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Effect of mild heat pre-treatment with alkaline electrolyzed water on the efficacy of acidic electrolyzed water against *Escherichia coli* O157:H7 and *Salmonella* on Lettuce

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Abstract

Cut lettuce dip-inoculated with *Escherichia coli* O157:H7 and *Salmonella* was treated with alkaline electrolyzed water (AlEW) at 20°C for 5 min, and subsequently washed with acidic electrolyzed water (AcEW) at 20°C for 5 min. Pre-treatment with AlEW resulted in an approximate 1.8 $\log_{10}$ cfu/g reduction of microbial populations, which was significantly ($p \leqslant 0.05$) greater than microbial reductions resulting from other pre-treatment solutions, including distilled water and AcEW. Repeated AcEW treatment did not show a significant bacterial reduction. Mildly heated (50°C) sanitizers were compared with normal (20°C) or chilled (4°C) sanitizers for their bactericidal effect. Mildly heated AcEW and chlorinated water (200 ppm free available chlorine) with a treatment period of 1 or 5 min produced equal reductions of pathogenic bacteria of 3 $\log_{10}$ and 4 $\log_{10}$ cfu/g, respectively. The procedure of treating with mildly heated AlEW for 5 min, and subsequent washing with chilled (4°C) AcEW for period of 1 or 5 min resulted in 3–4 $\log_{10}$ cfu/g reductions of both the pathogenic bacterial counts on lettuce. Extending the mild heat pre-treatment time increased the bactericidal effect more than that observed from the subsequent washing time with chilled AcEW. The appearance of the mildly heated lettuce was not deteriorated after the treatment. In this study, we have illustrated the efficacious application of AlEW as a pre-wash agent, and the effective combined use of AlEW and AcEW.

Keywords: Mild heat treatment; Acidic electrolyzed water; Alkaline electrolyzed water; Lettuce; *Escherichia coli* O157:H7; *Salmonella*

1. Introduction

Electrolyzed water is produced by the electrolysis of a dilute (ca. 0.1%) sodium chloride (NaCl) solution utilizing a commercially available electrolysis apparatus. The electrolysis apparatus usually electrolyses at a low level of 10–20 V of DC in either a single-cell chamber (Venczel et al., 1997) or a two-cell chamber separated by a diaphragm. Using the two-cell chamber, a strongly acidic electrolyzed water (AcEW) containing hypochlorous acid (HOCl) (Nakagawara et al., 1998), dissolved chlorine gas, and some activated chemicals species such as OH radicals (Suzuki et al., 2002b) is produced in the anode compartment. AcEW is reported to have strong bactericidal effects on most pathogenic bacteria in vitro (Kim et al., 2000; Venkitanarayanan et al., 1999). AcEW show also the decontaminative effects on the surface of lettuce and raw tuna (Koseki et al., 2001; Yoshida et al., 2001). AcEW has effectively inactivated *Escherichia coli* O157:H7, *Salmonella enteritidis* and *Listeria monocytogenes* on lettuce (Park et al., 2001), alfalfa seeds, sprouts (Kim et al., 2003), tomato (Bari et al., 2003), egg surfaces (Russell, 2003) and *Campylobacter jejuni* on poultry (Park et al., 2002). AcEW has also inactivated Staphylococcal enterotoxin-A and aflatoxin (Suzuki et al., 2002a, b). A strongly alkaline electrolyzed water (AlEW), considered akin to a dilute sodium hydroxide, is produced in the cathode compartment but has not yet been fully characterized. Miyashita et al. (1999) reported antioxidative effects of AlEW on highly unsaturated fats

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and oils. Shirahata et al. (1997) reported superoxide dismutase-like and catalase-like activities of the AlEW. However, the effective use of AlEW has not yet been investigated with respect to practical usage as a washing or sanitizing agent.

Mild heat treatment of fresh produce is reported to enhance the bactericidal effect of sanitizers and the physiological and sensory quality of the produce. Delaquis et al. (1999) demonstrated the effects on microbial reduction, using shredded iceberg lettuce in chlorinated water (100 ppm) at 47°C for 3 min. Initial aerobic mesophilic counts were reduced by 3 $\log_{10}$ cfu/g, compared to a reduction of 1 $\log_{10}$ cfu/g at 4°C. The efficacy of a hydrogen peroxide and lactic acid combination in killing microorganisms is greatly enhanced by an increase in temperature (Lin et al., 2002; Venkitanarayanan et al., 2002). Additionally, the sensory quality of lettuce could be improved by the treatment at around 50°C that is referred to mild heat treatment. The treatment delays the onset of discoloration, improves the retention of texture, and reduces the development of bitterness (Delaquis et al., 2000). Similar improvements in quality have been reported for persimmons (Burmeister et al., 1997), apples (Lurie and Klein, 1990), and broccoli (Tian et al., 1997).

The studies reported here were performed to examine the effect of pre-treatment with AlEW on the efficacy of AcEW as a sanitizing agent, and the effect of mild heat treatment on bactericidal effectiveness. We focused on developing an effective application of AlEW for practical use, and on discovering an efficacious produce-washing procedure using a combination of AlEW, AcEW and mild heat.

2. Materials and methods

2.1. Experimental design

We have conducted three experiments. Firstly, effect of pre-treatment with AlEW on the efficacy of subsequent treatment with AcEW against pathogens on lettuce was conducted. Then, we examined the effect of treatment temperatures of sanitizers on lettuce. Finally, the effect of combination of mild heat pre-treatment with AlEW and subsequent treatment with AcEW on the efficacy of bacterial reduction of lettuce was conducted.

2.2. Bacterial strains

Two strain suspensions of *Escherichia coli* O157:H7 (020153 and IID959) and three strain suspensions of *Salmonella* (T010150, SL3770 and E02023) were used in this study. Dr. J. Terajima of the National Institute of Infectious Diseases (NIID), Tokyo, Japan, provided *E. coli* O157:H7 (020153), *Salmonella typhimurium* (T010150), *Salmonella enteritidis* (E020203). *E. coli* O157:H7 (IID959) strain was obtained from the Laboratory of Culture Collection of the University of Tokyo, Tokyo, Japan. Dr. A. Yokota of Hokkaido University, Sapporo, Japan provided *S. typhimurium* (SL3770). All strains of *E. coli* O157:H7 and *Salmonella* were grown in tryptic soy broth (TSB, pH 7.3; Merck, Germany). Each bacterial strain was individually cultured in 10 ml of TSB at 37°C, with transfer using loop inocula at three successive 24-h intervals immediately before use as inocula. Cells of each bacterial strain were collected by centrifugation (2000 g, 15 min, 20°C) and the resulting pellet resuspended in 5 ml of sterile phosphate buffer saline (PBS). Equal volumes of cell suspensions of two or three strains of each pathogen were combined to give approximately equal populations of each strain. The inoculum was maintained at 22±2°C and applied to the lettuces within 1 h of preparation. The inoculum were suspended in 300 ml of sterile PBS, with the final cell concentration approximately 10^8 cfu/ml. Duplicate 0.1 ml quantities of appropriately diluted suspensions of *E. coli* O157:H7 and *Salmonella* were surface plated on tryptic soy agar (TSA) plates that were then incubated at 37°C for 24 h.

2.3. Lettuce evaluated

Head lettuce (variety unknown) was purchased from a local supermarket. The outer three or four leaves and the core were removed from the lettuce head and discarded. Each experiment required the cutting of intact leaves into 5 cm × 5 cm pieces using a sterile surgical knife. Lettuce leaves (ca. 50 g) were submerged in 300 ml of inoculum suspension (approximately 10^8 cfu/ml) at room temperature for 2 h and then drained in a sterile petri dish at room temperature for 1 h under a safety cabinet.

2.4. Pre-treatment with AlEW

AcEW was generated using a flow type electrolysis apparatus (ROX-20TA, HOSHIZAKI Electric Co. Ltd., Toyoake, Aichi, Japan). The current passing through the electrolysis apparatus was set at 16 A, and the voltage between the electrodes was set at 18 V. AcEW (40 ppm free available chlorine) was prepared within the anode compartment of an electrolytic cell and AlEW was prepared within the cathode compartment. The properties of each solution were determined, including pH and free available chlorine concentration. The pH of the tested solution was measured with a pH meter (HM-11P, TOA Electronics Ltd., Tokyo, Japan). Within 1 h prior to treating lettuces the initial concentration of the free available chlorine was determined with chlorine test kits (HACH Co., Loveland, CO, USA), which are EPA approved.

Pre-treatment of inoculated lettuce leaves was performed by immersing 10 pieces of lettuce leaf in 1.5 l of AlEW, distilled water (DW), or AcEW, at 20°C in a 2-l glass beaker that was subsequently agitated vigorously at 150 rpm (MMS-3010 Multi Shaker, EYELA, Tokyo, Japan) for 5 min. The lettuce leaves were removed from the pre-treatment solution, and then immediately immersed in 1.5 l of AcEW at 20°C for 5 min with agitation (150 rpm). When treating only with AcEW, the same procedure was used, but without a pre-treatment. After the treatment, the treated lettuce was rinsed twice with 1 l sterile DW (20°C) with agitation (150 rpm).

2.5. Treatment of lettuce with sanitizers at different temperatures

Treatment of inoculated lettuce leaves was performed by immersing 10 pieces of lettuce leaf into 1.5 l of the appropriate treatment solution (AcEW, chlorinated water and DW) at 4°C, 20°C, and 50°C, in a 2-l glass beaker that was subsequently agitated vigorously at 150 rpm for 1 or 5 min. The treated lettuce was subsequently rinsed twice with 1 l of sterile DW (20°C). Chlorinated water (200 ppm free available chlorine) was prepared by adding sodium hypochlorite (NaOCl 5%, Kanto chemical, Tokyo, Japan) to DW. Sterile DW was used as a control. Properties of each solution were determined as previously described.

2.6. Mild heat pre-treatment with AlEW

Lettuce leaves were pre-treated by immersing 10 pieces of lettuce leaf in 1.5 l of AlEW or DW at 20°C or 50°C in a 2-l glass beaker with vigorous agitation (150 rpm) for 1 or 5 min. The lettuce leaves were subsequently taken out of the pre-treatment solution and immediately immersed in 1.5 l of AcEW at 4°C for 1 or 5 min with agitation (150 rpm). After treatment, the treated lettuce was rinsed twice with 1 l of sterile DW (20°C).

2.7. Bacterial analysis

Levels of *E. coli* O157:H7 or *Salmonella* were determined for the resulting treatment solution used for the washing of lettuce. Undiluted wash solution was surface plated in quadruplicate (0.25 ml), serially diluted in 0.1% peptone water and plated in duplicate (0.1 ml) on appropriate enumeration media.

The population of *E. coli* O157:H7 or *Salmonella* in the homogenate was determined. The 10 pieces of lettuce leaves (15–25 g) treated in each solution were serially weighed and combined with 200 ml of 0.1% peptone water in a sterile polyethylene bag, and then macerated for 2 min at high speed in a Stomacher® (Seward, London, UK). Since the weight varied for each sample, the dilution factors were properly calculated based on the actual weights used. Undiluted lettuce homogenate was surface plated in quadruplicate (0.25 ml), serially diluted in 0.1% peptone water and plated in duplicate (0.1 ml) on appropriate enumeration media.

E. coli O157:H7 was enumerated on sorbitol MacConkey agar (SMAC, Merck, Germany) supplemented with CT selective supplement (Merck, Germany), and incubated at 37°C for 24 h. The presence of *E. coli* O157:H7 was confirmed using the latex agglutination test (*E. coli* single-path, Merck, Germany). *Salmonella* was enumerated on bismuth sulfate agar (BSA, Merck, Germany) incubated at 37°C for 24 h. The presence of *Salmonella* was confirmed by testing reactions on triple sugar iron (Merck) slants.

2.8. Statistical analysis

Three replicate trials for each pathogen were performed. Each trial contained three samples (10 pieces of lettuce per sample), making a total of nine samples analysed for each combination of test parameters. Reported plate count data are expressed as the mean $\pm$ standard error (SE). The data was subjected to Statview (SAS Campus Drive, Cary, NC) for Tukey–Kramer's multiple comparison test to determine statistical significance ($P \leqslant 0.05$).

3. Results and discussion

3.1. Effect of pretreatment on the efficacy of subsequent treatment with AcEW

Estimated pH values were 2.6 ± 0.1, 11.4 ± 0.1, and 6.2 ± 0.1 for AcEW, AlEW and sterile DW, respectively. Free available chlorine concentration of AcEW was 40.3 ± 1.5 ppm.

The effect of pretreatment on the efficacy of AcEW as a sanitizing agent is summarized in Table 1. Pretreatment with AlEW and subsequent AcEW treatment reduced levels of *E. coli* O157:H7 and *Salmonella* on lettuce by approximately 1.8 and 1.7 $\log_{10}$ cfu/g, respectively. This reduction exceeds that resulting from other pretreatments ($P \leqslant 0.05$). AlEW thus enhanced the efficacy of AcEW as a sanitizing agent. The treatment with only AcEW exhibited a bacterial reduction (1.3–1.4 $\log_{10}$ cfu/g) that was equal to the reduction observed in trials involving pretreatment with DW or AcEW. Multiple washing with AcEW did not result in greater bacterial reduction.

Very few effective applications of AlEW have been examined with respect to the use of electrolyzed water as a sanitizing agent. This study revealed that levels of

Table 1
Effect of pretreatment with some solutions at 20°C for 5 min on the efficacy of subsequent treatment with AcEW at 20°C for 5 min against *E. coli* O157:H7 and *Salmonella* on lettuce

Prewash (20°C, 5 min)	Subsequently with AcEW (20°C, 5 min)	
	E. coli O157:H7[a] ($\log_{10}$ cfu/g)	*Salmonella* ($\log_{10}$ cfu/g)
Control[b]	7.14±0.12A	7.07±0.14A
None[c]	5.78±0.22B	5.82±0.19B
AcEW[d]	5.61±0.30B	5.74±0.24B
AlEW[e]	5.32±0.13C	5.42±0.12C
DW[f]	5.85±0.21B	5.91±0.27B

[a] Results are mean±standard error of mean, $n = 9$. Values with different letter in each column are significantly different ($P \leqslant 0.05$).
[b] No treatment.
[c] Without prewash, treated with only AcEW.
[d] Wash twice with AcEW.
[e] Prewash with alkaline electrolyzed water.
[f] Prewash with distilled water.

pathogens inoculated on lettuce have been reduced by pre-treatment with AlEW. Pre-treatment with AlEW was more effective than pre-treatment with DW or AcEW. Repeated treatment with AcEW also failed to demonstrate a remarkable reduction of bacterial populations, and yielded reductions that were less than those observed in trials involving pretreatment with AlEW. AlEW is considered to act like a dilute sodium hydroxide aqueous solution (Ryoo et al., 2002). Accordingly, AlEW would act like a surfactant, and the hydrophobicity of the fruit surface would therefore be decreased when washed with such a solution. Consequently, any microorganisms on the surface of the fruit could be easily accessed with an AcEW solution, and a high reduction of levels of microbial populations would thus be achieved. The efficacy of pretreatment with AlEW has been demonstrated, but its effectiveness is limited: it yields a maximum reduction of 1.8 $\log_{10}$ cfu/g and we have estimates of 10^5 cfu/g pathogens surviving after such treatment. A more effective procedure for reducing levels of pathogens is therefore necessary.

3.2. Effect of temperature on the efficacy of a sanitizer

The efficacy of sanitizers, including AcEW (pH 2.6, 40 ppm), chlorinated water (pH 9.1, 200 ppm), DW (pH 6.2), and AlEW (pH 11.4), in reducing populations of *E. coli* O157:H7 and *Salmonella* are shown in Tables 2 and 3, respectively. Washing with AcEW and chlorinated water at 4°C and 20°C for 1 min was no more effective than washing with DW and AlEW at 4°C and 20°C for 1 min, with an observed bacterial population reduction of 0.6–0.9 $\log_{10}$ cfu/g for *E. coli* O157:H7 and *Salmonella*, respectively. Washing with AcEW and chlorinated water at 50°C for 1 min resulted in a bacterial reduction of 2.7–3.0 $\log_{10}$ cfu/g for both pathogens. Further significant ($P \leqslant 0.05$) reductions of approximately 4.0 $\log_{10}$ cfu/g for both pathogens were achieved by 5 min treatment with AcEW and chlorinated water at 50°C. There was no significant difference between the efficacy of AcEW and chlorinated water. Washing with DW and AlEW at 50°C for 1 min resulted in a bacterial reduction of 2.2 $\log_{10}$ cfu/g, and further significant ($P \leqslant 0.05$) reductions of 2.8 $\log_{10}$ cfu/g for both pathogens were achieved with a 5 min treatment.

There were no surviving bacteria in the AcEW or chlorinated water solutions after washing lettuce samples in such trials. However, surviving pathogenic bacteria were detected in the DW and AlEW. Both pathogens survived at an approximate level of 3 $\log_{10}$ cfu/ml in DW regardless of treatment temperature. Survival levels of populations of both pathogens were 2.3–3.0 $\log_{10}$ cfu/ml in AlEW at 4°C and 20°C. In the meantime surviving populations were reduced by 1.4–2.0 cfu/ml in AlEW at 50°C, and exhibited levels of bacterial population survival were significantly ($P \leqslant 0.05$) less than all other treatments.

A conventional technique used for the improvement of the efficacy of a sanitizer is to use a higher temperature during the treatment. There have been reports on the effect of a heated sanitizer in killing or removing bacteria on produce. Delaquis et al. (1999) have studied the effect of treating shredded lettuce in chlorinated water for 3 min at 47°C on microbiological quality. Initial aerobic bacterial counts were reduced by 3 $\log_{10}$ cfu/g compared to a reduction of 1 $\log_{10}$ cfu/g on lettuce treated at 4°C. Mildly heated (50°C) 2% hydrogen peroxide reduced the *E. coli* O157:H7 and *S. enteritidis* inoculated on lettuce by approximately 4 $\log_{10}$ cfu/g (Lin et al., 2002). Our results also presented a remarkable reduction of ca. 4 $\log_{10}$ cfu/g using a treatment with mildly heated (50°C) AcEW or chlorinated water for 5 min. Furthermore, dipping in water at 45–55°C would extend the shelf life and maintain the visual quality of cut lettuce (Delaquis et al., 2000). However, a treatment involving mildly heated AcEW and chlorinated water has a serious problem in that the

Table 2
Effect of temperature on the efficacy of sanitizers against *E. coli* O157:H7 on lettuce

Treatment	Temp. (°C)	Time (min)	*E. coli* O157:H7[a]	
			Lettuce ($\log_{10}$ cfu/g)	Solution ($\log_{10}$ cfu/ml)
None	—	–	7.26±0.19A	—
AcEW[b]	4	1	6.51±0.12B	ND[f]
		5	5.98±0.21C	ND
	20	1	6.64±0.30B	ND
		5	6.01±0.37C	ND
	50	1	4.38±0.11E	ND
		5	3.23±0.14F	ND
Chlorine[c]	4	1	6.41±0.14B	ND
		5	6.03±0.24C	ND
	20	1	6.49±0.33B	ND
		5	5.98±0.42C	ND
	50	1	4.22±0.31E	ND
		5	3.34±0.24F	ND
DW[d]	4	1	6.57±0.17B	3.41±0.21A
		5	6.48±0.11B	3.56±0.30A
	20	1	6.66±0.19B	3.34±0.22A
		5	6.51±0.19B	3.29±0.18A
	50	1	4.98±0.08D	3.11±0.23AB
		5	4.53±0.20E	2.98±0.17B
AlEW[e]	4	1	6.65±0.18B	2.50±0.16C
		5	6.53±0.09B	2.33±0.21C
	20	1	6.71±0.16B	2.42±0.22C
		5	6.57±0.22B	2.28±0.14C
	50	1	5.03±0.15D	1.79±0.21D
		5	4.47±0.08E	1.41±0.32E

[a] Values are mean±standard error of mean, $n = 9$. Values in the same column that are not followed by the same letter showed significantly difference ($P \leqslant 0.05$).
[b] Acidic electrolyzed water.
[c] Chlorinated water containing 200 ppm free available chlorine.
[d] Sterile distilled water.
[e] Alkaline electrolyzed water.
[f] No colonies were detected. Minimum level of detection was 10 cfu/ml of solution.

chlorine gas easily volatilizes at higher temperatures. Gases are generally difficult to dissolve in water or other solutions with elevated temperature. Therefore, AcEW must be used at the lowest possible temperatures to prevent volatilization of the chlorine gases in AcEW treatments.

3.3. Effect of mild heat pre-treatment with AlEW

The pre-treatment effect of mildly heated AlEW and DW on the efficacy of AcEW as a sanitizing agent is summarized in Table 4. The mildly heated (50°C) pre-treatment with AlEW or DW for 1 min with a subsequent treatment of AcEW (4°C) resulted in a 2.7 $\log_{10}$ cfu/g reduction for both pathogens, regardless of the duration of the subsequent treatment with AcEW. This result was revealed as 1.5–1.7 $\log_{10}$ cfu/g greater reductions of bacterial populations in such trials relative to reductions observed for pre-treatment with AlEW or DW at normal temperature (20°C), regardless of the duration of the subsequent treatment with AcEW (4°C). Further significant ($P \leqslant 0.05$) reductions of approximately 4.0 $\log_{10}$ cfu/g for both pathogens were achieved by mildly heated (50°C) pre-treatment with AlEW or DW for 5 min, irrespective of the subsequent treatment time (1 or 5 min) with AcEW (4°C). This treatment resulted in at least a 2.2 $\log_{10}$ cfu/g greater reduction of bacterial populations relative to the pre-treatment trials involving AlEW or DW at a normal temperature (20°C). Trials involving the mildly heated pre-treatment for 5 min with a subsequent AcEW (4°C) treatment exhibited a bactericidal effectiveness equal to that observed for the 5 min treatment of heated (50°C) AcEW and chlorinated water (Tables 2 and 3). The appearance of the mildly heated lettuce regardless of the treatment time (1 or 5 min) was not deteriorated by the macroscopic evaluation just after the treatment and 4 days after storage at 10°C (data not shown).

Table 3
Effect of temperature on the efficacy of sanitizers against *Salmonella* on lettuce

Treatment	Temp. (°C)	Time (min)	*Salmonella*[a]	
			Lettuce ($\log_{10}$ cfu/g)	Solution ($\log_{10}$ cfu/ml)
None	—	—	6.99±0.17A	—
AcEW[b]	4	1	6.22±0.22B	ND[f]
		5	5.85±0.27BC	ND
	20	1	6.19±0.28B	ND
		5	5.77±0.37C	ND
	50	1	4.13±0.16E	ND
		5	3.16±0.11F	ND
Chlorine[c]	4	1	6.23±0.17B	ND
		5	5.88±0.27BC	ND
	20	1	6.18±0.24B	ND
		5	5.79±0.15C	ND
	50	1	4.21±0.25E	ND
		5	3.19±0.16F	ND
DW[d]	4	1	6.41±0.19B	3.57±0.17A
		5	6.26±0.33B	3.48±0.11A
	20	1	6.37±0.23B	3.66±0.19A
		5	6.21±0.28B	3.51±0.19A
	50	1	4.86±0.21D	2.98±0.08B
		5	4.18±0.17E	2.83±0.20B
AlEW[e]	4	1	6.50±0.16B	2.98±0.18B
		5	6.33±0.21B	2.83±0.29B
	20	1	6.42±0.22B	2.71±0.16BC
		5	6.28±0.14B	2.57±0.22C
	50	1	4.79±0.21D	2.03±0.25D
		5	4.10±0.13E	1.57±0.28E

[a] Values are mean±standard error of mean, $n = 9$. Values in the same column that are not followed by the same letter showed significantly difference ($P \leqslant 0.05$).
[b] Acidic electrolyzed water.
[c] Chlorinated water containing 200 ppm free available chlorine.
[d] Sterile distilled water.
[e] Alkaline electrolyzed water.
[f] No colonies were detected. Minimum level of detection was 10 cfu/ml of solution.

AcEW used at low temperatures diminishes the bactericidal effect compared to its use with mild heat (Tables 2 and 3). In this study we have met the challenging task of developing a new concept of washing procedure that consists of a mild heat pre-treatment with AlEW and a subsequent treatment with chilled (4°C) AcEW. The bacterial reduction resulting from our new procedure displayed an equal level of reduction as compared to that observed for treatments involving AcEW or chlorinated water at mild heat (50°C). Extending the pre-treatment time of the mildly heated solution increased the bactericidal effect of the subsequent AcEW treatment, regardless of the duration of treatment with AcEW. Pathogens inoculated onto lettuce would have been susceptible to the sanitizer by our mild heat treatment method. Accordingly, after the mild heat treatment, 1 min AcEW treatment would be sufficient to yield an efficacious sanitizing effect. The advantage of using AlEW as a pretreatment compared to DW is it diminishes at 50°C on lettuce. However, the bacterial population in the AlEW after treatment at 50°C showed lower population in DW. Since pretreatment with AlEW at 50°C would reduce the risk of cross contamination more than with DW, there would be an advantage for use of AlEW.

Our suggested washing procedure includes the advantages of a mild heat treatment and the greater bactericidal effectiveness of treatments utilizing heated sanitizers. The use of chilled (4°C) AcEW has more advantages that control chlorine gas volatilization in AcEW and reduce the temperature of produce. Washing lettuce in chilled chlorinated water (4°C) limited the growth of *E. coli* O157:H7 during subsequent storage, whereas washing at 47°C had the opposite effect (Delaquis et al., 2002). The mild heat pretreatment and a subsequent treatment with chilled AcEW would

Table 4
Effect of temperature of pretreatment solution and treatment time on the efficacy of subsequent treatment with AcEW at 4°C

		Prewash		With AcEW (4°C)	
		Temp. (°C)	Time (min)	1 min Population[a] (log_{10} cfu/g)	5 min Population (log_{10} cfu/g)
E. coli O157:H7	Control[b]	—	—	7.16±0.08A	7.16±0.08A
	AlEW[c]	20	1	6.05±0.21B	5.95±0.22B
			5	5.81±0.19B	5.51±0.15C
		50	1	4.38±0.14C	4.42±0.21D
			5	3.23±0.16D	3.29±0.13E
	DW[d]	20	1	6.15±0.16B	5.97±0.22B
			5	5.92±0.22B	5.84±0.27B
		50	1	4.29±0.24C	4.38±0.21C
			5	3.35±0.17D	3.11±0.13D
Salmonella	Control	—	—	7.20±0.11A	7.20±0.11A
	AlEW	20	1	6.16±0.22B	6.03±0.24B
			5	5.98±0.18B	5.69±0.28C
		50	1	4.29±0.15C	4.17±0.12D
			5	3.36±0.20D	3.24±0.17E
	DW	20	1	6.25±0.26B	6.18±0.18B
			5	6.02±0.17B	5.92±0.23B
		50	1	4.38±0.13C	4.27±0.11D
			5	3.28±0.19D	3.19±0.14E

[a] Values are mean±standard error of mean, $n = 9$. Values in the same column of each pathogen that are not followed by the same letter showed significantly difference ($P \leqslant 0.05$).
[b] No treatment.
[c] Alkaline electrolyzed water.
[d] Sterile distilled water.

also be expected to achieve bacterial growth control. However, the pathogenic bacteria survived in the AlEW solutions involving prewashing with mild heat. For the prevention of cross contamination of the pathogen during the washing procedure, fresh solution of AlEW during the prewashing period should be continuously supplied. The washing procedure suggested in this study makes combined use of both electrolyzed waters (AcEW and AlEW). Since these two solutions are generated by one apparatus simultaneously and continuously, an effective washing system could be built with an electrolysis apparatus.

It is investigated that the results and new methodologies presented in this study will help to create effective sanitizing agents for produce and foodstuffs that will benefit manufacturers and consumers alike.

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