc}	0.01 ± 0.08^{a}	4.63 ± 0.17^{b}	4.61 ± 0.28^{b}	0.15 ± 0.10^{a}
NEW0	3.56 ± 0.19^{b}	3.49 ± 0.20^{b}	0.06 ± 0.18^{a}	4.77 ± 0.19^{b}	4.47 ± 0.23^{b}	0.11 ± 0.34^{a}

Note: *Means with different small case letters are significantly different ($p < .05$) among different treatments; **DI: Deionized water. NEW0: neutral pH electrolyzed water produced by the commercial unit. NEW1: neutral pH electrolyzed water produced by reintroducing catholyte liquid to the anode part. NEW2: neutral pH electrolyzed water produced by NaCl and $NaHCO_3$ as electrolyte.

TABLE 3 Surviving and sublethal injured *Escherichia coli* ATCC 25922 after treated by different 4 mg/L of neutral pH electrolyzed water before and after 75 hr storage*

Treatment	Before storage			After storage		
	Non-selective medium (log CFU/ml)	Selective medium (log CFU/ml)	Sublethally injured cells (log CFU/ml)	Non-selective medium (log CFU/ml)	Selective medium (log CFU/ml)	Sublethally injured cells (log CFU/ml)
DI**	$8.4\ 2 \pm 0.12^{a}$	8.33 ± 0.11^{a}	0.09 ± 0.12^{a}	8.31 ± 0.08^{a}	8.28 ± 0.10^{a}	0.03 ± 0.02^{a}
NEW1	3.10 ± 0.15^{c}	3.09 ± 0.15^{b}	0.01 ± 0.11^{a}	4.43 ± 0.13^{b}	4.28 ± 0.14^{b}	0.15 ± 0.23^{a}
NEW2	3.28 ± 0.19^{bc}	3.27 ± 0.20^{b}	0.01 ± 0.08^{a}	4.53 ± 0.22^{b}	4.34 ± 0.17^{b}	0.19 ± 0.18^{a}
NEW0	3.45 ± 0.15^{b}	3.39 ± 0.13^{b}	0.06 ± 0.25^{a}	4.46 ± 0.35^{b}	4.30 ± 0.14^{b}	0.16 ± 0.34^{a}

*Means with different small case letters are significantly different ($p < .05$) among different treatments; **DI: Deionized water. NEW0: neutral pH electrolyzed water produced by the commercial unit. NEW1: neutral pH electrolyzed water produced by reintroducing catholyte liquid to the anode part. NEW2: neutral pH electrolyzed water produced by NaCl and $NaHCO_3$ as electrolyte.

also showed the populations of sublethal injured cells of *E. coli* increased slightly after the storage. A similar trend can be observed in *Listeria*. These results agreed with the NEWs' properties (4 mg/L) before and after the storage, as after the 75 hr storage, the ORP of diluted 4 mg/L of NEWs decreased and the pH all increased (Table 2). Similarly, Rahman et al. (2012) reported that bactericidal activities of slightly acidic electrolyzed water (SAEW) against *E. coli* O157:H7 and *L. monocytogenes* under closed storage conditions significantly decreased by 0.3 log CFU/ml after storage (less than 2 mg/L loss with initial FAC of 10 mg/L).

In addition to investigating the bactericidal activities of different NEWs, we also evaluated the process-induced damages to *E. coli* and *Listeria* by comparing the sublethal injured cells after different treatments by selective medium (TSA supplemented with 3% sodium chloride). For the selective medium, only vital cells can grow on it because of the intact outer membrane of these cells. But for the non-selective medium (without the supplementation of sodium chloride), both vital cells and injured cells were able to grow on. Therefore, the difference between selective and non-selective media was described as the sublethal injured cells (Ghate et al., 2013; Ukuku et al., 2008). It was observed that the differences between the NEWs treatments and DI control were negligible before storage. However, after the 75 hr storage of NEWs, there was a slight increase in injured cells population compared with control group of (Tables 3 and 4). A similar trend was found in Liao et al.'s study (2017) that with the increase in SAEW exposure time, the inactivation efficiency increased but the population of injured cells declined, as such condition may induce complete death of microorganisms and avoid the sublethal injury state. Li et al.(2016) also observed no sublethal injury *S. aureus* cells after SAEW treatment. George et al. demonstrated that the use of EO water with chlorine concentrations lower than 5 mg/L can induce a viable but nonculturable state (Afari & Hung, 2018).

3.4 | Efficacy of NEWs on the reduction of E. coli and Listeria inoculated on fresh-cut organic lettuce

The sanitization efficacy of NEWs against inoculated *E. coli* and *Listeria* on organic lettuce is shown in Table 5. The populations of *E. coli* and *Listeria* inoculated on organic lettuce were 7.76 ± 0.16 and 7.43 ± 0.30 log CFU/g, respectively. Soaking of inoculated lettuce in 4 mg/L of commercial NEW for 5 min significantly reduced the population of *E. coli* and *Listeria* (1.40 and 0.92 log CFU/g, respectively) compared with that treated with DI water. In addition, there was no significant difference in the sanitizing efficacies between the commercial NEW and NEW produced by the portable unit ($p < .05$). *E. coli* or *Listeria* was undetected in the NEWs solution after treating the lettuce.

Previous studies revealed that the resistance of the natural microflora on fresh produce to sanitizer is stronger than that of the artificially inoculated microorganisms because of the bacterial cells' weak attachment onto the surface of the product (Kim et al., 1999; Ölmez, 2010; Singh et al., 2002). This should be the reason why in this study the 4 mg/L of NEWs showed a significant effect on inoculated bacteria but no effect on natural microflora on fresh organic lettuce in our previous study (Zhang & Yang, 2017).

Immersing the inoculated lettuce in 45 mg/L of EW for 1 min significantly reduced the surviving populations of *E. coli* O157:H7 and *L. monocytogenes* (2.78 and 2.38 log CFU/leaf reductions compared with the DI control, respectively) (Park et al., 2001). Issa-Zacharia et al. (2011) using dip-inoculation method and treated by 20 mg/L of NEW with 5 min achieved 2.24 log CFU/g reduction (compared with DI) of *E. coli* O157:H7 on lettuce. Furthermore, in our study air-drying the spot-inoculated lettuce under a laminar flow hood for 30 min at room temperature (22°C) reduced both bacterial populations on lettuce by around 1 log CFU/leaf, which is in consistent with a previous study (Park et al., 2001).

TABLE 5 Efficacy of different neutral pH electrolyzed water treatments (5 min) on reducing *Escherichia coli* ATCC 25922 and *Listeria innocua* Seeliger ATCC 33090 populations on artificially inoculated fresh-cut organic lettuce (*Lactuca sativa* Var. *crispa* L)*

	E. coli			*Lister*		
	Surviving population (log CFU/ml in solution or log CFU/on lettuce)			Surviving population (log CFU/ml in solution or log CFU/g on lettuce)		
Treatment	solution	lettuce	Inactivation on lettuce (log CFU/g)	solution	lettuce	Inactivation on lettuce (log CFU/g)
DI**	5.71 ± 0.13[a]	5.88 ± 0.12[a]		5.40 ± 0.18[a]	5.86 ± 0.09[a]	
NEW0	N.D.***	4.49 ± 0.42[b]	1.40 ± 0.32[a]	N.D.	4.95 ± 0.20[b]	0.92 ± 0.16 [a]
NEW1	N.D.	4.69 ± 0.21[b]	1.19 ± 0.19 [a]	N.D.	4.65 ± 0.38[b]	1.21 ± 0.31 [a]
NEW2	N.D.	4.59 ± 0.25[b]	1.31 ± 0.12 [a]	N.D.	4.71 ± 0.40[b]	1.16 ± 0.33 [a]

*Means with different small case letters are significantly different ($p < .05$) among different treatments; **DI: Deionized water. NEW0: neutral pH electrolyzed water produced by the commercial unit. NEW1: neutral pH electrolyzed water produced by reintroducing catholyte liquid to the anode part. NEW2: neutral pH electrolyzed water produced by NaCl and $NaHCO_3$ as electrolyte; ***N.D: not detectable by direct plate count or negative on enrichment media.

Treatments		DI**	NEW0	NEW1	NEW2
Firmness (*N*)		16.4 ± 1.72[a]	15.4 ± 2.49[a]	15.7 ± 2.55[a]	15.9 ± 2.71[a]
Color	*L**	49.30 ± 1.25[a]	49.56 ± 1.63[a]	49.75 ± 2.03[a]	48.96 ± 1.45[a]
	*a**	−10.08 ± 0.42[a]	−9.51 ± 0.76[a]	−9.97 ± 0.65[a]	−10.01 ± 0.54[a]
	*b**	29.05 ± 0.72[a]	29.41 ± 1.15[a]	28.57 ± 1.98[a]	30.46 ± 1.26[a]
	△*E*	58.11 ± 1.25[a]	58.42 ± 1.67[a]	58.25 ± 2.49[a]	58.54 ± 1.42[a]

*Means with different small case letters are significantly different ($p < .05$) among different treatments; **DI: Deionized water. NEW0: neutral pH electrolyzed water produced by the commercial unit. NEW1: neutral pH electrolyzed water produced by reintroducing catholyte liquid to the anode part. NEW2: neutral pH electrolyzed water produced by NaCl and $NaHCO_3$ as electrolyte.

TABLE 6 Effects of different neutral pH electrolyzed water treatments on firmness (*N*) and color (*L**, *a**, *b**, △*E*) of fresh-cut organic lettuce*

3.5 | Firmness and color analysis

The effects of NEW treatments on the textural and color of organic lettuce are shown in Table 6. The results showed that there was no significant difference between different treatments. A similar result has been reported in our previous study that there were no significant differences of firmness and color between H_2O_2 based sanitizers (1% H_2O_2 and the combination with 4 mg/L of EW and 6 g/L of citric acid) and control with a 15 min treatment time (Zhang & Yang, 2017). Xuan and others (2016) also reported that SAEW (pH of 7, FAC of 20 mg/L) effectively disinfected lettuce without effect on the quality of lettuce.

4 | CONCLUSION

In summary, the present study showed the greatest stability of the neutral pH electrolyzed water produced by NaCl and $NaHCO_3$ as electrolyte during the 75 hr storage at 7°C. Moreover, during storage the decay of free available chlorine of neutral pH electrolyzed water produced by redirecting of the catholyte solution back to the anode chamber and neutral pH electrolyzed water produced by the commercial unit could be described mathematically by a first-order kinetic model, which indicated the lower self-decomposition rate of neutral pH electrolyzed water produced by redirecting of the catholyte solution back to the anode chamber than neutral pH electrolyzed water produced by the commercial unit during the initial period. In addition, the changes in the physiochemical properties of neutral pH electrolyzed waters after the storage reduced the 4 mg/L dilution's bactericidal effect after the 75 hr storage. Furthermore, all the three neutral pH electrolyzed waters, (with free available chlorine of 4 mg/L) were effective against *E. coli* ATCC 25922 and *L. innocua* Seeliger ATCC 33090 inoculated on organic lettuce, causing 1.3 and 1.1 log CFU/g reductions, respectively. Thus, this study could provide a valuable reference for neutral pH electrolyzed waters regarding their stability during storage and the reduction of the risk from foodborne pathogens in the future.

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CONFLICTS OF INTEREST

The authors have declared no conflicts of interest for this article.

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