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Research Note

Efficacy of Acidic Electrolyzed Water for Microbial Decontamination of Cucumbers and Strawberries

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ABSTRACT

An examination was made of the efficacy of acidic electrolyzed water (AcEW, 30 ppm free available chlorine), ozonated water (5 ppm ozone), and a sodium hypochlorite solution (NaOCl, 150 ppm free available chlorine) for use as potential sanitizers of cucumbers and strawberries. AcEW and NaOCl reduced the aerobic mesophiles naturally present on cucumbers within 10 min by 1.4 and 1.2 log CFU per cucumber, respectively. The reduction by ozonated water (0.7 log CFU per cucumber) was significantly less than that of AcEW or NaOCl ($P \leq 0.05$). Cucumbers washed in alkaline electrolyzed water for 5 min and then treated with AcEW for 5 min showed a reduction in aerobic mesophiles that was at least 2 log CFU per cucumber greater than that of other treatments ($P \leq 0.05$). This treatment was also effective in reducing levels of coliform bacteria and fungi associated with cucumbers. All treatments offered greater microbial reduction on the cucumber surface than in the cucumber homogenate. Aerobic mesophiles associated with strawberries were reduced by less than 1 log CFU per strawberry after each treatment. Coliform bacteria and fungi associated with strawberries were reduced by 1.0 to 1.5 log CFU per strawberry after each treatment. Microbial reduction was approximately 0.5 log CFU per strawberry greater on the strawberry surface than in the strawberry homogenate. However, neither treatment was able to completely inactivate or remove the microorganisms from the surface of the cucumber or strawberry.

The sanitization of produce plays an important role in the preservation of food quality and safety of consumption. The control of spoilage 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 (4)*. Washing produce with tap water cannot be relied on to completely remove pathogenic and naturally occurring bacteria *(6)*. Chlorinated water 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 CFU/g reduction of bacteria on produce *(1, 5, 11, 28)*. Although other sanitizers, including chlorine dioxide (ClO_2), hydrogen peroxide (H_2O_2), organic acid, and calcinated calcium solution, have been evaluated *(2, 10, 14, 16)*, these sanitizers have a minimal sanitizing effect that is equal to that of chlorinated water. Moreover, most of these sanitizers are made from the dilution of condensed solutions, the handling of which involves some risk and troublesomeness. 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 (ca. 0.1%) sodium chloride (NaCl) solution using a commercially available apparatus. The electrolysis apparatus usually electrolyzes at a low level of 10 to 20 V of direct current in either a single-cell chamber *(24)* or a two-cell chamber separated by a diaphragm. Using the two-cell chamber, a strongly acidic electrolyzed water (AcEW) containing hypochlorous acid (HOCl) *(18)*, dissolved chlorine gas, and some activated chemical species are produced in the anode compartment. AcEW is reported to have strong bactericidal effects on the most pathogenic bacteria in vitro *(12, 25)*. The decontaminative effects of AcEW on the surface of lettuce and raw tuna were reported *(15, 26)*. AcEW has effectively inactivated *E. coli* O157:H7, *Salmonella* Enteritidis, and *L. monocytogenes* on lettuce *(19)*, alfalfa seeds, sprouts *(13)*, tomato *(3)*, egg surfaces *(22)*, and *Campylobacter jejuni* on poultry *(20)*. Because most of these investigated surfaces are relatively smooth, AcEW was highly effective in killing or removing the bacteria. The efficacy of AcEW in sanitizing fruits such as cucumber and strawberry has not been investigated. Furthermore, several studies have reported the difficulty of killing or removing the surface bacteria of cucumbers and strawberries using several other sanitizers *(7, 8, 17, 21, 27)*.

We focused on the efficacy of AcEW as a sanitizer that does not require dilution for use on cucumbers and strawberries. Additionally, we examined the differences in mi-

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crobial reduction between homogenates and the surfaces of the fruit.

MATERIALS AND METHODS

Plant materials. Cucumbers were purchased at a local supermarket in Tsukuba, Ibaraki, Japan. All fruits were in good condition, free of mechanical damage, and not waxed. Each cucumber was cut into smaller sticks of 5 cm height (weighing 25 to 30 g, ranging 2.6 to 3.4 cm in diameter). Strawberries were also purchased at a local supermarket in Tsukuba, Ibaraki, Japan. Undamaged strawberries, weighing 25 to 30 g, were selected for use in experiments.

Washing protocol. Five pieces of cucumber (ca. 150 g) or five pieces of strawberry (ca. 150 g) were dipped into an AcEW or ozonated water (2 liters each) for 10 min in an overflowing system. Because these solutions are generated by a flow-type apparatus and do not require dilution, samples were washed using the overflowing technique. A flow-type electrolysis apparatus (ROX-20TA; Hoshizaki Electric Co., Ltd., Toyoake, Aichi, Japan) was used to prepare the electrolyzed water. This apparatus generates electrolyzed water by the electrolysis of a dilute (0.1%) saline solution in an electrolytic cell separated into an anode and cathode region with a diaphragm. The current passing through the electrolysis apparatus and voltage between the electrodes was set at 14 A and 18 V, respectively. AcEW was prepared within the anode region of the electrolytic cell, and alkaline electrolyzed water (AlEW) was prepared within the cathode region. A flow-type ozone-generating apparatus (MCX-2000; Silver Reed, Tokyo, Japan) was used to prepare the ozonated water. This apparatus generated ozonated water by electrolysis of deionized water. The flow rate was set at 2 liters/min. Another treatment was conducted for the effective use of AlEW. Five pieces of cucumber or strawberry were washed by dipping in AlEW (2 liters) in an overflowing system for 5 min and then dipping in AcEW (2 liters) in an overflowing system for 5 min (5+5 treatment). As a comparison with a conventional sanitizer treatment, five pieces of cucumber or five pieces of strawberry were also dipped into 2 liters of a sodium hypochlorite (NaOCl) solution that contained 150 ppm available chlorine for 10 min. The NaOCl solution was prepared by adding NaOCl (5%, Wako, Tokyo, Japan) to distilled water. Tap-water washing with an overflowing technique was conducted using the same procedure as that of AcEW and ozonated water to form a control. After each treatment, the treated cucumber and strawberry was rinsed twice with 1 liter of sterile distilled water (20°C).

The pH of the tested solution was measured with a pH meter (D-22, HORIBA, Kyoto, Japan). The oxidation reduction potential (ORP) was measured with an ORP meter (HM-60V, TOA Electronics, Ltd., Tokyo, Japan). The initial concentration of available chlorine in AcEW, NaOCl, and tap water was determined with a chlorine test kit (HACH Co., Loveland, Colo.). The concentration of ozone in the ozonated water used in the present study was quantified by spectrophotometric analysis at 254 nm.

Enumeration of microorganisms. The populations of aerobic mesophilic bacteria, coliform bacteria, and fungi naturally present on the cucumber or strawberry tissues were enumerated by general procedures. To enumerate the microorganisms associated with cucumber or strawberry, one piece of cucumber or strawberry (25 to 30 g) was weighed, combined with 200 ml of sterile phosphate-buffered saline (PBS; 10 mM, pH 7.2) in a sterile polyethylene bag and pummeled with a Stomacher (Seward, London, UK) for 2 min at high speed. The homogenate was serially diluted for each medium. The surface of the cucumber or strawberry was swabbed with sterile absorbent cotton that contained 1 ml of PBS to enumerate the surface microorganisms. The absorbent cotton was then blended with 20 ml of PBS in a sterile polyethylene bag and pummeled with a Stomacher for 2 min at high speed. Wash fluid was serially diluted for each medium.

Aerobic mesophilic bacterial counts were determined from duplicate pour plates with standard plate count agar (Nissui, Tokyo, Japan) and incubated at 35°C for 48 h. Coliform bacteria counts were determined from duplicate pour plates with violet red bile agar (Difco, Becton Dickinson, Franklin Lakes, N.J.) with a medium overlay incubated at 35°C for 24 h. Fungal counts were determined from duplicate pour plates with YM agar (Difco, Becton Dickinson) supplemented with 0.1 g/liter of chloramphenicol (Wako, Osaka, Japan) and incubated at 25°C for 5 days. Both the homogenate and surface swabbed microbial counts were expressed as log CFU per cucumber or log CFU per strawberry. Because the homogenate and the surface swabs could not be directly compared with either a CFU/g or CFU/cm^2 rendering, we adopted an expression to allow for uniform denominator when evaluating all trials.

Statistical analysis. Five replicate trials were done for each fruit. Because each treatment condition included 5 pieces of cucumber or strawberry in one trial, a total of 25 pieces of cucumber or strawberry were analyzed for each treatment. All plate-count values are given as the mean ± standard deviation of 5 independent trials. Data were analyzed using StatView software (SAS, Cary, N.C.) for Tukey-Kramer's multiple comparison tests to determine statistical significance ($P \leq 0.05$).

TABLE 1. *Physicochemical properties of tested solutions*[a]

Solution	pH	ORP (mV)[b]	Concentration (ppm)[c]
AcEW[d]	2.6 ± 0.1	1,130 ± 8	32.1 ± 3.2
Ozone[e]	6.4 ± 0.1	1,250 ± 30	5.0 ± 0.2
NaOCl[f]	9.3 ± 0.2	640 ± 20	151.2 ± 4.1
Water[g]	7.0 ± 0.1	420 ± 25	0.3 ± 0.1
AlEW[h]	11.3 ± 0.1	−870 ± 15	—

[a] Values are mean ± standard deviation; $n = 5$.
[b] Oxidation reduction potential.
[c] Available chlorine concentration for AcEW, NaOCl and tap water, ozone concentration for ozonated water.
[d] Acidic electrolyzed water.
[e] Ozonated water.
[f] Sodium hypochlorite solution containing 150 ppm free available chlorine.
[g] Tap water.
[h] Alkaline electrolyzed water.

RESULTS

Efficacy of each treatment on microbial reduction of cucumbers. The physicochemical properties of the tested solution are shown in Table 1. The microbial populations in the homogenate or swabs of cucumber 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 homogenate by 1.4 and 1.2 log CFU per cucumber, respectively. Ozonated water reduced the aerobic mesophilic bacteria by 0.7 log CFU per cucumber. Populations of aerobic mesophilic bacteria were

TABLE 2. *Efficacy of various sanitizers against naturally present microorganisms associated with cucumber*

Tested site Treatment	Population (log CFU/cucumber)[a]		
	Aerobic mesophilic bacteria	Coliform bacteria	Fungi
Homogenate			
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[c]	<2.4
NaOCl[d]	5.9 ± 0.3 C	<2.4	<2.4
Ozone[e]	6.4 ± 0.2 B	3.0 ± 0.5 B	3.8 ± 0.4 B
5+5[f]	5.0 ± 0.5 D	<2.4	<2.4
Water[g]	6.8 ± 0.3 A	4.1 ± 0.4 A	4.4 ± 0.5 A
Swab			
None	5.3 ± 0.3 A	3.5 ± 0.4 A	4.0 ± 0.5 A
AcEW	3.8 ± 0.4 C	1.7 ± 0.6 B	1.9 ± 0.8 B
NaOCl	3.8 ± 0.3 C	1.9 ± 0.5 B	1.8 ± 0.9 B
Ozone	4.3 ± 0.4 B	1.7 ± 0.4 B	2.4 ± 0.5 B
5+5	3.3 ± 0.3 D	1.7 ± 0.5 B	1.7 ± 0.6 B
Water	5.1 ± 0.5 A	3.2 ± 0.3 A	3.8 ± 0.4 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] No colonies were detected in the homogenate.
[d] NaOCl solution containing 150 ppm free available chlorine, 10 min.
[e] Ozonated water containing 5 ppm ozone, 10 min.
[f] Alkaline electrolyzed water, 5 min, and then acidic electrolyzed water, 5 min.
[g] Tap water, 10 min.

TABLE 3. *Efficacy of various sanitizers against naturally present microorganisms associated with strawberry*

Tested site Treatment	Population (log CFU/strawberry)[a]		
	Aerobic mesophilic bacteria	Coliform bacteria	Fungi
Homogenate			
None	4.9 ± 0.4 A	2.7 ± 0.3 A	5.3 ± 0.5 A
AcEW[b]	4.0 ± 0.4 C	<2.4[c]	3.7 ± 0.4 C
NaOCl[d]	4.0 ± 0.2 C	<2.4	3.6 ± 0.4 C
Ozone[e]	4.6 ± 0.3 B	<2.4	4.4 ± 0.7 B
5+5[f]	4.1 ± 0.4 C	<2.4	4.3 ± 0.7 B
Water[g]	4.8 ± 0.3 A	2.5 ± 0.4 A	4.9 ± 0.5 A
Swab			
None	4.8 ± 0.2 A	2.4 ± 0.5 A	3.7 ± 0.4 A
AcEW	3.2 ± 0.2 C	<1.3[h]	2.0 ± 0.6 C
NaOCl	3.2 ± 0.2 C	<1.3	2.1 ± 0.7 C
Ozone	4.0 ± 0.4 B	<1.3	3.1 ± 0.8 B
5+5	3.7 ± 0.3 B	<1.3	2.9 ± 0.9 B
Water	4.6 ± 0.4 A	2.1 ± 0.7 A	3.6 ± 0.4 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] No colonies were detected in the homogenate.
[d] NaOCl solution containing 150 ppm free available chlorine, 10 min.
[e] Ozonated water containing 5 ppm ozone, 10 min.
[f] Alkaline electrolyzed water, 5 min, and then acidic electrolyzed water, 5 min.
[g] Tap water, 10 min.
[h] No colonies were detected in the swabbed solution.

reduced by 2.1 log CFU per cucumber for 5+5 treatment. The 5+5 treatment showed significantly greater bacterial reduction than the other treatments ($P \leq 0.05$). Coliform bacterial populations in the homogenates of cucumber were reduced by less than 2.4 log CFU per cucumber in all treatments except those involving ozonated and tap water. Fungal populations were also reduced by less than 2.4 log CFU per cucumber for trials involving AcEW, NaOCl, or the 5+5 treatment. Treatment with ozonated water reduced fungal levels by 0.8 log CFU per cucumber. No bacteria and fungi were detected in the AcEW, NaOCl, and ozonated water after treatment.

The microbial populations were 0.5 to 1.8 log smaller on the swabbed surface of the cucumber than in the homogenate. AcEW and NaOCl reduced the number of aerobic mesophilic bacteria on the surface of the cucumber by 1.5 log CFU per cucumber. Ozonated water reduced aerobic mesophilic bacteria by 1.0 log CFU per cucumber. The 5+5 treatment reduced aerobic mesophilic bacteria on the surface of the cucumber by 2.0 log CFU per cucumber. Coliform bacterial populations on the surface of the cucumber were reduced by approximately 1.7 log CFU per cucumber with all treatments. The 5+5 treatment did not show greater coliform bacterial reduction than the other treatments. Fungal populations were reduced by more than 2 log CFU per cucumber after AcEW, NaOCl, or 5+5 treatment. Treatment with ozonated water reduced fungal levels by 1.6 log CFU per cucumber.

Efficacy of each treatment on microbial reduction of strawberries. The microbial populations in the homogenate or on the surface of strawberries treated with various sanitizers are summarized in Table 3. The appearance of the strawberries was not affected by all treatments. AcEW, NaOCl, and 5+5 treatment reduced levels of aerobic mesophilic bacteria in the homogenate of the strawberry by 0.8 to 0.9 log CFU per strawberry. Ozonated water reduced levels of aerobic mesophilic bacteria by 0.4 log CFU per strawberry. Coliform bacterial populations were reduced to levels below the minimum detectable level (2.4 log CFU per strawberry) in all treatments. AcEW and NaOCl reduced fungal populations by 1.6 and 1.7 log CFU per strawberry, respectively. The ozonated water and 5+5 treatments resulted in a smaller reduction of fungal populations (0.9 and 1.0 log CFU per strawberry, respectively) relative to that observed for either AcEW or NaOCl treatments ($P \leq 0.05$). No bacteria and fungi were detected in the AcEW, NaOCl, and ozonated water after treatment. AcEW and NaOCl reduced levels of aerobic mesophilic bacteria and fungi on the surface of the strawberry by 1.6 log CFU per strawberry. Ozonated water and 5+5 treatments reduced levels of aerobic mesophilic bacteria and fungi by approximately 1 log CFU per strawberry. All sanitizers reduced coliform bacterial populations to levels below the minimum detectable level (1.3 log CFU per strawberry). Tap water did not reduce the microbial load of the strawberry.

DISCUSSION

Investigations of the effectiveness of acidic electrolyzed water have been conducted mainly for lettuces and tomatoes *(3, 15, 19)*. Because lettuce and tomato have a relatively smooth surface, AcEW was highly effective in killing or removing surface microorganisms. However, investigations have demonstrated that cucumbers are hard to sanitize using sodium hypochlorite or chlorine dioxide *(8, 21)*. Chlorine dioxide (5.13 ppm) reduced aerobic mesophilic bacteria by 2 log CFU/g when applied for 30 min *(21)*. Chlorine (250 ppm) reduced aerobic mesophilic bacteria by 2 log CFU/g when applied for 4 h *(8)*. Even blanching at 80°C for 120 s reduced aerobic mesophilic bacteria by 2.5 log CFU/g *(7)*. The results of our present surface-swabbing tests also indicated that the surface microorganisms were not completely eliminated by the sanitizers, including AcEW. However, a prewash with AlEW followed by treatment with AcEW gave a 2-log CFU per cucumber bacterial reduction within 10 min. AlEW is considered to act like a dilute sodium hydroxide aqueous solution *(23)*. Because AlEW would act like a surfactant, the hydrophobicity of the surface of the fruit would be decreased. Accordingly, AcEW would easily come into contact with the surface microorganisms, and a significant reduction in levels of microbial populations would be achieved.

Strawberries are mainly eaten raw as an important source of ascorbic acid (vitamin C) in the human diet; however, they are also highly perishable because of their high susceptibility to fungal infection. For this reason, microbial control plays an important role in rendering strawberries safe for human consumption. In the present study, AcEW and other sanitizers did not achieve a remarkable microbial reduction either in trials involving a tissue homogenate or the surface of strawberries. Chlorinated water (100 to 300 ppm chlorine) has also been shown to reduce *E. coli* O157:H7–inoculated strawberries by less than 1.5 log CFU/g *(17, 27)*. Moreover, chlorinated water (800 ppm chlorine) was reported to reduce feline calicivirus (FCV) by only 1 log FCV/g on the strawberry *(9)*. 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. In contrast to the present results for cucumber, treatment of the surface of the strawberry with AcEW and NaOCl for 10 min resulted in a greater microbial reduction than the 5+5 treatment. This suggests that sanitizers or other reagents would require at least 10 min to permeate and infiltrate the complex details of the strawberry's surface. Dipping for a longer duration (10 min) in AcEW or NaOCl in the case of strawberries would therefore be more effective in reducing microbial levels than prewashing with AlEW.

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 the 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 prewash reagent for producing greater microbial reduction with cucumbers.

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