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Changes in physicochemical properties and bactericidal efficiency of acidic electrolyzed water ice and available chlorine decay kinetics during storage

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ABSTRACT

Acidic electrolyzed water ice (AEW ice) is a new kind of bactericide used in preservation or cold sterilization of food products. The aim of this study was to investigate changes in the physicochemical properties (oxidation reduction potential (ORP), pH value and available chlorine concentration (ACC)), bactericidal efficiency, and decay kinetics of available chlorine in AEW ice during 10 h of storage time. Results indicated that pH changes of AEW ice did not have a significant difference ($p > 0.05$) during the first 6-h storage, after 6 h, the pH of AEW ice prepared with ≤1 g/l NaCl solution changed more slowly than that of AEW ice prepared with >1 g/l NaCl solution. Both ORP and ACC decreased with storage time. The ACC of AEW ices prepared from >1.5 g/l NaCl solutions decreased faster and in a greater extent than those prepared from ≤1.5 g/l NaCl solutions. According to the correlation analysis, the correlation coefficients between pH, ORP, and ACC and *Vibrio parahaemolyticus* inactivation were −0.831, 0.787 and 0.944, respectively, and those between the above parameters and *Listeria monocytogenes* inactivation were −0.814, 0.701 and 0.97, respectively. Based on the kinetic study, the decay of ACC fitted the first order kinetics.

1. Introduction

Listeria monocytogenes and *Vibrio parahaemolyticus* are two widespread food-borne pathogens that cause food-borne disease and severe symptoms that are fatal to people's health in some cases. These organisms can survive in various environments, such as soil and water as well as food processing facilities (Ribeiro, Manha, & Brito, 2006; Su & Liu, 2007). Aquatic products are also known as carriers of *L. monocytogenes* and *V. parahaemolyticus* (Liu, Duan, & Su, 2006; McCarthy, 1997; Xie, Sun, Pan, & Zhao, 2012a, 2012b). In order to preserve the freshness of products and extend their shelf life, ice made by freezing tap water is extensively used as an ideal substance because it can provide both low temperature and high humidity (Koseki, Fujiwara, & Itoh, 2002). However, bacteria cannot be inactivated effectively in tap water ice. In contrast to tap water ice, ice made from sanitized water such as electrolyzed water holds the potential to serve as bactericidal against microorganisms (Feliciano, Lee, Lopes, & Pascall, 2010).

Acidic electrolyzed water (AEW), with low pH (2.3–2.7), high oxidation reduction potential (ORP, >1000 mV), high dissolved oxygen and free chlorine was initially developed in Japan (Shimizu & Hurusawa, 1992) and has been widely used as a sanitizing agent in both research and practice (Wang et al., 2014). AEW is a novel nonthermal bactericidal technology and has less adverse impact on human body as well as the environment (Huang, Hung, Hsu, Huang, & Hwang, 2008; Katayose, Yoshida, Achiwa, & Eguchi, 2007). AEW ice is a new kind of bactericide for preservation or cold sterilization in recent years (Lin et al., 2013). It not only has the advantage of tap water ice but also the potential to be bactericidal. Up to now, a few studies have indicated that AEW ice can inactivate bacteria and preserve the freshness of food products. Koseki et al. (2002)

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reported that AEW ice could reduce the populations of aerobic bacteria associated with lettuce. Koseki, Isobe, and Itoh (2004) also showed that AEW ice exhibited notable bactericidal effect against microorganisms, including *L. monocytogenes* and *Escherichia coli* O157:H7. Kim et al. (2006) reported that AEW ice markedly inhibited the growth of both aerobic and psychrotrophic bacteria in saury flesh during refrigerated storage. Phuvasate & Su (2010) studied the efficacy of electrolyzed water ice in reducing histamine-producing bacteria on fish skin. It is believed that AEW ice has the potential use of keeping the freshness of products through inactivating bacteria (Lin et al., 2013; Wang et al., 2014; Xie et al., 2012b). Therefore, it is of great importance to comprehend the physicochemical properties and bactericidal efficiency of AEW ice which contribute to the application of AEW ice in food storage.

At present, there are a few researches on decay kinetics models of available chlorine concentration (ACC) in AEW during storage. Similar studies include chlorine decay kinetics model for water distribution systems by Zhou, Zhao, and Xue (2003) and decay kinetics model of free chlorine and monochloramine by Wang, Lu, and Zhang (2005).

Although many researchers have investigated the physicochemical properties of electrolyzed water, including pH, oxidation–reduction potential (ORP), and ACC (Cui, Shang, Shi, Xin, & Cao, 2009; Hsu & Kao, 2004; Len, Hung, & Chung, 2002; Tatsumi, Umimoto, Kumayama, & Jokei, 2007), there are few studies about the dynamic changes in physicochemical properties and bactericidal efficiency of AEW ice during storage. Here, we investigate the changes in pH, ORP, ACC, and bactericidal efficiency of AEW ice, as well as chlorine decay within 10 h of storage time.

2. Materials and methods

2.1. Bacterial culture

V. parahaemolyticus (ATCC 33847) and *L. monocytogenes* (ATCC 19115) were used in this study. Strain *L. monocytogenes* was directly grown in tryptic soy broth (TSB, Beijing Land Bridge Technology Company Ltd., Beijing, P. R. China) at 30 °C for 18–24 h, and *V. parahaemolyticus* strain was grown in TSB supplemented with 3% NaCl at 37 °C for 18–24 h. Cultures were pooled into a sterile centrifuge tube and centrifuged for 10 min (3000 g; Eppendorf, Centrifuge 5810R, Germany). Pelleted cells were resuspended with phosphate buffered saline (PBS, 135 mM NaCl, 2.7 mM KCl, 1.5 mM KH_2PO_4, and 8 mM K_2HPO_4, pH 7.2) in a sterile centrifuge tube so that the population in each cell suspension of *V. parahaemolyticus* and *L. monocytogenes* was approximately 8–9 log CFU/ml.

2.2. Preparation and storage conditions of AEW ice

AEW was prepared with different concentrations of sodium chloride (NaCl) solutions (0.75, 1, 1.25, 1.5 and 1.75 g/l) using a strongly acidic electrolyzed water generator (FW-200, AMANO, Japan). pH and ORP were determined using a pH/ORP meter (Mettler-Toledo, Switzerland). ACC was determined colorimetrically using a digital chlorine test kit (RC-2Z, Kasahara Chemical Instruments Corp., Saitama, Japan). AEW was poured in a 2-L-sealed-plastic bag and frozen at −20 °C for 24 h immediately after production. The obtained AEW ice was crushed using a hammer before use, and the approximate dimension of the crushed ices was 2.0 cm × 1.5 cm × 1.0 cm. AEW ice was poured into a sterile stainless steel tray with 2 blocks (72 × 48 × 9.5 cm) and stored for 10 h under open conditions at air conditioning ambient temperature of 22 ± 1 °C (Lin et al., 2013). The crushed ices were sampled randomly from the remaining stored ice, and pH, ACC, and ORP of AEW ice were measured every 2 h after the AEW ices melted completely in a sealed bag in a 70 °C water bath. Tap water (TW) ice was generated and used as control in the bactericidal efficiency experiment. Both the temperatures of the melted AEW ice and TW ice were 0 °C. All measurements were carried out in triplicate.

2.3. Treatment and bacteriological analysis

At the 0th, 2nd, 4th, 6th, 8th and 10th hour, each bacteria suspension and melted AEW ice were mixed together (1:1, v/v) in a sterile centrifuge tube for 15 s, and then 1 ml of the mixture was serially diluted (1:10) in 9 ml of sterile neutralizing buffer solution (0.85% physiological saline solution containing 0.5% $Na_2S_2O_3$). Diluted solution of 0.1 ml was spread onto TCBS (thiosulfate citrate bile salts sucrose, TCBS, Beijing Land Bridge Technology Company Ltd., Beijing, China) for *V. parahaemolyticus* or PALCAM agar plates for *L. monocytogenes*. Colonies were counted after the plates were incubated at 30 °C or 37 °C for 24 h. For controls, TW ice instead of AEW ice was used.

To investigate if AEW ice would cause secondary pollution to the environment, an enrichment experiment was performed to detect survivors in the dipping solution as they were reported to be at too low a level to be counted by direct plating (Cui et al., 2009; Huang et al., 2006). One ml of dipping solution was added into 9 ml of sterile TSB supplemented with 3% NaCl for *V. parahaemolyticus*, and then incubated at 37 °C for 24 h. Susceptive colonies of *V. parahaemolyticus* from enrichment were further detected on TCBS agar plates. For *L. monocytogenes*, 1 ml of dipping solution was added into 9 ml of sterile TSB, then incubated at 30 °C for 24 h and further detected on PALCAM agar plates. The tests were carried out in triplicate.

2.4. Chlorine decay assays

ACC was determined using the method described in section 2.2. In order to model chlorine decay as a function of time, the following equation was used (the first order kinetics):

$$\frac{dC}{dt} = -k'C$$

where C is the available chlorine fraction, k' is the chlorine decay kinetics coefficient. Chlorine decay can also be described by:

$$y = C_0 \cdot \exp(-k't)$$

where y is the available chlorine fraction, C_0 is the initial available chlorine fraction.

2.5. Statistical analyses

Values were expressed as mean ± standard deviation (SD). Statistical analyses were performed using SPSS statistical package 17.0 (SPSS Inc., Chicago, IL). One way analysis of variance was conducted to compare the bactericidal efficiency of AEW ice prepared from different concentrations of NaCl solution. The least significance difference (*LSD*) test was used to determine differences at $\alpha = 0.05$. Bactericidal efficiency of AEW ice on *L. monocytogenes* and *V. parahaemolyticus* was expressed as logarithmic viable cell reduction ($\log(N/N_0)$, N and N_0 are the number of bacteria surviving treatment with TW ice and AEW ice, respectively) under different storage durations. Pearson correlation coefficients between the three variables (pH, ORP, ACC) of AEW ice prepared with 1.5 g/l NaCl solution and population reduction of *V. parahaemolyticus* and *L. monocytogenes* during storage were calculated, respectively.