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Research Note

Decontaminative Effect of Frozen Acidic Electrolyzed Water on Lettuce

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ABSTRACT

We investigated the effects of frozen acidic electrolyzed water (AcEW) on lettuce during storage in a styrene-foam container. The lettuce was kept at 2 to 3°C for 24 h. Populations of aerobic bacteria associated with lettuce packed in frozen AcEW were reduced by 1.5 log CFU/g after storage for 24 h. With frozen tap water, no microorganism populations tested in this study were reduced. A frozen mixture of AcEW and alkaline electrolyzed water (AlEW) also failed to reduce populations of microorganisms associated with lettuce. Although chlorine gas was produced by frozen AcEW, it was not produced by the AcEW-AlEW mixture. This result indicates that the main factor in the decontaminative effect of frozen AcEW was the production of chlorine gas. Accordingly, low-temperature storage and decontamination could be achieved simultaneously with frozen AcEW during distribution.

Acidic electrolyzed water (AcEW) has been reported to have a strong bactericidal effect on most pathogenic bacteria *(2, 4, 11, 15, 16)*. Agricultural applications of AcEW for crop and seed disinfection have also been reported *(1, 12)*. AcEW is produced by the electrolysis of an aqueous sodium chloride solution with an instrument in which an anode and a cathode are separated by a membrane to form two compartments. AcEW is produced by the anode side and has a pH of $<$2.7, an oxidation reduction potential of $>$1,100 mV, and a free available chlorine concentration of 10 to 100 ppm *(13)*. A major advantage of the use of AcEW for the inactivation of bacteria is that it has a less adverse impact on the environment because it is produced from water with no added chemicals except sodium chloride. Furthermore, AcEW can be modified to reduce the available chlorine concentration to reduce health concerns about the use of chlorinated water while maintaining its effectiveness against microbes. The efficacy of AcEW as a disinfectant for fresh vegetables has recently been reported *(5, 6, 8)*.

Vegetables and fish have long been packed in ice for temperature control during distribution. Ice is ideally suitable for preserving fresh produce because it can provide both a low temperature and high humidity *(9)*. Since the specific heat of water is greater than that of air, fresh produce can be effectively cooled with ice. Furthermore, an ice-cooling system could be more cost-effective than gas compression refrigeration.

In this study, we examined the use of frozen AcEW 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 frozen AcEW would also be effective. Moreover, packing in frozen AcEW was expected to have a decontaminative effect. Thus, it may be possible to apply frozen AcEW to a simultaneous cooling and decontamination system.

The objective of this study was to investigate the storage characteristics of lettuce packed in frozen AcEW. We examined the decontaminative effect of frozen AcEW on lettuce and the changes in the temperature of lettuce when it was packed in frozen AcEW. Moreover, we used pH-induced changes in the form of available chlorine to investigate its effect on decontamination. From these results, we confirmed the decontaminative effect of frozen AcEW on lettuce and the possibility of using frozen AcEW as both a decontaminant and a cold insulator for lettuce.

MATERIALS AND METHODS

Preparation of ice. A batch-type electrolysis apparatus, the Super Oxeed Labo (Model JED-020, AOI Engineering, Shizuoka, Japan), was used to prepare the electrolyzed water. The electrolyzed water was produced by the electrolysis of a 0.05% sodium chloride solution at 9 to 12 DC volts for 10 min at room temperature. The AcEW (ca. 2 liters) was poured into a stainless steel ice tray (20 by 30 by 4 cm) divided into 40 blocks (3.5 by 3.5 cm), covered with polyethylene film, and then frozen at −40°C for 4 h. For controls, we prepared frozen tap water and a frozen mixture of AcEW (1 liter) and alkaline electrolyzed water (AlEW; 1 liter) produced by the electrolysis of a 0.1% sodium chloride solution at 9 to 12 DC volts for 10 min at room temperature. The tap water and the AcEW-AlEW mixture were frozen by the same protocol as that used for AcEW.

Procedure for storing lettuce in packed ice. Each type of ice (ca. 4 kg) was put into a styrene-foam container (wall thickness, 2.5 cm; effective volume, 14 liters) with two entire heads of lettuce (ca. 400 g). The heads of lettuce were placed on the ice

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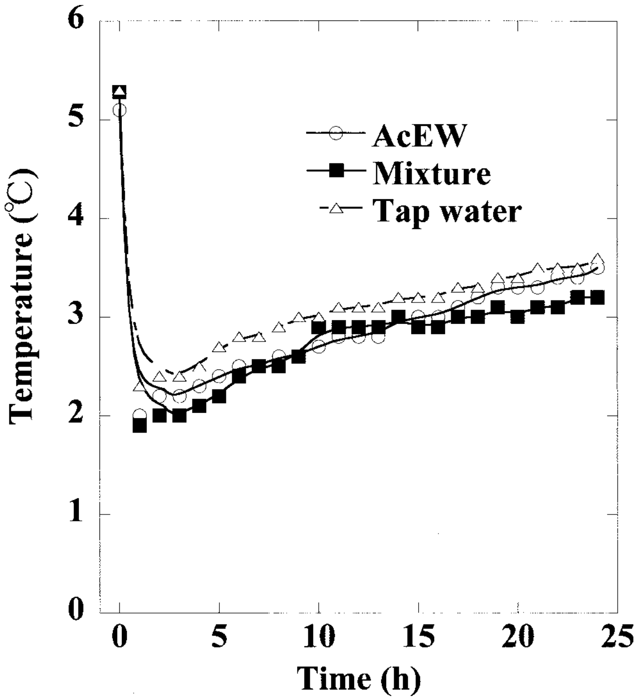

FIGURE 1. *Changes in the temperature of lettuce packed with frozen AcEW, a frozen mixture of AcEW and AlEW, and frozen tap water in a styrene-foam container at 20℃ for 24 h.*

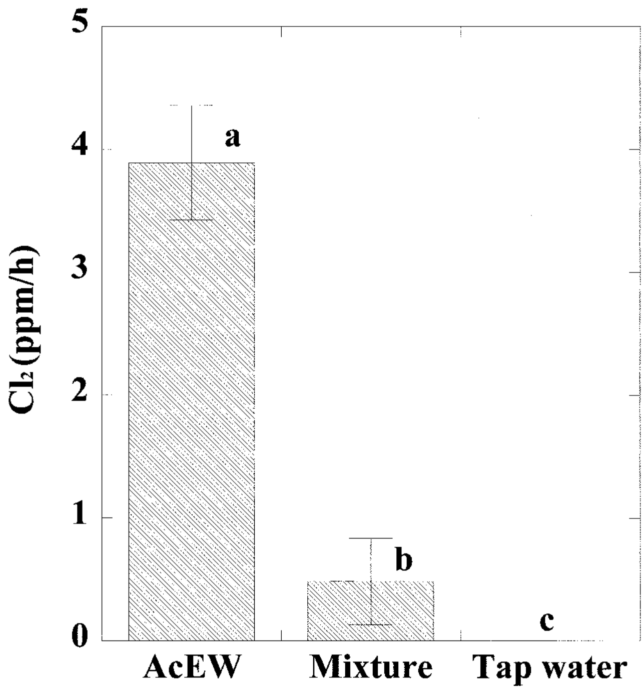

FIGURE 2. *Comparison of chlorine gas concentrations in the styrene-foam containers packed with AcEW, a frozen mixture of AcEW and AlEW, and frozen tap water for 24 h of storage. Results are expressed as means ± standard deviations;* n *= 5. Values with different letters differ significantly* (P ≤ *0.05).*

and were not covered. Lettuce used in this study was purchased at a local supermarket in Sapporo, Hokkaido, Japan. The lettuce was stored in a refrigerator set at 5°C for 1 day before it was used for the experiment. The container was sealed and stored at 20°C for 24 h. The temperature of the lettuce was measured with type T thermocouples. Three thermocouples were inserted into the core of the lettuce. The chlorine gas (Cl_2) concentration in the container was measured with a gas-detecting passive tube (no. 8D, GASTEC, Kanagawa, Japan) set on the inner wall of the container.

Microbiological analysis. To enumerate the microorganisms associated with the lettuce, a sample of lettuce (25 g) was combined with 225 ml of sterile 0.85% sodium chloride solution in a sterile polyethylene bag and pummeled with a Stomacher for 2 min at high speed. (Duplicate lettuce samples were used in each experiment.) The wash fluid was then serially diluted. All microbiological media used in this study were purchased from Merck (Darmstadt, Germany). Total aerobic bacterial counts were determined by pouring 1 ml of diluted sample into plate count agar. Plates were incubated at 35°C for 48 h, and the colonies were counted. Coliform counts were determined by pouring 1 ml of diluted sample into violet red bile agar. Plates were incubated at 35°C for 24 h, and the colonies were counted. *Bacillus cereus* counts were determined by direct plating 0.1 ml of diluted sample onto the surface of a mannitol–egg yolk–polymyxin agar plate. Plates were incubated at 35°C for 48 h, and the colonies were counted. Mold and yeast counts were determined by pouring 1 ml of diluted sample into potato dextrose agar containing 0.1 g/liter of chloramphenicol (Sigma Chemical Co., St. Louis, Mo.). Plates were incubated at 25°C for 120 h, and the colonies were counted. All pour and spread plates used for quantitative analysis were duplicated at each relevant dilution.

Statistical analysis. Microbial and gas concentration analyses were conducted in duplicate for each experiment. Five independent replications of each experiment were conducted. Data for microbial analysis were obtained from 10 lettuce samples and analyzed using statistical analysis software (MS-Excel 2000, Microsoft, Redmond, Wash.). Statistically significant differences ($P \leq 0.05$) between treatments were determined by the least-significant-difference method.

RESULTS

Changes in temperature and changes in Cl_2 concentration. Changes in the temperature of the lettuce packed with ice are illustrated in Figure 1. The temperature of the lettuce was kept at around 3°C for 24 h regardless of the kind of ice used. There were no differences in temperature changes among types of ice. Integral gas concentrations were measured for 24 h with a gas-detecting tube. Results are expressed as gas concentration per hour. The Cl_2 concentration increased by 3.9 ± 0.5 ppm/h in the container packed with frozen AcEW. In the container packed with the frozen AcEW-AlEW mixture, the Cl_2 concentration increased by 0.5 ± 0.4 ppm/h. No increases in the Cl_2 concentration were noted with frozen tap water (Fig. 2). There was no apparent degradation in the surface appearance of the lettuce after storage for 24 h under any packing conditions.

Decontaminative effects on lettuce. Physicochemical properties of the tested solutions before freezing are shown in Table 1. The pHs of AcEW, the AcEW-AlEW mixture, and tap water before freezing were 2.5, 7.0, and 7.0, respectively. The available chlorine concentration for the AcEW-AlEW mixture was 20 ppm, which was equivalent to that for the frozen AcEW.

The microorganisms associated with the lettuce stored

TABLE 1. *Physicochemical properties of tested solutions after freezing*

	pH	ORP (mV)	ACC (ppm)
AcEW	2.5 ± 0.1	1,148 ± 6	20.5 ± 2.2
AcEW-AlEW	7.0 ± 0.1	724 ± 18	20.1 ± 1.9
Tap water	7.0 ± 0.1	425 ± 28	0.3 ± 0.1

[a] ORP, oxidation reduction potential; ACC, available chlorine concentration; AcEW, acidic electrolyzed water; AlEW, alkaline electrolyzed water.

in frozen AcEW, in the frozen AcEW-AlEW mixture, and in frozen tap water were enumerated after 24 h (Fig. 3). The viable aerobic bacteria associated with lettuce packed in frozen AcEW were reduced by 1.5 log CFU/g within 24 h. On the other hand, the populations of viable aerobic bacteria associated with lettuce stored in the frozen AcEW-AlEW mixture and in frozen tap water remained at prestorage levels (10^6 CFU/g).

Populations of coliform bacteria were reduced by 10^1 CFU/g for lettuce packed in frozen AcEW. The lettuce stored in the frozen AcEW-AlEW mixture and in frozen tap water showed no changes from prestorage levels of coliform bacteria (10^2 CFU/g). After storage, there was a significant difference in numbers of coliform bacteria between the lettuce packed in frozen AcEW and that packed in the other types of ice ($P \leq 0.05$).

B. cereus populations associated with lettuce packed in the frozen AcEW-AlEW mixture and with that packed in AcEW were reduced by 0.3 and 0.7 log CFU/g, respectively, from prestorage levels. These populations were significantly smaller after storage than they were before storage ($P \leq 0.05$). There was also a significant difference in poststorage *B. cereus* populations between the lettuce packed in frozen AcEW and that packed in the AcEW-AlEW mixture ($P \leq 0.05$). Although frozen AcEW showed a decontaminative effect on *B. cereus,* this effect was minimal.

Packing with the frozen AcEW-AlEW mixture did not reduce mold and yeast populations. Packing with frozen AcEW, however, reduced mold and yeast populations by 0.6 log CFU/g from prestorage levels; this reduction was significant ($P \leq 0.05$). However, there were no differences between the populations associated with lettuce packed in the frozen AcEW-AlEW mixture and those associated with lettuce packed in frozen AcEW. The efficacy of frozen AcEW in reducing molds and yeasts was limited.

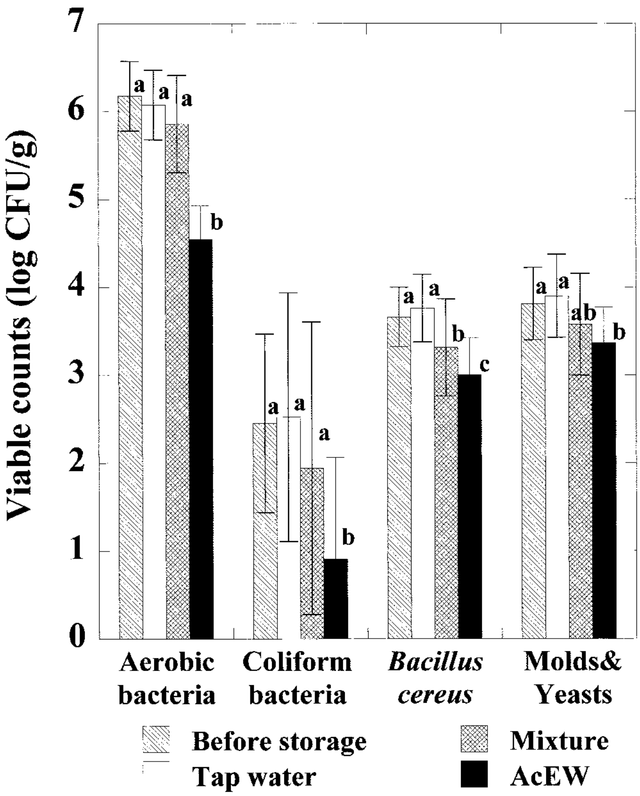

FIGURE 3. *Decontaminative effects on the microorganisms associated with lettuce stored with frozen tap water, a mixture of AcEW and AlEW, and AcEW in a styrene-foam container at 20°C for 24 h. Results are expressed as means ± standard deviations;* n *= 10. Values with different letters in the same medium differ significantly* ($P \leq 0.05$).

DISCUSSION

The reduction in numbers of aerobic bacteria associated with lettuce packed in frozen AcEW for 24 h was almost equivalent to that for lettuce treated with AcEW or sodium hypochlorite (NaOCl) solution containing 150 ppm of available chlorine for 10 min *(5, 6, 8).* Coliform bacteria and *B. cereus* populations associated with lettuce packed in frozen AcEW also showed reductions similar to those for lettuce treated with AcEW *(7).* Mold and yeast populations associated with lettuce packed in frozen AcEW did not show reductions similar to those for lettuce treated with AcEW *(8).* Although the decontaminative effects of frozen AcEW differ according to the type of microorganism concerned, frozen AcEW reduces the populations of most bacteria associated with lettuce.

AlEW has a pH of >11.0, an oxidation reduction potential of less than −800 mV, and an available chlorine concentration of 0 ppm. Since the frozen mixture consisted of AcEW and AlEW, which has a pH of ca. 7.0 and a free available chlorine concentration of ca. 20 ppm, the available chlorine concentration in the AcEW-AlEW mixture was equivalent to that of the AcEW before freezing in this study. The decontaminative effects on bacteria differed between frozen AcEW and the frozen AcEW-AlEW mixture regardless of almost equivalent available chlorine concentrations. The pH of the solution prior to freezing would have played a key role in this difference in the decontaminative effect. The form of available chlorine varies depending on its environmental pH *(10, 14).* Available chlorine exists as about 15% Cl_2 and 85% hypochlorous acid at a pH of <2.7 *(14).* As the pH rises, the percentage of Cl_2 decreases, and in theory available chlorine does not exist as Cl_2 at a pH of 7. Our Cl_2 concentration results generally agreed with this theory. Because the lettuce was put on top of the frozen AcEW and was not covered with frozen AcEW, the lettuce had little contact with melted AcEW; nevertheless, the decontaminative effect of frozen

AcEW was observed. Therefore, frozen AcEW would decontaminate the lettuce via the Cl_2 it produced. Hotta et al. *(3)* reported that the bactericidal effect of Cl_2 produced by AcEW on *Streptomyces griseus* was apparent. The AcEW-AlEW mixture did not reduce the microbial load associated with lettuce because of the lack of Cl_2. Thus, the Cl_2 concentration is considered an important factor in the bactericidal effect of frozen AcEW. Although the concentration of Cl_2 observed in this study did not affect the appearance of lettuce, a higher concentration of Cl_2 (e.g., $>$6 ppm/h) in a container caused a deterioration in appearance (e.g., browning; data not shown).

Packing in ice also allowed the maintenance of a low temperature. The humidity in the container was expected to be high *(9),* although it was not measured in this study. Low-temperature and high-humidity storage is suited for the preservation of most vegetables. Additionally, low-temperature storage and decontamination can be achieved simultaneously with frozen AcEW during distribution. A more detailed study of the effective Cl_2 concentration and the volume or weight ratio of vegetables to frozen AcEW is necessary for the optimization of frozen AcEW packing technology. However, this technology would be an effective new method for use in the distribution of fresh vegetables.

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