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Physicochemical properties and bactericidal efficiency of neutral and acidic electrolyzed water under different storage conditions

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ABSTRACT

Neutral (NEW) and acidic (AEW) electrolyzed water were stored in open or closed glass bottles under light or dark conditions at 20 °C for 30 days. The pH, oxidation–reduction potential (ORP), electrical conductivity (EC), available chlorine concentration (ACC), dissolved oxygen (DO), and bactericidal efficiency of NEW and AEW were determined during storage or before and after storage, respectively. The pH and EC of NEW and AEW remained unchanged in storage. The ORP, ACC and DO of AEW decreased 22%, 100% and 52% under open storage conditions, respectively. Light had no significant effects on the physicochemical properties of NEW ($P > 0.05$). Bactericidal efficiency was not markedly affected by storage conditions for NEW, but decreased significantly for AEW under open storage conditions. Electrolyzed water should be stored in closed containers or used immediately to prevent the loss of available chlorine that is one of the main contributing factors for antimicrobial activity.

1. Introduction

Acidic electrolyzed water (AEW), also known as electrolyzed oxidizing water, has been regarded as a novel antimicrobial agent in recent years. It is usually generated by electrolysis of a dilute NaCl solution in a chamber with anode and cathode electrodes separated by a membrane, and obtained from the anode side. The resultant AEW has lower pH values (<3.0), higher ORP values (>1000 mV) and contains available chlorine. AEW has been reported to have strong bactericidal activity against many food-borne pathogens, including *Escherichia coli* O157:H7, *Salmonella enteritidis*, *Salmonella typhimurium*, *Listeria monocytogenes* (Venkitanarayanan et al., 1999; Kim et al., 2000; Fabrizio and Cutter, 2003; Park et al., 2004; Liao et al., 2007). Several studies have shown that AEW can be effective in reducing pathogenic bacteria on the surface of fruits and vegetables (Koseki et al., 2004a, b, c), eggs (Russell, 2003; Park et al., 2005), poultry carcasses (Kim et al., 2005), pork (Fabrizio and Cutter, 2005), seafood (Ozer and Demirci, 2006), and food processing facilities (Ayebah and Hung, 2005; Huang et al., 2008). However, the utilization of AEW has limited potential for long-term applications because of its strong acidity ($pH < 2.7$) (Guentzel et al., 2008). At this low pH, dissolved Cl_2 gas can be rapidly lost due to volatilization, decreasing the bactericidal activity of the solution with time (Len et al., 2000) and adversely affecting human health and the environment. Moreover, the strong acidity of AEW may cause corrosion of equipment and consequently limit its practical application (Guentzel et al., 2008).

Neutral electrolyzed water (NEW) referred to as mixed oxidants, are commonly produced by two different types of NEW generators. One type of NEW generators electrolyzes diluted hydrochloric acid (HCl) or NaCl solution in a non-membrane electrolytic cell (Gómez-López et al., 2007), which is also used in this study. It is more effective, convenient and less expensive than other electrolyzed water systems. Another type electrolyzes dilute NaCl solution in a cell with a separating membrane, and part of the product formed at the anode is then redirected into the cathode chamber during electrolysis (Pernezny et al., 2005; Guentzel et al., 2008). These NEW generators produce a neutral solution (pH 5.0–6.5) in which the most effective form of chlorine compounds is almost hypochlorous acid HOCl (approximately 95%) having strong antimicrobial activity. Anonymous (1997) reported that hypochlorous acid is 80 times more effective as a sanitizer than an equivalent concentration of the hypochlorite ion (ClO^-) for inactivating *E. coli* at a set contact time. Due to its neutral pH, NEW does not contribute as aggressively as AEW to the corrosion of processing equipment or irritation of hands (Abadias et al., 2008), phytotoxicity in plant and the safety issues from Cl_2 off-gassing (Guentzel et al., 2008). Therefore, NEW is particularly attractive for utilization in agriculture like plant disease management and livestock management. Several researchers have investigated the bactericidal efficiency of NEW for inactivation of food-borne pathogens. Deza et al. (2003) demonstrated that the viable counts of four strains of microorganisms were reduced by more than

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4 log_{10} CFU/g on the surface of tomato using NEW containing 89 mg/L of active chlorine without affecting sensory qualities. The viable counts were reduced by about 65%. Pernezny et al. (2005) reported that mixed-oxidant electrolyzed oxidizing water with a pH of 7.0, ORP of −5.1 mV and available chlorine of 50 mg/L reduced the bacteria on the leaf of vegetables from log_9 to log_{10} CFU/mL to undetectable levels after 1 min exposure. Gómez-López et al. (2007) observed that the shelf-life of minimally processed cabbage treated with NEW containing 40 mg/L of free chlorine for 5 min could be extended more than 5 and 3 days at 4 °C and 7 °C, respectively. Guentzel et al. (2008) treated five pure cultures of pathogenic organism for 10 min using NEW with total residual chlorine concentrations of 20, 50, 100 and 120 mg/L, and reported 100% inactivation (reduction of 6.1–6.7 log_{10} CFU/mL) of all the pathogens.

Storage conditions are important factors influencing the physicochemical properties and bactericidal activity of electrolyzed water. Hsu and Kao (2004) observed that pH, oxygen–reduction potential (ORP) and electrical conductivity (EC) of AEW did not change appreciably, whereas total residual chlorine concentration and dissolved oxygen (DO) in AEW decreased significantly during storage. Similar results were observed by Koseki and Itoh (2000). The strong bactericidal activity of NEW and AEW has been proven by previous studies, but little information is available concerning the effects of storage conditions on physicochemical properties of NEW, and the bactericidal activity of NEW and AEW before and after storage.

The objectives of this study are: (1) to determine the effects of storage conditions on pH, ORP, EC, available chlorine concentration (ACC), DO and bactericidal efficiency of NEW and AEW; and (2) to compare stability and bactericidal efficiency of NEW *vs.* AEW. Understanding the physicochemical properties and bactericidal activity of NEW before and after storage is important to the practical application in inactivating harmful microorganisms in food industries and agriculture.

2. Materials and methods

2.1. Bacterial cultures

Freeze-dried pure cultures of *S. enteritidis* and *E. coli* O157:H7 were obtained from China Veterinary Culture Collection (CVCC, Beijing, China). Cultures were hydrated according to the manufacturer's directions and enriched in sterile tryptic soy broth (TSB, CVCC, Beijing, China) at 37 °C for 24 h. The viable counts were enumerated by plating 0.1 mL tenfold serial dilution of broth cultures onto sterile tryptic soy agar (TSA, CVCC, Beijing, China) and incubated at 37 °C for 48 h. The population in each culture of *S. enteritidis* and *E. coli* O157:H7 was approximately 6.0–7.5 log_{10} CFU/mL.

2.2. Preparation of NEW and AEW

NEW and AEW were produced from two different generators in this study. NEW was prepared using a NEW generator (model CWD-A, Shenyang Dongyu Xinbor Technology Company Ltd., Shenyang, China) that consisted of an electrolytic cell with anode and cathode electrodes and no separating membrane (Fig. 1A). NEW with different ACC can be produced in this device by regulating the concentration of hydrochloric acid (HCl) or salt solution, the current and voltage supplied to the electrodes, and electrolysis time. In this study, NEW1 with a pH of 6.34, ORP of 265.1 mV and an ACC of 21 mg/L were obtained in the cell by electrolysis of 1.2 mM HCl solution at 40 V for 30 min, and NEW2 with pH 6.51, ORP 512.6 mV and 25 mg/L of ACC generated by electrolysis of 17 mM NaCl solution at 20 V for 30 min.

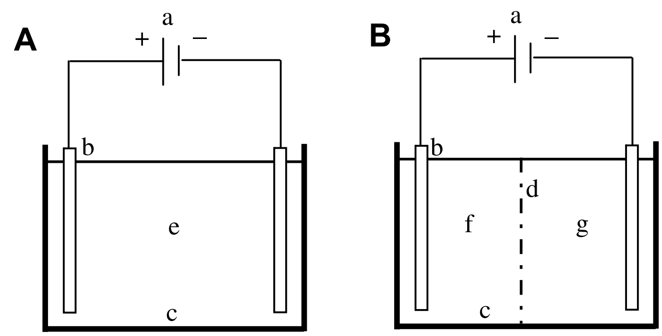

Fig. 1. Schematic diagram of electrolyzed water generators. (A) NEW generator; (B) AEW generator; (a) DC power; (b) electrodes; (c) electrolytic cell; (d) membrane; (e) NEW; (f) AEW; (g) alkaline electrolyzed water.

AEW was produced by electrolysis of 17 mM NaCl solution at 20 V for 15 min using an experimental AEW generator (model ZSJ-1, Shenyang Dongyu Xinbor Technology Company Ltd., Shenyang, China). The AEW generator basically consisted of an electrolytic cell where the anode and cathode electrodes are separated by a membrane (Fig. 1B). Two types of water were generated simultaneously. From the anode side of the generator, AEW with a pH of 3.01, ORP of 1079.1 mV and ACC of 25 mg/L were collected for the experiments, and from the cathode side, alkaline electrolyzed water (pH 11.07, ORP value of −762.9 mV and containing sodium hydroxide NaOH) was produced. All chemicals used were an analytical grade.

The physicochemical properties of AEW and NEW were measured immediately after preparation. The pH and ORP values were measured using a dual scale pH/ORP meter (HM-30 R, DKK-TOA Corporation, Tokyo, Japan) with a pH electrode (GST-5741C) or an ORP electrode (PST-5721C), and ranged from pH 0.0–14.0 and 0.0 to ±2000.0 mV, respectively. The pH meter was calibrated using commercial standard buffers pH 4.01 and 6.86 supplied by the manufacturer, and the calibration of the ORP electrode was conducted using the above standard buffers and quinhydrone $C_6H_4(OH)_2$ solution according to manufacturer's instruction. The available chlorine concentration in AEW and NEW was determined by a colorimetric method using a digital chlorine test kit (RC-2Z, Kasahara Chemical Instruments Corp., Saitama, Japan). The detection limit is 0–300 mg/L. The DO was measured using a DO portable meter (Orion 3-star, Thermo Electron Corporation, Beverly, MA, USA) and ranged from 0.0–90.0 mg/L. The DO meter was calibrated using the water-saturated air method according to the manufacturer's direction. The EC was measured by a conductivity meter (DDS-11A, Shanghai Rex Instrument Factory, Shanghai, China) with a range from 0.0–20.0 ms/cm. All measurements were made in triplicate.

2.3. Procedure of storage experiments

Four different storage conditions were designed for the experiments, including open or closed in light or dark, namely, a 2 × 2 factorial arrangement. Two types of 1 L glass bottles (clear and dark-brown) were used to collect the NEW and AEW samples described above. The 1 L sample-laden clear and dark-brown glass bottles were stored in open and closed states at a room temperature of 20 °C for 30 days. For exposure to light, sunlight was used for the experiments. The pH, ORP, EC, ACC and DO of the samples were measured on storage day 0, 1, 2, 3, 6, 13, 20 and 30. The measurements were completed within 30 min. Each experimental regimen was repeated in triplicate.