JARQ 41 (4), 273 – 282 (2007) http://www.jircas.affrc.go.jp

REVIEW
Microbial Control of Fresh Produce using Electrolyzed Water

Shigenobu KOSEKI* **and Seiichiro ISOBE**

National Food Research Institute (Tsukuba, Ibaraki 305–8642, Japan)

Abstract
The demand for fresh salad vegetables, such as iceberg lettuce, has increased worldwide in recent years. Numerous sanitizers have been examined for their effectiveness in killing or removing pathogenic bacteria on fresh produce. However, most of the sanitizers are made from the dilution of condensed solutions which involves some risk in handling and is troublesome. A sanitizer that is not produced from the dilution of a hazardous condensed solution is required for practical use. Electrolyzed water and ozonated water were investigated for bactericidal effects on fresh-cut produce as a convenient and safe alternative sanitizer. Although the efficacy of acidic electrolyzed water (AcEW) as a sanitizing agent was dependent on the kind of produce treated, AcEW could be sufficiently effective to offer an alternative solution to conventional sanitizers, such as sodium hypochlorite solution (150 ppm). A novel produce-washing procedure using a combination of alkaline electrolyzed water (AlEW), AcEW and mild heat demonstrated significant bactericidal effect compared with the treatment with ambient temperature. Besides the bactericidal effect, the progress of browning on lettuce was suppressed by using mild heat treatment. Furthermore, as a novel usage of AcEW, we examined the use of AcEW-ice for preserving vegetables. AcEW-ice inactivated the spoilage and pathogenic bacteria on lettuce and reduced the temperature of lettuce during storage.

Disciplines: Food / Postharvest technology
Additional key words: browning, food-borne pathogen, ozonated water, sanitization

Introduction

The demand for fresh salad vegetables and fruit has increased in recent years worldwide. Rising consumption levels have resulted in a higher frequency of outbreaks of foodborne illness associated with raw produce[1,6,10,37]. Sanitization of produce plays an important role in the preservation of food quality and safety of consumption. The control of spoilage bacteria and pathogenic bacteria is a requirement for both distributors and consumers. Numerous sanitizers have been examined for their effectiveness in killing or removing pathogenic bacteria on fresh produce, such as *Escherichia coli* O157:H7, *Salmonella* spp. and *Listeria monocytogenes*[7]. Washing produce with tap water cannot be relied upon to completely remove pathogenic and naturally occurring bacteria[8,40]. Chlorinated water (mainly sodium hypochlorite, NaOCl) is the most frequently used sanitizer for the washing of produce. This treatment, however, has a minimal sanitizing effect and results in less than a 2 $\log_{10}$ CFU/g reduction of bacteria on produce[2,7,19,56]. Although other sanitizers including chlorine dioxide (ClO_2), hydrogen peroxide (H_2O_2), organic acid, and calcinated calcium solution have been evaluated[4,17,23,35], these sanitizers have a minimal sanitizing effect which is equal to that of chlorinated water. Moreover, most of these sanitizers are made from the dilution of condensed solutions, which in handling involves some risk and is troublesome. A sanitizer that is not produced from the dilution of a hazardous condensed solution is required for practical use.

Electrolyzed water is produced by the electrolysis of a dilute (0.1–0.2%) sodium chloride (NaCl) solution utilizing a commercially available apparatus. The electrolysis apparatus usually electrolyzes at a low level of 10–20 V of DC in a two-cell chamber separated by a diaphragm (Fig. 1). In the anode cell, water reacts on the anodic electrode and produces oxygen and hydrogen ion. Chlorine ion also reacts on the electrode and generates chlorine gas. Chlorine gas reacts with water, and generates hypochlorous acid (HOCl). As a result, a low pH solution containing a low concentration of HOCl is pro-

*Corresponding author: e-mail koseki@affrc.go.jp
Received 15 January 2007; accepted 17 April 2007.

duced in the anode cell. This solution is called acidic electrolyzed water (AcEW). AcEW contains hypochlorous acid (HOCl)[39], dissolved chlorine gas and some activated chemical species. On the other hand, in the cathode cell, water reacts on the cathode electrode and produces hydrogen and hydroxide ion. A high pH solution is produced in the cathode cell. This solution is called alkaline electrolyzed water (AlEW). AcEW is reported to have strong bactericidal effects on most pathogenic bacteria in vitro[21,51]. Decontaminative effects of AcEW on the surface of lettuce and raw tuna were reported[26,54]. AcEW has effectively inactivated *E. coli* O157:H7, *S. enteritidis* and *L. monocytogenes* on lettuce[42], alfalfa seeds and sprouts[22], tomato[5], and egg surfaces[44], as well as *Campylobacter jejuni* on poultry[43].

Ozone is a strong oxidant and strong antimicrobial agent with high reactivity. Since ozone decomposes spontaneously to oxygen, it leaves no toxic residue. Therefore, ozone has been proposed as an alternative sanitizer to chlorine that can produce toxic compounds such as trihalomethane[20,24]. Ozone has been shown to inactivate bacteria on various produce, including lettuce[23,26], carrot[34], bean sprouts[38], and alfalfa seeds and sprouts[45,46]. The effects recorded were almost equal to that of chlorinated water. However, these trials showed little effectiveness compared to in vitro experiments on the same microorganisms. The presence of organic matter and the accessibility limit of ozone to the surface of the object treated would influence the potential bactericidal effect[25]. Some researchers have also reported a negative effect of ozone treatment on the quality of the fruits and vegetables, such as the altered surface color of peaches[3] and carrots[34].

In this review, the application of electrolyzed water and ozonated water for sanitization of fresh-cut produce is described. Besides the effect of electrolyzed water and ozonated water as a sanitizer, the quality of the cut-produce treated with electrolyzed water during storage is discussed. Furthermore, a different approach for the use of electrolyzed water as an ice to preserve fresh produce at low temperature and control microbial growth during storage has been reported.

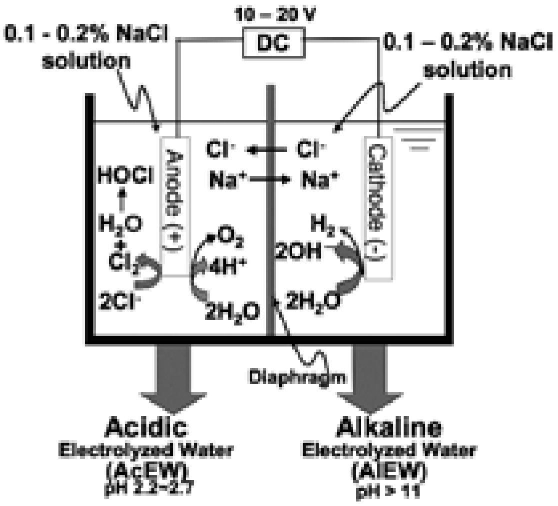

Fig. 1. A schematic representation on generation of electrolyzed water

Sanitization of fresh-cut produce using electrolyzed water

1. Decontamination of fresh-cut produce[26,30]

Fresh-cut lettuce was treated with various sanitizers. The microorganisms on the lettuce treated with various sanitizers were enumerated (Fig. 2). The physicochemical properties of the tested solutions are shown in Table 1. AcEW and NaOCl solution reduced the viable aerobic bacteria on the lettuce by 2 $\log_{10}$ CFU/g within 10 min. For lettuce washed with AlEW for 1 min and then treated with AcEW for 1 min (this treatment is referred to as 1+1 treatment), viable aerobic mesophilic bacteria on the lettuce were reduced by 2 $\log_{10}$ CFU/g. On the other hand, treatment with ozonated water reduced the viable aerobic mesophilic bacteria on the lettuce by 1.5 $\log_{10}$ CFU/g

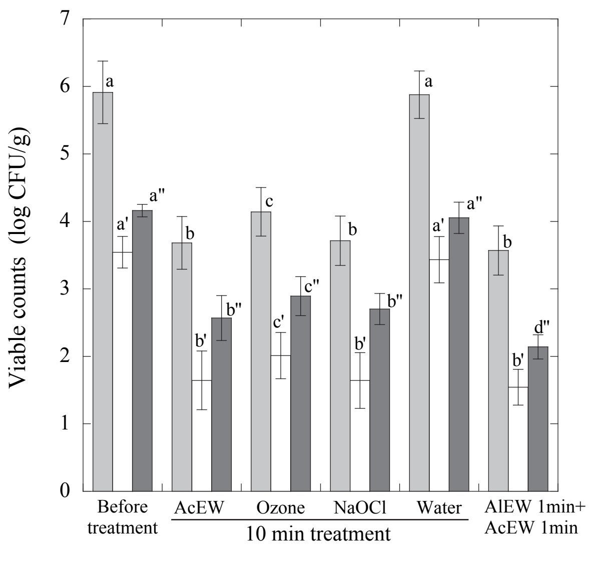

Fig. 2. Comparison of bactericidal effect on the lettuce treated with acidic electrolyzed water (AcEW), ozonated water (Ozone), NaOCl, and tap water (Water) for 10 min, and washed with alkaline electrolyzed water (AlEW) for 1 min and then treated with acidic electrolyzed water (AcEW) for 1 min

□ : Aerobic bacteria, □ : Coliform, ■ : Fungi.
Results are means ± SD, n = 5. Values with different letters in the same medium differ significantly at $P < 0.05$.

within 10 min. Tap water did not decrease aerobic bacteria in the lettuce.

Coliform bacteria populations were reduced to less than 10^2 CFU/g by all treatments except for tap water. There were little differences in the bactericidal effect among the treatments. Although the difference in bactericidal effect between AcEW and NaOCl solution was not significant, the effect of ozonated water was smaller than the other two treatments significantly at $P < 0.05$. Fungal populations were reduced by 1.5 log_{10} CFU/g by the treatment with AcEW, NaOCl and 1+1. Treatment with ozonated water reduced molds and yeasts by about 1 log_{10} CFU/g.

The surfaces of the lettuce were observed by scanning electron microscope (SEM) for microorganisms and surface structure. The surfaces of the untreated lettuce and lettuce treated with AcEW are shown in Figs. 3(A) and 3(B). There were many bacteria widely distributed on the surface of untreated lettuce, whereas no bacteria were seen on the surface of lettuce treated with AcEW. The residual microorganisms after treatment in the lettuce were supposed to be either inside the cellular tissue such as the stomata, or making the biofilm on the surface of lettuce[47–49].

Furthermore, we examined the bactericidal effect of AcEW on cucumber and strawberry. The microbial populations of cucumber and strawberry treated with the various sanitizers are summarized in Table 2. No changes in appearance of cucumber were shown after all treatments. AcEW and NaOCl reduced the number of aerobic mesophilic bacteria in the cucumber by 1.4 and 1.2 log_{10} CFU/cucumber, respectively. Ozonated water reduced the aerobic mesophilic bacteria by 0.7 log_{10} CFU/cucumber. Populations of aerobic mesophilic bacteria were reduced by 2.1 log_{10} CFU/cucumber for the 5+5 treatment (5 min treatment with AlEW followed by 5 min treatment with AcEW). The 5+5 treatment showed significantly greater bacterial reduction than the other treatments ($P \leq 0.05$).

The appearance of the strawberries was not affected by all treatments. The AcEW, NaOCl and 5+5 treatment reduced levels of aerobic mesophilic bacteria of the strawberry by 0.8 to 0.9 log_{10} CFU/strawberry. Ozonated water

Table 1. Physicochemical properties of tested solutions

	pH	ORP (mV)	ACC[a)] or OC[b)] (ppm)
AcEW[c)]	2.6 ± 0.1	1,140 ± 7	30.3 ± 3.1
Ozonated water	6.7 ± 0.1	1,256 ± 28	5.1 ± 0.3
Tap water	7.0 ± 0.1	414 ± 25	0.3 ± 0.1
NaOCl[d)]	9.3 ± 0.2	638 ± 18	155.2 ± 5.8
AlEW[e)]	11.4 ± 0.1	−870 ± 12	–

a): Available chlorine concentration.
b): Ozone concentration.
c): Acidic electrolyzed water.
d): Sodium hypochlorite.
e): Alkaline electrolyzed water.

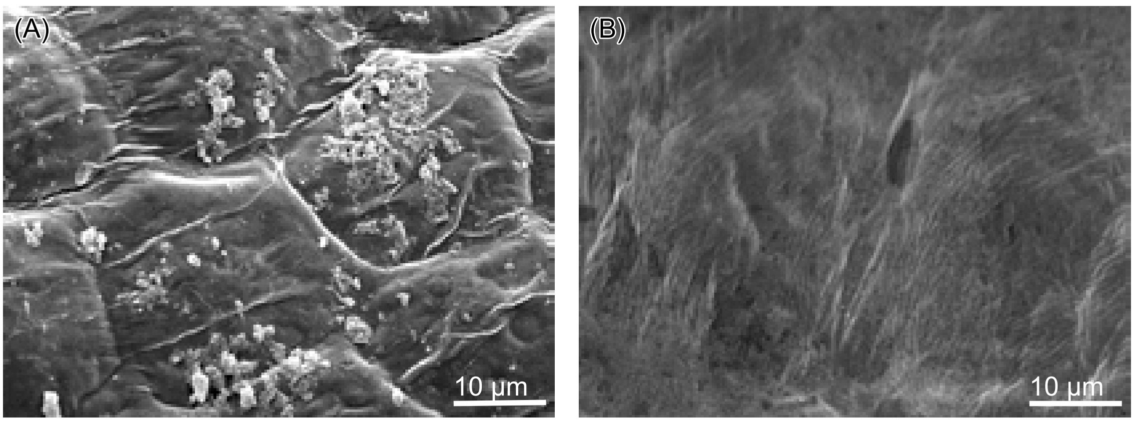

Fig. 3. Scanning electron micrographs of the surface on the lettuce treated with acidic electrolyzed water
(A): no treatment, (B): treatment with acidic electrolyzed water.

Table 2. Efficacy of various sanitizers against naturally present microorganisms on cucumber and strawberry

	Treatment	Population ($\log_{10}$ CFU/cucumber or strawberry)[a)]		
		Aerobic mesophilic bacteria	Coliform bacteria	Fungi
Cucumber	None	7.1 ± 0.4 A	4.5 ± 0.5 A	4.6 ± 0.4 A
	AcEW[b)]	5.7 ± 0.3 C	< 2.4[g)]	< 2.4
	Chlorine[c)]	5.9 ± 0.3 C	< 2.4	< 2.4
	Ozone[d)]	6.4 ± 0.2 B	3.0 ± 0.5 B	3.8 ± 0.4 B
	AlEW+AcEW[e)]	5.0 ± 0.5 D	< 2.4	< 2.4
	Water[f)]	6.8 ± 0.3 A	4.1 ± 0.4 A	4.4 ± 0.5 A
Strawberry	None	4.9 ± 0.4 A	2.7 ± 0.3 A	5.3 ± 0.5 A
	AcEW	4.0 ± 0.4 C	< 2.4	3.7 ± 0.4 C
	Chlorine	4.0 ± 0.2 C	< 2.4	3.6 ± 0.4 C
	Ozone	4.6 ± 0.3 B	< 2.4	4.4 ± 0.7 B
	AlEW+AcEW	4.1 ± 0.4 C	< 2.4	4.3 ± 0.7 B
	Water	4.8 ± 0.3 A	2.5 ± 0.4 A	4.9 ± 0.5 A

a): Values are mean ± standard deviation, n = 5. Values in the same column of each tested site that are not followed by the same letter differ significantly at $P \leq 0.05$.
b): Acidic electrolyzed water, 10 min.
c): Chlorinated water containing 150 ppm free available chlorine, 10 min.
d): Ozonated water containing 5 ppm ozone, 10 min.
e): Alkaline electrolyzed water, 5 min and then acidic electrolyzed water, 5 min.
f): Tap water, 10 min.
g): No colonies were detected.

reduced levels of aerobic mesophilic bacteria by 0.4 $\log_{10}$ CFU/strawberry. AcEW and NaOCl reduced fungal populations by 1.6 and 1.7 $\log_{10}$ CFU/strawberry, respectively. Ozonated water and 5+5 treatments resulted in a smaller reduction of fungal populations (0.9 and 1.0 $\log_{10}$ CFU/strawberry, respectively) relative to that observed for either AcEW or NaOCl treatments ($P \leq 0.05$). In this study, AcEW and other sanitizers did not achieve a remarkable microbial reduction on strawberry, especially fungi. Chlorinated water (100–300 ppm chlorine) has also been shown to reduce *E. coli* O157:H7 inoculated strawberries by less than 1.5 $\log_{10}$ CFU/g[36,55]. Moreover, chlorinated water (800 ppm chlorine) was reported to reduce Feline calicivirus (FCV) by only 1 $\log_{10}$ FCV/g on the strawberry[16]. These results can be attributed to the surface structure of the strawberry fruit. The strawberry has numerous achenes (seeds) that render its surface structure uneven and complex.

Although the efficacy of AcEW as a sanitizing agent was dependent on the kind of fruit treated, AcEW could be sufficiently effective to offer an alternative solution to conventional sanitizers, such as NaOCl (150 ppm). The advantages of using AcEW as a sanitizer are that it can be prepared by the electrolysis of a dilute saline solution, without the use of any chemicals other than sodium chloride or dilutants that are currently needed to prepare conventional concentrated sanitizers. Moreover, the AlEW produced simultaneously within the cathode region of the electrolysis process could be used as a pre-wash reagent for producing greater microbial reduction with lettuce and cucumber.

2. Inactivation of pathogenic bacteria on fresh-cut lettuce using electrolyzed water[28,31]

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. 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.[12] demonstrated the effects on microbial reduction, using shredded iceberg lettuce in chlorinated water (100 ppm) at 47ºC 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 4ºC. The efficacy of a hydrogen peroxide and lactic acid combination in killing microorganisms is greatly enhanced by an increase in temperature[35,52]. Additionally, the sensory quality of lettuce could be improved by the treatment at around 50ºC that is referred to as a mild heat treatment.

The treatment delays the onset of discoloration, improves the retention of texture and reduces the development of bitterness[13]. In this study we have met the challenging task by developing a new concept of a washing procedure that consists of a mild heat pre-treatment with AlEW and a subsequent treatment with chilled (4ºC) AcEW.

The mildly heated (50ºC) pre-treatment with AlEW for 1 min with a subsequent treatment of AcEW (4ºC) resulted in a 2.7 $\log_{10}$ CFU/g reduction for both pathogens of *E. coli* O157:H7 and *Salmonella* spp. inoculated on lettuce by a dipping procedure, regardless of the duration of the subsequent treatment with AcEW (Table 3). This result was revealed as 1.5 to 1.7 $\log_{10}$ CFU/g greater reductions in bacterial populations in such trials relative to reductions observed for pre-treatment with AlEW at normal temperature (20ºC), regardless of the duration of the subsequent treatment with AcEW (4ºC). Further significant ($P \leq 0.05$) reductions of approximately 4.0 $\log_{10}$ CFU/g for both pathogens were achieved by mildly heated (50ºC) pre-treatment with AlEW for 5 min, irrespective of the subsequent treatment time (1 or 5 min) with AcEW (4ºC). This treatment resulted in at least a 2.2 $\log_{10}$ CFU/g greater reduction in bacterial populations relative to the pre-treatment trials involving AlEW at a normal temperature (20ºC). Pathogens inoculated onto lettuce would have been susceptible to the sanitizer by the mild heat treatment. Accordingly, after the mild heat treatment, 1 min AcEW treatment would be sufficient to yield an efficacious sanitizing effect. The appearance of the mildly heated lettuce regardless of the treatment time (1 or 5 min) was not deteriorated by the macroscopic evaluation immediately after the treatment and 4 days after storage at 10ºC (data not shown).

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 (4ºC) AcEW has other advantages such as controlling chlorine gas volatilization in AcEW and reducing the temperature of produce. Washing lettuce in chilled chlorinated water (4ºC) limited the growth of *E. coli* O157:H7 during subsequent storage, whereas washing at 47ºC had the opposite effect[11]. The mild heat pre-treatment and a subsequent treatment with chilled AcEW would also be expected to achieve bacterial growth control. 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.

Quality of fresh-cut lettuce after treatment with ozonated water[32]

Besides the microbiological safety, good appearance of fresh-cut vegetables is required by consumers. In order to satisfy both the requirements, we examined the combined treatment of mildly heated water followed by ozon-

Table 3. Effect of pre-treatment solution temperature and treatment time on the efficacy of subsequent treatment with acidic electrolyzed water (AcEW) at 4ºC

	Pre-wash			Followed with AcEW (4ºC) 1 min	Followed with AcEW (4ºC) 5 min
		Temp. (ºC)	Time (min)	Population[a)] ($\log_{10}$ CFU/g)	Population ($\log_{10}$ CFU/g)
E. coli O157:H7	Control[b)]	–	–	7.16 ± 0.08 A	7.16 ± 0.08 A
	AlEW[c)]	20	1	6.05 ± 0.21 B	5.95 ± 0.22 B
			5	5.81 ± 0.19 B	5.51 ± 0.15 C
		50	1	4.38 ± 0.14 C	4.42 ± 0.21 D
			5	3.23 ± 0.16 D	3.29 ± 0.13 E
Salmonella	Control	–	–	7.20 ± 0.11 A	7.20 ± 0.11 A
	AlEW	20	1	6.16 ± 0.22 B	6.03 ± 0.24 B
			5	5.98 ± 0.18 B	5.69 ± 0.28 C
		50	1	4.29 ± 0.15 C	4.17 ± 0.12 D
			5	3.36 ± 0.20 D	3.24 ± 0.17 E

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 ($P \leq 0.05$).
b): No treatment.
c): Alkaline electrolyzed water.

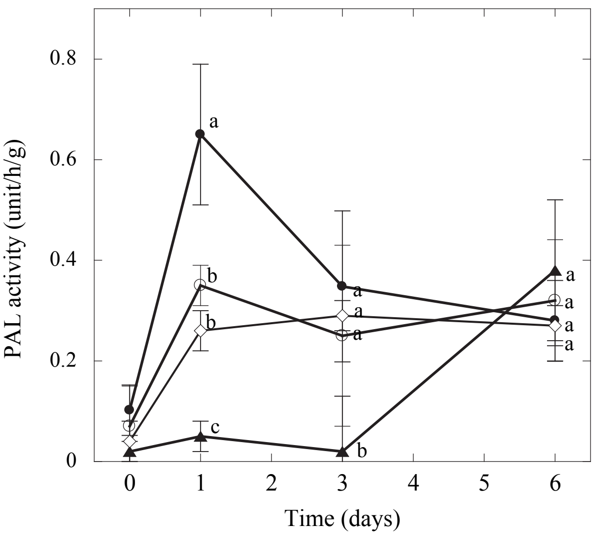

Fig. 4. Changes in phenylalanine ammonia lyase (PAL) activity for the lettuce treated with distilled water (Water), ozonated water (5 ppm) (Ozone), sodium hypochlorite solution (chlorine 200 ppm) (NaOCl) for 5 min, and hot water (50ºC, 2.5 min) followed by ozonated water (5 ppm, 2.5 min) (HW + Ozone) and subsequently stored at 10ºC for 6 days

—●—: Ozone, —▲—: HW + Ozone,
—◇—: NaOCl, —○—: Water.

Results are the mean value of five replicates ± standard deviation (SD). Values with different letters for each day show statistical significance at $P \leq 0.05$.

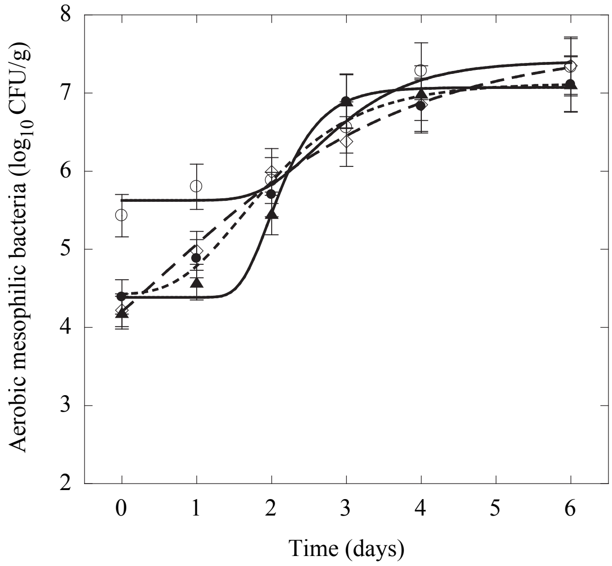

Fig. 6. Bacterial growth on lettuce treated with distilled water (Water), ozonated water (5 ppm) (Ozone), sodium hypochlorite solution (chlorine 200 ppm) (NaOCl) for 5 min, and hot water (50ºC, 2.5 min) followed by ozonated water (5 ppm, 2.5 min) (Hw + Ozone) during storage at 10ºC for 6 days

—●—: Ozone, —▲—: HW + Ozone,
—◇—: NaOCl, —○—: Water.

Results are the mean value of five replicates ± standard deviation (SD).

Fig. 5. Comparison of the appearance of the lettuce treated with ozonated water (Ozone) for 5 min, distilled water (Water) for 5 min, sodium hypochlorite solution (chlorine 200 ppm) (NaOCl) for 5 min and hot water (50ºC, 2.5 min) followed by ozonated water (5 ppm, 2.5 min) (HW → ozone), and subsequently stored at 10ºC for 6 days

ated water for the preparation of high quality fresh-cut lettuce.

The combination treatment of mild-heat water (50ºC, 2.5 min) followed by ozonated water (5 ppm, 2.5 min) had the same bactericidal effect as treatment with ozonated water alone (5 ppm, 5 min) or NaOCl (200 ppm, 5 min). Bacterial populations were reduced by 1.2 to 1.4 log_{10} CFU/g.

The combination treatment greatly inhibited the phenylalanine ammonia lyase (PAL) activity, which is associated with the browning of cut lettuce, after 3 days storage compared to other treatments (Fig. 4). The NaOCl treatment showed similar changes in PAL activity as the water-wash treatment. Ozonated water treatment increased the PAL activity compared with other treatments after 1 day of storage. The inhibition of browning was also apparent from macroscopic observation (Fig. 5). Although hot ozonated water could be used to simplify processing, it is only possible to dissolve extremely small amounts of ozone in hot water (50ºC, 0.006 mg/L)[18]. Moreover, undissolved, gaseous ozone would be detrimental to the working environment and human health. This combination of hot water treatment followed by ozonated water treatment will be suitable for practical use in the lettuce washing process for preserving both the microbiological and visual quality of lettuce.

However, the number of bacteria on the lettuce treated with sanitizers were initially reduced but then increased rapidly compared to the water-wash treated lettuce during storage at 10ºC (Fig. 6). Bacterial growth on lettuce treated with sanitizers is more rapid than that on untreated lettuce. This would be due to an initial decrease in the bacterial population, which reduces the number of the competing bacteria and allowing the remaining bacteria to thrive. Similar findings have been reported for *L. monocytogenes* growing on endive[9] and alfalfa sprouts[41], *Listeria innocua* growing on lettuce and coleslaw[14,15], and *E. coli* O157:H7 on ground beef[50,53]. Fresh-cut lettuce is often treated with sanitizers, such as chlorine, to reduce the bacterial counts during processing. Reduced background levels of native bacteria might also be caused by human pathogenic bacterial growth either through cross-contamination or due to the persistence of pathogenic bacteria after treatment. Moreover, hot water treatment causes the enhancement of the growth of pathogenic bacteria[33]. Care must therefore be taken when handling cut lettuce that has been treated with sanitizers and hot water, and it will be necessary to control bacterial growth by low temperature management.

Application of acidic electrolyzed water ice[27,29]

Our results as described above indicated that appropriate low temperature management is indispensable for maintaining the microbiological quality of fresh-cut vegetables after treatment with sanitizers. As a novel usage of AcEW, we examined the use of AcEW-ice for preserving fresh vegetables. Since packing in ice allows fresh produce to be kept at a low temperature during distribution, we speculated that packing in AcEW-ice would also be effective. Moreover, packing with AcEW-ice was expected to have a bactericidal effect. Thus, it may be possible to apply AcEW-ice to a simultaneous cooling and microbial control system. The microorganisms associated with the cut-lettuce stored with AcEW-ice and tap-water-ice in polystyrene-foam containers were enumerated after 5 days (Fig. 7). The viable aerobic mesophilic bacteria associated with lettuce packed with AcEW-ice were reduced by 1.5 log_{10} CFU/g within 24 h. On the other hand, the viable aerobic bacteria populations associated with lettuce stored with tap-water-ice maintained the pre-storage levels (10^6 CFU/g). This bactericidal effect is related to the emitted chlorine gas (Cl_2) from the AcEW-ice[27]. Furthermore, the relationship between the treatment time of AcEW-ice and the bactericidal effect was determined (Fig. 8). AcEW-ice did not reduce *L. monocytogenes* following treatment for 1 h, however a 1.3 log_{10}

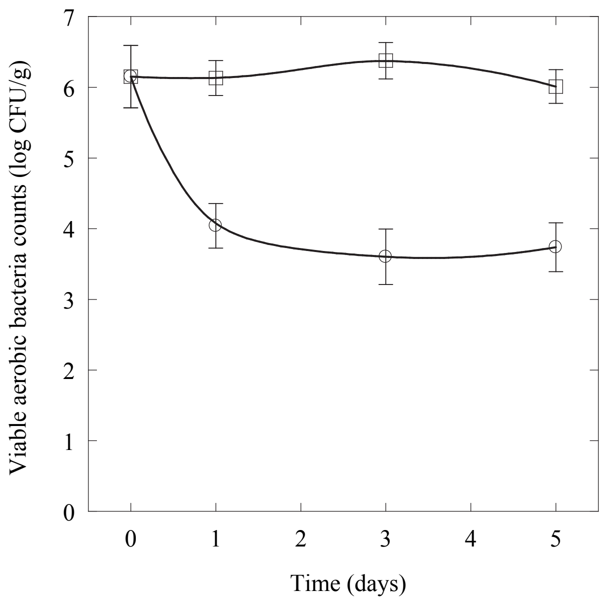

Fig. 7. Changes in aerobic bacteria counts in/on the lettuce stored with frozen acidic electrolyzed water (AcEW-ice) or frozen tap water (TW-ice) at 0ºC for 5 days
—○— : AcEW-ice, —□— : TW-ice.
Results are mean ± SD, n = 6.

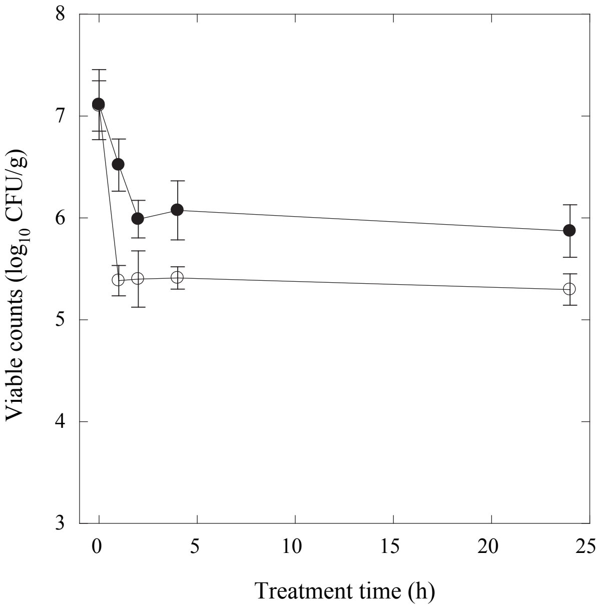

Fig. 8. Changes in pathogenic bacterial counts on the lettuce treated with acidic electrolyzed water ice (AcEW-ice) by different treatment times

—●— : *L. monocytogenes*, —○— : *E. coli* O157:H7. Results are mean ± standard error, n = 5. Values with different letters in the same pathogen are significantly difference ($P < 0.05$).

CFU/g reduction was observed following treatment for 2 h. Extending the treatment time did not result in any significant further reductions. *E. coli* O157:H7 was reduced by 1.7 log_{10} CFU/g following treatment for 1 h with AcEW-ice. Increased treatment of up to 24 h with AcEW-ice did not result in any significant further *E. coli* O157:H7 reduction.

The use of AcEW-ice will serve to maintain low storage temperatures and to decontaminate the raw foods during distribution. The use of AcEW-ice can be extended to the distribution of various raw foods that require large amounts of ice such as seafood. AcEW-ice can serve simultaneously as a refrigerant and inactivation of bacteria during distribution. AcEW-ice, therefore, will be an effective, new method for use in the distribution of raw foods.

Conclusion

This review described the applications of electrolyzed water and ozonated water for sanitizing fresh-cut produce. Electrolyzed water is a relatively new sanitizing technique for fresh-cut produce. We are focusing on the prevention of food poisoning and the prolongation of fresh produce shelf life by using electrolyzed water or ozonated water. Since electrolyzed water as well as ozonated water will readily become inert after contact with organic matter, little residue would be left. Therefore, electrolyzed water and ozonated water have a less adverse impact on the environment. From these features and the results of our study, it is suggested that electrolyzed water and ozonated water have a great potential as an alternative sanitizer for fresh produce and other food commodities.

References

1. Ackers, M. L. et al. (1998) An outbreak of *Escherichia coli* O157:H7 infections associated with leaf lettuce consumption. *J. Infect. Dis.*, **177**, 1588–1593.
2. Adams, M. R., Hartley, A. D. & Cox, L. J. (1989) Factors affecting the efficacy of washing procedures used in the production of prepared salad. *Food Microbiol.*, **6**, 69–77.
3. Badiani, M. et al. (1996) Deriving critical levels for ozone effects on peach trees (*Prunus persica* (L) Batsch) grown in open-top chambers in central Italy. *Fresenius Environ. Bull.*, **5**, 594–603.
4. Bari, M. L. et al. (1999) Inhibition of growth of *Escherichia coli* O157:H7 in fresh radish (*Raphanus sativus* L.) sprout production by calcinated calcium. *J. Food Prot.*, **62**, 128–132.
5. Bari, M. L. et al. (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.
6. Beuchat, L. R. (1996) Pathogenic microorganisms associated with fresh produce. *J. Food Prot.*, **59**, 204–216.
7. Beuchat, L. R. (1998) Surface decontamination of fruits and vegetables eaten raw: a review, *WHO/FSF/FOS/98.2*. Food Safety Unit, WHO, Geneva, pp. 42. Available online at http://www.who.int/foodsafety/publications/fs_management/en/surface_decon.pdf.
8. Brackett, R. E. (1992) Shelf stability and safety of fresh produce as influenced by sanitation and disinfection. *J. Food Prot.*, **55**, 808–814.
9. Carlin, F., Nguyen-The, C. & Morris, C. E. (1996) Influence of background microflora on *Listeria monocytogenes* on minimally processed fresh broad-leaved endive (*Cichorium endivia var. latifolia*). *J. Food Prot.*, **59**, 698–703.
10. De Roever, C. (1998) Microbiological safety evaluations and recommendations on fresh produce. *Food Control*, **9**, 321–347.
11. Delaquis, P. et al. (2002) Survival and growth of *Listeria monocytogenes* and *Escherichia coli* O157:H7 in ready-to-eat iceberg lettuce washed in warm chlorinated water. *J. Food Prot.*, **65**, 459–464.
12. Delaquis, P. J. et al. (1999) Effect of warm, chlorinated water on the microbial flora of shredded iceberg lettuce. *Food Res. Int.*, **32**, 7–14.
13. Delaquis, P. J. et al. (2000) Sensory quality of ready-to-eat lettuce washed in warm, chlorinated water. *J. Food Qual.*, **23**, 553–563.
14. Francis, G. A. & O'Beirne, D. (1998) Effects of the indigenous microflora of minimally processed lettuce on the survival and growth of *Listeria innocua*. *Int. J. Food Sci.*

Technol., **33**, 477–488.
15. Francis, G. A. & O'Beirne, D. (2002) Effects of vegetable type and antimicrobial dipping on survival and growth of *Listeria innocua* and *E. coli*. *Int. J. Food Sci. Technol.*, **37**, 711–718.
16. Gulati, B. R. et al. (2001) Efficacy of commonly used disinfectants for the inactivation of calicivirus on strawberry, lettuce, and a food-contact surface. *J. Food Prot.*, **64**, 1430–1434.
17. Han, Y. et al. (2000) Inactivation of *Escherichia coli* O157:H7 on surface-uninjured and -injured green pepper (*Capsicum annuum* L.) by chlorine dioxide gas as demonstrated by confocal laser scanning microscopy. *Food Microbiol.*, **17**, 643–655.
18. Japan Chemical Society (1993) Handbook of Chemistry, 4th ed. Maruzen, Tokyo, pp.621.
19. Jaquette, C. B., Beuchat, L. R. & Mahon, B. E. (1996) Efficacy of chlorine and heat treatment in killing *Salmonella stanley* inoculated onto alfalfa seeds and growth and survival of the pathogen during sprouting and storage. *Appl. Environ. Microbiol.*, **62**, 2212–2215.
20. Khadre, M. A., Yousef, A. E. & Kim, J. -G. (2001) Microbiological aspects of ozone applications in food: a review. *J. Food Sci.*, **66**, 1242–1252.
21. Kim, C., Hung, Y. -C. & Brackett, R. E. (2000) Efficacy of electrolyzed oxidizing (EO) and chemically modified water on different types of foodborne pathogens. *Int. J. Food Microbiol.*, **61**, 199–207.
22. Kim, C. et al. (2003) Efficacy of electrolyzed oxidizing water in inactivating *Salmonella* on alfalfa seeds and sprouts. *J. Food Prot.*, **66**, 208–214.
23. Kim, J. G., Yousef, A. E. & Chism, G. W. (1999) Use of ozone to inactivate microorganisms on lettuce. *J. Food Saf.*, **19**, 17–34.
24. Kim, J. G., Yousef, A. E. & Dave, S. (1999) Application of ozone for enhancing the microbiological safety and quality of foods: a review. *J. Food Prot.*, **62**, 1071–1087.
25. Kim, J. G. & Yousef, A. E. (2000) Inactivation kinetics of foodborne spoilage and pathogenic bacteria by ozone. *J. Food Sci.*, **65**, 521–528.
26. Koseki, S. et al. (2001) Decontamination of lettuce using acidic electrolyzed water. *J. Food Prot.*, **64**, 652–658.
27. Koseki, S., Fujiwara, K. & Itoh, K. (2002) Decontaminative effect of frozen acidic electrolyzed water on lettuce. *J. Food Prot.*, **65**, 411–414.
28. Koseki, S. et al. (2003) Influence of inoculation method, spot inoculation site, and inoculation size on the efficacy of acidic electrolyzed water against pathogens on lettuce. *J. Food Prot.*, **66**, 2010–2016.
29. Koseki, S., Isobe, S. & Itoh, K. (2004) Efficacy of acidic electrolyzed water ice for pathogen control on lettuce. *J. Food Prot.*, **67**, 2544–2549.
30. Koseki, S. et al. (2004) Efficacy of acidic electrolyzed water for microbial decontamination of cucumbers and strawberries. *J. Food Prot.*, **67**, 1247–1251.
31. Koseki, S. et al. (2004) 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. *Food Microbiol.*, **21**, 559–566.
32. Koseki, S. & Isobe, S. (2006) Effect of ozonated water treatment on microbial control and on browning of iceberg lettuce (*Lactuca sativa* L.). *J. Food Prot.*, **69**, 154–160.
33. Li, Y. et al. (2002) Mild heat treatment of lettuce enhances growth of *Listeria monocytogenes* during subsequent storage at 5 degrees C or 15 degrees C. *J. Appl. Microbiol.*, **92**, 269–275.
34. Liew, C. L. & Prange, R. K. (1994) Effect of ozone and storage-temperature on postharvest diseases and physiology of carrots (*Daucus-Carota* L). *J. Am. Soc. Hortic. Sci.*, **119**, 563–567.
35. Lin, C. -M. et al. (2002) Inactivation of *Escherichia coli* O157:H7, *Salmonella enterica* serotype Enteritidis, and *Listeria monocytogenes* on lettuce by hydrogen peroxide and lactic acid and by hydrogen peroxide with mild heat. *J. Food Prot.*, **65**, 1215–1220.
36. Lukasik, J. et al. (2003) Reduction of poliovirus 1, bacteriophages, *Salmonella* Montevideo, and *Escherichia coli* O157:H7 on strawberries by physical and disinfectant washes. *J. Food Prot.*, **66**, 188–193.
37. Mead, P. S. et al. (1999) Food-related illness and death in the United States. *Emerg. Infect. Dis.*, **5**, 607–625.
38. Naito, S. & Shiga, I. (1989) Studies on utilization of ozone in feed preservation. 9. Effect of ozone treatment on elongation of hypocotyl and microbial counts of bean sprouts. *Nippon Shokuhin Kagaku Kogaku Kaishi* (*J. Jpn. Soc. Food Sci. Technol.*), **36**, 181–188 [In Japanese with English summary].
39. Nakagawara, S. et al. (1998) Spectroscopic characterization and the pH dependence of bactericidal activity of the aqueous chlorine solution. *Anal. Sci.*, **14**, 691–698.
40. Nguyen-The, C. & Carlin, F. (1994) The microbiology of minimally processed fresh fruits and vegetables. *Crit. Rev. Food Sci. Nutr.*, **34**, 371–401.
41. Palmai, M. & Buchanan, R. L. (2002) Growth of *Listeria monocytogenes* during germination of alfalfa sprouts. *Food Microbiol.*, **19**, 195–200.
42. Park, C. M. et al. (2001) Pathogen reduction and quality of lettuce treated with electrolyzed oxidizing and acidified chlorinated water. *J. Food Sci.*, **66**, 1368–1372.
43. Park, H., Hung, Y. C. & Brackett, R. E. (2002) Antimicrobial effect of electrolyzed water for inactivating *Campylobacter jejuni* during poultry washing. *Int. J. Food Microbiol.*, **72**, 77–83.
44. Russell, S. M. (2003) The effect of electrolyzed oxidative water applied using electrostatic spraying on pathogenic and indicator bacteria on the surface of eggs. *Poult. Sci.*, **82**, 158–162.
45. Sharma, R. R. et al. (2002) Inactivation of *Escherichia coli* O157:H7 on inoculated alfalfa seeds with ozonated water and heat treatment. *J. Food Prot.*, **65**, 447–451.
46. Sharma, R. R. et al. (2003) Application of ozone for inactivation of *Escherichia coli* O157:H7 on inoculated alfalfa sprouts. *J. Food Process. Preserv.*, **27**, 51–64.
47. Takeuchi, K. & Frank, J. F. (2000) Penetration of *Escherichia coli* O157:H7 into lettuce tissues as affected by inoculum size and temperature and the effect of chlorine treatment on cell viability. *J. Food Prot.*, **63**, 434–440.
48. Takeuchi, K. et al. (2000) Comparison of the attachment of *Escherichia coli* O157:H7, *Listeria monocytogenes*, *Salmonella* Typhimurium, and *Pseudomonas fluorescens*

to lettuce leaves. *J. Food Prot.*, **63**, 1433–1437.
49. Takeuchi, K. & Frank, J. F. (2001) Expression of red-shifted green fluorescent protein by *Escherichia coli* O157:H7 as a marker for the detection of cells on fresh produce. *J. Food Prot.*, **64**, 298–304.
50. Tamplin, M. L. (2002) Growth of *Escherichia coli* O157:H7 in raw ground beef stored at 10ºC and the influence of competitive bacterial flora, strain variation, and fat level. *J. Food Prot.*, **65**, 1535–1540.
51. Venkitanarayanan, K. S. et al. (1999) Efficacy of electrolyzed oxidizing water for inactivating *Escherichia coli* O157:H7, *Salmonella enteritidis*, and *Listeria monocytogenes. Appl. Environ. Microbiol.*, **65**, 4276–4279.
52. Venkitanarayanan, K. S. et al. (2002) Inactivation of *Escherichia coli* O157:H7, *Salmonella enteritidis*, and *Listeria monocytogenes* on apples, oranges, and tomatoes by lactic acid with hydrogen peroxide. *J. Food Prot.*, **65**, 100–105.
53. Vold, L. et al. (2000) High levels of background flora inhibits growth of *Escherichia coli* O157:H7 in ground beef. *Int. J. Food Microbiol.*, **56**, 219–225.
54. Yoshida, K. et al. (2001) Sterilization effect and influence on food surface by acidic electrolyzed water treatment. *Nippon Shokuhin Kagaku Kogaku Kaishi* (*J. Jpn. Soc. Food Sci. Technol.*), **48**, 827–834 [In Japanese with English summary].
55. Yu, K. et al. (2001) Survival of *Escherichia coli* O157:H7 on strawberry fruit and reduction of the pathogen population by chemical agents. *J. Food Prot.*, **64**, 1334–1340.
56. Zhang, S. & Farber, J. M. (1996) The effects of various disinfectants against *Listeria monocytogenes* on fresh-cut vegetables. *Food Microbiol.*, **13**, 311–321.