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Sanitizer efficacy in preventing cross-contamination during retail preparation of whole and fresh-cut cantaloupe

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ABSTRACT

The objective of this study was to evaluate the efficacy of tap water (TW), commercial electrolyzed water (EW), and a commercial acid-based sanitizer (AS) in preventing cross-contamination of cantaloupe during processing in retail settings. A whole cantaloupe was dip-inoculated with a cocktail of *Salmonella* or *L. monocytogenes* to achieve approximately 5 log CFU/cm^2. One inoculated and two non-inoculated whole cantaloupes were treated in 76 L of TW, EW (free chlorine: 50–60 ppm), or AS (pH 2.8, combination of lactic acid and phosphoric acid) for 5 min. Subsequently, fresh-cut cantaloupe flesh from the inoculated and non-inoculated cantaloupes were soaked together in 76 L of TW, EW, or AS for 90 s. EW treatment resulted in an approximately 1.5 log reduction in both *Salmonella* and *L. monocytogenes* on the rind of whole cantaloupe, which was significantly greater than with the TW treatment (0.5 log reduction) ($P < 0.05$). Cross-contamination of non-inoculated whole cantaloupes occurred when washed with inoculated whole cantaloupe in TW (four of four cantaloupes positive for *Salmonella* and *L. monocytogenes*) or AS (four of four cantaloupes positive for *Salmonella* and two of four positive for *L. monocytogenes*). Cross-contamination did not occur when whole cantaloupes were washed in EW. Additional washing of mixed fresh-cut cantaloupe flesh from the inoculated and non-inoculated cantaloupes prepared after washing of whole cantaloupes demonstrated that the EW treatment reduced the likelihood of cross-contamination compared with TW and AS. No viable *Salmonella* or *L. monocytogenes* were detected from 100 mL sample of EW processing water, but were detected in TW and AS (*L. monocytogenes* only). The addition of a sanitizing agent to water used for the processing of whole and fresh-cut cantaloupe in a retail setting is recommended to prevent cross-contamination and reduce microbial load.

1. Introduction

Under the FDA Food Code, fresh-cut cantaloupe is considered a potentially hazardous food because it is consumed raw and is a good substrate for microbial growth (US FDA, 2013). Cantaloupe is at risk of being contaminated since it grows on the ground and is in contact with soil and rainwater runoff. In addition, cantaloupe provide a favorable environment for microbial survival and growth due to its low acidity (pH 5.2 to 6.7) and high water activity (0.97–0.99) (Del Rosario and Beuchat, 1995; Golden, Rhodehamel, & Kautter, 1993; Ukuku & Fett, 2002c). More significantly, its netted surface facilitates the attachment of pathogens from soil and/or contaminated surfaces and provides a barrier to removal of surface associated bacteria (Parnell, Harris, & Suslow, 2005; Richards & Beuchat, 2004). *Salmonella,* pathogenic *E. coli,* and *L. monocytogenes* can survive on cantaloupe for days, which increases the opportunity for cross-contamination of the edible flesh during processing (Ukuku et al., 2006). Spoilage and pathogenic bacteria are reported to grow rapidly on cantaloupe flesh (Golden et al., 1993).

The microbial safety of cantaloupe is a global concern since outbreaks linked to cantaloupe have been reported in a number of countries including the United States, Australia, and Canada (CDC, 2002; Munnoch et al., 2009). According to Centers for Disease Control and Prevention (CDC), at least 2 outbreaks linked to cantaloupe have occurred every year from 1999 to 2014 (CDC, 2016). A cantaloupe-related *Listeria* outbreak in 2011 is considered one of the deadliest multistate outbreaks in the United States to date, resulting in 147 illnesses and 33 deaths (Huang, Luo, & Nou, 2015; McCollum et al., 2013). In 2001, the FDA reported that 5.3%

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and 2.0% of cantaloupes exported from 9 countries (151 samples in total) tested positive for *Salmonella* and *Shigella*, respectively (US FDA, 2001). The severity of cantaloupe-linked outbreaks worldwide highlights the importance of improving postharvest processing practices especially those at retail establishments.

The antimicrobial efficacy of various sanitizers has been widely examined for use by the food industry, such as chlorinated water (sodium hypochlorite), hydrogen peroxide, hot water (96 °C), and nisin-based products (Ukuku, Bari, Kawamoto, & Isshiki, 2005; Ukuku, Huang, & Sommers, 2015). Currently, washing with sodium hypochlorite is one of the most common practices for whole or fresh-cut fruits and vegetables (Beuchat, 1996; Weng et al., 2016). Concerns associated with the use of chlorine include its low bactericidal efficacy in the presence of organic matter (Allende, McEvoy, Tao, & Luo, 2009; Gil, Selma, López-Gálvez, & Allende, 2009; Abadias, Usall, Oliveira, Alegre, & Viñas, 2008). Similar to sodium hypochlorite, hydrogen peroxide and ethanol are not as effective as may be expected since organic matter (cellular exudate on the surface of cut flesh) on cantaloupes can potentially interfere with activity (Beuchat & Ryu, 1997; Park & Beuchat, 1999). Research has focused predominantly on the industrial processing of cantaloupe and not practices at the retail level. In retail settings such as supermarkets or local farmers' markets, whole melons are generally washed with potable water alone (Ukuku et al., 2005). Under the FDA Food code (section: 3–302.15), melons shall be washed in water prior to cutting and preparing fresh-cut melon and may be washed with a chemical antimicrobial agent as specified under §7–204.12 (FDA, 2013). Practices used in washing whole cantaloupes and preparing fresh-cut cantaloupe at retail level should be evaluated. More importantly, it is prudent to evaluate antimicrobial agents which are safe, effective, and cost appropriate for use in fresh produce wash water.

Electrolyzed Water (EW) is generated by the electrolysis of a dilute salt solution passing through the anode of a membrane electrolyzer. A voluminous amount of research demonstrates that EW can inactivate Gram-positive and Gram-negative pathogens, fungi and viruses (Kim, Hung, Brackett, & Lin, 2003; Koseki, Yoshida, Kamitani, Isobe, & Itoh, 2004, Koseki, Isobe, & Itoh, 2004; Park, Hung, Doyle, Ezeike, & Kim, 2001). It has also been shown to inactivate biofilm formation on stainless steel (Kim, Hung, Brackett, & Frank, 2001). The active antimicrobial compounds are generated at the phase boundary between the electrodes and the water, either from the water itself (e.g., ozone) or from components dissolved in water (e.g., chloride is oxidized to free chlorine) (Graca, Abadias, Salazar, & Nunes, 2011). EW is easy to generate, less expensive than other sanitizers (Sakurai, Nakatsu, Sato, & Sato, 2003), environmentally friendly, and safer for employee work environments compared with sodium hypochlorite (Huang, Hung, Hsu, Huang, & Hwang, 2008). EW can be categorized into acidic EW (AEW or electrolyzed oxidizing water, ~ pH 2.5), neutral EW (NEW, pH 5.0–6.5), and basic EW (BEW or electrolyzed reducing water, ~ pH 11.4) (Nagamatsu, Chen, Tajima, Kakigawa, & Kozono, 2002). A comprehensive review summarized the research on the antimicrobial effects of AEW and BEW when used to treat food commodities pre- and post-harvest (Al-Haq, Sugiyama, & Isobe, 2005). Since the high acidity of AEW makes it potentially corrosive for equipment, researchers now focus more on NEW (Len et al., 2002; Nagamatsu et al., 2002). The active antimicrobial component of NEW is mainly hypochlorous acid (HOCl, 97%). Studies show that NEW has an equivalent or higher bactericidal activity against foodborne pathogens compared to AEW and sodium hypochlorite (NaClO), both of which are widely used by the food industry (Issa-Zacharia, Kamitani, Tiisekwa, Morita, & Iwasaki, 2010; Rahman, Ding, & Oh, 2010). Consequently, NEW is very promising for use post-harvest particularly for minimally processed fruits and vegetables.

In this study, the retail practice of submersion washing of cantaloupe was evaluated. The objective of the research was to determine the efficacy of commercially available sanitizers (electrolyzed water and acid-based sanitizer) to prevent foodborne pathogen cross-contamination during the washing process of whole and fresh-cut cantaloupe prepared in a retail setting. The surface of whole cantaloupe was inoculated with a cocktail of foodborne pathogens, and change in population and cross-contamination determined following simultaneous washing of non-inoculated whole cantaloupe and fresh-cut flesh.

2. Materials and methods

2.1. Bacterial strains and preparation of inoculum

Three strains of *Salmonella* and *Listeria monocytogenes* were obtained from Dr. Joshua Gurtler (Eastern Region Research Center, USDA, Wyndmoor, PA): *Salmonella* Newport H1275 (sprout outbreak), *S.* Stanley H0558 (sprout outbreak), *S.* Montevideo G4639 (raw tomato outbreak), *L. monocytogenes* L008 (serotype 4b, Canadian coleslaw/cabbage outbreak), L2624 (serotype 1/2b, cantaloupe outbreak), and L2625 (serotype 1/2a, cantaloupe outbreak). All strains were stepwise exposed to nalidixic acid; generating strains resistant to nalidixic acid at the concentration of 100 μg/mL. Stock cultures of each strain were stored in tryptic soy broth (TSB; Difco, Becton Dickinson, Sparks, MD) containing 20% glycerol at −80 °C. Working cultures were maintained on tryptic soy agar (TSA; Difco, Becton Dickinson, Sparks, MD) plates supplemented with nalidixic acid (100 μg/mL, Alfa Aesar, England) stored at 4 °C. Prior to each experiment, one isolated colony of the working culture was transferred into fresh 30 mL of TSB for *Salmonella* or brain heart infusion (BHI; Difco, Becton Dickinson, Sparks, MD) broth for *L. monocytogenes* and incubated at 37 °C for 20 h. All media was supplemented with nalidixic acid (100 μg/mL). The inoculum was centrifuged at 4500 rpm for 15 min (Allegra™ 21R, Beckman Coulter, Palo Alto, CA) and washed with a 0.1% sterile peptone water (SPW; Difco, Becton Dickinson, Sparks, MD). Washed cultures were then mixed and added to 6 L of sterile tap water, resulting in approximately 7.4 log CFU *Salmonella* and 7.9 log CFU *L. monocytogenes* per milliliter of inoculum.

2.2. Inoculation of cantaloupes

Fresh netted whole cantaloupes (1641 ± 132 g, Product of California) were purchased from a wholesale retail store and kept at 4 °C prior to use. A whole cantaloupe was dip-inoculated in 6 L of sterile tap water containing a cocktail of three strains of *Listeria monocytogenes* or *Salmonella* for 5 min to achieve approximately 5 log CFU per cm^2. Dip-inoculation ensured the entire surface of the cantaloupe was exposed to the inoculum. Inoculated cantaloupes were then placed on trays covered with stainless steel mesh and allowed to drain/dry for one hour in a laminar flow biosafety cabinet.

2.3. Preparation of wash solutions

Prior to use commercial stainless steel sinks were treated with a commercial sanitizer (Steramine quaternary sanitizer, Edwards-Councilor Co.) and residual microbes on surfaces determined. In short, a sterile cotton swab tip was moistened with 0.1% SPW and two 100 cm^2 areas in each sink swabbed. The swab tip was immersed in TSB and incubated at 37 °C for 20 h to check the presence of *L. monocytogenes* and *Salmonella* on the sinks. Each sink (60 × 60 × 35 cm^3) was filled with 76 L of tap water, commercial