Food Control 96 (2019) 212–218

Contents lists available at ScienceDirect

Food Control

journal homepage: www.elsevier.com/locate/foodcont

# Bactericidal activity of neutral electrolyzed water against *Bacillus cereus* and *Clostridium perfringens* in cell suspensions and artificially inoculated onto the surface of selected fresh produce and polypropylene cutting boards

Hamzah M. Al-Qadiri[a,b,*], Stephanie Smith[c], Aleksandra Checinska Sielaff[c], Byju N. Govindan[d], Mohamed Ziyaina[e], Nivin Al-Alami[f], Barbara Rasco[e]

[a] School of Food Science, University of Idaho, Moscow, ID, 83844, USA
[b] Department of Nutrition and Food Technology, School of Agriculture, The University of Jordan, Amman, 11942, Jordan
[c] Youth and Families Program Unit, Washington State University, Spokane, WA, 99202, USA
[d] Department of Entomology, CFANS, University of Minnesota, Saint Paul, MN, 55108, USA
[e] School of Food Science, Washington State University, Pullman, WA, 99164, USA
[f] Water, Energy and Environment Center, The University of Jordan, Amman, 11942, Jordan

ARTICLE INFO

*Keywords:*
Neutral electrolyzed water
Endospore forming bacteria
Fresh produce

ABSTRACT

Bactericidal activity of neutral electrolyzed water (NEW) was investigated against endospore forming *Bacillus cereus* and *Clostridium perfringens* in cell suspensions and artificially inoculated onto the surface of selected fresh produce items (cherry tomato, miniature cucumber, carrot and parsley) and polypropylene cutting boards at ambient temperature (22 °C). Viable counts of survivors were determined within 0 (untreated control), 1, 3 and 5 min of treatment at ambient temperature using NEW solutions of 60 and 120 mg/L free available chlorine FAC. All treatments showed significant differences ($P < 0.05$) in bacterial reductions with regard to contact time and concentration used and which maximized after 5 min of treatment at 120 mg/L FAC. For cell suspensions, the extent of reduction ($\log_{10}$ CFU/mL) after 5 min of treatment ranged from 2.11 to 3.03 for *B. cereus* and 2.46–3.62 for *C. perfringens* at 60 and 120 mg/L FAC, respectively. However, when the bacteria inoculated onto the produce items and cutting boards showed greater resistance to NEW treatments compared to cell suspensions. Sterile deionized water did not contribute any significant reduction ($P > 0.05$) after 5 min of treatment, whereas bacterial viability of the inoculated produce was reduced by 2.11–2.30 and 2.41–3.16 $\log_{10}$ CFU/g when NEW used at 120 mg/L FAC for *B. cereus* and *C. perfringens*, respectively. When inoculated cutting boards were sprayed with NEW at 120 mg/L FAC and after 5 min of treatment, cell viability was reduced by 2.33 and 3.06 $\log_{10}$ per 100 $cm^2$ for *B. cereus* and *C. perfringens*, respectively. This study showed that NEW could be used as an effective bactericidal treatment alternative to commonly used chemical sanitizers against endospore forming bacteria.

## 1. Introduction

Bacterial contamination during food processing and handling pose a risk for foodborne outbreaks and imposing significant health and economic impacts. Using chemical agents as food sanitizers is the most common and economical method to reduce bacterial counts to acceptable levels that reduce the risk of food borne illness. Approved food sanitizers must be safe for use on food contact surfaces, do not require a rinse after the sanitizing step (rated by the USDA as D2 sanitizers), free of dyes and fragrances and be EPA registered for sanitizing food contact surfaces (Gaulin, Le, Shum, & Fong, 2011; U.S. Department of Agriculture, 2013; U.S. Environmental Protection Agency, 1999; U.S. Food and Drug Administration, 2009).

Due to its antibacterial properties, electrolyzed water (EW) has been applied to control bacterial contamination on food products, non-food contact surfaces, and food processing surfaces, including the equipment and utensils used in food preparation (Al-Qadiri et al., 2016a, 2016b; Fraser & Pascall, 2010; Hricova, Stephan, & Zweifel, 2008; Monnin, Lee, & Pascall, 2012; Ovissipour et al., 2015). Neutral electrolyzed water (NEW) that combines both acidic and alkaline EW has several advantages compared to acidic or alkaline EW by reducing corrosion of equipment and utensils and minimizing skin and mucous irritation. Further advantages are that NEW can penetrate bacterial cell membranes, has a high oxidation reduction potential, availability of chlorine

* Corresponding author. Department of Nutrition and Food Technology, School of Agriculture, The University of Jordan, Amman, 11942, Jordan.
*E-mail address:* h.qadiri@ju.edu.jo (H.M. Al-Qadiri).

https://doi.org/10.1016/j.foodcont.2018.09.019
Received 30 June 2018; Received in revised form 14 September 2018; Accepted 16 September 2018
Available online 17 September 2018
0956-7135/ 

with the presence of $OH^-$ as an active surfactant, and its longer storage life at neutral pH which reduces chlorine loss (Al-Qadiri et al., 2016b; Len et al., 2002; Monnin et al., 2012).

*Clostridium perfringens* is the third most common cause of foodborne disease in the United States, with gastrointestinal illness mainly caused by enterotoxin produced by the most abundant *C. perfringens* type A strains (Li & McClane, 2006; Sarker, Shivers, Sparks, Juneja, & McClane, 2000; Scallan et al., 2011); these are highly resistant to heating (Novak & Yuan, 2003) and have a short generation time (10 min) (Al-Qadiri et al., 2015). *Bacillus cereus* is a Gram-positive, facultative anaerobic endospore forming bacterium that is widely distributed in the environment with prevalence in several food types due to its sporulating ability (Sood, Sahota, & Hunjan, 2017). *B. cereus* may cause diarrheal and emetic foodborne illnesses (Ceuppens et al., 2011) and can be introduced from soil, irrigation water and decaying organic materials causing pre- and post-harvest contamination of fruits and vegetables (Jensen, Hansen, Ellenberg, & Mahillon, 2003).

Foodborne illnesses associated with the consumption of raw and minimally processed vegetables have been reported in recent years, in which potential sources of microbial contamination from the environment are very significant (Abadias, Usall, Oliveira, Alegre, & Viñas, 2008). Accordingly, and as a food safety measure, application of effective sanitation procedures is an essential requirement to minimize the risk of microbial contamination of fresh produce to guarantee their safety for human consumption.

Several researchers have shown the antibacterial effect of EW against different non-spore forming pathogens as pure culture suspensions (Ovissipour et al., 2015), in food products (Al-Holy & Rasco, 2015; Al-Qadiri et al., 2016a), fruits and vegetables (Abadias et al., 2008; Koseki, Yoshida, Isobe, & Itoh, 2004), and in controlling bacterial contamination of food contact surfaces (Al-Qadiri et al., 2016b). However, there is a lack of studies investigating EW as a promising disinfection method to control microbial contamination by endospore forming bacteria which may exhibit a significant resistance to the most common used food sanitizers. Thus, the objective of the current study was to investigate the bactericidal activity of NEW against endospore forming *B. cereus* and *C. perfringens* in cell suspensions and artificially inoculated onto the surface of selected fresh produce (cherry tomato, miniature cucumber, carrot and parsley) and polypropylene cutting boards.

## 2. Materials and methods

### 2.1. Preparation of NEW solutions

A commercial neutral electrolyzed water NEW (Aquaox™ Disinfectant 275) was provided from Aquaox Industries Inc. Fontana, CA 92336. The active ingredient of the Aquaox NEW stock solution is hypochlorous acid (0.0275%, 275 mg/L free available chlorine FAC) that is generated electrochemically by electrolysis of a dilute sodium chloride solution passing through an electrolytic cell at neutral pH. In the current study, Aquaox NEW was diluted in sterile deionized water to obtain a final FAC content of 60 and 120 mg/L, a pH of 6.6 and an oxidation-reduction potential (ORP) of 805 mV. Treatment solutions were kept refrigerated and used within 3 h of preparation. The pH, ORP and FAC content were measured by a pH meter (FE20, Mettler-Toledo, Columbus, OH, USA), a pocket sized redox meter (HI 98201, HANNA® Instruments, Ann Arbor, Michigan, USA) and a digital colorimeter (Colorimeter™ Analysis System, Hach Co., Loveland, CO, USA), respectively, according to the manufacturer instructions.

### 2.2. Enumeration of aerobic plate count and coliform bacteria

Samples of fresh cherry tomato, miniature cucumber, carrot and parsley (30 samples each) were collected from three different local retail stores using sterile bags and examined within 24 h of their purchasing date. A composite sample of every produce (100 g) was placed in a sterile strainer-filter bag and immersed with manual shaking for 1 h in 300 mL sterile 0.1% peptone water (Bacto™, BD, Franklin Lakes, NJ) at ambient temperature to detach microorganisms from the surface of the produce. One mL of the homogenized suspension was then serially diluted (dilution range: $10^0$-$10^{-5}$) in sterile 0.1% peptone water. For aerobic plate count (APC), samples were then examined in duplicate using the spread plate technique. One mL aliquot of each dilution was divided into 5 aliquots of 0.2 mL and cultured on tryptic soy agar TSA (Bacto™, BD, Franklin Lakes, NJ). Plates were incubated at 30 °C for 72 h and the number of viable cells was determined and reported as $\log_{10}$ CFU/g sample. For coliform bacteria, the spread plate technique described above to enumerate APC was used with cells plated onto violet red bile agar (VRBA) (CM0978, Oxoid Ltd., Basingstoke, United Kingdom). Plates were incubated at 37 °C for 24 h. Typical coliform colonies were then streaked and isolated onto VRBA for further verification using lauryl tryptose broth (CM0451, Oxoid Ltd., Basingstoke, United Kingdom) and the number of viable cells was determined as $\log_{10}$ CFU/g sample. For samples treated with deionized water as a washing solution, a composite sample of 100 g was placed in a sterile strainer-filter bag and immersed with manual shaking in 300 mL of sterile deionized water (repeated three times, 15 min each). Enumeration of APC and coliform was then conducted as described above.

### 2.3. Bacterial strains and growth conditions

American Type Culture Collection (ATCC) strains were obtained from Microbiologics, Inc. (St. Cloud, MN). The *C. perfringens* ATCC 12915 strain was chosen for its antigenic properties as a serovar type A, since most reported *C. perfringens* food poisoning cases were caused by type A strains (Al-Qadiri et al., 2015; McClane, 2007). This strain was cultured and activated by inoculating the Kwik-Stik swab into 50 mL of sterile fluid thioglycollate (FTG) medium (CM0173, Oxoid Ltd., Basingstoke, United Kingdom). The cultures were then anaerobically incubated at 37 °C for 24 h to yield a cell count of approximately $10^8$–$10^9$ CFU/mL (bacterial suspensions were enumerated in duplicate using a pour plate technique in which a 1-mL aliquot of each dilution was cultured on perfringens agar [OPSP; oleandomycin, polymixin, sulphadiazine, perfringens]; CM0543, Oxoid Ltd., Basingstoke, United Kingdom). All culture manipulations of *C. perfringens* were conducted using sterile FTG medium to maintain anoxic conditions. The cultures were incubated in an anaerobic jar with an anoxic atmosphere (< 0.1% O2) using AnaeroGen sachets (AN0025, Oxoid Ltd., Basingstoke, United Kingdom) (Al-Qadiri et al., 2015). *B. cereus* ATCC 11778 strain was activated by inoculating the Kwik-Stik swab into 50 mL of sterile tryptic soy broth TSB (Bacto™, BD, Franklin Lakes, NJ) (Ryu, Kim, & Beuchat, 2005) and incubated at 37 °C for 24 h to yield a cell count of approximately $10^8$–$10^9$ CFU/mL. Bacterial suspensions of *B. cereus* were enumerated in duplicate using a spread-plate technique in which a 1-mL aliquot of each dilution was divided into 5 aliquots of 0.2 mL and cultured on MYP agar (mannitol egg yolk polymyxin agar) (CM0929, Oxoid Ltd., Basingstoke, United Kingdom) (Tallent, Kotewicz, Strain, & Bennett, 2012).

### 2.4. Inoculum preparation

After the appropriate incubation of bacterial cultures, 50 mL broth of each strain was transferred under aseptic conditions to a sterile centrifuge tube and centrifuged for 15 min at 5000 rpm (3380 × *g*) to harvest bacterial cells (AccuSpin centrifuge, Thermo Fisher Scientific, Waltham, MA). To eliminate any effect of broth components and bacterial metabolites, the resultant pellets of *B. cereus* and *C. perfringens* were resuspended in 50 mL of sterile saline solution (0.85%; wt/vol NaCl) and sterile FTG medium, respectively. The tubes were then centrifuged as before, and the resulting pellets were then resuspended