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Application of slightly acidic electrolyzed water as a potential non-thermal food sanitizer for decontamination of fresh ready-to-eat vegetables and sprouts

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ABSTRACT

The sanitization efficacy of slightly acidic electrolyzed water (SAEW) against food pathogens on selected fresh ready-to-eat (RTE) vegetables and sprouts was evaluated and compared to sodium hypochlorite (NaOCl) solution. RTE vegetables and sprouts were dip-inoculated with *Escherichia coli* (*E. coli*) and *Salmonella* spp. and dip-treated with SAEW, NaOCl solution for 5 min. SAEW treatment significantly ($p < 0.05$) reduced the total aerobic mesophilic bacteria from Chinese celery, lettuce and daikon sprouts by 2.7, 2.5 and 2.45 $\log_{10}$CFU/g, respectively relative to un-treated. Pathogens were significantly ($p < 0.05$) reduced from Chinese celery, lettuce and daikon sprouts by 2.7, 2.8 and 2.8 $\log_{10}$CFU/g (*E. coli*) and 2.87, 2.91 and 2.91 $\log_{10}$CFU/g (*Salmonella* spp.), respectively following a SAEW treatment. SAEW and NaOCl solution showed no significant sanitization difference ($p > 0.05$). Results demonstrate that SAEW at low chlorine concentration and a near neutral pH is a potential non-thermal food sanitizer that could represent an alternative to NaOCl solution and would reduce the amount of free chlorine used in fresh-cut vegetables industry, since the same microbial reduction as NaOCl solution is obtained.

1. Introduction

Fresh produce at harvest has a natural epiphytic micro-flora much of which is non-pathogenic. During any of the steps in the farm to consumer continuum (growth, harvest, processing, packaging, transportation, handling, retail) further microbial contamination can occur from a variety of sources such as environmental, animal or human. Therefore, fresh produce can be a vehicle for the transmission of bacterial, parasitic and viral pathogens capable of causing human illness and a number of reports refer to raw vegetables harboring potential food-borne pathogens (Beuchat, 1996; Nguyen-the & Carlin, 1994). For instance, fresh produce and sprouts have been implicated in a number of documented outbreaks of illness in countries such as Japan (Gutierrez, 1997; Nat'l. Inst. Inf. Dis., 1997), the USA (FDA, 2006) and EU (Gilbert et al., 2000; Pezzoli et al., 2007). Despite these facts, the consumption of minimally-processed and fresh ready-to-eat (RTE) fruits and vegetables has undergone a sharp increase as a result of the advocacy of health benefits associated with fresh vegetables combined with the on-going consumer trend toward eating out, that have in turn increased the incidence of food-borne outbreaks caused by contaminated fresh fruit and vegetables in recent years (Mukherjee, Speh, Jones, Buesing, & Diez-Gonzalez, 2006). Pathogens most frequently associated with fresh vegetables include *Listeria monocytogenes*, *Salmonella* spp., *Shigella* spp., enteropathogenic strains of *Escherichia coli* and *hepatitis A virus*. Fresh RTE vegetables may therefore pose a food safety risk because they are consumed raw and are susceptible to be contaminated by fecal material and soil on the farm (Mukherjee, Speh, Dyck, & Diez-Gonzalez, 2004). According to the 2007 epidemiological data of food-borne diseases in Japan, 1289 outbreaks and 33,477 cases of food-borne disease were reported. Among the 33,477 cases, 3939 cases were associated with composite ready-to-eat foods and 1242 cases with vegetables and vegetable products (Inspection and Safety Division, Department of Food Safety, Japan Ministry of Health, Labor and Welfare, 2008). To minimize microbiological hazards by reducing the microbial load, effective sanitization of RTE vegetables must be ensured before reaching the ultimate consumer. There is no single sanitization treatment so far that has been shown to completely eliminate food pathogens on fresh vegetables, seeds or sprouts without affecting the product quality.

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Currently, chlorine solution prepared from sodium hypochlorite (50–200 mg/l) is the most used sanitizing agent for washing fresh produce and has been authorized for use with food by Japanese Ministry of health, Labour and Welfare. However, if used in authorized concentration, this treatment has minimal effect and results in small reduction of pathogens on fresh produce (Beuchat, Nail, Adler, & Clavero, 1998; Jaquette, Beuchat, & Mahon, 1996; Zhang & Farber, 1996). Besides, a higher concentration for increased effectiveness may cause product tainting (Adam, Hartley, & Cox, 1989) and results in deposition of sodium residues on the product. These facts together with increasing public health concerns about the possible formation of chlorinated organic compounds from sodium hypochlorite solution as sanitizer (Singh, Singh, Bhunia, & Stroshine, 2002) has brought into attention for the need of an alternative method much more friend to both use and environment. For this reason, acidic electrolyzed water that was first authorized as food additive and allowed to be used directly to food in Japan by the Japanese Ministry of Health, Labor and Welfare in 2002, has been applauded as a potential and an emerging non-thermal food sanitizer effective against a number of food pathogens (Huang, Hung, Hsu, Huang, & Hwang, 2008). Acidic electrolyzed water exists as Strong acidic electrolyzed water (StAEW; pH 2.5 ± 0.2) and slightly acidic electrolyzed water (SAEW; pH 5.0–6.5). SAEW has been an authorized food additive in Japan since 2002 because of its proven biological safety and characterization as an effective bactericide even at low available chlorine concentrations (ACC) of 10–30 mg/l and pH 5.0–6.5 (Suzuki, Nakamura, Doi, Kokubo, & Tomita, 2005). Most of the literatures and reviews related to the application of acidic electrolyzed water in sanitization of fresh vegetables deals with inactivation of pathogens using a StAEW type (Huang et al., 2008; Koseki & Itoh, 2001). In contrast, very limited reports for sanitization efficacy of RTE vegetables using slightly acidic electrolyzed water type are available to date. This study aimed at assessing the sanitization efficacy of slightly acidic electrolyzed water on reduction of indigenous aerobic mesophilic bacteria and the inoculated *E. coli* on daikon sprouts, lettuce and Chinese celery.

2. Materials and methods

2.1. Preparation of plant material

Selected 3 ready-to-eat vegetables commonly consumed in Japan (daikon sprouts, lettuce and Chinese celery) were used for this trial. Samples of daikon sprout (*Raphanus sativus* L.), lettuce (*Lactuca sativa* L.) and Chinese celery (*Apium graveolens* L.) were purchased at a local supermarket in Kagoshima city, stored at 10 °C and used within 2 days. For the microbial challenge study (simulated cross-contamination), the outer 2 leaves of the lettuce head were discarded. Chinese celery and lettuce leaves were aseptically cut into approximately 5-by 5-cm square size, while daikon sprout samples were cut into a length of about 4 cm pieces using a sterile knife before being used for experiment.

2.2. Preparation of treatment solutions and analytical measurements

SAEW was produced by electrolysis of a mixture of aqueous dilute solution of HCl (2%) and tap water using Apia60 generator (Apia60, HOKUTY Co., Kanagawa, JAPAN) at 5.0 V, 3.0 A and produced at a rate of 1.0 l/min. SAEW generator basically consists of an electrolytic cell with anode and cathode electrodes and no separating membrane between them. NaOCl solution ($\sim$100 mg/l) was prepared by diluting 10% sodium hypochlorite solution using distilled water to obtain a final sodium hypochlorite (NaOCl) solution (Wako Pure Chemicals Ind., Ltd., Osaka, Japan). Tap water (TW) was used as control for this experiment. The oxidation–reduction potential (ORR), pH and ACC of treatment solutions were measured in duplicate immediately after preparation and before each sanitization experiment. 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 was determined by spectrophotometric method using a spectrophotometer (DR/4000 V, 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.

2.3. Bacteria and preparation inocula

Pure cultures of *E. coli* (NBRC3301) and *Salmonella* spp. (NBRC 13245) were used in a microbial challenge study for the selected RTE vegetables. Liquid-dried (L-dried) cultures of *E. coli* and *Salmonella* spp. were obtained from NITE Biological Resource Center (NBRC), revived soon after arrival according to L-dried culture reactivation procedures provided by manufacturer and as described in details by Issa-Zacharia, Kamitani, Morita, and Iwasaki (2010). The viable cell count of *E. coli* and *Salmonella* spp. cultures was verified by pour plate count methodology using standard method agar (NISSUI Pharmaceutical Co., Ltd, JAPAN). The colonies from plated pure culture were propagated once after every 4 d using a 4 by 4 looping out method on solidified standard method agar for preservation. Original *E. coli* and *Salmonella* spp. suspensions were prepared by transferring several colonies to a 10 ml of 0.1% peptone water using a sterile inoculation loop, vortexed using a thermal mixer (TM-100, Tokyo Thermonics Co.Ltd, JAPAN) and transferred to a 50 ml beaker that was filled up to a final volume of 50 ml by sterile 0.1% peptone water. The prepared original bacterial suspensions were continuously stirred using a magnetic stirrer (REXIM RS-6DR, AS-ONE Corporation, Osaka JAPAN) at 500 rpm to maintain the uniform distribution and applied to RTE vegetables within 30 min of preparation. The final prepared bacterial suspension used for dip-inoculation onto the vegetables contained bacteria concentration of ca. 10 $\log_{10}$CFU/ml that was determined by plating 1 ml of portion of appropriately diluted *E. coli* and *Salmonella* spp. suspension on standard method agar plates and incubating plates at 37 ± 2 °C for 24 ± 2 h.

2.4. Sample inoculation for microbial challenge study and treatments

For microbial challenge study, prepared samples of Chinese celery, lettuce and daikon sprout were dip-inoculated by submerging each vegetable sample separately into a prepared *E. coli* and *Salmonella* spp. suspensions (ca. 10 $\log_{10}$CFU/ml, 20 ± 2 °C) at a vegetable to bacterial suspension ratio of 1:5 for 15 min. The suspensions were decanted and vegetable samples were sterilely air-dried on a wire screen under a bio-safety chamber for 1 h. Treatments of inoculated vegetable samples were performed by submerging each vegetable sample in a 500-ml glass beaker containing SAEW, NaOCl or tape water (control) with continuously stirring using a magnetic stirrer (REXIM RS-6DR, AS-ONE Corporation, Osaka JAPAN) at 500 rpm for 5 min at a vegetable to treatment solution ratio of 1:5. After treatment, four 30-g vegetable samples for each treatment were immediately transferred separately into sterile stomacher bags using