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Efficacy of Acidic Electrolyzed Water Ice for Pathogen Control on Lettuce

SHIGENOBU KOSEKI,[1]* SEIICHIRO ISOBE,[1] AND KAZUHIKO ITOH[2]

[1]*Food Processing Laboratory, National Food Research Institute, 2-1-12 Kannondai, Tsukuba 305-8642, Japan; and* [2]*Graduate School of Agriculture, Hokkaido University, Kita 9, Nishi 9, Kita-ku, Sapporo 060-8589, Japan*

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ABSTRACT

Acidic electrolyzed water (AcEW) was used as frozen AcEW (AcEW-ice) for inactivation of *Listeria monocytogenes* and *Escherichia coli* O157:H7 on lettuce. AcEW-ice was prepared from AcEW with 20, 50, 100, and 200 ppm of available chlorine by freezing at −40°C and generated 30, 70, 150, and 240 ppm of chlorine gas (Cl_2), respectively. The AcEW-ice was placed into styrene-foam containers with lettuce samples at 20°C for 24 h. Although AcEW-ice generating 30 ppm Cl_2 had no effect on *L. monocytogenes* cell counts, AcEW-ice generating 70 to 240 ppm of Cl_2 significantly ($P < 0.05$) reduced *L. monocytogenes* by ca. 1.5 log CFU/g. *E. coli* O157:H7 cell counts were reduced by 1.0 log CFU/g with AcEW-ice generating 30 ppm of Cl_2. AcEW-ice generating 70 and 150 ppm of Cl_2 reduced *E. coli* O157:H7 by 2.0 log CFU/g. Further significant reduction of *E. coli* O157:H7 (2.5 log CFU/g) was demonstrated by treatment with AcEW-ice generating 240 ppm of Cl_2. However, treatment with AcEW-ice generating 240 ppm of Cl_2 resulted in a physiological disorder resembling leaf burn. AcEW-ice that generated less than 150 ppm of Cl_2 had no effect on the surface color of the lettuce. AcEW-ice, regardless of the concentration of the emission of Cl_2, had no effect on the ascorbic acid content in the lettuce. The weight ratio of lettuce to AcEW-ice required was determined to be over 1:10. The bactericidal effect of AcEW-ice appeared within the first 2 h. The use of AcEW-ice provides simultaneously for low temperature storage and inactivation of bacteria.

Consumers are demanding that fresh whole and cut produce exhibit high quality and microbiological safety. Inhibition of respiration plays an important role in extending the shelf life of fresh produce during storage and distribution. Modified atmosphere packaging is increasingly being used as a means of controlling respiration and extending the shelf life of fresh produce *(7, 8, 17, 21)*. For the most part, however, bacterial growth on produce has not been affected by these technologies *(3–6, 23)*. Bacterial growth on produce is mainly affected on storage temperature *(15, 22)*. For maintaining low temperatures, raw foods such as produce or seafood are often distributed in containers with ice.

Bacterial control on fresh produce using various sanitizers has been investigated. Chlorinated water, chlorine dioxide, hydrogen peroxide, organic acids, calcinated calcium solution, and ozonated water have been evaluated for their action against pathogens on produce *(1, 10, 13, 18)*. Acidic electrolyzed water (AcEW), which is produced by the electrolysis of an aqueous sodium chloride solution in an anode cell, has also been reported to have a strong bactericidal effect on most pathogenic bacteria on lettuce *(20)*, alfalfa seeds and sprouts *(12)*, and tomato *(2)*. Decontaminative effects of AcEW on the surface of lettuce and raw tuna were also reported *(16, 25)*. These treatments, however, have minimal effect and result in a <2 log CFU/g reduction.

Frozen AcEW (AcEW-ice) exhibits a bactericidal effect against microorganisms naturally present on lettuce *(14)*. This bactericidal effect is related to the chlorine gas (Cl_2) emitted from the AcEW-ice *(14)*. AcEW-ice may be used to maintain low storage temperatures and to decontaminate raw foods during distribution. To date, the characteristics of AcEW-ice treatment have not been determined in detail, including the effective Cl_2 concentration required for bactericidal protection and the ratio of produce to AcEW-ice.

In the present study, we examined the effect of AcEW-ice preparation method on the emission of Cl_2 from AcEW-ice. The effective Cl_2 concentration emitted from AcEW-ice has also been investigated for *Listeria monocytogenes* and *Escherichia coli* O157:H7 on lettuce. The optimal ratio of lettuce to AcEW-ice and treatment time also was examined. We also noted changes in lettuce appearance and in vitamin C (ascorbic acid [ASA]) content before and after treatment with AcEW-ice. These results will assist industry in the practical use of AcEW-ice in various raw food distribution systems.

MATERIALS AND METHODS

Preparation of AcEW-ice. 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 16 V. AcEW (50 ppm free available chlorine) was prepared within the anode compartment of an electrolytic cell. The properties of each solution were determined, including pH and the free available chlorine concentration. The pH

* Author for correspondence. Tel: +81-29-838-8029; Fax: +81-29-838-8122; E-mail: koseki@nfri.affrc.go.jp.

of the tested solution was measured with a pH meter (D-22, Horiba, Kyoto, Japan). Within 1 h before freezing, the initial concentration of the free available chlorine was determined with chlorine test kits (Hach Co., Loveland, Colo.). The AcEW (ca. 500 ml) was poured into a plastic ice tray divided into 14 blocks (40 by 40 by 25 mm), covered with polyethylene film, and then frozen at −40, −30, −20, and −10°C in the freezer (ULT-390-3JA, Revco, Asheville, N.C.). The freezing time was set for 6, 24, 48, 72, 96, and 120 h at each freezing temperature. The AcEW-ice (ca. 500 g) was placed into glass gas-tight containers (effective volume, 5 liters) at 20°C for 2 h. The concentration (ppm) of Cl_2 in the headspace gas in the container was measured with a chlorine detector tube (No. 8H, Gastec, Kanagawa, Japan) using a gas sampling pump kit (GV-100S, Gastec) through a small hole (7 mm diameter) covered with a silicone rubber septum (2 by 2 cm) on the upper cover of container.

Bacterial strains. A five-strain suspension of *L. monocytogenes* (ATCC 49594, ATCC 43256, JCM 7672, JCM 7676, and JCM 7671) and a three-strain suspension of *E. coli* O157:H7 (DT-66, MN-28, and MY-29) were used in this study. All strains of *L. monocytogenes* and *E. coli* O157:H7 were grown in tryptic soy broth (TSB, pH 7.3; Merck, Darmstadt, Germany). Each bacterial strain was individually cultured in 10 ml of TSB at 37°C, with loop transfer at three successive 24-h intervals immediately before use as inocula. Cells of each bacterial strain were collected by centrifugation (2,000 × *g*, 15 min, 20°C), and the resulting pellet was resuspended in 5 ml of sterile 0.1% peptone water (pH 7.1). Equal volumes of cell suspensions of three or five strains of each pathogen were combined to give approximately equal populations of each strain. The final cell concentrations were approximately 10^9 CFU/ml. The inoculum was maintained at 22 ± 2°C and applied to the lettuces within 1 h of preparation.

Preparation of inoculated lettuce. Iceberg lettuce was purchased from a local supermarket. The outer three or four leaves and core were removed from the lettuce head and discarded. Each experiment required the cutting of intact leaves into 5- by 5-cm pieces using a sterile surgical knife. A 100-g sample of lettuce leaves was placed into a plastic bag (25 by 20 cm) to facilitate the inoculation of the lettuce. Each bag was inoculated with a suspension of 1 ml *L. monocytogenes* or *E. coli* O157:H7 and then shaken gently 30 times to ensure an even distribution of the cells on the lettuce. Inoculated leaves were air-dried under a safety cabinet at room temperature for 1 h before being used in studies.

AcEW-ice treatment of lettuce. AcEW-ice was prepared from AcEW that contained different level of available chlorine concentration (ACC). The AcEW with different ACCs (20, 50, 100, and 200 ppm) was generated using a flow type electrolysis apparatus (ROX-LAB, Hoshizaki). The ACC of AcEW was adjusted by controlling the voltage between the electrodes. These AcEW solutions (ca. 500 ml) were then poured into separate plastic ice trays divided into 14 blocks (40 by 40 by 25 mm), covered with polyethylene film, and then frozen at −40°C for 24 h. The AcEW-ice (500 g) was then placed into styrene-foam containers (wall thickness, 2.0 cm; effective volume, 5.8 liters) with lettuce samples (25 g) at 20°C for 24 h. Inoculated lettuce samples were placed on the ice uncovered.

Quality of lettuce leaves. The color changes of the lettuce were determined objectively by color measurement of 10 pieces lettuce in each treatment using a colorimeter (Model CR300, Minolta Co., Tokyo). The color was represented by L*, a*, and b*. Color measurements were determined before and after treatment with AcEW-ice prepared from AcEW with 20, 50, 100, and 200 ppm of ACC by freezing at −40°C for 24 h.

The ASA content in the lettuce samples was determined by high-pressure liquid chromatography (HPLC) according to the method of Yamaguchi et al. *(24)*. Lettuce samples (10 to 20 g) were cut into small pieces and homogenized for 20 to 30 s using a homogenizer in 20 ml of 5% metaphosphoric acid. The resulting homogenate was centrifuged at 27,000 × *g* for 10 min at room temperature, and the supernatant was filtered through a 0.45-μm filter. Lettuce extracts (100 μl) were mixed with or without 0.2% 2,6-dichloroindophenol (50 μl). A 1% stannous chloride solution in 5% metaphosphoric acid (50 μl) and a 2% 2,4-dinitorophenylhydrazine solution (120 μl) in 4.5 M sulfuric acid were then added. The mixture was incubated in a water bath at 37°C for 3 h, and then ethyl acetate (1 ml) and water (1 ml) were added. After shaking and centrifugation (1,500 × *g*) for 5 min, 300 μl of the ethyl acetate layer was removed and dried under nitrogen. The residue was dissolved in 200 μl of acetonitrile and used for HPLC. A Cosmosil 5C18-MS column (4.6 by 150 mm; Waters, Milford, Mass.) with the UV-VIS detector (UV-1575, JASCO, Tokyo, Japan) set at 505 nm was used for HPLC. The mobile phase consisted of acetonitrile:water (50:50, vol/vol) adjusted to pH 3.5 with 0.1% triethylamine and phosphoric acid, and the flow rate was 1.0 ml/min. The ASA content was calculated by subtracting the value of the sample mixed with 2,6-dichloroindophenol from the value of the sample not containing 2,6-dichloroindophenol. The data were expressed as mg per 100 g of fresh lettuce.

Bactericidal characteristics of AcEW-ice treatment. The influence of the weight ratio of lettuce to AcEW-ice on the bactericidal effect was examined. Lettuce samples (25, 50, 100, 125, and 165 g) were placed on AcEW-ice (500 g) uncovered in styrene-foam containers and then stored at 20°C for 24 h. The AcEW-ice was prepared from AcEW with an ACC of 50 ppm by freezing at −40°C for 24 h. The viable cell counts of *L. monocytogenes* and *E. coli* O157:H7 on lettuce were determined before and after treatment.

The influence of AcEW-ice treatment duration on the bactericidal effect was also examined. AcEW-ice (500 g) was placed into styrene-foam containers with a lettuce sample (25 g) at 20°C for 1, 2, 4, and 24 h. The lettuce samples were placed on the AcEW-ice uncovered. Following treatment, viable cell counts of *L. monocytogenes* and *E. coli* O157:H7 on the lettuce were determined.

Bacterial analysis. A 25-g lettuce sample treated under each condition was combined with 225 ml of 0.1% peptone water in a sterile polyethylene bag and then pummeled for 2 min in a blender (TC82, CQC, Venice, Italy). 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. *L. monocytogenes* was enumerated on Oxford Listeria selective agar supplemented with Oxford Listeria selective supplement (Merck), which was incubated at 37°C for 24 h. The presence of *L. monocytogenes* was confirmed using a Listeria diagnosis kit (Singlepath Listeria, Merck). *E. coli* O157:H7 was enumerated on sorbitol MacConkey agar supplemented with CT selective supplement (CT-SMAC, Merck) which was incubated at 37°C for 24 h. The presence of *E. coli* O157:H7 was confirmed using the latex agglutination test (Singlepath E. coli O157, Merck).

Statistical analysis. Five replicate trials for each experiment were performed. Reported plate count data are expressed as the mean ± standard error. The data were analyzed using the Statview

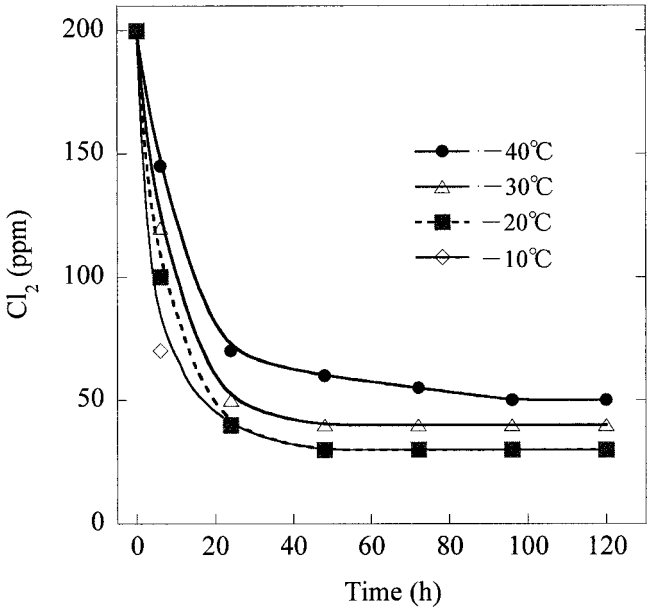

FIGURE 1. *Changes in concentration of chlorine gas emitted from acidic electrolyzed water during freezing.*

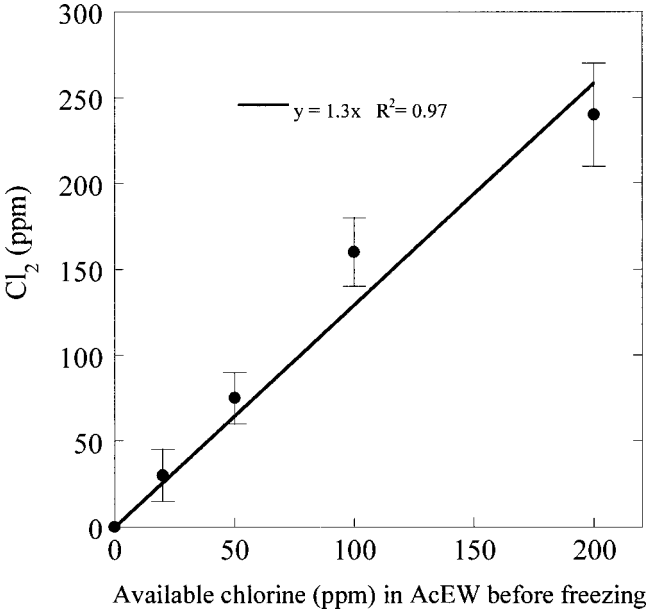

FIGURE 2. *Relationship between available chlorine concentration in acidic electrolyzed water (AcEW) before freezing, and concentration of chlorine gas emitted from AcEW-ice prepared by freezing at −40℃ for 24 h. Values are mean ± standard deviation,* n = *3.*

(SAS, Cary, N.C.) Tukey-Kramer multiple comparison test to determine statistical significance ($P \leq 0.05$).

RESULTS

Relationship between preparation temperature of AcEW-ice and emission of Cl_2. The pH value used for the AcEW was 2.6 ± 0.1. The concentration of the Cl_2 emitted from the AcEW (before freezing) was 200 ppm. After freezing, this concentration rapidly decreased by 40 to 70 ppm within 24 h at every preparation temperature (Fig. 1). The Cl_2 emitted from AcEW-ice prepared at −30, −20, and −10°C decreased by 40 to 50 ppm after 24 h. The Cl_2 emitted from AcEW-ice prepared at −40°C was 70 ppm after 24 h. After 24 h of freezing time, the Cl_2 concentration was almost stable up to 120 h at each temperature. The AcEW-ice prepared at −40°C generated a higher concentration of chlorine than did the AcEW-ice prepared at higher temperatures.

Bactericidal effect of AcEW-ice on lettuce. The concentration of Cl_2 emitted from the AcEW-ice was in proportion to the initial (prior to freezing) ACC of the AcEW (Fig. 2). AcEW-ice prepared from AcEW containing 20, 50, 100, and 200 ppm of ACC generated 30, 70, 150, and 240 ppm of Cl_2, respectively. Hereinafter, the AcEW-ice used is described based on the Cl_2 emission, e.g., AcEW-ice (Cl_2 30 ppm).

The bactericidal effect on lettuce of AcEW-ice prepared from different ACCs is shown in Figure 3. *L. monocytogenes* cell counts were not significantly reduced by AcEW-ice (Cl_2 30 ppm). AcEW-ice (Cl_2 70 to 240 ppm) reduced *L. monocytogenes* on lettuce by ca. 1.5 log CFU/g. For AcEW-ice (Cl_2 70 to 240 ppm), there was no significant difference in bacterial reduction. *E. coli* O157:H7 cell counts were reduced by 1.0 log CFU/g with AcEW-ice (Cl_2 30 ppm). AcEW-ice (Cl_2 70 and 150 ppm) yielded a 2.0 log CFU/g reduction. This reduction was significantly

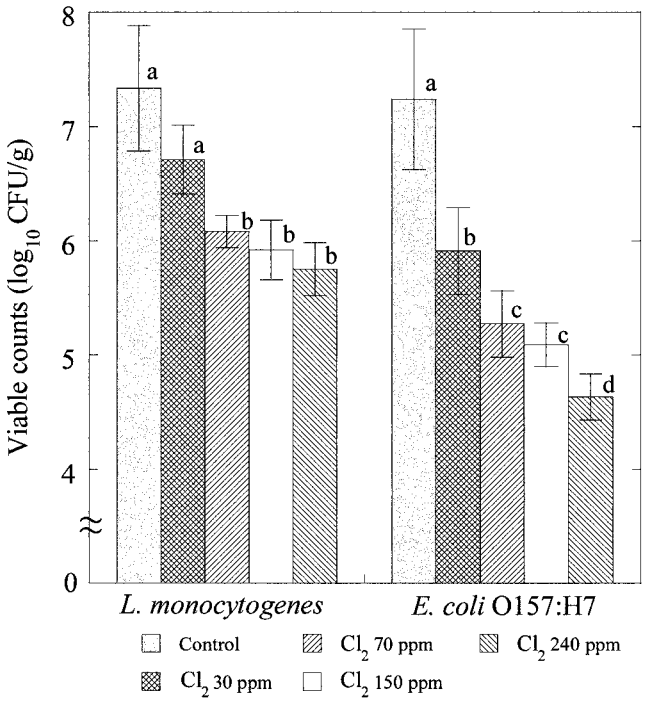

FIGURE 3. *Comparison of pathogenic bacterial population on lettuce treated with acidic electrolyzed water ice (AcEW-ice) generating different concentrations of chlorine gas. The lettuce was stored with AcEW-ice in styrene-foam containers for 24 h at 20℃. Results are mean ± standard error,* n = *5. Values with different letters within the same pathogen are significantly different (*P < *0.05).*

TABLE 1. *Surface color of lettuce stored for 24 h with acidic electrolyzed water ice (AcEW-ice) prepared with different available chlorine concentrations*[a]

Treatment	L*	a*	b*
Control	65.2 ± 3.6 A	−12.7 ± 2.7 A	32.0 ± 3.2 A
AcEW-ice			
Cl_2 240 ppm	63.2 ± 5.0 A	−8.0 ± 3.5 B	24.4 ± 2.2 B
Cl_2 150 ppm	65.6 ± 2.7 A	−9.1 ± 2.2 AB	26.8 ± 3.6 AB
Cl_2 70 ppm	64.8 ± 4.4 A	−11.2 ± 2.9 A	29.9 ± 2.8 A
Cl_2 30 ppm	66.2 ± 3.7 A	−13.1 ± 3.6 A	30.8 ± 3.3 A

[a] Values are mean ± standard deviation, $n = 5$. Values with different letters are significantly different ($P < 0.05$).

greater than that observed with AcEW-ice (Cl_2 30 ppm). Further bacterial reduction was observed by treatment with AcEW-ice (Cl_2 240 ppm). The AcEW-ice reduced *E. coli* O157:H7 by 2.5 log CFU/g, a reduction larger than that observed under other conditions ($P < 0.05$). The AcEW-ice showed a greater bactericidal effect against *E. coli* O157:H7 than against *L. monocytogenes.*

Quality of lettuce leaves. Results detailing the surface color of the lettuce treated with AcEW-ice (Cl_2 30 to 240 ppm) are shown in Table 1. Treatment with AcEW-ice (Cl_2 240 ppm) resulted in a physiological disorder resembling leaf burn. According to this macroscopic change, the a* value (redness [+] to greenness [−]) increased but the b* value (yellowness [+] to blueness [−]) decreased following treatment with AcEW-ice (Cl_2 240 ppm). However, AcEW-ice (Cl_2 70 and 30 ppm) did not affect the surface color of the lettuce.

AcEW-ice regardless of the Cl_2 concentration did not affect the ASA content in the lettuce (Fig. 4).

Bactericidal characteristics of AcEW-ice treatment. The influence of the weight ratio of lettuce to AcEW-ice on the bactericidal effect was also determined (Fig. 5). The use of AcEW-ice did not result in a significant reduction in *L. monocytogenes* when the weight of AcEW-ice used was within five times the weight of the lettuce. However, when 10 times the weight of AcEW-ice was used relative to the weight of the lettuce, *L. monocytogenes* was significantly reduced ($P < 0.05$) by 1.5 log CFU/g. A similar reduction was observed when using 20 times the weight of AcEW-ice relative to the weight of the lettuce. *E. coli* O157:H7 was reduced by 1.0 log CFU/g when three and four times the weight of AcEW-ice was used relative to the weight of the lettuce. Further reductions were observed when using over five times the weight of AcEW-ice relative to the weight of the lettuce. The use of 5, 10, and 20 times the weight of AcEW-ice relative to that of the lettuce reduced *E. coli* O157:H7 by ca. 2.0 log CFU/g.

The relationship between AcEW-ice treatment time and

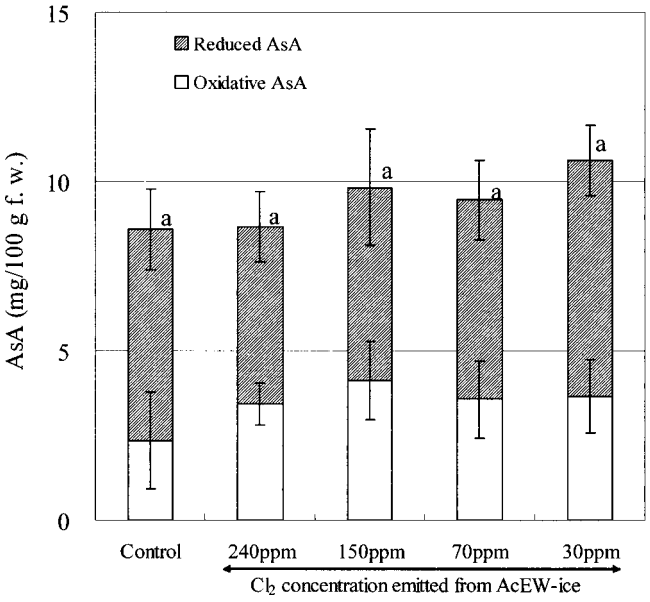

FIGURE 4. *Comparison of ascorbic acid (ASA) content in lettuce treated with acidic electrolyzed water ice (AcEW-ice) generating different concentrations of chlorine gas. The lettuce was stored with AcEW-ice in styrene-foam containers for 24 h at 20°C. Results are mean ± standard deviation,* n *= 5. Values with different letters are significantly different (*P *< 0.05).*

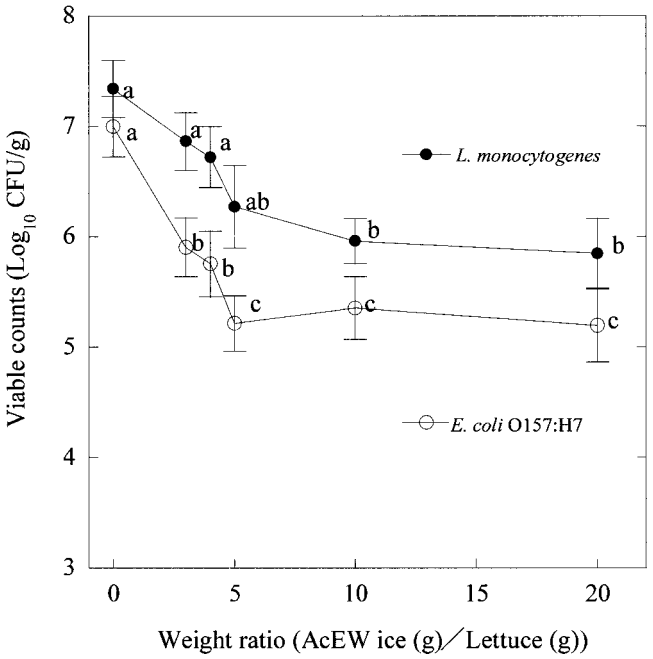

FIGURE 5. *Effect of weight ratio of acidic electrolyzed water ice (AcEW-ice) to lettuce on the bactericidal properties of AcEW-ice on lettuce. Results are mean ± standard error,* n *= 5. Values with different letters within the same pathogen are significantly different (*P *< 0.05).*

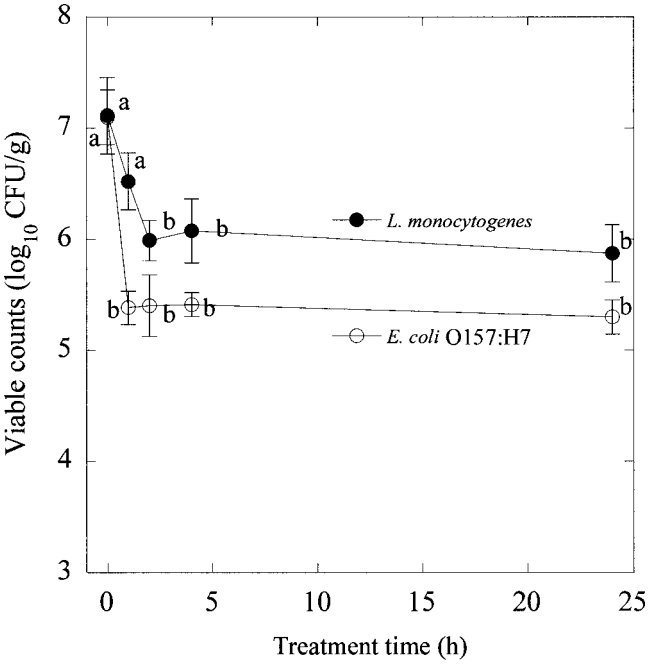

FIGURE 6. *Changes in pathogenic bacterial counts on lettuce treated with acidic electrolyzed water ice (AcEW-ice) for different amounts of time. Results are mean ± standard error,* n = *5. Values with different letters within the same pathogen are significantly different (*P < *0.05).*

the bactericidal effect was determined (Fig. 6). AcEW-ice did not reduce *L. monocytogenes* following treatment for 1 h, however a 1.3 log 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 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.

DISCUSSION

AcEW has a pH of 2.4 to 2.7 and an ACC of 20 to 100 ppm. The form of available chlorine varies depending on its environmental pH. Available chlorine consists of about 15% chlorine gas (Cl_2) and 85% hypochlorous acid (HOCl) in the pH range of AcEW *(19).* The main bactericidal effect of AcEW-ice has been attributed to the emitted Cl_2 from AcEW-ice *(14).* Hotta et al. *(11)* also demonstrated this causal relationship between the emitted Cl_2 from AcEW and the bactericidal effect on *Streptomyces griseus.* Thus, it seems that the Cl_2 concentration plays an important role in the bactericidal effect of AcEW-ice. In crystalline solids such as clathrate hydrates or gas hydrates, the formation of water lattices is promoted by the presence of rare gases such as chlorine *(9).* The Cl_2 in AcEW, therefore, could get into AcEW-ice. However, the boiling point of chlorine is −34°C. The Cl_2 is emitted in the freezing process regardless of the preparation temperature. More Cl_2 is emitted at relatively higher temperatures, such as −30, −20, and −10°C. According to the results of this study, an improved method for the preparation of AcEW-ice would be to subject AcEW to a temperature of −40°C.

Although *L. monocytogenes* was not significantly reduced on lettuce after exposure to AcEW-ice (Cl_2 30 ppm), significant reductions (by ca. 1.5 log CFU/g) were observed after exposure to AcEW-ice with >70 ppm Cl_2 emission. This result was comparable to that observed when chlorinated water or chlorine dioxide were used *(26).* AcEW-ice generating higher concentrations of Cl_2 (such as 240 ppm), however, did not reduce *L. monocytogenes* more effectively than did AcEW-ice with lower Cl_2 concentrations (70 ppm). *E. coli* O157:H7 was reduced after exposure to AcEW-ice (Cl_2 240 ppm) at a magnitude far greater than that observed under other conditions in this study. However, this treatment affected the appearance of the lettuce, resulting in a physiological disorder resembling leaf burn. AcEW-ice at lower Cl_2 concentrations (70 to 150 ppm) can be used successfully to achieve the desired bactericidal effect while maintaining produce appearance. Moreover, the ASA content of the lettuce, which is an important nutritional component, will not be changed when using this type of AcEW-ice (70 to 150 ppm).

Both *L. monocytogenes* and *E. coli* O157:H7 on lettuce were significantly reduced when >10 times the weight of AcEW-ice was used relative to the weight of the lettuce. Therefore, the use of at least 10 times the weight of AcEW-ice relative to that of lettuce is required. Although AcEW-ice plays a significant role as a refrigerant, its bactericidal effect was demonstrated within 2 h against both pathogens on lettuce. The Cl_2 emitted from AcEW-ice eliminated the bacteria on the surface of the lettuce within 2 h. However, the Cl_2 gas emitted from AcEW-ice would not be able to penetrate tissues and eliminate the bacteria there, just as in the case of conventional sanitizers.

The results of this study revealed some important characteristics relating to the treatment of lettuce with AcEW-ice. Effective Cl_2 emission resulted from the use of AcEW-ice (Cl_2 70 to 100 ppm), the weight ratio of AcEW-ice to lettuce required was >10, and the bactericidal effect of AcEW-ice appeared within 2 h after treatment. According to these results, 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 as an inactivator of bacteria during distribution. AcEW-ice, therefore, is an effective new treatment for use during the distribution of raw foods.

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REFERENCES

1. Bari, M. L., H. Kusunoki, H. Furukawa, H. Ikeda, K. Isshiki, and T. Uemura. 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.
2. Bari, M. L., Y. Sabina, S. Isobe, T. Uemura, and K. Isshiki. 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.

3. Bennik, M. H. J., H. W. Peppelenbos, C. Nguyenthe, F. Carlin, E. J. Smid, and L. G. M. Gorris. 1996. Microbiology of minimally processed, modified-atmosphere packaged chicory endive. *Postharvest Biol. Technol.* 9:209–221.
4. Berrang, M. E., R. E. Brackett, and L. R. Beuchat. 1989. Growth of *Aeromonas hydrophila* on fresh vegetables stored under a controlled atmosphere. *Appl. Environ. Microbiol.* 55:2167–2171.
5. Berrang, M. E., R. E. Brackett, and L. R. Beuchat. 1990. Microbial, color and textual qualities of fresh asparagus, broccoli, and cauliflower stored under controlled atmosphere. *J. Food Prot.* 53:391–395.
6. Brackett, R. E. 1990. Influence of modified atmosphere packaging on the microflora and quality of fresh bell peppers. *J. Food Prot.* 53:255–257.
7. Church, I. J., and A. L. Parsons. 1995. Modified atmosphere packaging technology—a review. *J. Sci. Food Agric.* 67:143–152.
8. Exama, A., J. Arul, R. W. Lencki, L. Z. Lee, and C. Toupin. 1993. Suitability of plastic films for modified atmosphere packaging of fruits and vegetables. *J. Food Sci.* 58:1365–1370.
9. Franks, F. 1983. The physics and physical chemistry of water, p. 117. *In* F. Franks (ed.), Water: a comprehensive treatise, vol. 1. Plenum Press, New York.
10. Han, Y., D. M. Sherman, R. H. Linton, S. S. Nielsen, and P. E. Nelson. 2000. The effects of washing and chlorine dioxide gas on survival and attachment of *Escherichia coli* O157:H7 to green pepper surfaces. *Food Microbiol.* 17:521–533.
11. Hotta, K., K. Kawaguchi, F. Saitoh, N. Saitoh, K. Suzuki, K. Ochi, and T. Nakayama. 1994. Antimicrobial activity of electrolyzed NaCl solutions: effect on the growth of *Streptomyces* spp. *Actinomycetology* 8:51–56.
12. Kim, C., Y. C. Hung, R. E. Brackett, and C. S. Lin. 2003. Efficacy of electrolyzed oxidizing water in inactivating *Salmonella* on alfalfa seeds and sprouts. *J. Food Prot.* 66:208–214.
13. Kim, J. G., A. E. Yousef, and G. W. Chism. 1999. Use of ozone to inactivate microorganisms on lettuce. *J. Food Saf.* 19:17–34.
14. Koseki, S., K. Fujiwara, and K. Itoh. 2002. Decontaminative effect of frozen acidic electrolyzed water on lettuce. *J. Food Prot.* 65:411–414.
15. Koseki, S., and K. Itoh. 2002. Effect of nitrogen gas packaging on the quality and microbial growth of fresh-cut vegetables under low temperatures. *J. Food Prot.* 65:326–332.
16. Koseki, S., K. Yoshida, S. Isobe, and K. Itoh. 2001. Decontamination of lettuce using acidic electrolyzed water. *J. Food Prot.* 64:652–658.
17. Lee, K. S., I. S. Park, and D. S. Lee. 1996. Modified atmosphere packaging of a mixed prepared vegetable salad dish. *Int. J. Food Sci. Technol.* 31:7–13.
18. Lin, C. M., S. S. Moon, M. P. Doyle, and K. H. McWatters. 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.
19. Nakagawara, S., T. Goto, M. Nara, Y. Ozawa, K. Hotta, and Y. Arata. 1998. Spectroscopic characterization and the pH dependence of bactericidal activity of the aqueous chlorine solution. *Anal. Sci.* 14:691–698.
20. Park, C. M., Y. C. Hung, M. P. Doyle, G. O. I. Ezeike, and C. Kim. 2001. Pathogen reduction and quality of lettuce treated with electrolyzed oxidizing and acidified chlorinated water. *J. Food Sci.* 66: 1368–1372.
21. Phillips, C. A. 1996. Review: modified atmosphere packaging and its effects on the microbiological quality and safety of produce. *Int. J. Food Sci. Technol.* 31:463–479.
22. Riva, M., L. Franzetti, and A. Galli. 2001. Microbiological quality and shelf life modeling of ready-to-eat cicorino. *J. Food Prot.* 64: 228–234.
23. Segall, K. I., and M. G. Scanlon. 1996. Design and analysis of a modified-atmosphere package for minimally processed romaine lettuce. *J. Am. Soc. Hortic. Sci.* 121:722–729.
24. Yamaguchi, T., T. Mizobuchi, R. Kajikawa, H. Kawashima, F. Miyabe, J. Terao, H. Takamura, and T. Matoba. 2001. Radical-scavenging activity of vegetables and the effect of cooking on their activity. *Food Sci. Technol. Res.* 7:250–257.
25. Yoshida, K., K. Lim, H. Chung, K. Uemura, S. Isobe, and T. Suzuki. 2001. Sterilization effect and influence on food surface by acidic electrolyzed water treatment. *J. Jpn. Soc. Food Sci. Technol.* 48:827–834.
26. Zhang, S., and J. M. Farber. 1996. The effects of various disinfectants against *Listeria monocytogenes* on fresh-cut vegetables. *Food Microbiol.* 13:311–321.