Food Microbiology 58 (2016) 121–127

Contents lists available at ScienceDirect

Food Microbiology

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

Inactivation of *Listeria monocytogenes*, *Salmonella* spp. and *Escherichia coli* O157:H7 on cantaloupes by octenidine dihydrochloride

Abhinav Upadhyay [a], Chi-hung Chen [a], Hsinbai Yin [a], Indu Upadhyaya [a], Samantha Fancher [a], Yanyan Liu [a], Meera Surendran Nair [a], Leanne Jankelunas [a], Jitendra R. Patel [b], Kumar Venkitanarayanan [a, *]

[a] Department of Animal Science, University of Connecticut, Storrs, CT 06269, USA
[b] Environmental Microbial & Food Safety Lab, USDA-ARS, 10300 Baltimore Avenue, Building 201 BARC-East, Room 101, Beltsville, MD 20705, USA

ARTICLE INFO

Article history:
Received 31 December 2015
Received in revised form
21 April 2016
Accepted 24 April 2016
Available online 26 April 2016

Keywords:
Octenidine hydrochloride
Chitosan
Cantaloupes
Listeria monocytogenes
Escherichia coli O157:H7
Salmonella spp

ABSTRACT

The efficacy of a new generation disinfectant, octenidine dihydrochloride (OH), as wash and coating treatments for reducing *Listeria monocytogenes* (LM), *Salmonella* spp. (SAL), and *Escherichia coli* O157:H7 (EC) on cantaloupe was investigated. Cantaloupe rind plugs inoculated separately with the three bacterial species (~8 log CFU/cm^2) were washed for 1, 3, 5 min at 25 °C in water, or chlorine (200 ppm), ethanol (1%), OH (0.01, 0.05, 0.1%) and surviving populations were measured after treatment. Additionally, inoculated cantaloupe rind plugs were coated with 2% chitosan or chitosan containing OH (0.01, 0.05, 0.1%) and sampled for surviving pathogens. Subsequently, the antimicrobial efficacy of OH wash and coating (0.1, 0.2%) on whole cantaloupes was determined. All OH wash reduced LM, SAL, and EC on cantaloupe rinds by > 5 log CFU/cm^2 by 2 min, and reduced populations to undetectable levels (below 2 log CFU/cm^2) by 5 min ($P < 0.05$). Similarly, OH coating on cantaloupe rinds reduced the pathogens by 3 −5 log /cm^2 ($P < 0.05$). Washing and coating whole cantaloupes with OH reduced the three pathogens by at least 5 log and 2 log CFU/cm^2, respectively ($P < 0.05$). Results suggest that OH could be used as antimicrobial wash and coating to reduce LM, SAL, and EC on cantaloupes.

1. Introduction

The United States is one of the leading producers and consumers of cantaloupes in the world. The farm value of cantaloupe production in the US amounts to ~ $314 million, with approximately 1.8 billion pounds of the fruit produced annually from over 45 actively participating states (USDA-ERS, 2012a, 2012b, 2012c). However, over the last decade, reports of multi-state disease outbreaks due to the consumption of cantaloupes contaminated with major foodborne pathogens such as *Salmonella* Panama (CDC, 2012a), *S.* Poona (CDC, 2002), *S.* Litchfield (CDC, 2012b), *S.* Typhimurium and *S.* Newport (CDC, 2012c) have been reported. In addition, the first outbreak of cantaloupe-associated listeriosis that affected more than 150 people in 28 states and resulted in 30 deaths was documented in 2011 (CDC, 2012d). Taken together, these outbreaks highlight the increasing role of cantaloupes as a vehicle of foodborne pathogens, and justify the need for investigating effective and practical decontamination procedures for preventing human infections.

Several factors potentially contribute to pathogen contamination of cantaloupes, including proximity of the fruit to soil, irrigation water, and contact with animals or humans during cultivation, harvesting or processing (Shewfelt, 1987). Contamination of the fruit could also occur due to poor hygiene and unsanitary measures, including inadequate washing of the processing equipment or melons during post-harvest stage (Beuchat, 1995; Brackett, 1992; NACMCF, 1999; Nguyen-The and Carlin, 1994). Analysis of previous disease outbreaks associated with cantaloupes have highlighted contaminated rinds (Mohle-Boetani et al., 1999), wash water (Parnell et al., 2005), and cross contamination during shipping (Hedberg et al., 1994; Tauxe, 1997) as the major sources of cantaloupe contamination.

In the United States, the cantaloupe industry has traditionally used various chemical wash treatments to reduce bacterial load (Ray, 1992; Wei et al., 1985). Chlorine (200 ppm) has been

* Corresponding author. Department of Animal Science, 3636 Horse barn hill Rd Ext., Unit 4040, University of Connecticut, Storrs, CT, USA.
E-mail address: kumar.venkitanarayanan@uconn.edu (K. Venkitanarayanan).

http://dx.doi.org/10.1016/j.fm.2016.04.007
0740-0020/

commonly used for washing cantaloupes, but results in only minimal reductions (~1–1.5 log/cm^2) in pathogen counts (Richards and Beuchat, 2004; Rodgers et al., 2004; Ukuku et al., 2006; Wei et al., 1995). The inefficiency of chlorine could be attributed to the rough surface of the cantaloupes covered by the microscopic lenticellar netting that facilitates bacterial attachment and protection from aqueous disinfectants (Annous et al., 2004; Wang et al., 2009). Moreover, formation of harmful chemical byproducts such as chloramines, trihalomethanes and other organochlorine compounds when chlorine and organic materials interact is a concern due to potential health risks, including cancer (Donato and Zani, 2010; Richardson et al., 1998; Zhang and Farber, 1996). Other antimicrobial chemicals such as hydrogen peroxide (Ukuku et al., 2005; Ukuku and Fett, 2002), nisin (Ukuku and Fett, 2004), calcium sulfate, acidified sodium chlorite and peroxyacetic acid (Fan et al., 2008), or treatments such as hot water (Ukuku et al., 2005; Fan et al., 2008), irradiation (Lamikanra et al., 2005), chitosan coating (Chen et al., 2012) or their combinations have been investigated for reducing foodborne pathogens on cantaloupes with varying degrees of success. However, none of these treatments were effective in rendering the fruit free from pathogens. Thus, there is a need for novel disinfectants, which are effective, safe, and easily implemented at various stages of cantaloupe processing, handling and distribution system for enhancing the microbiological safety of the fruit.

Octenidine dihydrochloride (OH) (*N*, *N*′-(1,10-decanediyldi-1 [4*H*]-pyridinyl-4-ylidene)-bis-(1-octanamine) is a cationic, bispyridinamine used in mouth rinses in Europe that exhibits antimicrobial activity against a wide range of pathogenic microorganisms, including plaque forming bacteria (Bailey et al., 1984; Dogan et al., 2009; Hubner et al., 2010). Moreover, OH has been used in shampoos to effectively remove methicillin resistant *Staphylococcus aureus* (MRSA) colonization on the skin (Rohr et al., 2003). Further, OH was found effective against skin infections in humans, especially in neonates, where it was found to be superior to polyvidone-iodine, and pure alcohol based formulae. OH possesses a broad safety margin, and studies investigating its toxicity in various species have indicated that OH is not absorbed through mucous membrane and the gastrointestinal tract, with no reports of carcinogenicity, genotoxicity, or mutagenicity (Harke, 1989; Kramer et al., 1993; Spitzbart, 1994). The objective of this study was to investigate the efficacy of OH as post-harvest antimicrobial washing and coating treatments for reducing *Salmonella* spp., *Listeria monocytogenes* and *Escherichia coli* O157:H7 on cantaloupe surface.

2. Materials and methods

2.1. Bacterial strains and culture conditions

All culture media were procured from Difco (Becton Dickinson, Sparks, MD). Four strains each of *L. monocytogenes*, *Salmonella* spp., and *E. coli* O157:H7 from our culture collection were used in the study. The L. *monocytogenes* strains used were ATCC Scott A, ATCC 19115, 101, and Presque-598, whereas *Salmonella* spp. were *Salmonella enterica* serovar Poona (RM 2350, RM 1254), S. *enterica* serovar London (RM 6838), and *S. enterica* serovar San Diego (RM 6822). The *E. coli* O157:H7 strains included E16, E7927, E104, and E933. Each bacterial strain was cultured separately in 10 ml of sterile tryptic soy broth with 0.6% yeast extract (TSBYE), and incubated at 37 °C for 24 h. Following incubation, the cultures were sedimented by centrifugation (3600 × g for 15 min) at 4 °C. The pellet was washed twice, resuspended in 10 ml of sterile phosphate buffered saline (PBS, pH 7.0), and serial dilutions were applied to the surface of duplicate plates containing Oxford (for *L. monocytogenes*), MacConkey-Sorbitol (SMA, for *E. coli* O157:H7), or Xylose lysine deoxycholate (XLD, for *Salmonella*) agar, followed by incubation at 37 °C for 48 h. Equal portions of the four strains of each bacterial species were combined, diluted appropriately, and the resulting suspension was used as the inoculum for each pathogen. The bacterial count in the four-strain mixture of *L. monocytogenes*, *Salmonella* spp. or *E. coli* O157:H7 inoculum was also confirmed by spreading 100 μl of culture suspension from appropriate dilutions on Oxford, XLD and SMA agar plates, respectively with incubation at 37 °C for 48 h.

2.2. Cantaloupe rind plugs

Fresh cantaloupes, free of surface defects, were procured from a local farm. Cantaloupe rind plugs were prepared from whole cantaloupes, as described previously (Sapers et al., 2001; Ukuku, 2006; Ukuku and Sapers, 2007). Briefly, circular rind plugs (2.5 cm diameter) were obtained from cantaloupes using a sterile stainless steel cork borer, and the flesh portions were removed. Prior to inoculation, representative rind plug samples were placed in sterile stomacher bags (10 cantaloupe rind plug/bag) containing 100 ml of sterile TSBYE, and incubated at 37 °C for 24 h. The enriched culture was streaked on Oxford, SMA and XLD plates, incubated at 37 °C for 48 h and observed for typical *L. monocytogenes*, *E. coli* O157:H7 or *Salmonella* colonies, respectively. This was done to rule out that the cantaloupes were inherently contaminated with any of the pathogens.

2.3. Inoculation and wash treatment of cantaloupe rind plugs

Each cantaloupe rind plug was spot-inoculated with 200 μl (~8 log CFU) of the 4-strain mixture of *L. monocytogenes*, *E. coli* O157:H7 or *Salmonella* spp., as previously described (Beuchat et al., 2001; Chen et al., 2012). The inoculated rind plugs were air-dried for 2 h at 25 °C in a biosafety cabinet to facilitate bacterial attachment onto cantaloupe surface. Each cantaloupe rind plug was placed in a sterile stomacher bag (Fisher Scientific) containing 50 ml of sterile deionized water (control) or water containing OH (Dishman USA, Inc., Middlesex, NJ) at 0.01, 0.05 or 0.1%. Cantaloupe rind plugs inoculated with the pathogens, but not subjected to any wash treatment served as the baseline to determine the efficiency of bacterial attachment after inoculation. Ethanol (100%) was used to dissolve OH for treatment. Sterile deionized water containing ethanol at 1% final concentration was included as one of the controls to test if ethanol by itself exerted any antimicrobial effect on the pathogens. In addition, 200 ppm total chlorine treatment was included. The 200 ppm total chlorine treatment was prepared by adding appropriate volumes of 12.5% fresh Sodium hypochlorite (Fisher Scientific) to deionized water. Each inoculated rind plug was subjected to OH wash treatments at 25 °C for 1, 3, 5 min in a water bath shaker (Model R76; New Brunswick Scientific, Edison, NJ). After treatment, each rind plug was aseptically transferred to a stomacher bag containing 10 ml of Dey-Engley neutralizing broth and subjected to stomaching for 2 min. The broth was serially diluted (1:10 in PBS) and plated (100 μl) on duplicate Tryptic soy agar (TSA), oxford, SMA or XLD plates. The plates were incubated at 37 °C for 48 h before colonies were counted. In addition, 1 ml of the neutralizing broth was added to 50 ml of TSBYE, and incubated at 37 °C for 24 h. Following enrichment in TSBYE, the culture was streaked on Oxford, SMA or XLD plates, incubated at 37 °C for 48 h, and observed for *L. monocytogenes*, *E. coli* O157:H7 or *Salmonella* colonies. The limit of pathogen detection by the spread plate method and enrichment was 2 log CFU/plug (1.3 log CFU/cm^2) and 1 log/plug (0.3 log CFU/cm^2), respectively.