International Scholars Journals

Advanced Journal of Microbiology Research ISSN 2241-9837 Vol. 12 (3), pp. 001-007, March, 2018. Available online at www.internationalscholarsjournals.org © International Scholars Journals

Author(s) retain the copyright of this article.

Full Length Research Paper

Antimicrobial effect of slightly acidic electrolyzed water for inactivation of *Salmonella* spp. and *Escherichia coli* on fresh strawberries (*Fragaria* L.)

Abdulsudi Issa-Zacharia[1,2], Yoshinori Kamitani[3], Happiness Muhimbula[2] and Koichi Iwasaki[3]

[1]Laboratory of Food Biosystems and Environment, 1-21-24 Korimoto, United Graduate School of Agricultural Sciences, Kagoshima University, Kagoshima 890-0065, Japan.
[2]Department of Food Science and Technology, Sokoine University of Agriculture, P. O. Box 3006, Morogoro, Tanzania.
[3]Department of Environmental Science and Technology, Faculty of Agriculture, Kagoshima University, 1-21-24 Korimoto, Kagoshima 890-0065, Japan.

Accepted 24 January, 2018

Antimicrobial effect of slightly acidic electrolyzed water (SAEW: pH 5.6 ± 0.1, 20.5 ± 1.3 mg/L available chlorine concentration; ACC) against indigenous aerobic mesophiles and inoculated *Escherichia coli* and *Salmonella spp*. on fresh strawberry was assessed. The antimicrobial effect of SAEW was compared with that of strong acidic electrolyzed water (StAEW) and sodium hypochlorite (NaOCl) solution. SAEW effectively reduced total aerobic mesophilic bacteria from strawberries by 1.68 $\log_{10}$CFU/g and was not significantly different from that of NaOCl solution ($p > 0.05$). Antimicrobial effect of SAEW against *Salmonella spp.* and *E. coli* was indicated by a more than 2 $\log_{10}$CFU/g reduction of their population and the effect was not significantly different from that of NaOCl solution and StAEW at similar treatment conditions ($p > 0.05$). From these findings, SAEW with a near- neutral pH and low available chlorine concentration exhibits an equivalent bactericidal effectiveness to NaOCl solution and thus SAEW is a potential sanitizer that would be used as an alternative for StAEW and NaOCl solution in the fresh fruit and vegetables industry.

Key words: Slightly acidic electrolyzed water, strawberry, total aerobic mesophilic bacteria, *Escherichia coli*, *Salmonella spp*

INTRODUCTION

Fresh fruits are an important part of the human diet worldwide and consumers continue to eat more fruits partly because of reported health benefits (Beuchat, 1996). Strawberries are among the popular fruits and are mainly eaten raw as an important source of ascorbic acid (vitamin C). Fresh fruits can serve as a vehicle for many spoilage and food-borne pathogenic microorganisms with *Escherichia coli* O157:H7 and *Salmonella spp.* being the most frequent bacterial pathogens associated with fresh produce (Beuchat, 1996). Strawberries are reported to have a short postharvest life, mostly due to high metabolic bacterial activities and fungal decay. Studies have shown that *E. coli, Salmonella* spp. and *Listeria monocytogenes* are able to survive in fresh and frozen strawberries beyond the expected shelf-life of the fruit (Flessa et al., 2005) and therefore, their contamination with food-borne bacteria during harvesting or processing may pose a particular health hazard to consumers; this necessitate effective disinfection before they reach ultimate consumer. Washing produce with tap water cannot be relied upon to completely remove pathogenic and naturally occurring bacteria (Nguyen-The and Carlin, 1994; Yu et al., 2001; Koseki et al., 2004). Chemical compounds such as sodium hypochlorite (Adams et al., 1989), chlorine dioxide (Kim et al., 2009), sodium bisulfite (Krahn, 1977), sulfur dioxide (Bolin et al., 1977), organic acids (Adams et al., 1989), calcium chloride (Izumi and Watada, 1994, 1995), acidified sodium chlorite (Allende

---

*Corresponding author. E-mail: godson.issa@yahoo.co.uk. Tel: +81 80 3978 2632. Fax: +81 99 285 8606.

Abbreviations: **StAEW,** Strong acidic electrolyzed water; **SAEW,** slightly acidic electrolyzed water.

et al., 2009; Liao, 2009) and ozone (Nagashima and Kamoi, 1997) have been shown to reduce microbial populations on fresh produce. However, most of these sanitizers are made from the dilution of condensed solutions, which in handling involves some risk and is troublesome to the user and environment. A sanitizer that is not produced from the dilution of a hazardous condensed solution is required for practical use. For this reason, the use of electrolyzed water (EW) has been introduced as an alternative sanitizer in agriculture and food industry as it is safe for both environment and the user (Al-Haq et al., 2005). Strong acid electrolyzed water (StAEW), which is generated by the electrolysis of a dilute salt (NaCl) solution, has been proven to exhibit strong bactericidal activity for the inactivation of many pathogens (Venkitanarayanan et al., 1999; Park et al., 2004; Fabrizio and Cutter, 2005; Huang et al., 2008; Cao et al., 2009). However, the potential application of SAEW is limited because of its low pH values (2.7) and its corrosive characteristics. At this low pH, dissolved Cl_2 gas can be rapidly lost due to volatilization decreasing the bactericidal activity of the solution with time (Len et al., 2000) and adversely affecting human health and the environment. Moreover, the high acidity of SAEW may cause the corrosion of equipment and consequently limit its practical application (Abadias et al., 2008; Guentzel et al., 2008). Slightly acidic electrolyzed water (SAEW), a newly developed type of electrolyzed water with near-neutral pH value (5.0 - 6.5) is thought to be the best alternative for StAEW in disinfection of food and agricultural products. SAEW is produced by electrolysis of a dilute hydrochloric acid in a chamber without a membrane. At a pH of 5.0 - 6.5, the effective form of chlorine compounds in SAEW is almost (ca. 97%) the hypochlorous acid (HOCl) having strong antimicrobial activity (Honda, 2003; Cao et al., 2009). Therefore, the application of SAEW may improve the bactericidal activity with maximizing the use of hypochlorous acid, reduce corrosion of surfaces and minimize human health and safety issues from Cl_2 off-gassing (Guentzel et al., 2008). Despite these facts, the application of slightly acidic electrolyzed water (pH 5 - 6.5) as a food sanitizer has not been extensively studied in various types of fruits and vegetables as compared to strong acidic electrolyzed water (Koseki et al., 2001, 2004). The objectives of this study were to evaluate the antimicrobial effect of SAEW and compare its efficacy with that of StAEW and NaOCl solution in controlling the survival of indigenous bacteria as well as *Salmonella spp*. and *E. coli* inoculated onto strawberries at 20 ± 2°C.

MATERIALS AND METHODS

Strawberry sample preparation

Fresh strawberries of uniform size, color and maturity with a weight range of 25 - 30 g per fruit were purchased immediately after harvest from Katahira Kankou farm located in Kagoshima city and stored at 10 ± 2°C immediately after arrival at the laboratory. Strawberries were left whole, unhulled, or unwashed to evaluate bactericidal activity of SAEW against indigenous aerobic mesophilic bacteria associated with fresh strawberries. For simulated cross-contamination study, the calyx of the strawberries was removed.

Preparation of treatment solutions

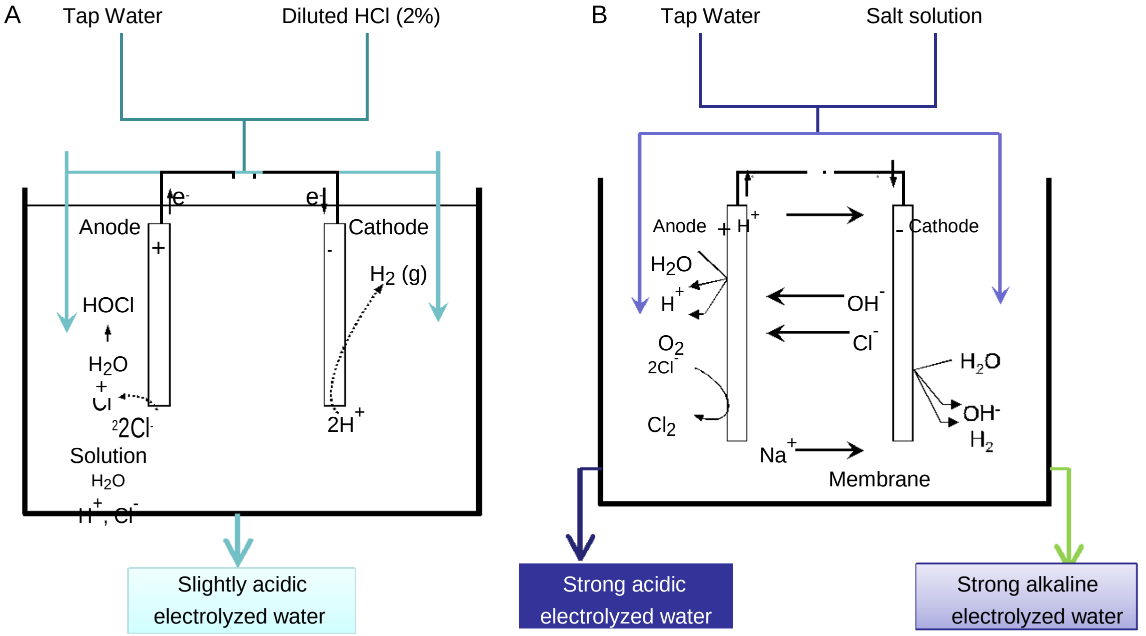
SAEW was generated by electrolysis of a mixture of aqueous dilute solution of HCl (2%) and tap water using Apia60 generator (Apia60, HOKUTY Co., Kanagawa, JAPAN) at 5.0 V, 3.0 A and produced at a rate of 1.0 l/min. SAEW generator basically consists of an electrolytic cell with anode and cathode electrodes and no separating membrane between them (Figure 1a). StAEW was generated by electrolysis of 0.15% sodium chloride (NaCl) solution using a ROX-20TA generator (base model ROX- 20TA, Hoshizaki Electric Co. Ltd., Japan) at 15.0 V, 14.5 A and at a rate of 1.5 l/min. The generator was left to run for 15 min before collecting water for the treatment. The StAEW generator consists of an electrolytic cell where the anode and cathode electrodes are separated by a diaphragm or membrane (Figure1b). With this type of apparatus, both StAEW and strong alkaline electrolyzed water are generated simultaneously. From the anode side of the generator, StAEW was produced and was collected to be used in this experiment. The cathode side produced strong alkaline electrolyzed water that was however, not collected. NaOCl solution was prepared by diluting 10% sodium hypochlorite solution (Wako Pure Chemicals Ind., Ltd., Osaka, Japan) using distilled water to obtain a final desired NaOCl solution. Tap water (TW) was used as control for this experiment.

Analytical measurements

The ORR, pH and ACC of treatment solutions were measured in duplicate immediately after preparation and before each bactericidal experiment. The pH was measured with a pH meter (HM-14P, TOA electronics Ltd., Tokyo, Japan) using a pH combination electrode (GST-2419C) and ORP was measured with ORP meter (RM-12P, TOA Electronics Ltd., Tokyo, Japan) using an ORP electrode (PST-2019C) . The pH meter was calibrated using commercial standard buffers pH 4.01 and 6.86 (Nacalai Tesque, Inc., Kyoto, Japan). Available chlorine concentration of treatment solutions were determined by spectrophotometric method using a spectrophotometer (DR/4000V, HACH Co., Loveland, U.S.A). The detection limit is 0.2 mg/l Cl_2. Therefore samples were first diluted to desired lower levels of ACC using deionized water prior to measurement.

Preparation of inoculum and inoculation of strawberry samples

The pure L-dried culture of *E. coli* NBRC 3301 and salmonella spp. (NBRC 13245) were obtained from NITE Biological Resource Center (NBRC, Japan), revived soon after arrival according to L-dried culture reactivation procedures provided by NBRC and as described in details by Issa-Zacharia et al. (2010) . The viable cell count of *E. coli* and *Salmonella* spp. cultures were verified by pour plate count methodology using standard method agar (NISSUI Pharmaceutical Co., Ltd, JAPAN). The colonies from plated pure culture were propagated once after every 4 days using a 4 by 4 looping out method on solidified standard method agar for preservation. Original *E. coli* and *Salmonella* spp. suspensions were prepared by transferring several colonies to a 10 ml of 0.1% peptone water using a sterile inoculation loop, vortexed using a thermal mixer (TM-100, Tokyo Thermonics Co. Ltd, JAPAN) and transferred to a 50 ml beaker that was filled up to a final volume of 50 ml by sterile 0.1% peptone water. The prepared original bacterial



Basic electrolysis reaction at anode (positive) and cathode (negative) poles

At Anode side

$2Cl^{-} \rightarrow Cl_2 - + 2e^{-}$

$Cl_2 + H_2O \rightarrow HOCl + H^{+} + Cl$

At Cathode side

$H^{+} + 2e^{-} \rightarrow H_2-$

At Anode side (positive)

$2H_2O \rightarrow 4H^{+} + O_2 - + 4e^{-}$

$2NaCl \rightarrow Cl_2 - + 2e^{-} + 2Na^{+}$

$Cl_2 + H_2O \rightarrow HCl + HOCl$

At Cathode side (negative)

$2H_2O + 2e^{-} \rightarrow 2OH^{-} + H_2 -$

$2NaCl + 2OH^{-} \rightarrow 2NaOH + Cl^{-}$

Figure 1. Schematic diagram of electrolyzed water generators resulting compounds during electrolysis. (A) is a SAEW generator and (B) is a StAEW generator. SAEW generator consists of an electrolytic cell with anode and cathode electrodes and no separating membrane between them (Figure 1A). StAEW generator consists of an electrolytic cell where the anode and cathode electrodes being separated by a diaphragm or membrane (Figure 1B). With this type of apparatus, both StAEW and strong alkaline electrolyzed water are generated simultaneously.

suspensions were continuously stirred using a magnetic stirrer (REXIM RS-6DR, ASONE Corporation, Osaka JAPAN) at 500 rpm to maintain the uniform distribution and applied to strawberries within 30 min of preparation. The final prepared bacterial suspension used for dip-inoculation onto the strawberry samples contained bacteria concentration of ca. 10 $\log_{10}$CFU/ml that was determined by plating 1 ml of portion of appropriately diluted *E. coli* and *Salmonella* spp. suspension on standard method agar plates and incubating plates at 37 ± 2°C for 24 ± 2 h. For a simulated cross-contamination study, strawberries were dip-inoculated into prepared *Salmonella spp.* and *E. coli* suspension at a fruit to bacterial suspension ratio (weight) of 1:3 with agitation on the rotary shaker at 150 rpm for 15 min to ensure uniform inoculation. The suspension was decanted and strawberries were placed on a sterile aluminum screen under a biosafety chamber and sterile air-dried for 30 min at room temperature (20 ± 1°C) before washing with different solutions.

Disinfection treatments of inoculated and un-inoculated samples

To evaluate the effectiveness of SAEW, StAEW and NaOCl solution on total aerobic mesophilic count and inoculated *E. coli* and *Salmonella* spp., both un-inoculated and inoculated strawberry samples received similar treatment. Four inoculated or un-inoculated strawberries (100 ± 20 g) were randomly selected and placed into a sterile 500 ml beaker with the aid of sterile forceps. SAEW, StAEW, NaOCl solution or TW (control) was added into the beaker at a fruit to treatment solution ratio of 1:3 by weight and treated for 5 min with agitation to facilitate the exposure between fruit and treatment solutions. The 5 min exposure time was selected to minimize product damage. Moreover, in the study by Udompijitkul et al. (2007) increase of exposure time from 5 to 15 min did not significantly increase the antibacterial effect of electrolyzed oxidizing water against *L. monocytogenes* and *E. coli* O157:H7 on strawberry, thus increase time would mean unnecessary loss of time. The antimicrobial effect of SAEW, StAEW and NaOCl solution against total aerobic bacteria, *E. coli* and *Salmonella spp.* was determined on the whole fruit tissue only.

Microbiological assay

Treated berries were put into sterile sampling bag with 99 ml Butterfield's phosphate buffer (BP) and homogenized in a stomacher (Model 22, TUL Instruments, Barcelona, Spain) at 230 rpm for 2 min. Fruit homogenate was serially diluted in BP, and 1 ml aliquots of appropriate serial dilution were pour-plated with plate standard plate count agar (NISSUI, Tokyo, JAPAN). Populations of mesophilic aerobic bacteria were counted after incubation at 35°C for 48 h and 37 ± 2°C for 24 h in case of *E. coli* and *Salmonella spp*.

Table 1. Physico-chemical properties of treatment solutions used for sanitization of strawberry.

	pH	ORP (mV)[a]	ACC (mg/L)[b]
TW[c]	6.8±0.4	632±6	<1
NaOCl[d]	9.7±0.1	655±7	125.3±1.7
SAEW[e]	5.6±0.1	940±7	20.5±1.3
StAEW[f]	2.5±0.1	1141±2	50.8±4.1

Values are the mean ± standard deviation of duplicate samples with n=10 for each solution; a: Oxidization reduction potential (mV) b: Available chlorine concentration (mg/L) c: Tap water. d: Sodium hypochlorite solution e: Slightly Acidic Electrolyzed water f: Strong Acidic electrolyzed water.

To obtain the initial population of *Salmonella spp.* or *E. coli* on strawberries, four inoculated but untreated strawberries were put into a sterile sampling bag with 99 ml of BP. Similarly, the baseline data for aerobic mesophilic bacteria were obtained by combining untreated samples with 99 ml BP and macerated in the stomacher for 2 min followed by plating procedures as previously described. Microbial counts were expressed as $\log_{10}$ CFU/g sample.

Statistical analysis

Mean of bacteria population reductions ($\log_{10}$ CFU/g) for each treatment was calculated from duplicate plates of each sample (n = 30 for each experiment). Data was expressed as mean ± standard deviation. The results were subjected to one way analysis of variance (ANOVA) and Tukey's HSD test was used to determine the differences at p 0.05 using SPSS 13.0 (SPSS software for Windows, release 13.0, SPSS, Inc., USA).

RESULTS AND DISCUSSION

The physicochemical properties of treatment solutions used in current study are presented in Table 1. SAEW (pH 5.6; 20.5 mg/L ACC; ORP of 940 mV), StAEW (pH 2.5; 50.8 mg/L ACC; ORP of 1141 mV), NaOCl solution (pH 9.7; 125 mg/L ACC; ORP of 655 mV) were used to inactivate indigenous aerobic mesophilic bacteria, *Salmonella spp*. and *E. coli* on strawberry. In this experiment, tap water was used as control. The bactericidal effect of SAEW against indigenous aerobic mesophilic bacteria on strawberry was assessed and compared with other treatment solutions. The surviving populations of aerobic mesophilic bacteria in the macerate of strawberries treated with various sanitizers are summarized in Table 2. SAEW, NaOCl solution and StAEW treatment reduced levels of aerobic mesophilic bacteria in the macerate of the strawberry by 1.68, 1.71 and 2.07 $\log_{10}$CFU/g, respectively. These sanitizers resulted into a significantly higher reduction of indigenous aerobic mesophilic bacteria from strawberries ($p < 0.05$) than tap water (control) which achieved only 0.25 $\log_{10}$CFU/g. The difference in bactericidal effect against indigenous aerobic mesophilic bacteria between NaOCl solution and SAEW was not significant ($p > 0.05$). However, the bactericidal effect of StAEW was significantly higher than that of NaOCl solution and SAEW ($p < 0.05$). Similar reductions were reported by Udompijitkul et al. (2007) who reported a more than 2$\log_{10}$CFU/g of aerobic mesophiles by using electrolyzed oxidizing water (pH 2.27, ACC 68 ppm and 1137 mV of ORP) from strawberries.

The populations of *Salmonella spp.* and *E*. *coli* in the macerate of strawberries treated with various sanitizers are respectively summarized in Tables 3 and 4. When compared with the initial population (untreated), SAEW, NaOCl solution and StAEW significantly decreased the population of *Salmonella spp.* ($p < 0.05$) by 2.12, 2.15 and 2.22 $\log_{10}$CFU/g, respectively. Washing with tap water alone achieved insignificant *Salmonella spp.* reduction. The antimicrobial effect of SAEW, NaOCl solution and StAEW against *Salmonella spp.* (expressed as log reduction) was not significantly different ($p > 0.05$) as shown in Table 3 despite of their differences in available chlorine concentration. The available chlorine in NaOCl solution was > 6 times higher than that of SAEW, while the concentration in StAEW was more twice of SAEW (Table 1). The initial population of *E.coli* on inoculated strawberries was 8.13$\log_{10}$CFU/g. Washing with tap water resulted into insignificant ($p > 0.05$) reduction of *E*. *coli* population and 0.32$\log_{10}$CFU/g reduction was achieved. All the tested sanitizers significantly reduced the population of *E*. *coli* from strawberries and more than 2$\log_{10}$CFU/g of their population was evident. SAEW, NaOCl solution and StAEW respectively achieved a 2.21, 2.29 and 2.77 $\log_{10}$CFU/g reduction of *E. coli* population from strawberries. In the present study, SAEW (pH 5.6, 20.5 mg/L ACC) showed an equivalent antimicrobial effect against *E*. *coli* to that of NaOCl solution (pH 9.7, 125 mg/L ACC) . StAEW (pH 2.5, 51 mg/L ACC) had a significantly higher antimicrobial effect against *E. coli* inoculated on strawberries than SAEW and NaOCl solution.

Strawberries are commonly eaten fresh without further heat treatment and therefore microbial control plays an important role in rendering them safe for human consumption. In the present study, SAEW and other tested sanitizers only achieved a 1.7- 2.0log reduction of indigenous aerobic mesophilic bacteria from strawberries. Higher log reduction in aerobic mesophilic bacteria was

Table 2. Efficacy of SAEW and other sanitizers against indigenous aerobic mesophilic bacteria associated with strawberries.

Treatments	Mean aerobic mesophilic bacteria in macerate (log $_{10}$CFU/g)		
	Before treatment	After treatment	log reduction
TW	7.82A	7.57A	0.25 ± 0.02^{a}
NaOCl	7.82A	6.11B	1.71 ± 0.05^{b}
SAEW	7.82A	6.17B	1.68 ± 0.04^{b}
StAEW	7.82A	5.75B	2.07 ± 0.03^{c}

Mean surviving population (log$_{10}$CFU/g) with different upper case letters (A, B) on the same row (before and after treatment) showed a significant difference at $p < 0.05$. Microbial reduction (log$_{10}$CFU/g) values are the means ± standard deviation and the mean log reduction (log$_{10}$CFU/g) with different lower case letters (a, b) on the same column for were significantly different at $p < 0.05$, Strawberries were dip-treated using NaOCl solution, SAEW and StAEW for 5 min to assess the antimicrobial effect of these sanitizers on the indigenous microorganism present on strawberries, TW: Tap water, NaOCl: Sodium hypochlorite solution, SAEW: Slightly Acidic Electrolyzed water and StAEW: Strong Acidic electrolyzed water.

Table 3. Efficacy of SAEW and other sanitizers against *Salmonella spp.* inoculated on strawberries

Treatments	Mean population of *Salmonella spp*. in macerate (log$_{10}$CFU/g)		
	Before treatment	After treatment	log reduction
TW	8.13A	7.64A	0.49 ± 0.03^{a}
NaOCl	8.13A	5.98B	2.15 ± 0.03^{b}
SAEW	8.13A	6.01B	2.12 ± 0.03^{b}
StAEW	8.13A	5.91B	2.22 ± 0.02^{b}

Mean surviving population of *Salmonella* spp. (log$_{10}$CFU/g) with different upper case letters (A, B) on the same row (before and after treatment) showed a significant difference at $p < 0.05$. Microbial reduction (log$_{10}$CFU/g) values are the means ± standard deviation and the mean log reduction (log$_{10}$CFU/g) with different lower case letters (a, b) on the same column for were significantly different at $p < 0.05$, Strawberry samples that were cross-contaminated by *Salmonella* spp. were dip-treated in NaOCl solution, SAEW and StAEW for 5 min. The antimicrobial effect was assessed by microbial log reduction (log$_{10}$CFU/g) as the result of a 5-min treatment and was calculated relative to un-treated samples, TW: Tap water, NaOCl: Sodium hypochlorite solution, SAEW: Slightly Acidic Electrolyzed water and StAEW: Strong Acidic electrolyzed water.

previously reported by Koseki et al. (2001) and Bari et al. (2003) when strong acidic electrolyzed water was used for decontamination of lettuce and tomato. This is due to the fact that lettuce and tomato have a relatively smooth surface, therefore strong acidic electrolyzed water was highly effective in killing or removing surface microorganisms (Koseki et al., 2004) . A relatively lower reductions observed in current study could be attributed to the surface structure of the strawberry fruit. The strawberry has numerous achenes (seeds) that render its surface structure uneven and complex.

Results of current study have shown that SAEW and StAEW significantly reduced the population of *E.coli* and *Salmonella spp.* by more than 2log$_{10}$ CFU/g. Udompijitkul et al. (2007) reported almost similar results in which washing with strong acidic electrolyzed water significantly decreased mean populations of *E. coli* O157:H7 and *L. monocytogenes* on fresh strawberry by 2log$_{10}$ CFU/ml of solution, indicating that SAEW could equally be used instead of StAEW which is limited by its chlorine loss with time rendering loss of its antimicrobial activity. The observed efficacy of SAEW at low ACC could be due to its high content of hypochlorous acid (HOCl) and high ORP. At a pH of 5.0 - 6.5, the effective form of chlorine compounds in SAEW is almost the hypochlorous acid (HOCl) having strong antimicrobial activity (Yoshifumi, 2003; Cao et al., 2009). Current study showed that the effect of SAEW on the indigenous microbiota (total aerobic mesophilic count) of strawberries was smaller than that obtained with artificially inoculated *Salmonella spp.* and *E. coli.* This was probably because native microbiota on strawberries could have produced biofilms and could have therefore been more attached to, or become trapped in, the strawberry's tissue. Also, the total aerobic mesophilic count would have included many bacteria some of which might be resistant to SAEW and other tested sanitizers. In addition, Seo and Frank (1999) suggested that the effectiveness of disinfectants depends on the accessibility between the active sanitizing agent and the target microorganisms. Therefore, microorganisms that may be embedded in cracks, crevices and stomata or penetrate into interior structures can be protected from the action of disinfectants.

Current study demonstrated that SAEW at lower available chlorine concentration (20.5 mg/l ACC) exhibits a similar level of antimicrobial efficacy to NaOCl solution (125.3 mg/l ACC) against indigenous bacteria and other tested food pathogens. Unlike NaOCl solution, the

Table 4. Efficacy of SAEW and other sanitizers against *E. coli* inoculated on strawberries.

Treatment	**Mean population of *E. coli* in macerate (log $_{10}$CFU/g)**		
	Before treatment	**After treatment**	**log reduction**
TW	8.25A	7.93A	0.32±0.04^a
NaOCl	8.25A	5.96B	2.29±0.07^b
SAEW	8.25A	6.04B	2.21±0.05^b
StAEW	8.25A	5.48B	2.77±0.02^c

Mean surviving population of *E. coli* (log$_{10}$CFU/g) with different upper case letters (A, B) on the same row (before and after treatment) showed a significant difference at $p < 0.05$, Microbial reduction (log$_{10}$CFU/g) values are the means ± standard deviation and the mean log reduction (log$_{10}$CFU/g) with different lower case letters (a, b) on the same column for were significantly different at $p < 0.05$, Strawberry samples that were cross-contaminated by *E. coli* were dip-treated in NaOCl solution, SAEW and StAEW for 5 min. The antimicrobial effect was assessed by microbial log reduction (log$_{10}$CFU/g) as the result of a 5-min treatment and was calculated relative to un-treated samples, TW: Tap water, NaOCl: Sodium hypochlorite solution, SAEW: Slightly Acidic Electrolyzed water and StAEW: Strong Acidic electrolyzed water.

available chlorine in SAEW at pH 5.5 - 6.5 is predominantly (~97%) HOCl (Parish et al., 2003; Sapers and Gorny, 2006; Yoshifumi, 2003). The electrochemically activated HOCl in electrolyzed water is reported to be over 400% more effective than that formed chemically in for example, bleach (Schaik, 2009). It is widely believed that the bactericidal effect of slightly acidic electrolyzed water at a near neutral pH against various strains of bacteria is due to the combined action high oxidation-reduction-potential (ORP-reactions) and dissolved chlorine (HOCl). First, ORP-reactions at the cell membrane damage the outer and inner membrane and inactivate the microbes' defense mechanism. Then HOCl can penetrate the cell and oxidize it. SAEW has ORP of + 900 mV which directly and irreparably damages the microbial cell wall. In addition, the arrangement of water molecules is electrochemically altered which allows better penetrability and interaction of the microbicidal ions, a feature not found in conventional disinfectants (Schaik, 2009). This could further justify that observed antimicrobial efficacy of SAEW at low available chlorine concentration in current study could be due to its high content of hypochlorous acid (HOCl) and high ORP as previously stated. The ORP of + 900 mV for the SAEW used in current study would have contributed to its observed aerobic mesophilic bacteria reduction efficacy. Also, since *E. coli* and *Salmonella spp*. are facultative anaerobes, it is very likely that the high ORP of SAEW too played an influential role, in combination with its high proportion of HOCl for the effective inactivation of these pathogens from contaminated strawberry samples.

Due to the sanitizing equivalence of SAEW and NaOCl solution and increasing public health concerns about the possible formation of chlorinated organic compounds (Singh et al., 2002) and the safety concern that raised doubts in relation to the use of chlorine by the fresh fruit and vegetable industry, SAEW stands a promising chance as a novel disinfectant that could represent an alternative to sodium hypochlorite solution in fresh-cut industry. Moreover, the application of widely used StAEW might be replaced by SAEW, which may improve the antimicrobial activity while maximizing the use of hypochlorous acid, reducing the corrosion of surfaces and minimizing human health and safety issues from Cl_2 off-gassing (Guentzel et al., 2008). SAEW would thus be a potential environmentally friendly food sanitizer to be used towards the current green world advocacy era.

Conclusions

Populations of indigenous aerobic mesophilic bacteria were significantly reduced by SAEW, NaOCl solution and StAEW. Washing with tap water alone did not significantly reduce their population. SAEW at low available chlorine concentration demonstrated equivalent bactericidal effect to NaOCl solution against aerobic mesophilic bacteria and *E. coli* and *Salmonella spp.* inoculated on strawberries. SAEW (pH 5.6, 20.5 mg/L available chlorine) is promising and has potentials as sanitizer that would be used as an alternative for StAEW and NaOCl solution in the fresh fruit and vegetables industry.

ACKNOLEDGEMENT

The authors want to thank HOKUTY Company (Kanagawa, JAPAN) for providing us with a SAEW generator (Apia60).

REFERENCES

Abadias M, Usall J, Oliveira M, Alegre I, Viñas I (2008). Efficacy of neutral electrolyzed water (NEW) for reducing microbial contamination on minimally processed vegetables. Int. J. Food Microbiol., 123: 151-158.

Adams MR, Hartley AD, Cox LJ (1989). Factors affecting the efficacy of washing procedures used in the production of prepared salads. Food

Parish ME, Beuchat LR, Suslow TV, Harris LJ, Garrett EH, Farber JN, Busta FF (2003). Methods of reduce/eliminate pathogens from freshParish ME, Beuchat LR, Suslow TV, Harris LJ, Garrett EH, Farber JN, Busta FF (2003). Methods of reduce/eliminate pathogens from fresh Microbiol., 6: 69-77.

Al-Haq MI, Sugiyama J, Isobe S (2005). Applications of Electrolyzed Water in Agriculture and Food Industries. Food Sci. Technol. Res., 11(2): 135-50.

Allende A, McEvoy J, Tao Y, Luo Y (2009). Antimicrobial effect of acidified sodium chlorite, sodium chlorite, sodium hypochlorite, and citric acid on Escherichia coli O157:H7 and natural microflora of fresh-cut cilantro. Food Control., 20: 230-234.

Bari ML, Sabina Y, Isobe S, Uemura T, Isshiki K (2003). Effectiveness of electrolyzed acidic water in killing *Escherichia coli* O157:H7, Salmonella Enteritidis, and Listeria monocytogenes on the surfaces of tomatoes. J. Food Prot., 66: 542-548.

Beuchat LR (1996). Pathogenic microorganism associated with fresh produce, J. Food. Prot., (59): 204-216.

Bolin HR, Stafford AE, King AD Jr, Huxsoll CC (1977). Factors affecting the storage stability of shredded lettuce. J. Food Sci., 42: 1319-1321.

Brackett RE (1987). Microbiological consequences of minimally processed fruits and vegetables. J. Food Quality., 10: 195-206.

Cao W, Zhu ZW, Shi ZX, Wang CY, Li BM (2009). Efficiency of slightly acidic electrolyzed water for inactivation of *Salmonella enteritidis* and its contaminated shell eggs. Int. J. Food Microbiol., 130: 88-93.

Fabrizio KA, Cutter CN (2003). Stability of electrolyzed oxidizing water and its efficacy against cell suspensions of *Salmonella Typhimurium* and *Listeria monocytogenes*. J. Food Prot., 66: 1379-1384.

Flessa S, Lusk DM, Harris LJ (2005). Survival of Listeria monocytogenes on fresh and frozen strawberries. Int. J. Food Microbiol., 101: 255-262.

Guentzel JL, Lam KL, Callan MA, Emmons SA, Dunham VL (2008). Reduction of bacteria on spinach, lettuce, and surfaces in food service areas using neutral electrolyzed oxidizing water. Food Microbiol., 25: 36-41.

Honda Y (2003). Improvement of the electrolysis equipment and application of slightly acidic electrolyzed water for dairy farming. J. JSAM*.,* 65: 27-29.

Huang YR, Hung YC, Hsu SY, Huang YW, Hwang DF (2008). Application of electrolyzed water in the food industry. Food Control. 19:329-345.

Issa-Zacharia A, Kamitani Y, Morita K, Iwasaki K (2010). Sanitization potency of slightly acidic electrolyzed water against pure cultures of *Escherichia coli* and *Staphylococcus aureus*, in comparison with that of other food sanitizers. Food Control, 21: 740-745.

Izumi H, Watada AE (1994). Calcium treatments affect storage quality of shredded carrots. J. Food Sci., 59: 106-109.

Izumi H, Watada AE (1995). Calcium treatment to maintain quality of zucchini squash slices. J. Food Sci., 60: 789-793.

Kim YJ, Kim MH, Song KB (2009). Efficacy of aqueous chlorine dioxide and fumaric acid for inactivating pre-existing microorganisms and Escherichia coli O157:H7, *Salmonella typhimurium*, and *Listeria monocytogenes* on broccoli sprouts. Food Control., 20: 1002-1005.

Koseki S, Yoshida K, Isobe S, Itoh K (2001). Decontamination of lettuce using acidic electrolyzed water. J. Food Prot., 64: 652-658.

Koseki S, Yoshida K, Isobe S, Itoh K (2004). Efficacy of acidic electrolyzed water for microbial decontamination of cucumbers and strawberries. J Food Prot., 67(6): 1247-1251.

Krahn TR (1977). Improving the keeping quality of cut head lettuce. Acta Horticulture., 62: 79-92.

Len SV, Hung YC, Erickson MC, Kim C (2000). Ultraviolet spectrophotometric characterizations and bacterial properties of electrolyzed oxidizing water as influenced by amperage and pH. J. Food Prot., 63: 1534-1537.

Liao CH (2009). Acidified sodium chlorite as an alternative to chlorine for elimination of Salmonella on Alfalfa Seeds. J. Food Sci., 74: 159-164.

Nagashima T, Kamoi I (1997). Sterilization and preservation of vegetables by ozonated water treatment. Food Preserv. Sci., 23: 127-131.

Nguyen-The C, Carlin F (1994). The microbiology of minimally processed fresh fruits and vegetables. Crit. Rev. Food Sci., 34: 371-401.

and fresh-cut produce. Compr. Rev. Food Sci. Food Safety, 2: 161-173.

Park H, Hung YC, Chung D (2004). Effects of chlorine and pH on efficacy of electrolyzed water for inactivating *Escherichia coli* O157:H7 and *Listeria monocytogenes*. Int. J. Food Microbiol., 91: 13-18.

Sapers GM, Gorny JR, Yousef AE editors (2006). Microbiology of fruits and vegetables, Boca Raton, Fla.: CRC Press, pp. 375-400.

Schaik M (2009). Food Safety, Chemicals, Toxins and Electrolyzed Water. Aquaox company publication. Available at http://aquaox.wordpress.com/2009/10/12/food-safety-chemicals-toxins-and-electrolyzed-water/. Accessed on June 28, 2010.

Seo KH, Frank JF (1999). Attachment of *Escherichia coli* O157:H7 to lettuce leaf surface and bacterial viability in response to chlorine treatment as demonstrated by using confocal scanning laser microscopy. J Food Prot., 62(1): 3-9.

Singh N, Singh RK, Bhunia AK, Stroshine RL (2002). Effect of inoculation and washing methods on the efficacy of different sanitizers against *Escherichia coli* O147:H7 on lettuce. Food Microbiol., 19: 183-193.

Udompijitkul P, Daeschel MA, Zhao Y (2007). Antimicrobial Effect of Electrolyzed Oxidizing Water against *Escherichia coli* O157:H7 and *Listeria monocytogenes* on Fresh Strawberries (*Fragaria* × *ananassa*). J Food Sci. 72(9): M397-M406.

Venkitanarayanan KS, Ezeike GO, Hung YC, Doyle MP (1999). Efficacy of electrolyzed oxidizing water for inactivating *Escherichia coli* O157:H7, *Salmonella enteritidis*, and *Listeria monocytogenes*. Appl. Environ. Microbiol., 65: 4276-4279.

Yu K, Newman MC, Archbold DD, Hamilton-Kemp TR (2001). Survival of *Escherichia coli* O157:H7 on strawberry fruit and reduction of the pathogen population by chemical agents. J. Food Prot., 64(9): 1334-40.