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

Running Title: Octenidine hydrochloride reduces pathogens on cantaloupe

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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/cm²) 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 $> 5 \log \text{ CFU/cm}^2$ by 2 min, and reduced populations to undetectable levels (below $2 \log \text{ CFU/cm}^2$) by 5 min ($P<0.05$). Similarly, OH coating on cantaloupe rinds reduced the pathogens by 3-5 $\log \text{ CFU/cm}^2$ ($P<0.05$). Washing and coating whole cantaloupes with OH reduced the three pathogens by at least 5 $\log$ and 2 $\log \text{ CFU/cm}^2$, respectively ($P<0.05$). Results suggest that OH could be used as antimicrobial wash and coating to reduce LM, SAL, and EC on cantaloupes.

Key words: Octenidine hydrochloride, Chitosan, Cantaloupes, *L. monocytogenes*, *E. coli* O157:H7, *Salmonella* spp.

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 commonly used for washing cantaloupes, but results in only minimal reductions (~1-1.5 log/cm²) 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-decanediyl-di-1[4H]-pyridinyl-4-ylidene)-bis-(1-octanamine) is a cationic, bipyridinamine 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., *L. monocytogenes* and *E. 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 *S. 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 ($3600 \times g$ 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, D.O., 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/cm²) and 1 log/plug (0.3 log CFU/cm²), respectively.

2.4 Preparation of OH coating solution and application on cantaloupe rind plugs

The coating of cantaloupe rind plugs with OH was done according to a previously published method (Chen et al., 2012). Two grams of low molecular weight chitosan (~5 to 15 kDa) (Sigma Aldrich) was dissolved in sterile deionized water (100 ml) containing 1% acetic acid (Sigma Aldrich), and stirred at room temperature for 12 h to obtain a concentration of 2% (pH 4.6) (Chen et al., 2012). Subsequently, OH (0.01, 0.05, 0.1%) was added to the chitosan solution and the solution was stirred for another 6 h to facilitate proper mixing of the compound. Cantaloupe rind plugs were prepared from fresh whole cantaloupes as described previously. The cantaloupe rind plugs were inoculated with *L. monocytogenes*, *E. coli* O157:H7 or *Salmonella* (~8 log CFU/cm²), as previously mentioned followed by OH coating treatments. For coating cantaloupe rind plugs, 200 µl of each OH coating solution maintained at 25°C was applied and spread uniformly using a sterile L- spreader. Coated plugs were allowed to dry in a laminar hood for 2 h. After coating treatment, the cantaloupe rind plugs were stored at 4°C for 3 days. Enumeration of *L. monocytogenes*, *E. coli* O157:H7 or *Salmonella* on cantaloupe rind plugs was done on days 0, 1, and 3 of storage, as previously described.

2.5 Inoculation and wash treatment of whole cantaloupes with OH

Each whole cantaloupe was dipped in 3 L of sterile PBS containing *L. monocytogenes*, *E. coli* O157:H7 or *Salmonella* spp. (~ 7 log CFU/ml) for 5 min followed by air-drying in a biosafety cabinet for 2 h at 25°C (Ukuku et al., 2005). Each inoculated cantaloupe was washed in 3 L of sterile deionized water (control) or water containing OH at 0.1 or 0.2% for 5 min at 25°C. Appropriate controls were included as before. After treatment, rind plugs were aseptically prepared from whole cantaloupes, and the surviving pathogens on the plugs were

enumerated, as described in section 2.3. The wash water was also tested for the presence of the three pathogens after washing the cantaloupes. The limit of pathogen detection by plating and enrichment was 2 log CFU/plug (1.3 log CFU/cm²) and 1 log/plug (0.3 log CFU/cm²), respectively.

2.6 Coating treatment of whole cantaloupe

Whole cantaloupes were inoculated with the pathogens as described in section 2.4. Each inoculated cantaloupe was coated by dipping in 3 L of 2% chitosan solution or chitosan solution containing 0.1 or 0.2% OH. The coating solutions were maintained at 25°C. The OH-coated cantaloupes were air-dried for 2 h in biosafety cabinet. Inoculated, but uncoated cantaloupes served as control. After coating, rind plugs were prepared from each whole cantaloupe, and stored for 3 days at 4°C. The pathogen population surviving on cantaloupe rind plugs was enumerated (6 plugs/time point) on days 0, 1 and 3, as described in section 2.3.

2.7 Statistical Analysis

For the OH wash experiment with cantaloupe rind plugs, a completely randomized design (CRD) was used with 6 X 3 factorial treatment structure. The factors included 6 treatments (0, 0.01, 0.05, 0.1% OH, 1% ethanol, 200 ppm chlorine) and 3 time points (1, 3, 5 min). The efficacy of OH coating in inactivating the pathogens on cantaloupe rind plugs was investigated using a CRD with 6 X 3 factorial structure. The factors included 5 treatments (0, 0.01, 0.05, 0.1% OH, 1% ethanol, 2% chitosan), and 3 time points (days 0, 1, 3). Three cantaloupe rind plugs were included for each treatment/time point/trial, and the study was repeated three times (n=9). In the experiments with whole cantaloupes, a CRD was also used with duplicate samples and repeated three times (n=6). Data from the three independent experiments were pooled and

analyzed using the mixed procedure of SAS (ver. 9.2, Cary, NC). Differences among the means were analyzed at $P < 0.05$ using Fisher's least significance difference test with appropriate corrections for multiple comparisons.

3. Results

Since selective culture media such as Oxford, XLD or SMA agar could exert potential inhibitory effect on the growth of antimicrobial-stressed *L. monocytogenes*, *Salmonella* spp. and *E. coli* O157:H7, we conducted preliminary experiments wherein the three pathogens on cantaloupe rind plugs following OH treatment were enumerated on a non-selective medium, TSA and the aforementioned respective selective media. However, since no significant differences ($P > 0.05$) were found between the pathogen counts recovered on TSA and the respective selective media (data not shown), Oxford, XLD and SMA agar plates were used for selective enumeration of the respective pathogens in this study. Random microbiological testing of cantaloupe rind plugs for the presence of inherent *L. monocytogenes*, *Salmonella* spp. and *E. coli* O157:H7 revealed that the fruits were not contaminated with the pathogens (data not shown).

The efficacy of wash treatments with 0.01, 0.05 or 0.1% OH at 25°C in reducing *L. monocytogenes*, *Salmonella* spp. and *E. coli* O157:H7 on cantaloupe rind plugs is presented in Fig. 1A-C. The average pathogen population recovered from unwashed inoculated cantaloupe rind plugs (baseline bacterial count) was $\sim 7.0 \log \text{CFU/cm}^2$ for *L. monocytogenes* (Fig. 1A), *Salmonella* spp. (Fig. 1B), or *E. coli* O157:H7 (Fig. 1C). Inoculated cantaloupe rind plugs washed with sterile deionized water (control) yielded average populations of 6-6.5 $\log \text{CFU/cm}^2$ for each bacterial species, which did not significantly change with increase in wash time from 1 to 3 or 5 min. Since ethanol was used as the diluent for OH, the antimicrobial effect of 1%

ethanol solution was also tested. However, at this concentration, ethanol wash treatment did not significantly reduce the populations of either species as compared to controls ($P>0.05$). Similarly, chlorine treatment (200 ppm) exerted only a minimal effect and reduced population by ~ 0.7 CFU/cm², with no significant differences in pathogen counts being observed among the samples subjected to washing in sterile water and chlorine ($P>0.05$).

Washing of cantaloupe rind plugs in OH treatment solutions resulted in a significant reduction of all three species compared to controls (Figs. 1A-C). The dipping of rind plugs in OH treatment solutions for 1 and 3 min reduced populations by at least 3.0 to 4.0 log CFU/cm². The efficacy of OH treatment increased with increase in concentration and treatment time, especially with 3 and 5 min wash times ($P<0.05$). For example, after 5 min of wash treatment, all OH treatments reduced pathogen counts to undetectable levels (< 2 log CFU/cm²; enrichment positive) on cantaloupe rind plugs ($P<0.05$). Moreover, no pathogen was detected in the wash water containing OH or chlorine, whereas ~ 4.0 log CFU/ml of the pathogens was recovered from the control solution after washing the inoculated rind plugs.

The efficacy of OH coating in reducing *L. monocytogenes*, *Salmonella*, and *E. coli* O157:H7 on cantaloupe rind plugs is depicted in Table 1. Populations on control samples after three days ranged between 7.2 to 7.6 log CFU/cm². Chitosan or chitosan with ethanol reduced the pathogen population by ~ 1.5 log on day 1, however, no further reduction was observed during storage. Consequently, all OH treatments decreased populations of *L. monocytogenes*, *Salmonella* and *E. coli* O157:H7 by at least 3 log CFU/cm² on day 1 of refrigerated storage. On day 3, 0.1% OH reduced populations of all three species to below detection limit ($P < 0.05$; enrichment positive), whereas 0.01 and 0.05% OH coatings led to reduction of at least 3 log CFU/cm² on the rind plugs ($P<0.05$).

Since 0.1% OH reduced all three pathogens on rind plugs to below detection limits in both wash and coating treatments (day 3), we used OH at 0.1% and a higher concentration of 0.2% OH for testing its antimicrobial efficacy on whole cantaloupes. The results revealed that both OH wash and coating treatments at 0.1 and 0.2% were effective in significantly reducing the three **bacterial species** on whole cantaloupes (Fig. 2, Table 2). The wash treatments with 0.1 and 0.2% OH decreased pathogen populations by $> 5 \log \text{ CFU/cm}^2$ after 5 min of treatment time (Fig. 2). Chlorine and ethanol wash treatments did not result in significant reduction in bacterial counts as compared to controls ($P>0.05$). In addition, chitosan-based OH coating at 0.1 and 0.2% was effective in reducing all three **microbial populations** on whole cantaloupes (Tables 2A-C). The OH coating at 0.1 and 0.2% reduced *L. monocytogenes* and *Salmonella* counts by 5 log CFU/cm^2 and to undetectable levels (enrichment positive), respectively on day 3 ($P<0.05$). However, *E. coli* O157:H7 population were reduced only by 2 log CFU/cm^2 ($P < 0.05$) on whole cantaloupes coated with OH.

4. Discussion

Since cantaloupes can **become** contaminated in the field (Beuchat and Ryu, 1997; CDC, 1991), or during post-harvest processing and shipping (Suslow 2004; Ukuku and Fett, 2002; Ukuku and Sapers, 2001), there is a need for safe and effective intervention strategies, which can be easily implemented at various stages of cantaloupe processing operations. **In commercial farms, melons are either field packed (Suslow, 2004) or treated with disinfectants to reduce contamination from spoilage microorganisms or pathogens (O'Connor-Shaw et al., 1994).** Chlorine-based sanitizers are commonly used by many cantaloupe growers in large dump tanks for **reducing undesirable microorganisms** on the fruit. **However, this results in only minimal reduction (1-2 logs) in initial level of contamination with bacterial pathogens.** In our study,

washing of cantaloupe rind plugs (Fig. 1) or whole cantaloupes (Fig. 2) in water, or water containing 200-ppm chlorine, or 1% ethanol did not significantly reduce pathogen populations from cantaloupe surface as compared to unwashed cantaloupes (baseline). This could be due to the presence of surface irregularities on cantaloupes that facilitate strong bacterial attachment and reduced ability of sanitizers to act on the bacteria (Ukuku and Fett, 2002; Annous et al., 2004; Wang et al., 2009). Moreover, *Salmonella*, *E. coli* O157:H7 and *L. monocytogenes* are known to possess high surface charge and hydrophobicity that further facilitate strong adherence to cantaloupe rind (Ukuku and Fett, 2002). Biofilm formation has also been reported to significantly contribute to colonization and persistence of enteric pathogens on plants and fruits (Yaron and Romling, 2014).

The wash and coating treatments of cantaloupes were conducted at 25°C since washing of cantaloupes in dump tanks maintained at room temperature is a relatively easy process that can be adopted by both small and large scale cantaloupe growers. Moreover, previous study by Richards and Beuchat (2004) has shown that attachment and infiltration of bacteria such as *Salmonella* Poona on cantaloupe rind increases when the cantaloupe is maintained at 4°C. Similarly, a higher bacterial load was recovered from the rind when the cantaloupe was immersed in inoculum maintained at 4°C as compared to 30°C. Therefore, we selected 25°C as the temperature of both wash and coating treatments.

We observed that wash treatments with OH were very effective in reducing ($> 5 \log \text{CFU/cm}^2$) all three pathogen populations on cantaloupe surface (Fig. 1, 2). Octenidine hydrochloride is a positively charged compound that exerts its bactericidal action by binding to the negatively charged bacterial cell membranes (Harke, 1989), thereby leading to membrane damage and cell death (Hubner et al., 2010; Brill et al., 2006). It is reported to exhibit high affinity to cardiolipin,

a phospholipid found primarily in bacterial membranes, thus making it selectively lethal to microbial cells without adversely affecting eukaryotic cells (Arias-Cartin et al., 2012; Gilbert and Moore, 2005). In addition, studies have reported significant anti-biofilm efficacy of OH against bacterial pathogens (Amalaradjou et al., 2009; Amalaradjou and Venkitanarayanan, 2014). The high antimicrobial efficacy observed in our results could be attributed to the aforementioned attributes of OH.

Several studies have shown that washing of cantaloupes could lead to damaged rind integrity, thereby increasing the risk of pathogen entry and contamination (Bennik et al., 1996; Ukuku et al., 2006). Edible coatings are a viable strategy that can act as an antimicrobial barrier to improve microbiological safety of fruits during storage or transport. Recent studies have investigated the efficacy of edible coatings on fruits (Chen et al., 2012; Vargas et al., 2006; Upadhyay et al., 2014). Chitosan is a polycationic, linear heteropolysaccharide comprising of B-1, 4-linked D-glucosamine and N-acetyl-D-glucosamine (Dutta et al., 2009), obtained by the deacetylation of chitin. The unique physico-chemical properties of chitosan, especially film formation and its non-toxic nature enable its application on foods. We observed that the coating of chitosan fortified with OH were effective in reducing significant pathogen loads on cantaloupe rind plugs and whole cantaloupe (Table 1, 2). Moreover, the antimicrobial effect persisted during refrigerated storage, which could be a result of increased contact time provided by the chitosan coating between bacteria and OH.

In general, OH was found to be somewhat more effective against *L. monocytogenes* than *Salmonella* and *E. coli* O157:H7. Since the OH primarily acts on bacterial outer membrane, the difference in its efficacy could be due to differences in the membrane composition and structure of Gram-positive and Gram-negative microbes. Zumtobel and coworkers (2009) had similar

findings, where OH was found to be more effective against *Staphylococcus aureus* than *Pseudomonas aeruginosa* when used as a coating on tracheotomy tubes. In conclusion, OH was effective in reducing the three major foodborne pathogens, *L. monocytogenes*, *Salmonella* spp. and *E. coli* O157:H7 on cantaloupes when applied as antimicrobial wash and coating treatments. Thus, OH could potentially be used to decontaminate cantaloupes, however follow up long-term storage studies and organoleptic investigations of washed and coated cantaloupes are warranted.

Acknowledgement

This study was funded by a grant (2012-04333) awarded to K. Venkitanarayanan from the USDA-NIFA critical and emerging food safety issues program.

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449 USDA-ERS, 2012a. Table 3--U.S. cantaloupe: Acreage, yield, production, and value, 1950-
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476

Figure legends

Fig.1. Inactivation of (A) *L. monocytogenes* (B) *Salmonella* spp. (C) *E. coli* O157:H7 on cantaloupe rind plugs by wash treatment with octenidine hydrochloride (OH) at 25°C for 1, 3, 5 min. The treatments included Control, Chlorine 200 ppm, Ethanol 1%, OH 0.01%, OH 0.05%, OH 0.1%. Error bars represent SEM (n=9). Values with different superscripts represent significant differences between treatments (P<0.05). * Below Detection Limit (1.3 log CFU/cm²).

Fig.2. Inactivation of (A) *L. monocytogenes* (B) *Salmonella* spp. (C) *E. coli* O157:H7 on whole cantaloupe by wash treatment with octenidine hydrochloride (OH) at 25°C for 5 min. The treatments included Control, Chlorine 200 ppm, Ethanol 1%, OH 0.1%, OH 0.2%. Error bars represent SEM (n=6). Values with different superscripts represent significant differences between treatments (P<0.05). *Below Detection Limit (1.3 log CFU/cm²).

Table 1. Effect of chitosan based coating with octenidine hydrochloride (OH 0.01, 0.05, 0.1%) on (A) *L. monocytogenes* (B) *Salmonella* spp. (C) *E. coli* O157:H7 on cantaloupe rind plugs. The treatments included Control, Chitosan 2%, Ethanol 1%, OH 0.01%, OH 0.05%, OH 0.1%. Error bars represent SEM (n=9). Values with different superscripts represent significant differences between treatments (P<0.05). *Below Detection Limit (1.3 log CFU/cm²).

Table 2. Effect of chitosan based octenidine hydrochloride coating (OH, 0.1, 0.2%) on (A) *L. monocytogenes*, (B) *Salmonella* spp. (C) *E. coli* O157:H7 on whole cantaloupes. The treatments included Control, Chitosan 2%, Ethanol 1%, OH 0.1%, OH 0.2%. Error bars represent SEM (n=6). Values with different superscripts represent significant differences between treatments (P<0.05). *Below Detection Limit (1.3 log CFU/cm²).

Fig. 1

(A)

(B)

(C)

Fig. 2

(A)

(B)

(C)

Table 1:

(A) *L. monocytogenes*

Coating treatments	Bacterial population (log CFU/cm ²) during storage at 4°C		
	Day 0	Day 1	Day 3
Control	7.3±0.6 ^a	7.5±0.5 ^a	7.6±0.3 ^a
Chitosan 2%	6.8±0.3 ^b	6.5±0.2 ^b	6.1±0.4 ^b
Ethanol	6.5±0.2 ^b	6.4±0.1 ^b	6.2±0.3 ^b
OH 0.01%	4.3±0.4 ^c	4.2±0.7 ^c	3.8±0.5 ^c
OH 0.05%	3.8±0.2 ^c	3.6±0.05 ^c	2.3±0.7 ^d
OH 0.1%	BDL*	BDL*	BDL*

(B) *Salmonella*

Coating treatments	Bacterial population (log CFU/cm²) during storage at 4°C		
	Day 0	Day 1	Day 3
Control	7.5±0.5 ^a	7.6±0.8 ^a	7.2±0.5 ^a
Chitosan 2%	6.5±0.2 ^b	6.7±0.3 ^{ab}	6.8±0.4 ^{ab}
Ethanol	6.4±0.3 ^b	6.8±0.4 ^{ab}	6.5±0.4 ^b
OH 0.01%	4.2±0.5 ^c	4.5±0.8 ^c	4.0±0.6 ^c
OH 0.05%	3.8±0.3 ^{cd}	4.0±0.4 ^c	3.0±0.8 ^d
OH 0.1%	3.2±0.8 ^d	2.5±0.5 ^d	BDL*

(C) *E. coli* O157:H7

Coating treatments	Bacterial population (log CFU/cm ²) during storage at 4°C		
	Day 0	Day 1	Day 3
Control	7.4±0.5 ^a	7.6±0.8 ^a	7.5±0.6 ^a
Chitosan 2%	6.5±0.4 ^b	6.9±0.3 ^{ab}	6.8±0.5 ^{ab}
Ethanol	6.6±0.4 ^b	6.8±0.5 ^{ab}	6.5±0.4 ^b
OH 0.01%	4.3±0.5 ^c	4.5±0.8 ^c	4.0±0.6 ^c
OH 0.05%	3.9±0.3 ^{cd}	4.0±0.4 ^c	3.3±0.3 ^d
OH 0.1%	3.3±0.3 ^d	3.2±0.3 ^d	BDL*

Table 2:

(A) *L. monocytogenes*

Coating treatments	Bacterial population (log CFU/cm ²) during storage at 4°C		
	Day 0	Day 1	Day 3
Control	5.3±0.1 ^a	5.4±0.05 ^a	6.3±0.1 ^a
Chitosan 2%	3.1±0.09 ^b	3.2±0.08 ^b	3.5±0.1 ^b
Ethanol	3.3±0.6 ^b	3.2±0.4 ^b	3.7±0.4 ^b
OH 0.1%	0.3±0.3 ^c	0.4±0.4 ^c	0.38±0.4 ^c
OH 0.2%	0.38±0.3 ^c	BDL*	BDL*

(B) *Salmonella*

Coating treatments	Bacterial population (log CFU/cm ²) during storage at 4°C		
	Day 0	Day 1	Day 3
Control	5.1±0.2 ^a	5.2±0.1 ^a	5.7±0.1 ^a
Chitosan 2%	4.0±0.5 ^b	4.2±0.7 ^b	3.9±0.4 ^b
Ethanol	4.0±0.7 ^b	4.3±0.3 ^b	4.1±0.3 ^b
OH 0.1%	BDL*	1.3±0.6 ^c	0.7±0.5 ^c
OH 0.2%	BDL*	BDL*	BDL*

(C) *E. coli* O157:H7

Coating treatments	Bacterial population (log CFU/cm ²) during storage at 4°C		
	Day 0	Day 1	Day 3
Control	4.8±0.6 ^a	3.6±0.2 ^a	4.2±0.2 ^a
Chitosan 2%	3.5±0.8 ^b	3.1±0.2 ^b	4.5±0.1 ^a
Ethanol	3.3±0.6 ^b	3.3±0.4 ^b	4.2±0.5 ^a
OH 0.1%	2.5±0.3 ^c	1.9±0.6 ^c	3.2±0.1 ^c
OH 0.2%	2.3±0.3 ^c	1.4±0.5 ^c	2.6±0.5 ^c