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Internalization of *Listeria monocytogenes* in cantaloupes during dump tank washing and hydrocooling

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ABSTRACT

Recent listeriosis outbreaks and recalls associated with cantaloupes urge for studies to understand the mechanisms of cantaloupe contamination by *Listeria monocytogenes*. Postharvest practices such as washing and hydrocooling were suggested to facilitate the contamination of fresh fruits by human pathogens. This study assessed the potential of *L. monocytogenes* internalization into cantaloupes during dump tank washing and immersion-type hydrocooling in water contaminated with *L. monocytogenes*. The effect of cantaloupe cultivar, water temperature, and harvesting technique on *L. monocytogenes* internalization was also evaluated. Full slip (cantaloupe without any residual stem) Western and Eastern cultivar cantaloupes were pre-warmed to 42 °C (to imitate peak-high field temperatures of freshly harvested cantaloupes) and then immersed in water at 6 °C and 18 °C containing 4 and 6 log CFU/ml of *L. monocytogenes*. Clipped (cantaloupe with short stem residues obtained by clipping the stem at harvest) Western and Eastern cantaloupes were pre-warmed to 42 °C and then immersed in water at 6 °C containing 6 log CFU/ml of *L. monocytogenes*. Additionally, full slip and clipped Western cantaloupes were equilibrated to 18 °C and then immersed in water at 18 °C containing 6 log CFU/ml of *L. monocytogenes* (isothermal immersion without temperature differential). Water containing *L. monocytogenes* infiltrated both full slip and clipped cantaloupes through the stems/stem scars and was then distributed along the vascular system in hypodermal mesocarp reaching the calyx area of the fruit. The current study demonstrated that, under experimental conditions, *L. monocytogenes* can internalize into cantaloupes during immersion in water contaminated by *L. monocytogenes*, both in the presence and absence of temperature differential, and that temperature differential moderately enhanced the internalization of *L. monocytogenes*. The incidence and levels of *L. monocytogenes* internalized in the middle-mesocarp were significantly affected by harvesting technique but not by cantaloupe cultivar.

1. Introduction

Melons have been frequently implicated in foodborne illnesses, resulting in 34 outbreaks in the United States (U.S.) between 1973 and 2011 with cantaloupes being the most common (19 outbreaks) melon type involved (Walsh et al., 2014). Considering the relatively long incubation period of listeriosis (Lorber, 2007) and the short shelf life of fresh produce commodities which are frequently not available for trace back investigations, the number of listeriosis cases linked to cantaloupes could be potentially underestimated.

Under conventional agricultural practices, cantaloupes are grown on the ground; this naturally increases their potential exposure and subsequent contamination by zoonotic pathogens. U.S. Food and Drug Administration (FDA) cantaloupe sampling efforts in 1999 and 2000/2001 revealed that 7.3% of imported and 3.0% of domestic cantaloupes were contaminated with either *Salmonella* or *Shigella* (FDA, 2001, 2003). A surveillance study on a total of nine cantaloupe farms and packing plants in South Central region of the U.S. and Mexico discovered that pre-harvest contamination with either *Salmonella* or *Escherichia coli* was present in 3.2% and 0% of U.S. and Mexico cantaloupes, respectively (Castillo et al., 2004). Three independent studies analyzed field–collected cantaloupes from > 25 different regions of California from 1999 to 2004 and in Texas in 2012 and did not recover *Salmonella* or *L. monocytogenes* from fruit rind (Burfield, 2001; Suslow, 2004; Dev Kumar et al., 2015). Together these surveillance data suggest that the pre-harvest incidence of human pathogens in cantaloupes is very low.

Unless cantaloupes are field packed, within a few hours of

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harvesting, they are transported to the packing facility for washing and cooling. Inadequate sanitation practices at the facilities can lead to contamination of cantaloupes by enteric microrganisms. For example, the contamination of cantaloupes by coliforms and fecal enterococci occurred during washing and hydrocooling operations at the packing facilities rather than in the field (Gagliardi et al., 2003). Similarly, an increase in the incidence of *Salmonella* and *E. coli* on cantaloupes occurred mainly at the packing facilities after fruit washing (Castillo et al., 2004). Likewise, Akins et al. (2008) observed a slight increase in aerobic bacterial and *E. coli* populations on cantaloupes collected after dump tank washings, suggesting possible contamination during washing. In another study on the prevalence of human enteric pathogens in cantaloupes (both in the field and after harvest) in Texas (South Central region of the U.S.A.), *Salmonella* was recovered only from processed (washed) cantaloupes (Duffy et al., 2005). The investigation of the 2011 listeriosis outbreak involving Colorado-grown (West region of the U.S.A.) cantaloupes traced the contamination back to the processing facility where postharvest washing was suggested, among other factors, to facilitate *L. monocytogenes* survival, growth, and potential contamination of a large number of cantaloupes (FDA, 2011). The investigation of the 2012 salmonellosis outbreak linked to cantaloupes produced in Indiana (Midwest region of the U.S.) demonstrated that all of the implicated cantaloupes were packed at a single on-farm packing house that used dump tank washing (FDA, 2013). The submersion of warm melons in cool dump tank water was suggested to facilitate the contamination of cantaloupes during packing operations by the infusion of dump tank water contaminated with *Salmonella* into the cantaloupes via their stem scars or rind netting (FDA, 2013). Furthermore, this investigation revealed that a large well providing water for the cantaloupes dump tank was open and not protected from surface contaminants and no records on the disinfection of wash water were available (FDA, 2013).

The infiltration of human enteric pathogens into fresh fruits during dump tank washing and hydrocooling was demonstrated in tomatoes, mangos, oranges, apple, and avocados (Bartz et al., 2015; Bordini et al., 2007; Buchanan et al., 1999; Chen et al., 2016; Eblen et al., 2004; Penteado et al., 2004). The temperature differential between the fruit and immersion water was suggested as one of the causes leading to pathogen internalization into whole fruits immersed in water (Bartz and Showalter, 1981; Buchanan et al., 1999; Eblen et al., 2004; Zhuang et al., 1995). However, the research on the effect of postharvest practices, such as dump tank washing and hydrocooling on the internalization of human enteric pathogens in cantaloupe is still limited.

Currently, cantaloupe growers practice two hand-harvesting methods, full slip and clipping. The highest quality cantaloupes are harvested when the fruit easily separates from the vine with a light twisting motion (full slip) (Suslow, 2004). Full slip causes a complete detachment of stems along the abscission zone leaving the fruit with a large stem scar. To extend the shelf-life, cantaloupes are also harvested at earlier maturity by cutting the peduncle (clipping) that leaves the fruit with a short stem. We hypothesized that during postharvest handling of cantaloupes, specifically dump tank washing or/and hydrocooling, water infiltration into fruit enables passive internalization of *L. monocytogenes* suspended in the water or present on the fruit surface. Therefore, the main objective of the current study was to evaluate the potential of *L. monocytogenes* to internalize cantaloupes and colonize edible portions of the mesocarp after immersion in water contaminated with this pathogen in the presence and absence of temperature differential (between the fruit and water). We also evaluated if cantaloupe cultivar and harvesting method have any effect on the *L. monocytogenes* internalization into fruit mesocarp.

2. Materials and methods

2.1. Preparation of L. monocytogenes inoculum

Three previously characterized strains of *L. monocytogenes* involved in the 2011 cantaloupe outbreak of listeriosis were used. Strains LIS007 [sequence type (ST) 5, clonal complex (CC) 5], 8, LIS0072 (ST7, CC7), and LIS0077 (ST561, CC7) were obtained from the Center for Food Safety and Applied Nutrition (CFSAN) *L. monocytogenes* culture collection, and they represented three different PFGE profiles, serotypes 1/2a and 1/2b, and epidemic clones VI and VII (Lomonaco et al., 2013). Stock cultures were streaked on Trypticase Soy Agar (TSA, Difco, BD, Sparks, MD) supplemented with 0.6% yeast extract (Yeast Extract [YE], Bacto, BD) and incubated at 37 °C for 24 h before a single colony of each strain was transferred to 10 ml of Trypticase Soy Broth (TSB, Bacto, BD) supplemented with 0.6% yeast extract. Three consecutive overnight suspensions were made via loop inoculation into 10 ml of TSBYE and incubation at 37 °C. A final transfer of 10 ml for each strain was made into 1000 ml of buffered *Listeria* enrichment broth (BLEB; Oxoid Ltd., Basingstoke, England), followed by incubation at 37 °C for 24 h. *L. monocytogenes* populations in each suspension were determined by spiral plating serial dilutions on RAPID'*L.mono* agars (BioRad, Hercules, CA) in duplicate.

An aliquot of three-strain cocktail of *L. monocytogenes* was transferred to 40 l of deionized water adjusted to 6 °C (to simulate hydrocooling) and 18 °C (to simulate dump tank washing), to attain 10^4 and 10^6 CFU/ml inoculum levels of *L. monocytogenes*. That resulted in a total of 4 inoculum level/temperature combinations as follows: 18 °C containing 10^4 CFU/ml (I); 18 °C containing 10^6 CFU/ml (II); 6 °C containing 10^6 CFU/ml (III); and 6 °C containing 10^4 CFU/ml (IV) (Fig. 1). All *L. monocytogenes* suspensions were also supplemented with 1% Acid Blue 9 dye (Chem-Impex Int'l Inc., Wood Dale, IL) to visualize water infiltration into cantaloupes. Each variant of inoculum was sampled prior and after the immersion of cantaloupes as described above to determine the bacterial concentration.

2.2. Preparation, dump tank washing and hydrocooling of cantaloupes

Clipped Western cantaloupes (cultivar (cv.) Rocky Ford) and Eastern (cv. Athena) were obtained from experimental fields of the United Stated Department of Agriculture, Beltsville Agricultural Research Center in Beltsville, Maryland. Cantaloupes growing conditions were described elsewhere (Nyarko et al., 2016). Physiologically mature cantaloupes of each cultivar were detached from the vine with clippers to leave short stems. Full slip Western and Eastern cantaloupes (the same cultivars) were purchased at retail.

To imitate peak-high field temperatures of the cantaloupes prior to harvest (up to 49 °C for external and 43.2 °C for internal) as previously described (Schroeder, 1965; Suslow, 2004, 2013), 32 full slip and 14 clipped cantaloupes, prior to immersion in inoculum, were pre-warmed to 42 °C by incubation for 24 h at 42 °C in New Brunswick™ Innova® 44R incubators (Eppendorf North America, Hauppauge, NY) at 75% humidity to prevent fruit wilting. Additionaly, 4 full slip and 7 clipped Western cantaloupes were equilibrated to 18 °C by incubation for 24 h at 18 °C in New Brunswick™ Innova® 44R at 75% humidity. Western and Eastern full slip cantaloupes, pre-warmed to 42 °C, were randomly divided into four groups of 4 fruits per group. Then, four fruits of each cultivar were immersed for 30 min in each of the 4 inoculum level/temperature combinations (I, II, III and IV) (Fig. 1). All Western cantaloupes equilibrated to 18 °C were immersed in 10^6 CFU/ml inocula at 18 °C (isothermal immersion). During fruit immersion, the inocula were vigorously agitated to maintain the temperatures at 6 °C (herein referred as hydrocooling) and 18 °C (herein referred as dump tank washing) with ANOVA® 40 refrigerated circulators (ANOVA Industries, Huston, TX). Internal temperature of one cantaloupe from each treatment was monitored using Traceable™ Hi-Accuracy Thermometers