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Disinfection efficacy of electrolyzed oxidizing water on brown rice soaking and germination

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ABSTRACT

Brown rice is prone to be contaminated by microorganism and it is a common problem during brown rice soaking and germination. In this study, efficacy of acidic electrolyzed water (AEW) and slightly acidic electrolyzed water (SAEW) with different available chorine concentrations (ACC) on inactivation of natural microbiota and inoculated *Bacillus cereus* spores on brown rice was determined. Effect of replacing treatment solution during soaking and properties of brown rice after treatment was also determined. Brown rice was treated with AEW and SAEW at ACC of 50, 100 and 150 mg/L for 30 or 60 min at the ratio of 1: 5 (w/v) to remove the natural microbiota and then soaked in sterilized deionized water for the following 24 h. The results showed AEW with ACC of 150 mg/L for 30 or 60 min treatment decontaminated natural microbiota completely with no significant impact on properties of brown rice and could be used to remove microbiota on brown rice for the following inoculation trials. AEW and SAEW with ACC of 50, 100 and 150 mg/L were used to soak brown rice for 24 h and achieved the reduction of *B. cereus* spores inoculated on brown rice about 1.6–3.3 logs. AEW and SAEW with ACC of 50 and 100 mg/L replaced for 5 times during brown rice soaking achieved additional 2.40, 2.87 and 2.80, 2.78 log reductions respectively, in comparison with electrolyzed oxidizing (EO) waters with the same ACC soaked brown rice without replacement. However, germination potential and germination rate of brown rice were negatively affected by AEW for 24 h soaking. Results suggested that the application of SAEW to soak brown rice could reduce the microorganism with no adverse effect or even promote the properties of brown rice and hence could be used safely in food industry for brown rice soaking. Other approaches will be needed to combine with EO water for brown rice germination to reduce microbiota.

1. Introduction

Rice is an important staple food worldwide and the primary dietary source of carbohydrates. Brown rice is the part of rice remaining after outer layer of the rice kernel is removed. Brown rice has been recognized a popular healthy product which is rich in nutrients and bioactive components (Adil, 2012; Tian, Nakamura, & Kayahara, 2004). Germinated brown rice soaked in water to initiate budding is healthier and consumed more since high molecular weight polymers are broken down by activated hydrolytic enzymes to form some bio-functional substances (Lin, Pao, Wu, & Chang, 2015; Moongngarm & Saetung, 2010; Sirisoontaralak, Nakornpanom, Koakietdumrongkul, & Panumaswiwath, 2015). However, rice and rice-based products is prone to be contaminated with *Bacillus cereus* which can be found in a wide variety of environments during growth, harvesting, transportation, milling and other operations (Fangio, Roura, & Fritz, 2010; Sarrias, Valero, & Salmeron, 2002; Yang, Tao, Liu, & Zhu, 2008). *B. cereus* is an aerobic, gram-positive, spore-forming *bacilli* which can cause two primary types of foodborne gastrointestinal disease: emesis and diarrhea (Agata, Ohta, & Yokoyama, 2002; Ankolekar, Rahmati, & Labbe, 2009). Spores could survive in the absence of exogenous nutrients in a dormant state for many years and return to life rapidly to cause disease as the presence of appropriate nutrients by germinating and resuming vegetative growth (Piggot & Hilbert,

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2004; Setlow, 2006). Brown rice is easily to be contaminated with *B. cereus* spores which could regrow or reproduce with the nutrient obtained from brown rice during soaking and germination. Spores, unlike bacterial vegetative cells, are much more resistant to a wide range of adverse conditions including heat, radiation, desiccation and chemicals (Logan, 2005; Nicholson, Munakata, Horneck, Melosh, & Setlow, 2000; Ryu & Beuchat, 2005; Setlow, 2006). It is a challenge to inactivate spores, and hence routine approaches are insufficient to be used to achieve antimicrobial target.

Electrolyzed oxidizing (EO) water treatments were reported in many fields and has been used to eliminate or inactivate foodborne pathogens (Hao, Li, et al., 2013; Hung, Tilly, & Kim, 2010; Jadeja & Hung, 2014; Park, Hung, & Brackett, 2002), fungi (Abbasi & Lazarovits, 2006), viruses (Hao, Shen, et al., 2013; Tamaki, Bui, Ngo, Ogawa, & Imai, 2014) and spores (Park, Guo, Rahman, Ahn, & Oh, 2009; Tang et al., 2011). EO water produced by electrolysis of dilute sodium chloride solution is a novel disinfectant for its environmentally friendly and broad spectrum antimicrobial effect. Slightly acidic electrolyzed water (SAEW) with a pH of 5.0–6.5 is produced by electrolysis of hydrochloride acid or mixture of anode solution and cathode solution. At the near neutral pH, hypochlorous acid is the dominant form of free chlorine that could enhance inactivation of microorganism. It was reported that *Escherichia coli* O157:H7 and *Salmonella enteritidis* could be effectively inactivated on mung bean seeds by SAEW and without affecting the viability of seeds (Zhang et al., 2011). EO water collected from anode side of the chamber containing chlorine gas, hypochlorous acid and hydrochloric acid is known as acidic electrolyzed water (AEW). AEW has pH < 2.7, oxidation reduction potential (ORP) > 1050 mV and high free chlorine concentration and hence possesses effective antimicrobial properties (Jadeja, Hung, & Bosilevac, 2013). AEW was reported as an effective disinfectant to reduce microbial load on brown rice and could even enhance the growth of germinated brown rice (Liu et al., 2013). In recent years, EO water has been widely used in the food industry, agricultural protection, and hospital disinfection and has been proven to be a promising agent to reduce or eliminate microorganism.

B. cereus or *B. cereus* spores are commonly associated with brown rice. The effectiveness of EO water against *B. cereus* spores on brown rice has not yet been reported. The objective of this present study was to determine the effectiveness of SAEW and AEW on decontamination of natural microbial load and artificially inoculated *B. cereus* spores on brown rice. The effect of SAEW and AEW with different ACC at different soaking time duration, as well as the properties of brown rice were investigated.

2. Materials and methods

2.1. Bacterial strains

Bacillus cereus (ATCC 14579) used in this study was obtained from American Type Culture Collection. Nutrient agar (Neogen, USA) supplemented with 50 mg/L manganese sulfate (NAMS agar, Alfa Aesar, England) was used as a sporulation medium (Kim, Hung, & Brackett, 2000). Two hundred microliter of overnight *B. cereus* culture grown in tryptic soy broth (TSB, Difco, USA) was spread evenly on the NAMS agar using sterilized bent glass rod. Plates were sealed with parafilm and then incubated at 30 °C for 72 h to allow spore forming. Spores were harvested by adding 20 mL of sterilized 0.85% NaCl on the surface of each plate and were gently rubbed with a sterilized glass rod. Spore suspension was collected into sterilized 50 mL plastic tube and was completely mixed by vortex, then centrifuged at 2600 × *g* for 20 min at 4 °C. Spore pellets was collected and resuspended in 30 mL of 0.85% NaCl, centrifuged (4 °C) another twice at 2600 × *g* for 20 min. The final spore pellets were resuspended in 0.85% NaCl and stored in 4 °C until used. For artificially inoculating brown rice trials, refrigerated spore suspension was brought to room temperature and heated at 80 °C for 5 min in water bath to kill their vegetative cells prior to use (Kim et al., 2000).

2.2. Preparation of EO water

SAEW and AEW were produced by electrolyzing 0.03% NaCl solution in the EO water generator (EAU, Model #P30HST44T, GA, USA). EO water was collected into an air-tight container and the ACC, pH and ORP were measured immediately prior to experiment. The ACC was determined by a DPD-FEAS method (Hach Co., Loveland, Co., USA). The pH and ORP were measured using an ACCUMET pH meter (AR 50, Fisher Scientific, Pittsburgh, PA, USA) with a pH electrode and an ORP electrode. SAEW with a pH of 5.92–6.04, ORP of 938–970 mV, AEW with a pH of 2.61–2.87, ORP of 1146–1185 mV and ACC of 50, 100 and 150 mg/L were used in this study.

2.3. Decontamination of natural microbiota on brown rice by EO water

Brown rice used in this study was obtained from local market (Griffin, GA, USA). Five grams of brown rice was used as a sample in this study. Brown rice sample transferred to 50 mL sterilized centrifuge tube and was pre-washed using 50 mL of treatment solution by vortexing. Samples were drained and soaked in 25 mL of treatment solution (1:5 w/v) in 50 mL centrifuge tubes for 30 min or 60 min. At the end of desired contact time, 1 mL of suspension was taken out and mixed with 1 mL of 0.5% $Na_2S_2O_3$ to terminate any residual chlorine reaction. Counts of viable bacteria were obtained by spread plating 0.1 mL of appropriate 10-fold serial dilution onto tryptic soy agar (TSA, Difco, Becton Dickinson, MD, USA). The plates were incubated at 37 °C overnight before enumeration. Another 1 mL mixed solution was taken into 9 mL of TSB and incubated at 37 °C shaking for enrichment. Treatment solutions were decanted after 30 or 60 min treatment and brown rice samples were washed with 50 mL of sterilized deionized water (DIW) and then re-soaked in 25 mL of DIW in biochemical incubator at 28 °C. At the end of 24 h soaking period, viable bacterial population was counted using the method described above. Samples after germination (described in 2.4) were collected into 50 mL tubes and washed with phosphate buffered solution (PBS) at the ratio of 1: 5 (w/v) by vortex. Counts of viable bacteria were obtained as previously described. Sample soaked in DIW for 24 h without additional wash acted as control. The detection limit is 1.0 in this study.

2.4. Germination of brown rice

One hundred grains of soaked brown rice were aseptically spread on the sterilized petri dish of 9 cm diameter with 8 layers cheese cloth and placed in incubator (28 °C, 90% RH) for 48 h germination. Each treatment solution was sprayed to maintain the humidity during brown rice germination.

2.5. Measurement of brown rice properties

Water absorption (WA): samples were drained after 24 h soaking and dried in the air for 30 min. Weight of samples was measured before and after soaking. WA (%) = (weight of sample after soaking-weight of sample before soaking)/weight of sample before soaking × 100%.

Germination potential (GP): GP (%) = (number of germinated