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Full Length Research Paper

Decontamination of ready-to-eat Japanese mustard green (*Brassica japonica*) from *Escherichia coli* using slightly acidic electrolyzed water

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In the present study, slightly acidic electrolyzed water (SAEW: pH 5.6 ± 0.21, 22 ± 1.4 mg/L available chlorine concentration; ACC) was evaluated for decontamination of Japanese mustard green (*Brassica japonica*) from *Escherichia coli* (*E. coli*) and its effectiveness was compared to that of sodium hypochlorite solution (NaOCl solution: pH 9.8±0.01, 110 ± 2.3 mg/L ACC). Decontamination of Japanese mustard green inoculated with *E. coli* was done by dipping samples into SAEW, NaOCl solution and Tap water (control) with stirring for 2, 5, 10 and 15 min. SAEW achieved *E. coli* reduction of 1.29 -1.64 $\log_{10}$ CFU/g and was not significantly different from that achieved by NaOCl solution ($P > 0.05$). Increasing the exposure time did not significantly affect its decontamination effectiveness. The results of current study indicate that SAEW with near neutral pH and low available chlorine concentration could be used as an alternative food sanitizer for NaOCl solution to reduce the population of pathogens from the fruits and vegetables in food industry.

Key words: Slightly acidic electrolyzed water, ready-to-eat vegetables, decontamination, *E. coli*, *Mizuna.*

INTRODUCTION

The health benefits associated with fresh fruits and vegetables combined with the on-going consumer trend toward eating out and consuming ready-to-eat (RTE) fruits and vegetables have contributed to a substantial increase in the popularity of ready-to-eat vegetables. RTE vegetables are those vegetables which have not been cooked and will not undergo cooking before consumption. Concomitant with this trend, an increased number of microbial infections associated with the consumption of fresh RTE fruit and vegetables have been documented in recent years, for example in Japan (Gutierrez, 1997), USA (FDA, 2006), England and Wales (PHLS, 2000) and Europe (Pezzoli et al., 2007).

The RTE can easily be contaminated from farm manure used as fertilizer (IFST, 1999) and plant workers. Some of the microbial pathogens associated with fresh vegetables include *Escherichia coli*, *Listeria monocytogenes*, *Salmonella* spp., and *Shigella* spp. Their high moisture content, the lack of lethal process to eliminate microbial pathogens, and the potential for temperature abuse during preparation, distribution and handling further intensify the risk of food-borne illness. To avoid risks of food-borne illness associated with RTE vegetables, their decontamination is inevitable in guaranteeing their quality and safety for human consumption. Chlorine based sanitizer especially sodium hypochlorite (NaOCl) solution that has long been used for this purpose has been criticized of having limited disinfection effectiveness on raw food and agricultural produce (Suslow, 2001) and high concentration for increased effectiveness may cause product tainting (Adams et al., 1989). For this reason, the use of electrolyzed water (EW), has been introduced as an alternative and a novel sanitize in agriculture and food industry as it is safe for both environment and the user (Al-Haq and Sugiyama, 2005). Acidic electrolyzed

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water (AEW) has been widely used for this purposed than any other form. It exists as strong acidic (StAEW, pH 2.5 ± 0.2) and slightly acidic (pH 5 - 6.5) electrolyzed water. Most of the literatures related to the application of AEW in food and agricultural produces deals with inactivation of pathogens using a StAEW type (Kim et al., 2000).

Although in vitro studies at a commercial level on the use of SAEW has shown strong bactericidal, fungicidal, and virucidal activities (Okamoto et al., 2006), its application in agriculture and food industry as sanitizer is a relatively new concept and is not much explored. This study therefore aimed at assessing the effectiveness of slightly acidic electrolyzed water (SAEW; pH 5 - 6.5, 10 - 30 mg/L available chlorine concentration) on decontamination of ready-to-eat Japanese mustard green (*Brassica japonica*) commonly known as *Mizuna* from *E. coli*. *Mizuna* has recently become popular as a leaf salad in Japan, China, Taiwan and Korea. It is frequently paired with *daikon* (giant white radish) in a fresh testing salad. It may also appear in soups or Japanese hot pot or as a garnish on various dishes.

MATERIALS AND METHODS

Preparation of treatment solutions

Slightly acidic electrolyzed water was produced by electrolysis of a mixture of aqueous dilute solution of HCl (2%) and tap water using SAEW generator (Apia60, HOKUTY Co., Kanagawa, JAPAN) at a rate of 1.0 $L.min^{-1}$. SAEW generator basically consists of an electrolytic cell with anode and cathode electrodes and no separating membrane between them. Sodium hypochlorite solution was prepared by diluting 10% sodium hypochlorite solution using distilled water to obtain a final sodium hypochlorite (NaOCl) solution (Wako, Tokyo, Japan) containing ca. 110 mg/L ACC (Beuchat, 1998). Tap water (TW) was used as control for this experiment.

Analytical measurements

The oxidation reduction potential (ORP), pH and ACC of treatment solutions were measured in duplicate immediately after preparation and before decontamination experiments. The pH was measured with a pH meter (HM-14P, TOA electronics Ltd., Tokyo, Japan) using a pH combination electrode (GST-2419C) and ORP was measured with ORP meter (RM-12P, TOA Electronics Ltd., Tokyo, Japan) using an ORP electrode (PST-2019C). The pH meter was calibrated using commercial standard buffers pH 4.01 and 6.86 (Nacalai Tesque, Inc., Kyoto, Japan). Available chlorine concentration of treatment solutions were determined by spectrophotometric method using a spectrophotometer (DR/4000V, HACH Co., Loveland, U.S.A). The detection limit is 0 - 2 mg/L Cl_2. Therefore samples were first diluted to desired lower levels of ACC using deionized water prior to measurement.

Inoculation of Japanese mustard green (*mizuna*) with *E. coli*

The pure L-dried culture of *Escherichia coli* NBRC 3301 was obtained from NITE Biological Resource Center (NBRC), revived soon after arrival according to L-dried culture reactivation procedures provided by NBRC and used for this experiment. Samples of Japanese mustard green (*Mizuna*) were purchased at a local supermarket in Kagoshima city, stored at 10°C and used within 2 days. Samples were cut into the length of about 4 cm pieces using a sterile kitchen knife. A 100 g sample of *mizuna* leaves was placed into a (30 by 20 cm) stomach bag to facilitate the inoculation process. Each bag containing *mizuna* sample was inoculated with 500 mL of prepared *E. coli* suspension (ca. 8 log_{10}CFU/mL) adequately shaken gently to ensure uniform distribution and left for 15 minutes. The bacterial suspension was decanted and vegetable samples were sterilely air-dried under a bio-safety chamber for 1 h.

Treatment of inoculated Japanese mustard green (*Mizuna*)

The inoculated vegetable samples were treated by dipping method in which inoculated sample was dipped into SAEW, NaOCl or tape water (control) with stirring for 2, 5, 10 and 15 min at a vegetable to treatment solution ratio of 1:5. At the end of exposure time, the treatment solution was drained off, and the samples were aseptically drip dried for 5 min before microbial analysis.

Microbiological analysis

The microbiological analysis was done according to Gómez-López *et al*. (2007) with some modification in which a 25 g of Japanese mustard green sample from each treatment time and untreated sample were combined with 225 ml of 0.1% peptone water in a sterile stomach bag and then homogenized for 2 min with a Stomacher (Model 22, TUL Instruments, Barcelona, SPAIN). Tenfold dilution series were made in 0.1% peptone water poured in duplicate standard methods agar plate (NISSUI Pharmaceutical Co., Ltd, JAPAN) and incubated at 37 ± 2°C for 24 ± 3 h to determine the total viable count on sample macerate. Microbial counts were expressed as log_{10} CFU/g sample.

Statistical analysis

The calculated bacterial log_{10} CFU/g sample reductions as the result of treatment using SAEW, NaOCl solution and TW were considered for further statistical analysis. The values reported for plate count are the mean values of 16 individual samples ± standard deviation with duplicate plates for each sample. Data were subjected to one way analysis of variance (ANOVA) and Tukey's HSD test was used to determine the differences at $P \leq 0.05$ using SPSS 13.0 (SPSS software for Windows, release 13.0, SPSS, Inc., USA).

RESULTS AND DISCUSSION

The physicochemical properties of treatment solutions are presented in Table 1. In current study, SAEW (pH 5.6, 22 mg/L ACC and 940 mV ORP) was used to decontaminate the Japanese mustard green from *E. coli* and its effectiveness was compared with that of commonly used food sanitizer (NaOCl; pH 9.8, 110 mg/L ACC and 650 mV ORP). Tap water was used as control.

Treatment of Japanese mustard green using SAEW, NaOCl solution and tap water (control) reduced the *E. coli* population as shown in Table 2. A comparison the decontamination effectiveness of SAEW with NaOCl and tap water was made. Washing with water alone can not achieve the general requirement of microbial safety. In current study, tap water achieved between 0.84 and 1.02 log_{10} CFU/g reduction of *E. coli* for 2 and 15 min,

Table 1. Physical chemical properties of tested solutions

	N	pH	ORP[a] (mV)	ACC[b] (mg/L)
SAEW[c]	10	5.6 ± 0.21	920 ± 10	22 ± 1.40
NaOCl[d]	10	9.8 ± 0.01	650 ± 6	110 ± 2.30
Tap water	10	6.9 ± 0.05	625 ± 2	<1

Values are the mean ± standard deviation of duplicate samples with n=10 for each solution;
a: Oxidization reduction potential (mV).
b: Available chlorine concentration (mg/L).
c: Slightly Acidic Electrolyzed water.
d: Sodium hypochlorite solution.

Table 2. Comparison of SAEW with NaOCl solution on their decontamination effectiveness at each treatment time expressed as log reduction (log_{10}CFU/g).

Treatment time (min)	*E. coli* log Reduction (log_{10}CFU/g)		
	TW	SAEW	NaOCl
2 min	0.84±0.06[a]	1.29±0.05[b]	1.51±0.05[b]
5 min	0.88±0.05[a]	1.46±0.04[b]	1.60±0.04[b]
10 min	0.98±0.06[a]	1.46±0.04[b]	1.59±0.03[b]
15 min	1.02±0.04[a]	1.64±0.09[b]	1.79±0.04[b]

Values are mean ± standard error of mean. Means with different lower case letter on the same low are significantly different at p<0.05.
Inoculated Japanese mustard green samples were treated by dipping into SAEW, NaOCl solution and TW for 2, 5, 10 and 15 min.
TW: Tap water.
SAEW: Slightly acidic electrolyzed water (pH 5.6 and 22 mg/L available chlorine concentration).
NaOCl: Sodium hypochlorite solution (pH 9.8 and 110 mg/L available chlorine concentration).

respectively. Koseki et al. (2004) reported that washing with tap water failed to reduce the microbial loads on cucumbers and strawberries. Similar results were reported by Izumi (1999) in which washing carrots, radish and potatoes with water could only achieve 0.4 - 0.6 log10 CFU/g microbial reduction.

At all treatment times, SAEW showed a significantly higher (P < 0.05) *E. coli* log_{10} CFU/g reduction than tape water (Table 2). SAEW achieved 1.29 log_{10} CFU/g at 2 min and 1.64 log_{10} CFU/g reductions at 15 min treatment. The results obtained in this study using SAEW (22 mg/L ACC) were similar to those obtained by Izumi (1999) in which dipping trimmed spinach in 500 mL neutral electrolyzed water (20 mg/L ACC, pH 6.8) for 3 min followed by rinsing with tap water for 1 min resulted in a 1.4 log_{10}CFU/g reduction of total microbial count on macerate. This suggests that SAEW might be as effective as other electrolyzed water and with the advantage of being less corrosive to metallic surfaces, having a longer storage life and being safer to the environment and operators because of its near neutral pH; it could be much applicable in agriculture and food industry.

Compared to NaoCl solution in terms of their decontamination effectiveness, SAEW showed no significant difference (P > 0.05) despite of the former having more than 5 times higher available chlorine concentration than the later. Adam et al. (1989) proposed 100 mg/L ACC to be adopted as working concentration since higher levels could cause product tainting and equipment corrosion. SAEW used in this study had only 22 mg/L ACC, therefore, potential problems mentioned by the authors would not be of concern when using SAEW in agriculture and food industry. Findings from the current study therefore, indicate that SAEW may be used as an alternative disinfectant for NaOCl solution to reduce the population of pathogens from the Japanese mustard green. The observed efficacy of SAEW at low ACC could be due to its high content of HOCl as reported by Sapers (2006) of about 97% at this pH and that was identified by Parish *et al.* (2003) as the most active antimicrobial component responsible for the bactericidal activity of chlorinated water. SAEW used in current study had higher positive ORP value of 940 mV. Higher positive ORP value is an indication of a stronger oxidizing ability (Jay, 1996). An ORP of +200 to +800 mV is optimal for growth of aerobic microorganisms, whereas an optimum range of +200 to +400 mV is favored for growth of anaerobic microorganisms (Jay, 1996). Since the ORP value of SAEW used in the current study was higher (ca. 940 mV) and *E. coli* is a facultative anaerobe, it is very likely that ORP too played an influential role, in combination with high proportion of HOCl, in killing *E. coli*. These facts suggest that chlorine-related substances, especially HOCl and high ORP, may be the main bactericidal factor in SAEW (Issa-Zacharia et al., 2009b).

Figure 1 shows the effect of treatment time on the decontamination effectives of each treatment solution. There was no significant difference in *E. coli* population reduction when the Japanese mustard green was washed with tap water at 2, 5, 10 or 15 min. However, SAEW and NaOCl solution showed a significantly higher (P < 0.05) reduction of *E. coli* only with 15 min of treatment, while there was no difference with other treatment time (P > 0.05).

Although 15 min of treatment time showed significantly higher decontamination effectiveness, treatment for such long time has been reported to be unsuitable for product quality and it is therefore discouraged. Also a conventional washing of vegetables at home normally would not take such long time. Since, 2, 5 and 10 min of treatment did not show any significant difference, washing the Japanese mustard green for 2 min would be economical and sufficient to eliminate or reduce its pathogens. Similar results were reported by Issa-Zacharia et al. (2009a) who evaluated the effectiveness of SAEW against aerobic microbes naturally present in spinach

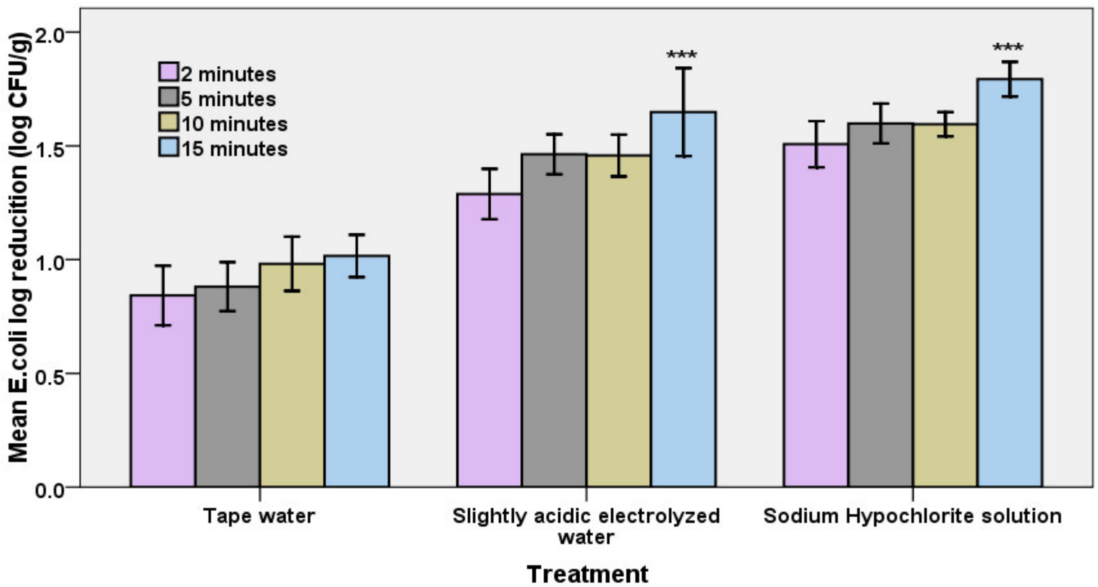

Figure 1. *E. coli* log reduction after a 2, 5, 10 and 15 min treatment of Japanese mustard (*mizuna*) using SAEW, NaOcl solution and Tape water (control). Mean values with asterisks showed a significant difference at $P < 0.05$.
A 15 min treatment time resulted to a significantly higher *E. coli* reduction (log_{10} CFU/g) than other treatment time with slightly acidic electrolyzed water and Sodium hypochlorite solution.

leaves. In their study, extending washing time from 5 - 20 min did not further decrease bacterial number with all tested solutions.

In conclusion, this study demonstrated that SAEW with an near-neutral pH value exhibits equivalent decontamination effectiveness for the Japanese mustard green (*Mizuna*) compared to sodium hypochlorite solution. The advantage of SAEW is non-corrosive, used without further dilutions and a less potential health hazard to the worker as no use of concentrated chemical solutions are needed for its preparation. Therefore, it could be used instead of sodium hypochlorite as an effective disinfectant for ready-to-eat vegetables.

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