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Application of electrolyzed oxidizing water in production of radish sprouts to reduce natural microbiota

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ABSTRACT

The objective of this study was to determine the efficacy of electrolyzed oxidizing (EO) water in reducing natural microbiota on radish seed and sprout during seed soaking and sprouting. EO water with different available chlorine concentrations (ACC, 15, 20, 28, 33 and 40 mg/L) and different pH (2.5, 3.5, 4.5, 5.5 and 6.5) were used to soak radish seeds for 12 h and the surviving population of total aerobic bacteria, yeast and mold, and germination rate were determined. On the other hand, EO water with ACC of 30 and 50 mg/L was applied to spray sprouts during seed sprouting and the antimicrobial efficacy of EO water, as well as length, gross weight and dry weight of sprout were evaluated. The results showed that the population of natural microbiota decreased with increasing ACC of EO water, while no significant difference was observed among EO waters with different pH levels that were applied while soaking the seeds. EO water with higher ACC and lower pH slightly reduced the germination percentage of radish seed during seed soaking. EO waters with ACC of 30 and 50 mg/L sprayed during seed sprouting resulted in 1.39 and 1.58 log reductions of total aerobic bacteria, yeast and mold, respectively, and improved the length, gross weight and dry weight of the sprouts. Therefore, EO water with low ACC and near neutral pH could be used to soak seeds and water sprouts throughout seed germination and sprouting to control the population of natural microbiota on seeds and sprouts.

1. Introduction

Seed sprouts such as alfalfa, soybean, mung bean and radish are commonly consumed raw or slightly cooked in many countries (Robertson, Johannessen, Gjerde, & Loncarevic, 2002). Sprouts have been associated with numerous foodborne outbreaks worldwide (Buchholz et al., 2011; Frank et al., 2011; Michino et al., 1999; Watanabe et al., 1999). *Escherichia coli* O157:H7, *Salmonella* and *Listeria* have been reported to contaminate sprouts (Dechet et al., 2014; Taormina, Beuchat, & Slutsker, 1999). Microbiological safety issues for fresh produce are important and highly concerned, since these products are susceptible to be contaminated with microbiota, especially pathogens from soil, water, hands of workers during harvesting, transportation, storage and processing. Seeds grow close to soil and are easily contaminated with natural soil microbiota which grows at very fast rate when seeds are sprouting in the environment with proper temperature and high humidity. Contaminated seeds have become the source of most sprout-associated foodborne diseases and are considered to be the most common source of contamination. It was reported that the microbial load in sprout after several days' germination was 2–4 logs greater than that in seeds (Kimanya et al., 2003; Martinez-Villaluenga, Frias, Gulewicz, Gulewicz, & Vidal-Valverde, 2008). The contamination of sprouts with foodborne pathogen has become a worldwide food safety concern and therefore, disinfection is critical during seed sprouting.

Various methods and technologies have been previously applied to inactivate and control microorganism in seed or sprout, including physical treatment such as dry heat, hot water, high hydrostatic pressure and irradiation (Bari, Nei, Enomoto, Todoriki, & Kawamoto, 2009; Neetoo, Pizzolato, & Chen, 2009; Pao, Kalantari, & Khalid, 2008; Schoeller, Ingham, & Ingham, 2002), biological interventions such as antagonistic microorganisms, antimicrobial metabolites, bacteriophages (Bennik, van Overbeek, Smid, & Gorris, 1999; Ye, Kostrzynska, Dunfield, & Warrineri, 2010) and chemical

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interventions such as chlorine-bases products, acid compounds, electrolyzed water (Nei, Latiful, Enomoto, Inatsu, & Kawamoto, 2011; Tornuk, Ozturk, Sagdic, & Yetim, 2011; Zhang et al., 2011). Although these technologies are effective in reducing microbial load; an easy, effective and economical approach which can be applied throughout the period of seed soaking to sprouting process will be more necessary.

Electrolyzed oxidizing (EO) water has been recognized as a novel, effective, relatively inexpensive, and environmentally friendly sanitizer (Huang, Hung, Hsu, Huang, & Hwang, 2008). The advantages of EO water including generally recognized as safe (GRAS) status, as well as its quick onsite production make EO water an economically as well as industrially feasible antimicrobial agent (Bosilevac, Shackelford, Brichta, & Koohmaraie, 2005). EO water is produced by electrolysis of a diluted salt solution in an electrolytic chamber which contains an anode and cathode separated by a diaphragm membrane. EO water produced from anode with low pH (<2.7), high oxidation reduction potential (ORP, >1000 mV), high dissolved oxygen is known as acidic electrolyzed water (AEW) (Huang et al., 2008). Slightly acidic electrolyzed water (SAEW) with a pH of 5.0–6.5 and high hypochlorous acid is produced by electrolysis of diluted NaCl and/or HCl solution in a non-membrane electrolytic cell (Zhang et al., 2011). Both types of EO water have been widely applied in many fields such as fruit and vegetable (Hung, Tilly, & Kim, 2010; Pangloli & Hung, 2013), meat products (Rahman, Park, Song, Al-Harbi, & Oh, 2012), and seafood (Lin et al., 2013) to reduce or eliminate pathogens (Jadeja & Hung, 2014; Zhang, Li, Jadeja, Fang, & Hung, 2016a), fungi (Abbasi & Lazarovits, 2006), viruses (Hao et al., 2013; Tamaki, Bui, Ngo, Ogawa, & Imai, 2014), spores (Park, Guo, Rahman, Ahn, & Oh, 2009; Tang et al., 2011; Zhang, Li, Jadeja, & Hung, 2016b), and biofilms (Sun, Zhang, Chen, & Han, 2012).

Limited number of study has reported application of EO water throughout the period of seed soaking to sprouting to reduce the natural microbiota on seeds and sprouts. Therefore, the objectives of present study were to evaluate: (1) The effect of different pH and available chlorine concentrations (ACC) on germination rate of radish seed after soaked in EO water. (2) The antimicrobial efficacy of EO water with different ACC and pH on total aerobic bacteria, yeast and mold when applied to soak radish seed. (3) The effect of EO water on controlling the natural microbiota during radish seed sprouting. (4) The properties of EO water treated sprout (sprout length, gross weight and dry weight) after harvest.

2. Materials and methods

2.1. Preparation of EO water

EO water with different ACC was produced by electrolysis of 0.1% NaCl and 0.02% HCl mixed solution at 20 V for different time periods using an EO water generator (Shenyang Dongyu Xinbor Technology Company Ltd., Shenyang, China). EO water with different ACC was obtained by electrolysis of mixed solution for different time periods and pH was obtained by adjusting pH using 1 mol/L HCl. The physicochemical properties of EO water were measured immediately after preparation. The ACC was determined by a colorimetric method using a digital chlorine test kit (RC-3F, Kasahara Chemical Instruments Corp., Saitama, Japan). The pH and ORP were measured using a dual scale pH/ORP meter (HM-30R, DKK-TOA Corporation, Tokyo, Japan) with a pH probe (GST-5741C) and an ORP probe (PST-5721C). EO water at pH of 2.5, 3.5, 4.5, 5.5, and 6.5, ACC of 15, 20, 25, 28, 30, 33, 40 and 50 mg/L, and ORP of 836.2–1067.4 mV were used in this study. Tap water with pH of 7.2, ORP of 426 mV and non-detectable ACC was used as a control.

2.2. Seed soaking and germination rate determination

Radish seeds (weight of 13.90 ± 0.05 g/1000 grains) were obtained from a commercial seed supplier (Yongxing Science and Technology Co., Ltd, Beijing, China). Uniform seeds based on shape and size were selected for this study. Hundred grams of seed were washed twice with 500 mL of tap water and then soaked in 500 mL of EO water with ACC of 25 mg/L at pH of 2.5, 3.5, 4.5, 5.5 and 6.5 at room temperature (22 °C) for 12 h. On the other hand, EO water at pH 6.5 and ACC of 15, 20, 28, 33 and 40 mg/L was used to soak seeds for 12 h, respectively. Seeds were gently agitated by sterile glass rod every 3 h during soaking period. Tap water was used as a control. Hundred grains of seed were randomly selected and spread on a sterile 90 mm-diameter petri dish covered with 2 layers of sterile filter paper and were incubated at 25 °C and 85% RH for germination. The humidity in each petri dish was maintained by spraying 2 mL of its own treatment solution every 4 h. Germinated seeds were counted at 10, 20, 30, 40 and 50 h to calculate germination rate. On the other hand, 5 g of seed after soaking was selected to determine the surviving population of total aerobic bacteria, yeast and mold using the method described in 2.4.

2.3. Seed sprouting

One hundred grams of radish seed were soaked in 500 mL of EO water with ACC of 15 mg/L at pH of 6.5 or in tap water (control) as described in 2.2. After 12 h soaking, seeds were drained and aseptically spread evenly onto seeding tray (60 cm × 25 cm × 5 cm) with 4 layers of sterile cheesecloth. Seeding trays with soaked seeds were stacked in between two trays without seeds. One tray without seeds was placed on the top and one was placed at the bottom to black out the light and maintain the moisture level. Every 8 h, 30 mL of EO water with the same properties as used for seed soaking (treated samples) or tap water (control samples) were applied and the trays in the stack were changed in order to allow them to stay in similar conditions with each other. After 50 h of pre-germination, seeding trays with germinated seeds were placed in a single-deck stack in a chamber covered with black plastic film. EO water with ACC of 30 and 50 mg/L (pH 6.5) (treatment) or tap water (control) was sprayed every 8 h during seed sprouting. Fifty milliliter of treatment solution or tap water was used for seed sprouting for the first day and 10 mL was increased every day until harvest. Black plastic film was uncovered for 30 min in the morning and afternoon every day to allow sprout under fluorescent light. The temperature for seed sprouting was maintained at 22–25 °C. Five grams of each sprout sample was randomly selected to determine the total aerobic bacteria and yeast and mold at day 1, day 3, day 5, and day 7 before solution was sprayed.

2.4. Microbiological analysis

Five grams of seed or sprout were selected and placed in a sterile Ziplock bag and mixed completely with 50 mL of sterile deionized water by vortex for 30 s. Obtained suspension was serially diluted using 0.85% NaCl solution. Total aerobic bacteria count was obtained by plating 0.1 mL of the appropriate dilution onto sterile nutrient agar (Aiboxing Bioscience Inc., Beijing, China) and plates were incubated at 37 °C for 24 h before enumeration. Yeast and mold counts were determined by pouring 0.1 mL of the diluted sample onto Rose Bengal medium (Aiboxing Bioscience Inc.) (Koide & Nonami, 2007). Plates were incubated at 28 °C for 48 h before counting.