

Electrolyzed Water as a Disinfectant for Fresh-cut Vegetables

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ABSTRACT

The effect of electrolyzed water on total microbial count was evaluated on several fresh-cut vegetables. When fresh-cut carrots, bell peppers, spinach, Japanese radish, and potatoes were treated with electrolyzed water (pH 6.8, 20 ppm available chlorine) by dipping, rinsing, or dipping/blowing, microbes on all cuts were reduced by 0.6 to 2.6 logs CFU/g. Rinsing or dipping/blowing were more effective than dipping. Electrolyzed water containing 50 ppm available chlorine had a stronger bactericidal effect than that containing 15 or 30 ppm chlorine for fresh-cut carrots, spinach, or cucumber. Electrolyzed water did not affect tissue pH, surface color, or general appearance of fresh-cut vegetables.

Key words: freshcut vegetables, electrolyzed water, disinfectant, chlorine, microbes

INTRODUCTION

FRESH-CUT VEGETABLES ARE HIGHLY SUSCEPTIBLE TO MICROBIAL spoilage, because processing steps by shredders and slicers may be principal sites of microbial contamination (Garg et al., 1990) and inner tissues are exposed to microbial contamination after cutting (Brackett, 1987). Bolin et al. (1977) reported that the initial microbial load of shredded lettuce influenced the storage life of the product. Chemical compounds such as sodium hypochlorite (Bolin et al., 1977; Adams et al., 1989; Zhang and Farber, 1996), chlorine dioxide (Zhang and Farber, 1996), sodium bisulfite (Krahn, 1997), sulfur dioxide (Bolin et al., 1977), organic acids (Adams et al., 1989; Zhang and Farber, 1996), calcium chloride (Izumi and Watada, 1994, 1995), and ozone (Nagashima and Kamoi, 1997) have been shown to reduce microbial populations on fresh-cut vegetables. A 50 to 125 ppm chlorine solution has been widely used in the food and dairy industry as a disinfectant, because the concentration at that level is not abusive to the product and the residual chlorine is not at the toxic level (Smith, 1962).

Sodium hypochlorite is a common food product disinfectant and effectiveness depends on the concentration, pH, temperature, organic matter, time of exposure, and growth stage of the pathogen (Boyette et al., 1993). The effectiveness increases with increasing concentration of available chlorine (El-Kest and Marth, 1988; Adams et al., 1989), but high concentrations may cause product tainting (Adams et al., 1989) and sodium residue on the product and equipment (Ritenour and Crisosto, 1996).

When sodium hypochlorite is added to water, it generates hypochlorous acid (HOCl), which is undissociated. Hypochlorous acid is the active antimicrobial component of hypochlorite solution. Adding sodium hypochlorite increases pH. The acid dissociates readily to hypochlorite ions (OCl⁻) at high pH, or chlorine gas (Cl₂) at low pH, thus the pH must be kept in the range of 6.5 to 7.5 for HOCl to be stable (Boyette et al., 1993). Sodium hypochlorite has been approved for use as a food additive by the Japanese Ministry of Health and Welfare, but hypochlorous acid was deleted from the lists of Japanese

food additives in 1991 due to lack of use at that time. Thus, it appears that use of electrolyzed water would be presently constrained legally.

Hypochlorous acid is present in electrolyzed water, which is generated from the reaction of Cl₂ and H₂O in an anode site when NaCl solution <10% is electrolyzed through a septum. This results in acid water with pH of 2 to 3. Neutral water at pH 6.8 is generated by electrolysis of NaCl solution without a septum, because HCl formed at the anode site neutralizes the NaOH at the cathode site (Hirano and Ueda, 1997). The objective of this work was to determine the effectiveness of electrolyzed neutral water as a source of hypochlorous acid to control microbial populations of fresh-cut vegetables.

MATERIALS & METHODS

Plant materials

Carrot (*Daucus carota* cv. Koyo), spinach (*Spinacia oleracea* L. cv. sunbest), bell pepper (*Capsicum annuum* L. cv. Kagayaki), Japanese radish (*Raphanus sativus* L. cv. aokubi), potato (*Solanum tuberosum* L. cv. Mayqueen), and cucumber (*Cucumis sativus* L. cv. unknown) were obtained from a farm or at the Wakayama Wholesale Market.

A food processor (Model CQM-62, Toshiba, Tokyo) was used to prepare carrot slices (35–40 mm dia and 3 mm thick), Japanese radish shreds (ca. 1 mm wide, 50 mm long, and 1 mm thick), and cucumber slices (25–30 mm dia and 3 mm thick). A knife was used to manually chop bell peppers (ca. 10 mm wide, 10 mm long, and 3 mm thick) and dice potatoes (ca. 15 mm wide, long, and thick). Scissors were used to cut medium sized spinach leaves and trim the petiole, leaving a 5-mm stem.

Generation of electrolyzed water

Electrolyzed water (pH 6.8) containing 20 ppm available chlorine was generated by electrolysis of 2.5% NaCl solution using an electrolyzed neutral water generator, Ameni Clean (Model FJ-W04F1, Matsushita Seiko, Osaka). Another generator, Ameni Clean (Model FJ-W25M1) was used to generate the electrolyzed water (pH 6.8) containing 15, 30, and 50 ppm available chlorine to investigate the effects of concentration of chlorine on disinfection. These generators were connected to a water faucet and the electrolyzed water of each chlorine concentration was automatically provided at a rate of 4 L/min. Concentration of available chlorine was confirmed by the sodium thiosulfate titration method (Asada et al., 1981).

Electrolyzed water treatments

A 50-g sample of each fresh-cut vegetable except cucumber was treated with tap water or electrolyzed water (20 ppm available chlorine) at room temperature (≈23°C) as follows: (a) Control = rinsed in running tap water at 2 L/min for 4 min; (b) rinsing treatment = rinsed in running electrolyzed water at 2 L/min for 3 min followed by rinsing in running tap water for 1 min; (c) dipping treatment = dipped in 500 mL electrolyzed water for 3 min followed by rinsing in running tap water for 1 min; (d) dipping/blowing treatment = dipped in 500 mL electrolyzed water while simultaneously blowing air at 25 L/min for 3 min, followed by rinsing in running tap water for 1 min, and then centrifuged for 30 sec to remove surface water. This was used on all vegetables, except trimmed spinach leaves, which were centrifuged for 3 min. A preliminary study indicated that a 3-min disinfectant

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treatment was sufficient to reduce the microbial count.

To determine if the last rinse in running water reduced the effectiveness of treatment, a 50-g sample from the above lot of samples was rinsed with tap water or electrolyzed water (20 ppm available chlorine) for 3 min and centrifuged to remove surface water. Each of the above treatments was replicated three times.

Concentrations of available chlorine

Effectiveness of electrolyzed water with different concentrations of chlorine was determined. A 50g sample of carrot slices, trimmed spinach leaves, or cucumber slices was rinsed in running tap water or running electrolyzed water containing 15, 30, or 50 ppm available chlorine for 4 min. After treatment, carrot and cucumber slices were centrifuged for 30 sec and spinach leaves for 3 min.

To determine if the rinse in running water affected disinfectant activity, a 50-g sample of each was rinsed with tap water for 4 min as a control or electrolyzed water containing the highest level of available chlorine (50 ppm) for 3 min followed by rinsing with tap water for 1 min. They were then centrifuged as described. The chlorine concentration of 50 ppm we used was based on results of this preliminary experiment. Each treatment was replicated three times.

Analytical determination

Microbial counts were made from the surface of a 10-g sample and of 10-g homogenate of the sample and were expressed as log₁₀ colony-forming units (CFU)/g sample. The surface sample was determined as previously described (Izumi and Watada, 1994) and the homogenate test was based on the enumeration of microbes after the homogenate was macerated in 90 mL of sterile physiological saline solution (0.85% NaCl-water) with a stomacher (Model 400D, Gunze, Tokyo).

The pH of samples used for microbial analysis was determined with a compact pH meter (Model B-113, Horiba, Tokyo). Surface color of samples was measured with a colorimeter (Model NR-3000, Nippon Denshoku, Tokyo) and was expressed as hue angle value (tan⁻¹b/a) with trimmed spinach leaves and cucumber slices and chroma value [(a² + b²)^{0.5}] with carrot slices.

Data were subjected to analysis of variance and the Duncan's Multiple Range Test (p≤0.05).

RESULTS

Electrolyzed water treatments

The total microbial count on the surface or in macerate of nontreated fresh-cut vegetables was the lowest with carrot slices and the highest with diced potatoes, ranging from 3.5 to 6.2 log CFU/g (Table 1). The count was 0.1 to 1.0 logs greater in the macerate than on the surface of fresh-cut vegetables. Rinsing with tap water (control) or electrolyzed water (20 ppm available chlorine) reduced the microbial count by about 0.4 to 1.4 logs or 0.8 to 2.1 logs, respectively, relative to nontreated samples. The electrolyzed water treatment reduced the microbial load by 0.3 log on the surface of Japanese radish shreds, 0.7 to 1.1 logs in spinach macerate, 0.4 to 0.7 logs on surface and macerate of carrot slices, and 0.7 to 0.8 logs on surface and macerate of diced potatoes when compared with water treatment (control). The population on chopped bell peppers was similar between the treatment and control. Rinsing or dipping/blowing treatments reduced the microbial count slightly more than dipping of carrots, Japanese radish, and potatoes. The electrolyzed water did not affect the tissue pH, which ranged from 5.7 to 6.4 among the fresh-cut vegetables (Table 1).

Electrolyzed water treatment by rinsing (20 ppm available chlorine) without subsequent water washing reduced the microbial population on the surface of all vegetables except potatoes, and in macerate of spinach leaves and bell peppers relative to water wash only (Table 2). The reductions in population ranged from 0.4 to 2.3 logs, with the maximum reduction occurring on trimmed spinach leaves. When compared with non rinsed samples water-rinsed samples after treatment de-

Table 1—Total microbial count and pH of fresh-cut vegetables treated with tap water or electrolyzed water containing 20 ppm available chlorine followed by rinsing with running tap water

Treatment ^d	Log ₁₀ CFU/g		pH	
	Surface ^e	Macerate ^f	Surface	Whole
Carrot slices				
Nontreatment	3.5 ^a	4.2 ^a	6.1 ^a	6.4 ^a
Control	2.7 ^b	3.8 ^b	6.1 ^a	6.4 ^a
Rinsing	2.2 ^c	3.1 ^c	6.1 ^a	6.3 ^a
Dipping	2.6 ^b	3.4 ^c	6.1 ^a	6.3 ^a
Dipping/Blowing	2.0 ^c	3.3 ^c	6.1 ^a	6.3 ^a
Trimmed spinach leaves				
Nontreatment	4.2 ^a	4.3 ^a	5.9 ^a	6.1 ^a
Control	3.0 ^b	4.0 ^a	5.9 ^a	6.1 ^a
Rinsing	2.7 ^b	3.0 ^b	5.8 ^a	6.0 ^a
Dipping	2.6 ^b	2.9 ^b	5.8 ^a	6.0 ^a
Dipping/Blowing	2.6 ^b	3.3 ^b	5.9 ^a	6.0 ^a
Chopped bell peppers				
Nontreatment	4.4 ^a	4.9 ^a	5.6 ^b	5.8 ^a
Control	3.1 ^b	3.5 ^b	5.9 ^a	5.9 ^a
Rinsing	2.9 ^b	3.6 ^b	5.8 ^a	5.9 ^a
Dipping	2.8 ^b	3.3 ^b	5.9 ^a	5.9 ^a
Dipping/Blowing	2.8 ^b	3.2 ^b	5.8 ^a	5.9 ^a
Japanese radish shreds				
Nontreatment	4.3 ^a	4.4 ^a	6.2 ^a	6.4 ^a
Control	3.6 ^b	3.9 ^b	5.7 ^b	6.0 ^b
Rinsing	3.3 ^c	3.7 ^b	5.7 ^b	6.1 ^{ab}
Dipping	3.5 ^{bc}	3.8 ^b	5.7 ^b	6.1 ^{ab}
Dipping/Blowing	3.3 ^c	3.7 ^b	5.7 ^b	6.1 ^{ab}
Diced potatoes				
Nontreatment	5.9 ^a	6.2 ^a	6.2 ^a	6.2 ^a
Control	4.5 ^b	5.0 ^b	5.9 ^{ab}	6.1 ^a
Rinsing	4.2 ^{bc}	4.8 ^{ab}	5.8 ^b	6.1 ^a
Dipping	4.5 ^b	4.6 ^b	5.9 ^{ab}	6.1 ^a
Dipping/Blowing	3.8 ^c	4.2 ^c	6.0 ^{ab}	6.1 ^a

^{a-c}Means with different letters within each fresh-cut vegetable in the same column are significantly different (p<0.05).

^dControl=rinsing with tap water 4 min; Rinsing=rinsing with electrolyzed water 3 min followed by rinsing with tap water 1 min; Dipping=dipping in electrolyzed water 3 min followed by rinsing with tap water 1 min; Dipping/Blowing=dipping and blowing air at 25 L/min in electrolyzed water 3 min followed by rinsing with tap water 1 min.

^eTotal CFU on tissue surface.

^fTotal CFU in tissue macerate.

Table 2—Total microbial count of fresh-cut vegetables rinsed with tap water as a control or electrolyzed water containing 20 ppm available chlorine (E.W.) for 4 min

Vegetable	Treatment	Log ₁₀ CFU/g	
		Surface ^a	Macerate ^b
Carrot	Control	3.3	3.8
	E.W.	2.3	3.5
Spinach	Control	4.2	4.4
	E.W.	1.9	2.6
Bell Pepper	Control	3.6	4.2
	E.W.	2.8	3.8
Japanese radish	Control	3.6	3.9
	E.W.	3.1	3.7
Potato	Control	4.5	5.0
	E.W.	4.4	4.6
		NS	NS

^aTotal CFU on tissue surface.

^bTotal CFU in tissue macerate.

NS, *: Nonsignificant or significant (p<0.05) between paired control and electrolyzed water treatments.

creased the reduction of microbial count on trimmed spinach leaves and chopped bell pepper, and did not have any deleterious effect on carrot slices or Japanese radish shreds (Tables 1, 2).

Concentrations of available chlorine

The concentration of available chlorine in electrolyzed water had an effect on the microbial population only on the surface of spinach leaves and cucumber slices (Table 3). Total microbial count on the surface of trimmed spinach leaves was below the detection level (2.4

Table 3—Total microbial count of fresh-cut vegetables rinsed with tap water as a control or electrolyzed water containing different concentrations of available chlorine for 4 min

Treatment	Log ₁₀ CFU/g		Surface color ^e
	Surface ^c	Macerate ^d	
Carrot slices			
Control	3.5 ^a	4.1 ^a	37.6 ^a
15 ppm	3.0 ^b	4.0 ^a	35.8 ^a
30 ppm	3.1 ^{ab}	3.8 ^a	35.6 ^a
50 ppm	2.9 ^b	3.8 ^a	34.9 ^a
Trimmed spinach leaves			
Control	2.9 ^a	4.3 ^a	102.2 ^a
15 ppm	<2.4 ^b	2.5 ^b	102.5 ^a
30 ppm	<2.4 ^b	2.5 ^b	104.2 ^a
50 ppm	ND	2.7 ^b	101.6 ^a
Cucumber slices			
Control	4.4 ^a	4.9 ^a	112.5 ^a
15 ppm	4.1 ^a	4.6 ^a	112.0 ^a
30 ppm	4.0 ^a	4.8 ^a	111.6 ^a
50 ppm	3.6 ^b	4.5 ^a	112.2 ^a

^{ab}Means with different letters within each fresh-cut vegetable in the same column are significantly different (p<0.05).
^cTotal CFU on tissue surface.
^dTotal CFU in tissue macerate.
^eHue angle value (tan⁻¹b/a) with spinach and cucumber and chroma value [(a²+b²)^{0.5}] with carrot.
 ND=Not detectable.

Table 4—Total microbial count of fresh-cut vegetables rinsed with tap water for 4 min (Control), electrolyzed water containing 50 ppm available chlorine for 4 min (E.W.), or electrolyzed water for 3 min followed by rinsing with tap water for 1 min (E.W.+W.)

Treatment	Log ₁₀ CFU/g	
	Surface ^d	Macerate ^e
Carrot slices		
Nonrinsing	4.6 ^a	5.3 ^a
Control	3.7 ^b	5.0 ^a
E.W.	2.8 ^c	4.0 ^b
E.W.+W.	2.6 ^c	3.9 ^b
Trimmed spinach leaves		
Nonrinsing	3.8 ^a	4.1 ^a
Control	3.0 ^a	3.6 ^a
E.W.	<2.4 ^b	<2.5 ^b
E.W.+W.	<2.4 ^b	2.8 ^b
Cucumber slices		
Nonrinsing	3.9 ^a	3.9 ^a
Control	3.1 ^b	3.5 ^a
E.W.	<2.4 ^c	2.9 ^b
E.W.+W.	<2.4 ^c	2.9 ^b

^{a-c}Means with different letters within each fresh-cut vegetable in the same column are significantly different (p<0.05).
^dTotal CFU on tissue surface.
^eTotal CFU in tissue macerate.

log CFU/g) when rinsed with electrolyzed water containing 15 or 30 ppm available chlorine and not detectable when rinsed with 50 ppm available chlorine. With cucumber slices, chlorine concentration of 15 and 30 ppm did not have an effect on reduction of microbes as compared to water-rinsed control, but the concentration of 50 ppm reduced the count by 0.8 log. The microbial counts in the macerate of all samples treated with electrolyzed water were similar.

Electrolyzed water did not affect surface color of carrot slices, trimmed spinach leaves, or cucumber slices irrespective of available chlorine concentration (Table 3). Rinsing only with water reduced the microbial count by 0.8 to 0.9 logs on the surface of carrot and cucumber slices when compared with the unrinsed samples (Table 4). Rinsing with electrolyzed water with 50 ppm available chlorine further reduced the microbial population when compared with controls. Water rinse after electrolyzed water treatment did not affect the microbial population.

DISCUSSION

MESOPHILIC AND PSYCHROTROPHIC GRAM-NEGATIVE RODS ARE the predominant microorganisms on fresh-cut vegetables, with the genus *Pseudomonas* being most numerous (Garg et al., 1990; King et al., 1991; Babic et al., 1996). Adams et al. (1989) reported that a standard washing in tap water produced about 90% reduction in mesophilic aerobic bacteria, *Pseudomonas spp.*, and psychrotrophic *Enterobacteriaceae* of lettuce leaf.

In this study, mesophilic aerobic microorganisms were reduced more by electrolyzed water treatment than by rinsing with tap water on several fresh-cut vegetables. The reduction by electrolyzed water treatment ranged from 0.6 to 2.6 logs CFU/g when compared with non-treated samples, which was comparable or more effective than other chemical disinfectants including sodium hypochlorite (Adams et al., 1989; Zhang and Farber, 1996).

The degree of reduction was dependent on the method of treatment, concentration of available chlorine, and type of fresh-cut vegetables. Among the 3 electrolyzed water treatments, rinsing or dipping/blowing seemed to be more effective than dipping. This may have been due to greater contact of the product surface with electrolyzed water by rinsing or dipping/blowing. The dipping/blowing procedure helped the sample move around in the water.

In the available chlorine concentration treatments from 15 to 50 ppm, the electrolyzed water containing 50 ppm chlorine had the strongest microbicidal effect. Increasing the chlorine concentration in sodium hypochlorite has resulted in a decreasing number of microorgan-

isms in the solution (El-Kest and Marth, 1998) and in salad vegetables. Adams et al. (1989) recommended that 100 ppm available chlorine should be adopted as the working concentration because higher levels could cause product tainting and equipment corrosion. Ritenour and Crisosto (1996) later reported that the high concentration of sodium hypochlorite accumulated sodium on the product and equipment to levels which could damage the product. The electrolyzed water used in this study had only 50 ppm available chlorine without sodium, which was only 50% of that suggested as the working chlorine concentration. Thus the potential problems with high concentration and sodium residue were not of concern here. We have found that the effectiveness of electrolyzed water containing 50 ppm chlorine corresponded to that of sodium hypochlorite solution containing 100–150 ppm chlorine with fresh-cut carrot, spinach, cucumber and potatoes (data not shown).

The effect of electrolyzed water also was influenced by the type and style of fresh-cut vegetables, which confirmed reports on hypochlorite solutions (Garg et al., 1990; Zhang and Farber, 1996). The effectiveness of electrolyzed water was the greatest with spinach leaves which had the maximum surface area/unit weight of tissue among the tested fresh-cut vegetables. The surface area, anatomy and microstructure of the tissues, which differ among vegetables as well as type of cuts, would affect the extent of contact of electrolyzed water with microorganisms. With chopped bell peppers and diced potatoes, the effectiveness of electrolyzed water was no greater than water rinse probably due to the low chlorine.

The microbicidal effect of electrolyzed water was greater on the surface of tissues than in macerate, which indicated that microbes inside of the tissue were more difficult to control. This may occur because the solution could not penetrate into the protective hydrophilic pockets or folds (Adams et al., 1989) or minute cracks of the surface (Babic et al., 1996). However, the limited control of microflora may be desirable, because Bennik et al. (1996) and Babic et al. (1997) reported that native microorganisms influenced the inhibitory effects of fresh-cut vegetables on growth of *Listeria monocytogenes*.

CONCLUSIONS

ELECTROLYZED WATER CONTAINING 15 TO 50 PPM AVAILABLE CHLORINE was effective as a disinfectant for fresh-cut vegetables without causing discoloration. The effectiveness appeared to be greater than that reported for other disinfectant chemicals with bench-scale experimentation. Rinsing of the product with tap water after treatment would be necessary because hypochlorous acid is not approved for use on

fresh-cut products in Japan. However, rinsing with tap water did not reduce the effectiveness of electrolyzed water containing 50 ppm available chlorine for reducing microbial population.

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