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. 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. 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

REVIEW
Microbial Control of Fresh Produce using Electrolyzed Water

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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.

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duced in the anode cell. This solution is called acidic electrolyzed water (AcEW). AcEW contains hypochlorous acid (HOCl), 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. Decontaminative effects of AcEW on the surface of lettuce and raw tuna were reported. AcEW has effectively inactivated E. coli O157:H7, S. enteritidis and L. monocytogenes on lettuce, alfalfa seeds and sprouts, tomato, and egg surfaces, as well as Campylobacter jejuni on poultry.

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. Ozone has been shown to inactivate bacteria on various produce, including lettuce, carrot, bean sprouts, and alfalfa seeds and sprouts. 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. 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 and carrots.

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.

Sanitization of fresh-cut produce using electrolyzed water

1. Decontamination of fresh-cut produce

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 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 CFU/g. On the other hand, treatment with ozonated water reduced the viable aerobic mesophilic bacteria on the lettuce by 1.5 log CFU/g.

![Fig. 1. A schematic representation on generation of electrolyzed water](image_url)

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

![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.](image_url)

<table>
<thead>
<tr>
<th>Viable counts (log CFU/g)</th>
<th>Before treatment</th>
<th>AcEW</th>
<th>Ozone</th>
<th>NaClO</th>
<th>Water</th>
<th>AlEW 1 min</th>
<th>AcEW 1 min</th>
</tr>
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<tbody>
<tr>
<td>0.1 - 0.2% NaCl solution</td>
<td>a</td>
<td>b</td>
<td>c</td>
<td>d</td>
<td>e</td>
<td>f</td>
<td>g</td>
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<tr>
<td>10 - 20 V</td>
<td>b</td>
<td>a</td>
<td>c</td>
<td>b</td>
<td>e</td>
<td>f</td>
<td>g</td>
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<tr>
<td>0.1 - 0.2% NaCl solution</td>
<td>a</td>
<td>b</td>
<td>c</td>
<td>b</td>
<td>e</td>
<td>f</td>
<td>g</td>
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</table>

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 AIEW 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>
<thead>
<tr>
<th>Table 1. Physicochemical properties of tested solutions</th>
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<tr>
<td>pH</td>
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<tr>
<td>-----------------</td>
</tr>
<tr>
<td>AcEW(^{c})</td>
</tr>
<tr>
<td>Ozonated water</td>
</tr>
<tr>
<td>Tap water</td>
</tr>
<tr>
<td>NaOCl(^{d})</td>
</tr>
<tr>
<td>AIEW(^{e})</td>
</tr>
</tbody>
</table>

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](image)

(A): no treatment, (B): treatment with acidic electrolyzed water.
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 ≤ 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\textsuperscript{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\textsuperscript{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 diluents 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.

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

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Population (log_{10} CFU/cucumber or strawberry)\textsuperscript{a})</th>
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<tbody>
<tr>
<td></td>
<td>Aerobic mesophilic bacteria</td>
</tr>
<tr>
<td>Cucumber</td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>7.1 ± 0.4 A</td>
</tr>
<tr>
<td>AcEW\textsuperscript{b})</td>
<td>5.7 ± 0.3 C</td>
</tr>
<tr>
<td>Chlorine\textsuperscript{d})</td>
<td>5.9 ± 0.3 C</td>
</tr>
<tr>
<td>Ozone\textsuperscript{e})</td>
<td>6.4 ± 0.2 B</td>
</tr>
<tr>
<td>AlEW+AcEW\textsuperscript{f})</td>
<td>5.0 ± 0.5 D</td>
</tr>
<tr>
<td>Water\textsuperscript{g})</td>
<td>6.8 ± 0.3 A</td>
</tr>
<tr>
<td>Strawberry</td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>4.9 ± 0.4 A</td>
</tr>
<tr>
<td>AcEW</td>
<td>4.0 ± 0.4 C</td>
</tr>
<tr>
<td>Chlorine</td>
<td>4.0 ± 0.2 C</td>
</tr>
<tr>
<td>Ozone</td>
<td>4.6 ± 0.3 B</td>
</tr>
<tr>
<td>AlEW+AcEW</td>
<td>4.1 ± 0.4 C</td>
</tr>
<tr>
<td>Water</td>
<td>4.8 ± 0.3 A</td>
</tr>
</tbody>
</table>

\textsuperscript{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 ≤ 0.05.
\textsuperscript{b}) Acidic electrolyzed water, 10 min.
\textsuperscript{c}) Chlorinated water containing 150 ppm free available chlorine, 10 min.
\textsuperscript{d}) Ozonated water containing 5 ppm ozone, 10 min.
\textsuperscript{e}) Alkaline electrolyzed water, 5 min and then acidic electrolyzed water, 5 min.
\textsuperscript{f}) Tap water, 10 min.
\textsuperscript{g}) No colonies were detected.

2. **Inactivation of pathogenic bacteria on fresh-cut lettuce using electrolyzed water\textsuperscript{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.\textsuperscript{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\textsuperscript{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. 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 (4°C) AcEW.

The mildly heated (50°C) pre-treatment with AlEW for 1 min with a subsequent treatment of AcEW (4°C) 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 (20°C), regardless of the duration of the subsequent treatment with AcEW (4°C). Further significant (P ≤ 0.05) reductions of approximately 4.0 log_{10} CFU/g for both pathogens were achieved by mildly heated (50°C) pre-treatment with AlEW for 5 min, irrespective of the subsequent treatment time (1 or 5 min) with AcEW (4°C). This treatment resulted in at least a 2.2 log_{10} CFU/g greater reduction in bacterial populations relative to the pre-treatment trials involving AlEW at a normal temperature (20°C). 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 10°C (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 (4°C) AcEW has other advantages such as controlling chlorine gas volatilization in AcEW and reducing the temperature of produce. Washing lettuce in chilled chlorinated water (4°C) limited the growth of E. coli O157:H7 during subsequent storage, whereas washing at 47°C had the opposite effect. The mild heat pretreatment 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

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-
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 (50°C, 2.5 min) followed by ozonated water (5 ppm, 2.5 min) (HW + Ozone) and subsequently stored at 10°C for 6 days.

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. 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 (50°C, 2.5 min) followed by ozonated water (5 ppm, 2.5 min) (HW → Ozone), and subsequently stored at 10°C for 6 days.

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 (50°C, 2.5 min) followed by ozonated water (5 ppm, 2.5 min) (HW + Ozone) during storage at 10°C for 6 days.

Results are the mean value of five replicates ± standard deviation (SD).
ated water for the preparation of high quality fresh-cut lettuce.

The combination treatment of mild-heat water (50°C, 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<sub>10</sub> 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 (50°C, 0.006 mg/L)<sup>18</sup>. 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 <i>L. monocytogenes</i> growing on endive<sup>9</sup> and alfalfa sprouts<sup>41</sup>, <i>Listeria innocua</i> growing on lettuce and coleslaw<sup>14,15</sup>, and <i>E. coli</i> O157:H7 on ground beef<sup>50,53</sup>. 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<sup>33</sup>. 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<sup>27,29</sup>

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<sub>10</sub> 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<sup>6</sup> CFU/g). This bactericidal effect is related to the emitted chlorine gas (Cl<sub>2</sub>) from the AcEW<sup>27</sup>. Furthermore, the relationship between the treatment time of AcEW-ice and the bactericidal effect was determined (Fig. 8). AcEW-ice did not reduce <i>L. monocytogenes</i> following treatment for 1 h, however a 1.3 log<sub>10</sub>
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 log10 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**

Microbial Control of Fresh Produce using Electrolyzed Water


