

Efficacy of Electrolysed Water as a Disinfectant for Fresh-cut Spinach

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Abstract

The effect of electrolysed water on bacterial count was evaluated on freshly prepared and stored fresh-cut spinach leaves. Electrolysed water (pH 6.8), containing 15 to 50 ppm available chlorine, was generated by electrolysis of a 2.5% NaCl solution using an electrolysed neutral water generator. Trimmed spinach leaves were treated with electrolysed water containing 20 ppm available chlorine by dipping, rinsing or dipping/blowing. These treatments reduced the total microbial count (mesophilic aerobic bacteria) by 1.0 to 1.6 log₁₀ colony forming units (CFU)/g when compared with untreated samples. Electrolysed water containing 50 ppm available chlorine had a stronger bactericidal effect than water containing 15 or 30 ppm chlorine, and was as effective as sodium hypochlorite solution containing 100 to 200 ppm chlorine. When the trimmed leaves were rinsed with electrolysed water containing 50 ppm chlorine and stored in normal air or 4% O₂ at 10 and 20°C, the increasing count of mesophilic aerobic bacteria was restricted when compared with the water-rinsed control. Electrolysed water did not affect the respiration rate nor surface colour of fresh-cut spinach during storage. Microbial populations increased on intact leaves stored at 0°C for 7, 14, 21 or 28 days. The electrolysed water was more effective in reducing the counts of mesophilic aerobic bacteria, psychrotrophic aerobic bacteria and coliform bacteria when the treatment was made using leaves stored for a brief period than for leaves stored for a long period.

FRESH-CUT vegetables are more perishable than intact vegetables mainly due to microbial contamination during and after processing (Brackett 1987; Watada et al. 1996; Zagory 1999). Microbial safety is of major concern in the production and distribution of fresh-cut vegetables in conjunction with the Hazard Analysis and Critical Control Points (HACCP) system (IFPA 1996). HACCP is a system of hazard prevention implemented by the food industry to produce safe food. It involves the systematic assessment of all steps involved in food manufacturing operations and the identification of those steps which are critical with respect to food safety (Wilcox et al. 1994). To reduce microbiological

hazards of fresh-cut vegetables, washing during processing is the only step taken to actively reduce microbial populations. A 50 to 150 parts per million (ppm) chlorine solution prepared from sodium hypochlorite has been widely used in the food industry as a disinfectant, but a high concentration of sodium hypochlorite for increased effectiveness may cause product tainting (Adams et al. 1989) as well as result in sodium residues on the product and the equipment (Ritenour and Crisosto 1996).

Alternatives to sodium hypochlorite, such as chlorine dioxide (Zhang and Farber 1996), sodium bisulfite (Krahn 1997), organic acid (Adams et al. 1989; Zhang and Farber 1996) and ozone (Nagashima and Kamoi 1997), have been studied to control microbial populations on fresh-cut vegetables. We previously reported the effect of electrolysed water on the microflora on several

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fresh-cut vegetables (Izumi 1999). Electrolysed water contains hypochlorous acid generated from the reaction of H_2O and Cl_2 produced by electrolysis of a NaCl solution of less than 10% using an electrolysed water generator. Our earlier study indicated that electrolysed water containing 15 to 50 ppm available chlorine was effective as a disinfectant for several fresh-cut vegetables, and the potential problems experienced using sodium hypochlorite—high concentration of available chlorine and sodium residue—were not of concern when using electrolysed water.

In this study, we initially determined the effect of electrolysed water on microbial populations on freshly prepared and stored fresh-cut spinach leaves in comparison to that of sodium hypochlorite. Treated, fresh-cut spinach was stored in normal air or 4% O_2 , as virtually all fresh-cuts are packaged in modified atmosphere packaging (MAP). Subsequently, our research was expanded to determine the effect of electrolysed water on the microflora on cut spinach prepared from intact leaves at 0°C, because raw vegetables for fresh-cuts are sometimes several days old by the time they are processed.

Materials and Methods

Generation of electrolysed water

Electrolysed water (pH 6.8) containing 15, 20, 30 or 50 ppm available chlorine was generated by electrolysis of a 2.5% NaCl solution using an electrolysed neutral water generator, Ameni Clean (Model FJ-W25M1; Matsushita Seiko, Osaka). The generator was connected to a water faucet and the electrolysed water of each chlorine concentration was automatically provided at a rate of 4 L/min. The concentration of available chlorine was confirmed by the sodium thiosulfate titration method (Asada et al. 1981).

Sample preparation and electrolysed water treatments

Spinach (*Spinach oleracea* L. 'Sunbest' or 'Joker') leaves were freshly harvested at a farm in Wakayama City, Japan. Sub-samples of the leaves were cut to have 0.5 cm petioles using scissors. Each 50 g sample was treated with tap water as a control or electrolysed water (20 ppm available chlorine) at room temperature. The treatments included: (a) control = rinsed in running tap water at 2 L/min for 4 min; (b) rinsing =

rinsed in running, electrolysed water at 2 L/min for 3 min followed by rinsing in running tap water for 1 min; (c) dipping = dipped in 500 mL electrolysed water for 3 min followed by rinsing in running tap water for 1 min; and (d) dipping/blowing = dipped in 500 mL electrolysed water while simultaneously blowing air at 25 L/min for 3 min, followed by rinsing in running tap water for 1 min. The water rinse after treatments was added because hypochlorous acid had not been approved for use as a food additive by the Japanese Ministry of Health and Welfare. The constraints on the use of electrolysed water had been waived as a special case. All samples were then centrifuged for 3 min to remove surface water.

50 g samples from a different lot were rinsed in running tap water or running electrolysed water containing 15, 30, or 50 ppm available chlorine for 4 min and centrifuged for 3 min to determine the effect of different concentrations of chlorine. A rinsing treatment using sodium hypochlorite solution containing 50, 100, 150 or 200 ppm available chlorine was also included to compare with the effect of electrolysed water containing 50 ppm available chlorine. All of the above treatments were replicated three times.

Total microbial counts (mesophilic aerobic bacteria) were made from the surface of a 10 g sample and of 10 g of macerated sample and the results expressed as \log_{10} colony forming units (CFU)/g sample as previously described (Izumi and Watada 1994; Izumi 1999).

Storage of trimmed spinach leaves

Nine trimmed spinach leaves (\approx 30 g) were rinsed with tap water or electrolysed water containing 50 ppm available chlorine for 4 min and centrifuged for 3 min and then placed in a 1.5 L plastic container containing 20 mL of distilled water in a beaker to maintain high relative humidity. Three replicated samples were stored at 10 or 20°C under a continuous stream of normal air or 4% O_2 at 10 or 15 mL/min, respectively. The balance of the gas mixture for the 4% O_2 atmosphere was N_2 . The carbon dioxide content of the inlet and outlet streams of each container was monitored with a CO_2 analyser (Model CD-3A; Ametek, PA) during storage. Three replicated samples of each treatment were taken periodically to measure counts of mesophilic aerobic bacteria and lactic acid bacteria in 10 g samples and surface colour (hue angle = $\tan^{-1}b^*/a^*$) with a chroma meter (Model NR-300; Nippon Denshoku, Tokyo), as previously described (Izumi et al. 1997).

Storage of intact spinach leaves

Intact spinach leaves were packaged in 30 µm low-density polyethylene film bags and stored at 0°C for 28 days. Sub-samples were periodically removed from the bag, and leaves were cut into sections, dipped in tap water or electrolysed water containing 50 ppm available chlorine for 3 min, and then centrifuged for 3 min. After treatment, counts of mesophilic aerobic bacteria, psychrotrophic aerobic bacteria and coliform bacteria were determined from the surface of a 10 g sample and of 10 g sample homogenate. Culture media and culture conditions for determination of microbial counts were standard method agar incubated at 37°C for 48 h for aerobic mesophiles, or incubated at 7°C for 10 days for aerobic psychrophiles, and desoxycholate agar incubated at 37°C for 24 h for coliforms.

Results and Discussion

Total counts of mesophilic aerobic bacteria of untreated fresh-cut spinach were similar between the surface and macerate of the sample (Table 1), which may be due to the style and anatomy of leafy vegetables. Rinsing with tap water (control) reduced the microbial load by 1.2 log₁₀ CFU/g on the leaf surface but not in the macerate, relative to untreated samples, while electrolysed water (20 ppm available chlorine) reduced the microbial load by about 1.6 log₁₀ CFU/g on the surface and 1.0 to 1.4 log₁₀ CFU/g in the macerate of samples. There was no difference in the bactericidal effect among the various treatments.

The concentration of available chlorine in electrolysed water had an effect on the microbial populations only on the surface of trimmed spinach leaves (Table 1). The microbial count on the surface was below the detection level (<2.4 log₁₀ CFU/g) when rinsed with electrolysed water containing 15 or 30 ppm available chlorine and not detectable when rinsed with 50 ppm available chlorine.

The number of mesophilic aerobic bacteria on the surface of trimmed spinach leaves which had been rinsed with sodium hypochlorite solution (available chlorine from 50 to 200 ppm) decreased as the chlorine concentration increased (Table 1). This decrease in microorganism numbers with increased chlorine concentration has been reported with raw apples and tomatoes (Beuchat et al. 1998) and within the sodium hypochlorite solution itself (El-Kest and Marth 1988). The effectiveness of electrolysed water containing 50 ppm chlorine corresponded to that of sodium hypochlorite solution containing 100–150 ppm

chlorine, which was comparable to or more effective than other chemical disinfectants (Adams et al. 1989; Zhang and Farber 1996).

The mesophilic aerobic bacterial count on cut spinach leaves treated with water or electrolysed water (50 ppm available chlorine) and stored at 10 or 20°C increased during storage at both temperatures regardless of the storage atmosphere, and was higher at 20°C than at 10°C (Table 2). The count was lower with electrolysed water-treated samples than water-treated controls during storage at 10 and 20°C except for samples stored in a 4% O₂ atmosphere at 10°C for 15 days. These results agree with those of Bolin et al. (1977) who reported that the initial microbial load of shredded lettuce influenced the storage stability of the product. Park and Lee (1995) reported that cut watercress and onion treated with sodium hypochlorite solution containing 50–1,000 ppm and 10–100 ppm, respectively, maintained lower microbial populations than the control samples for only the first 5 days of storage at 5°C. This indicated that the effectiveness of sodium hypochlorite treatment was limited to short-term storage. Thus the residual effect of electrolysed water on fresh-cuts during storage seems to be greater than that of sodium hypochlorite. Lactic acid bacterial counts on all samples were not detectable on day 0 and were below the detection level (2.5 log₁₀ CFU/g) during storage at 10 or 20°C.

Electrolysed water did not affect the respiration rate or the surface colour of trimmed spinach leaves held in normal air or 4% O₂ at 10 or 20°C (data not shown).

The mesophilic and psychrotrophic aerobic bacteria on the leaf surface and coliform bacteria in the macerate of fresh-cut spinach prepared from the stored, intact leaves increased by 1.4, 1.5, and 2.5 log₁₀ CFU/g, respectively, during the 28 days of storage at 0°C (Figure 1). Electrolysed water treatment (50 ppm available chlorine) of cut spinach leaves reduced the population of all microorganisms during storage, and the reduction was greater with leaves stored for a brief period than for a long period. Perhaps the population of microorganisms on spinach stored for a long time became too great for 50 ppm chlorine to remain effective.

Table 1. Total microbial count (mesophilic aerobic bacteria) of fresh-cut spinach treated with tap water (control), electrolysed water (EW) or sodium hypochlorite solution.

Treatment	Log ₁₀ colony forming units (CFU)/g	
	Surface ^e	Macerate ^f
Method^g		
Untreated	4.2 a	4.3 a
Control	3.0 b	4.0 a
Rinsing	2.7 b	3.0 b
Dipping	2.6 b	2.9 b
Dipping/Blowing	2.6 b	3.3 b
Concentration^h		
Control	2.9 a	4.3 a
15 ppm	<2.4 b	2.5 b
30 ppm	<2.4 b	2.7 b
50 ppm	ND	2.7 b
Sodium hypochloriteⁱ		
Untreated	4.4 a	Not measured
EW(50 ppm)	2.8 cd	
50 ppm	3.6 b	
100 ppm	3.2 bc	
150 ppm	3.0 bcd	
200 ppm	<2.4 d	

a–d Means with different letters within each treatment in the same column are significantly different ($p < 0.05$).

^e Mesophilic aerobic bacterial CFU on tissue surface.

^f Mesophilic aerobic bacterial CFU in tissue macerate.

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

^h Control = rinsing with tap water for 4 min; 15 ppm, 30 ppm, and 50 ppm = rinsing with electrolysed water containing 15 ppm, 30 ppm, and 50 ppm available chlorine, respectively, for 4 min.

ⁱ EW(50 ppm) = rinsing with electrolysed water containing 50 ppm available chlorine for 4 min; 50 ppm, 100 ppm, 150 ppm, and 200 ppm = rinsed with sodium hypochlorite solution containing 50 ppm, 100 ppm, 150 ppm, and 200 ppm available chlorine, respectively, for 4 min.

ND = Not detectable.

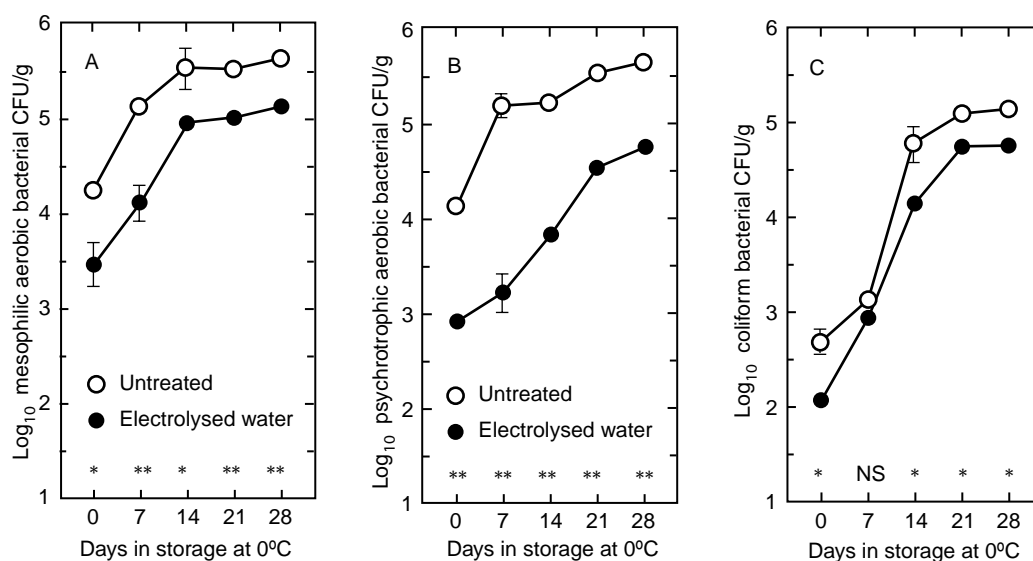


Figure 1. Counts of mesophilic aerobic bacteria (A), psychrotrophic aerobic bacteria (B) and coliform bacteria (C) of fresh-cut spinach prepared from intact leaves stored at 0°C, then dipped in electrolysed water containing 50 ppm available chlorine for 3 min. Vertical lines represent the standard error (SE). SE bars are not shown when masked by the graph symbol (CFU = colony forming units, NS = not significant, * = significant at $p < 0.05$, and ** = significant at $p < 0.01$).

Table 2. Counts of mesophilic aerobic bacteria (MAB) and lactic acid bacteria (LAB) of fresh-cut spinach treated with tap water (Water) or electrolysed water containing 50 ppm available chlorine (EW) for 4 min and stored at 10 or 20°C under normal air or 4% O₂ conditions.

Days	Treatment	Log ₁₀ colony forming units (CFU)/g	
		MAB ^d	LAB ^e
10°C storage			
0	Water	2.9 a	ND
	EW	<2.5 b	ND
8	Water-air	3.9 a	<2.5 a
	EW-air	3.7 b	<2.5 a
	Water-4% O ₂	3.9 a	<2.5 a
	EW-4% O ₂	3.5 c	<2.5 a
15	Water-air	4.4 a	<2.5 a
	EW-air	3.9 b	<2.5 a
	Water-4% O ₂	4.5 a	<2.5 a
	EW-4% O ₂	4.2 a	<2.5 a

abc Means with different letters within each day in the same column are significantly different ($p < 0.05$).

^d Mesophilic aerobic bacterial CFU in tissue macerate.

^e Lactic acid bacterial CFU in tissue macerate.

ND = Not detectable.

Table 2. (Cont'd) Counts of mesophilic aerobic bacteria (MAB) and lactic acid bacteria (LAB) of fresh-cut spinach treated with tap water (Water) or electrolysed water containing 50 ppm available chlorine (EW) for 4 min and stored at 10 or 20°C under normal air or 4% O₂ conditions.

Days	Treatment	Log ₁₀ colony forming units (CFU)/g	
		MAB ^d	LAB ^e
20°C storage			
0	Water	3.9 a	ND
	EW	<2.5 b	ND
3	Water-air	5.4 a	<2.5 a
	EW-air	3.6 c	<2.5 a
	Water-4% O ₂	4.9 b	<2.5 a
	EW-4% O ₂	3.8 c	<2.5 a
5	Water-air	6.6 a	<2.5 a
	EW-air	6.1 b	<2.5 a
	Water-4% O ₂	6.5 a	<2.5 a
	EW-4%O ₂	5.7 b	<2.5 a

abc Means with different letters within each day in the same column are significantly different ($p < 0.05$).

^d Mesophilic aerobic bacterial CFU in tissue macerate.

^e Lactic acid bacterial CFU in tissue macerate.

ND = Not detectable.

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