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Effect of water hardness on the production and microbicidal efficacy of slightly acidic electrolyzed water

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ABSTRACT

Slightly acidic electrolyzed water (SAEW) has been proved as an effective sanitizer against microorganisms attached to foods. However, its physical properties and inactivation efficacy are affected by several factors such as water hardness. Therefore, in this study the effect of water hardness on SAEW properties were studied. Pure cultures of foodborne bacteria were used in vitro and in vivo to evaluate the inactivation efficacy of the SAEWs produced. Results obtained showed water hardness to be an important factor in the production of SAEW. Low water hardness may result in the necessity of further optimization of production process. In this study the addition of 5% HCl and 2 M NaCl at 1.5 mL/min flow rate was found to be the best electrolyte concentration for the optimization of SAEW production from low hardness water (34 ± 2 mg/L). Furthermore, the results showed that pre-heating was a better approach compared to post-production heating of SAEW, resulting in higher ACC values and therefor better sanitization efficacy.

1. Introduction

Minimally processed fresh produce is an essential part of the diet of people around the world due to their health effects and changes in people lifestyles (Wang and OH, 2012) and the trend to consume fresh produce has been an ever growing phenomena over the last few decades (López-Gálvez et al., 2009). However, concerns about the safety of consumers have also risen, as presence of spoilage and pathogenic bacteria, yeasts and molds is common in these foods (Zhang and Farber, 1996; Seymour et al., 2002).

Despite the advances in food safety regulations and food processing methods, fresh produce have been implicated in many foodborne disease outbreaks caused by a variety of pathogen microorganisms (Farber and Peterkin, 1991; Elizaquível and Aznar, 2008), revealing that the present commercial sanitizing approaches are not enough to assure produce safety (José and Dantas Vanetti, 2012). Therefore, the complete inactivation of pathogens is still a challenge for the food industry resulting in the necessity of improving sanitizion techniques. There are numerous sanitizing methods applied for foodincluding but not limited to irradiation, warm water, chlorine dioxide, ultrasound, acidified sodium chlorite, hydrogen peroxide and finally different types of electrolyzed water such as acidic, slightly acidic and alkaline (Forghani and Oh, 2013).

Slightly acidic electrolyzed water (SAEW) is a type of electrolyzed water with a pH value of 5.0–6.5 that contains a high concentration of hypochlorous acid (HOCl). The antimicrobial effect of SAEW mainly caused by the presence of HOCl has been extensively studied and proved (Cao et al., 2009; Nan et al., 2010; Rahman et al., 2012). It is generated by electrolysis of a dilute hydrochloric acid (HCl) and/or NaCl solution in a non-membrane electrolytic cell (Forghani and Oh, 2013). Compared to acidic electrolyzed water SAEW has the advantage of possessing antimicrobial activity with low available chlorine, resulting in reduced corrosion of production electrodes and surfaces. Also the potential damage to human health and the environment is reduced. Therefore, there is growing interest in new applications of SAEW in the food industry as an environmental friendly sanitization method (Issa-zacharia et al., 2010; Koide et al., 2011).

Application of commercial SAEW generators in food industry is becoming more popular due to its advantages upon other types of electrolyzed water (EW). However, the properties of EW produced by the same EW generator using fixed settings may significantly differ due to the different properties of starting water such as pH

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and hardness (Pangloli and Hung, 2013). This becomes of even greater importance for the production of SAEW because of the strict properties such as pH (5.0–6.5), necessary for SAEW.This critical point seems to be neglected in all studies on SAEW until today since they all reported the production, application or sanitizing efficacy of SAEW under standardized production settings using one type of water,usually without considering the properties of starting water (Rahman et al., 2010a; Webby Soli et al., 2010; Zhang et al., 2011).

According to the US Geological Survey (USGS, 2012) water hardness varies widely in the USA and is classified into different categories: soft water (0–60 mg/L CaCO3), moderately hard (60–120 mg/L CaCO3), hard (120–180 mg/L CaCO3) and very hard (more than 180 mg/L CaCO3). A similar high variety has been reported in Korea by the Korea Water Resources Corporation (Water, 2011). These two samples clearly show that there is a high chance of having different properties of the starting water regarding water hardness for the production of SAEW. Authors found only one report on the effects of water hardness on the efficacy of EW (Pangloli and Hung, 2013) while there was no report found on the effects of water hardness on the production of SAEW, its properties and efficacy in inactivating foodborne pathogens.Thus, the objective of this study was to investigate the effects of water hardness on the production of SAEW, its properties and efficacy in inactivating foodborne pathogens. Also, methods for the optimization of SAEW production from inappropriate water sources by the addition of electrolytes were investigated.

2. Materials and methods

2.1. Bacteria and preparation of inocula

Escherichia coli O157:H7 (B0265), *Bacillus cereus* (ATCC 11778), *Listeria monocytogenes* (ATCC 19115), *Staphylococcus aureus* (ATCC 25923), *Salmonella* Typhimurium (ATCC 14028) and *Vibrio parahaemolyticus* (ATCC 17802) were used in this study. All strains were obtained from the Department of Food Science and Biotechnology, Kangwon National University, South Korea. Stock cultures were transferred into tryptic soy broth (TSB; Becton Dickinson Diagnostic Systems, Sparks, MD, USA) and incubated for 24 h at 35 °C. For culturing *V. parahaemolyticus* TSB medium was supplied with 2% NaCl. Following incubation, 10 mL of each bacterial culture was sedimented by centrifugation (3000 × g for 10 min at 4 °C), washed and resuspended in 10 mL of 0.1% peptone water (pH 7.2) (BD). The final bacterial concentration was approximately 9 log CFU/mL. These cultures were used in subsequent experiments. The bacterial population was checked by plating 0.1 mL portions of appropriately diluted cultures on tryptic soy agar (TSA; BD) plates and incubation at 35 °C for 24 h followed by enumeration.

2.2. SAEW primary preparation

The initial SAEW was generated by electrolysis of a diluted hydrochloric acid (HCl; 6%) in a chamber without membrane using a self-developed device at a setting of 2.9 A and 24 V (Fig. 1). The electrolytic cell (8 × 10 × 8 cm) contained both anode (IrO_2+ SnO_2) and cathode (Ti). The high concentration hypochlorous acid (HOCl) containing electrolyzed water was diluted by water at a flow rate of 4 L/min to produce the final SAEW using a Barnant masterflex tube pump (L/S standard pump, Barnant Co., IL, USA). The developed device was also equipped with an instant water heater to increase the starting water temperature if needed. The SAEW was collected 20 min after starting the EW generator when stable amperage was reached. Two different types of water were separately supplied to the generator for the production of SAEW under same conditions: 1) the groundwater from Jinbu food Co. Ltd. site (Chuncheon, Kangwon-do, Korea) with a hardness of 98 ± 2 mg/L and 2) the water from Kangwon National University (KNU) campus (Chuncheon, Kangwon-do, Korea) with 34 ± 2 mg/L hardness. The sanitization efficacy of EW produced from each water type was assessed in vitro using *E. coli* O157: H7and *L. monocytogenes* broth cultures.

2.3. Determination of water hardness, pH, ORP and ACC

Water hardness was measured using a total hardness test kit (Model HA-71A, Hach Company, Loveland, CO, USA) by drop count

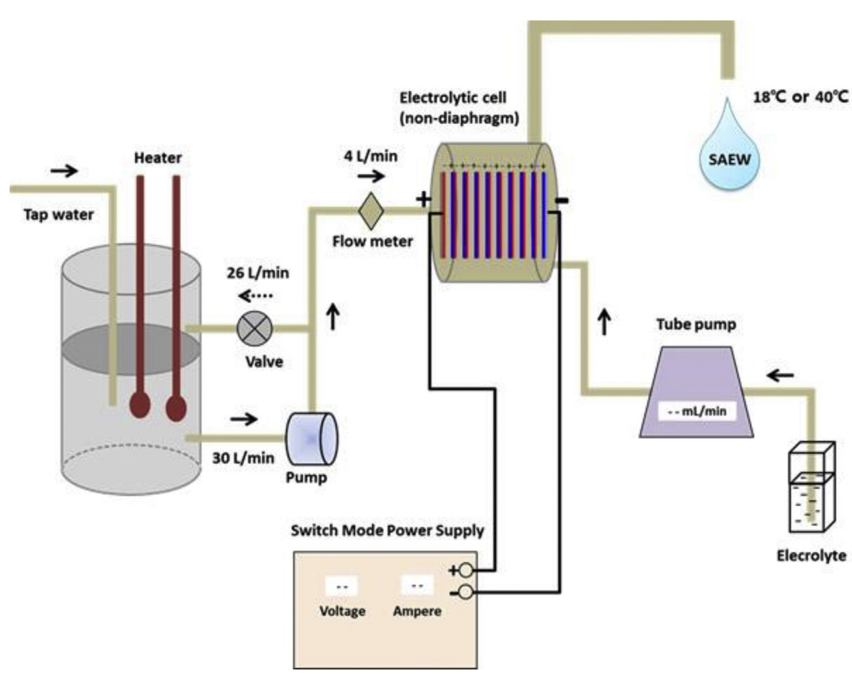

Fig. 1. Schematic view of the electrolyzed water generator system used for the production of SAEW in the study.