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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

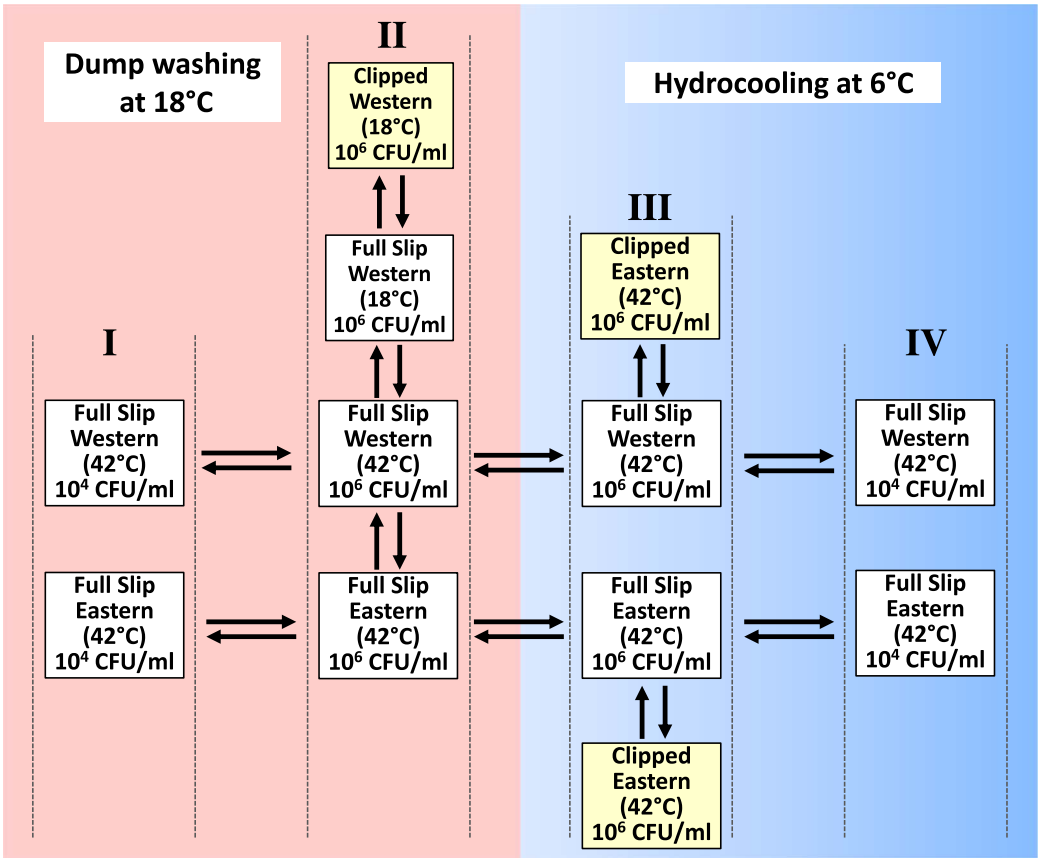


Fig. 1. The schematic presentation of the treatment of cantaloupes and comparative analysis of *L. monocytogenes* populations internalized in corresponding areas of the mesocarp. Cantaloupes, pre-warmed to 42 °C or equilibrated to 18 °C, were immersed in *L. monocytogenes* suspensions at 6 °C (blue background) or 18 °C (pink background) for 30 min. Two inoculum levels, 10^4 and 10^6 CFU/ml, of *L. monocytogenes* were used, that resulted in 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). Full slip cantaloupes (white squares) and clipped cantaloupes (yellow squares) were analyzed on day 7 after immersion. Reciprocal arrows indicate the treatment groups from which *L. monocytogenes* populations intern to determine the effect of cultivar, harvesting method, inoculum temperature, and inoculum level.

(Fisher Scientific, Pittsburg, PA).

Clipped Western and Eastern cantaloupes (7 each), pre-warmed to 42 °C, were immersed for 30 min in inoculum level/temperature combination III; containing 10^6 CFU/ml *L. monocytogenes* inoculum at 6 °C (Fig. 1). During the immersion of clipped cantaloupes, the temperature of the inocula was controlled and internal temperatures of cantaloupes were monitored as described above. After immersion, cantaloupes were removed from the inoculum tank, air-dried for 45 to 60 min in the biosafety hood to remove the excess water, and then stored at 4 °C until analyzed. The cantaloupes used for fruit internal temperature monitoring were disposed of after fruit immersion, and thus, 3 full slip cantaloupes and 6 clipped cantaloupes from each group were subsequently analyzed for *L. monocytogenes* internalization.

2.3. Detection and enumeration of L. monocytogenes in cantaloupe mesocarp

Prior to microbiological analysis cantaloupe surface was sterilized by transferring individual fruit in Ziploc bags containing 2 l of freshly prepared 1% sodium hypochlorite (Clorox®) for 15 min with a periodic hand massaging. The pH of the sodium hypochlorite solution was measured before and after surface-disinfection of the cantaloupes. After surface disinfection, fruits were washed three times in sterile deionized water. The rinsates from the third wash were centrifuged at 3500 × *g* for 15 min and the resulting pellets were re-suspended in buffered peptone water and plated onto both Agar *Listeria* Ottavani & Agosti (ALOA; bioMeriéux, St Louis, MO) and RAPID'*L.mono* agar plates to confirm that the fruit surfaces were free of *L. monocytogenes*.

As the ability of *L. monocytogenes* strains from the 2011 Cantaloupe outbreak to grow in cantaloupe mesocarp at 4 °C has been recently characterized by Martinez et al. (2016), our objective was to assess the potential of *L. monocytogenes* infiltration into edible portions of cantaloupes during dump washing and hydrocooling, not the quantification of the growth of these strains internalized in cantaloupe mesocarp. Considering that the majority of cantaloupes reach the retail at least a week after harvest, herein all cantaloupes were analyzed on day 7 after immersion into inocula to estimate *L. monocytogenes* levels present in the mesocarp (if internalization occurred) had contaminated cantaloupes been distributed to consumers. Sampling immediately after cantaloupe immersion into inocula (time 0) was not conducted. Three full slip cantaloupes of each cultivar per inoculum level/temperature combination were analyzed. Because we expected less water infiltration in the clipped cantaloupes and therefore lower probability for *L. monocytogenes* internalization, six clipped cantaloupes of each cultivar were analyzed on day 7 after immersion. Cantaloupes were cut on their transversal plane starting from the blossom-end (calyx) of the fruit towards the stem end using sterilized stainless steel knives. To prevent potential transfer of the *L. monocytogenes* from one area of the mesocarp to another, a different sterilized stainless steel knife was used for each area. Because dye solution permeated the entire cantaloupe, multiple mesocarp portions were sampled in three major areas of the fruit: 1) stem, the area of the mesocarp located 1 cm below the stem scar; 2) equator, the central portion of the fruit; and 3) calyx, the portion of mesocarp located 1 cm above the blossom end of the cantaloupe (Fig. 2). Three mesocarp portions containing the dye were collected from each area (stem, equator and calyx) using disposable sterile scoops

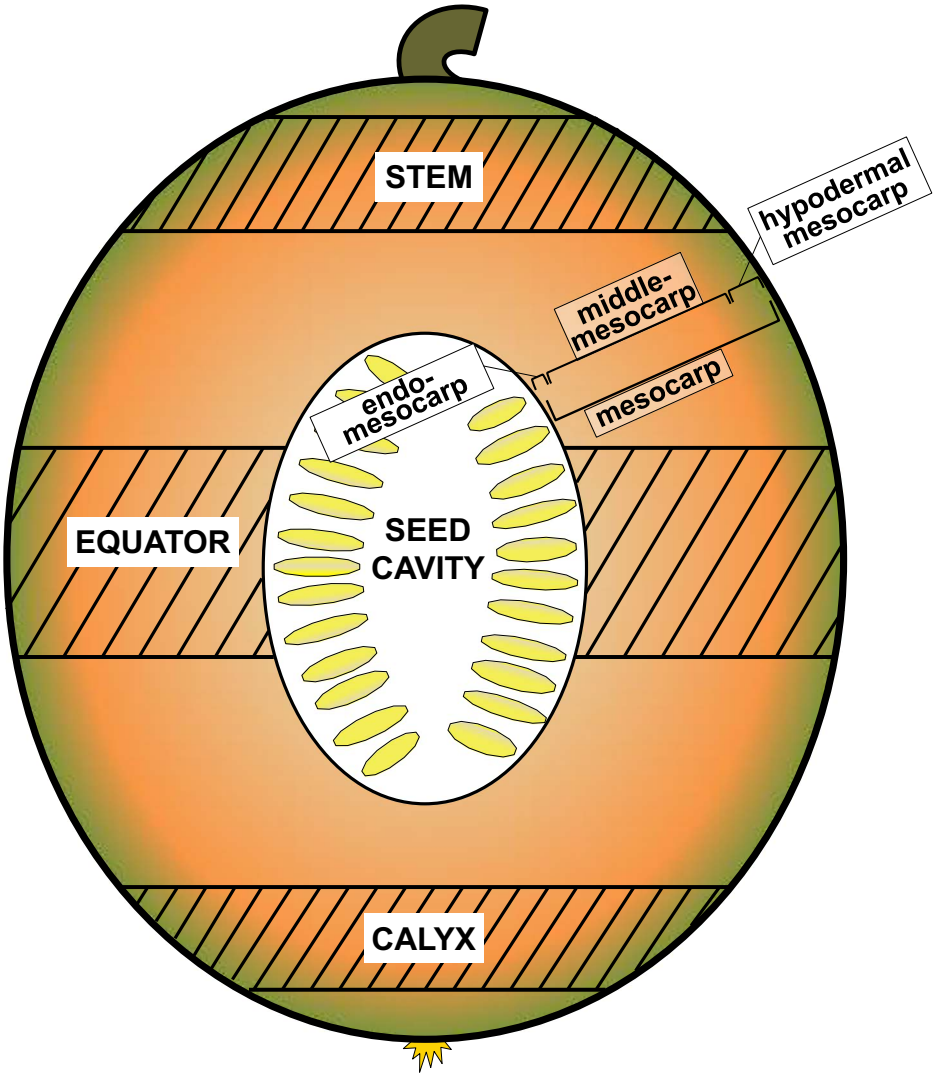


Fig. 2. Schematic presentation of the longitudinal section through the cantaloupe, outlining anatomical layers of the mesocarp. Dashed areas outline the portions of the mesocarp sampled for *L. monocytogenes* enumeration. "Stem" is the area of the mesocarp located 1 cm below the stem scar, "Equator" is the central portion of the fruit, and "Calyx" is the portion of mesocarp located 1 cm beneath the blossom end of the cantaloupe.

(1 tsp. styrene disposable spoons, Bel-Art, Wayne, NJ). Each sampled mesocarp portion was weighed and then analyzed using the FDA *Bacteriological Analytical Manual* (BAM) method (Hitchins et al., 2013). For *L. monocytogenes* enumeration mesocarp samples were homogenized (Polytron PT2500E Homogenizer, Kinematica, Bohemia, NY) in BLEB (1:10) and 100 μl of the mesocarp-broth mixture was then spread plated onto four ALOA plates. This direct plating scheme had a lower limit of detection (LOD) of 25 CFU/g. A subset of presumptive colonies of *L. monocytogenes* were confirmed by real time PCR and/or API® *Listeria* (bioMérieux Inc. St. Louis, MO) as described in the *L. monocytogenes* chapter of FDA's BAM (Hitchins et al., 2013). The remainder of the mesocarp-broth mixture was enriched as described in the *L. monocytogenes* chapter in FDA's BAM (Hitchins et al., 2013) to determine if any of the samples that were negative by direct plating were still be positive. Additional mesocarp portions without dye were also collected as controls to evaluate the efficiency of surface disinfection in 1% sodium hypochlorite and to determine if an inadvertent transfer of residual *L. monocytogenes* from the rind to mesocarp took place during fruit dissections. These portions were analyzed by enrichment as described in BAM (Hitchins et al., 2013).

2.4. Statistical analysis

Some mesocarp samples were positive for *L. monocytogenes* after enrichment, but the levels of *L. monocytogenes* were below the LOD (25 CFU/g) for direct plating enumeration. For statistical analysis, the level of *L. monocytogenes* in these samples was assumed as half of the LOD (12.5 CFU/g). One-way analysis of variance (ANOVA) was conducted on the log (CFU) values to compare levels of *L. monocytogenes* internalized in various areas of mesocarp. If differences were significant ($p < 0.05$), a two-tailed *t*-test post hoc analysis of independent data sets was conducted with a Bonferroni correction for the significance threshold at $p < 0.0167$. Additionally, populations of *L. monocytogenes* internalized in various areas of mesocarp of cantaloupes immersed in 10^6 CFU/ml inoculum were analyzed using two-way ANOVA to determine the effect of cultivar, harvesting method, and inoculum temperature. If differences were significant ($p < 0.05$), a two-tailed *t*-test post hoc analysis of independent data sets for the corresponding areas of the mesocarp was conducted with a Bonferroni correction for the significance threshold at $p < 0.0167$. The percentage of mesocarp samples positive for *L. monocytogenes* was used to calculate the incidence of *L. monocytogenes* internalization in stem, equator, and calyx areas of the fruit. One-way ANOVA was conducted to compare the differences in average percentage of internalization incidence between cantaloupes immersed in 10^4 CFU/ml and 10^6 CFU/ml inoculum levels, between cantaloupes immersed in inoculum at 6 °C and 18 °C, between Eastern and Western cantaloupes, and between clipped and full slip cantaloupes. Microsoft Excel software was used for these analyses.

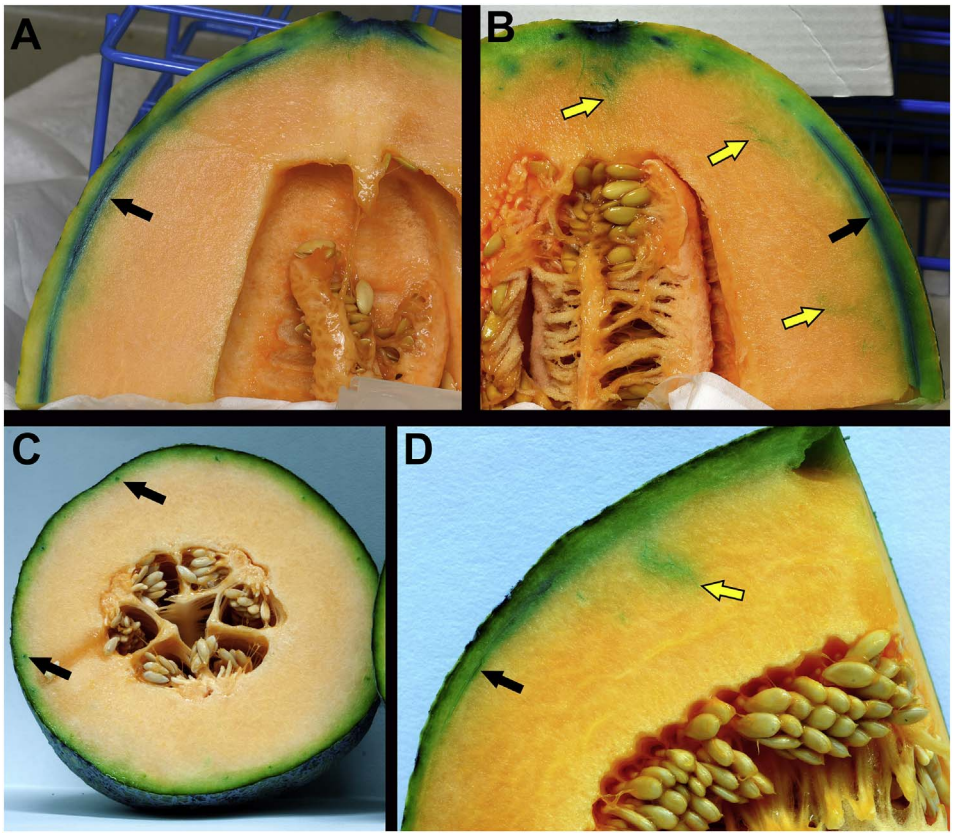


Fig. 3. Dye uptake and distribution within full slip cantaloupes. Dye solution infiltrated through the stem scars in Eastern (A) and Western (B) cantaloupes. An intensive dye uptake and distribution in hypodermal mesocarp was observed along the vascular system across the entire fruit (black arrows). A transversal cut through the center of the fruit (C) showing cross section of the major vascular bundles caring the dye (black arrows) from the stem to the calyx area. Panel D illustrates the longitudinal section of the cantaloupe from the central towards calyx area. Vascular bundles in the hypodermal mesocarp (black arrows) from the calyx area of the fruit are less intensively stained than those located in the stem area. Yellow arrows point to the secondary smaller vessels spreading the dye solution into the middle mesocarp.

3. Results

3.1. Changes in cantaloupe internal temperature during hydrocooling

Internal temperature of cantaloupes pre-warmed to 42 °C gradually decreased during the immersion in cool water. After 30 min immersion in 18 °C inocula, the internal temperature of the fruits decreased by 5 ± 0.8 °C (average ± standard error) from 42 °C to 37 °C. After 30 min immersion in 6 °C inocula, the internal temperature of the fruits decreased by 18 ± 2.0 °C, from 42 °C to 24 °C. Internal temperature of the fruits equilibrated to 18 °C did not change during the immersion in inoculum at 18 °C.

3.2. Water infiltration and distribution within the fruit as visualized by dye uptake

Water infiltration into mesocarp was observed during immersion of pre-warmed cantaloupes in both 6 °C and 18 °C inoculum. Fig. 3A–D shows dye uptake and distribution within Eastern and Western full slip cantaloupes. Dye infiltration primarily took place through the stem scar and was then distributed via the vascular system in the hypodermal mesocarp from the stem to the calyx area. Water influx spread through the middle-mesocarp via secondary (smaller) vascular bundles (Fig. 3B and D) and in some instances reached the seed cavity (picture not shown). Fig. 4 illustrates the dye uptake in clipped Eastern cantaloupe (part of the peduncule remaining attached). The intensity of dye permeation in clipped cantaloupes was lower than that in full slip cantaloupes; however, its distribution pattern within the fruit was identical to that observed in full slip cantaloupes. Lesser dye staining of the tissue was observed in cantaloupes equilibrated to 18 °C than those pre-warmed to 42 °C prior to immersion (picture not shown).

3.3. Detection and enumeration of L. monocytogenes in various areas of the mesocarp in full slip cantaloupes

3.3.1. Surface disinfection of cantaloupes

No *L. monocytogenes* was detected in the pelleted rinsates recovered from cantaloupes washed in 1% sodium hypochlorite for 15 min. The pH value of the sodium hypochlorite solution was 7.0 and did not change after a 15 min immersion of the cantaloupes. No *L. monocytogenes* was detected in mesocarp portions without dye, further suggesting that any positive samples detected below were not a result of transfer of residual *L. monocytogenes* from the rind to mesocarp during fruit dissections.

3.3.2. Full slip Eastern cantaloupes dump tank washed (18 °C) and hydrocooled (6 °C) in 10^6 CFU/ml inocula

Water infiltration into cantaloupes during dump tank washing (18 °C) and hydrocooling (6 °C) in water inoculated with *L. monocytogenes* led to bacterial internalization in all 3 areas of the mesocarp. One week after full slip Eastern cantaloupes were immersed in 10^6 CFU/ml inocula, *L. monocytogenes* populations recovered from stem areas were larger than those in the other two tested areas of the mesocarp. Average population levels in stem areas of fruits immersed in inocula at 18 °C and 6 °C were 3.30 and 3.99 log CFU/g, respectively (Table 1). Significantly ($p < 0.0167$) lower levels of *L. monocytogenes* were recovered from equator areas, with average populations of 1.83 and 2.61 log CFU/g, after immersion in 18 °C and 6 °C inocula, respectively (Table 1). Even lower numbers of *L. monocytogenes* were recovered from calyx areas, with average population levels of 1.34 and 1.69 log CFU/g after immersion in 18 °C and 6 °C inocula, respectively (Table 1). All mesocarp samples not permeated by dye were devoid of *L. monocytogenes*.

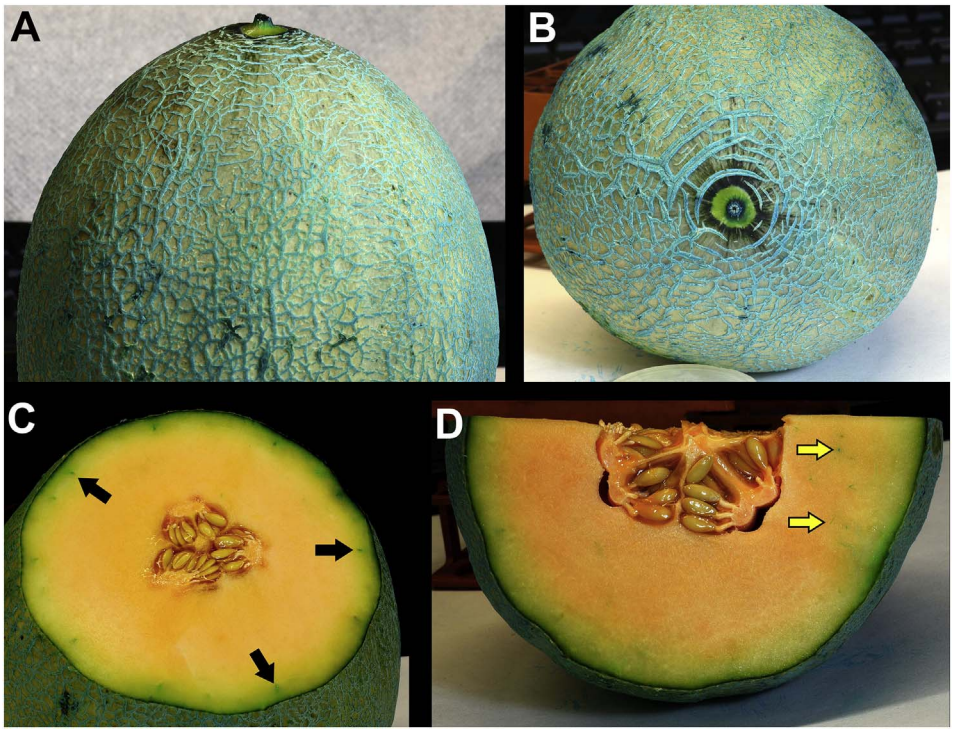


Fig. 4. The infiltration and distribution of dye solution within clipped cantaloupes. Eastern cantaloupe showing a short stem (A) and intensive staining of the stems section (B). A transversal cut through the cantaloupe (C) showing cross section of the major vascular bundles caring the dye (black arrows). Panel D illustrates a transversal section of the cantaloupe through the equator area highlighting secondary smaller vessels permeated by dye in the middle mesocarp (yellow arrows).

3.3.3. Full slip Eastern cantaloupes dump tank washed (18 °C) and hydrocooled (6 °C) in 10^4 CFU/ml inocula

L. monocytogenes internalization was also detected in full slip Eastern cantaloupes immersed in 10^4 CFU/ml inocula at 18 °C and 6 °C. At 7 days after fruit immersion in the 18 °C inocula, populations of *L. monocytogenes* recovered from stem areas were not significantly different from those from equator and calyx areas of the mesocarp (Table 1). In cantaloupes immersed in 6 °C inocula, the differences in average *L. monocytogenes* populations levels between the stem and other areas of the mesocarp were significant ($p < 0.0167$) (Table 1). All mesocarp samples not permeated by dye were devoid of internalized *L. monocytogenes*.

3.3.4. Full slip Western cantaloupes dump tank washed (18 °C) and hydrocooled (6 °C) in 10^6 CFU/ml inocula

In full slip Western cantaloupes *L. monocytogenes* internalization was observed in all three areas of the mesocarp after both dump tank washing and hydrocooling in 10^6 CFU/ml inocula. One week after immersion, the highest ($p < 0.0167$) levels of *L. monocytogenes* were recovered from stem areas of the mesocarp (Table 1). Average *L. monocytogenes* populations in stem areas of pre-warmed Western cantaloupes immersed in inocula at 18 °C and 6 °C were 4.16 and 3.96 log CFU/g, respectively and those in cantaloupes equilibrated to 18 °C and immersed in inocula at 18 °C were 3.31 log CFU/g (Table 1). *L. monocytogenes* levels recovered from equator areas of all full slip Western cantaloupes were significantly ($p < 0.0167$) lower than in stem areas with average populations not exceeding 2.86 log CFU/g (Table 1). *L. monocytogenes* populations in calyx areas of pre-warmed cantaloupes immersed in inocula at 18 °C (1.39 log CFU/g) and 6 °C (2.13 log CFU/g) were even lower than those in equator areas; the difference, however, was statistically significant only in fruits after immersion at 18 °C (Table 1). In calyx area of cantaloupes equilibrated

Table 1
Average populations [a] of *L. monocytogenes* internalized in cantaloupe mesocarp on day 7 after immersion in 10^4 CFU/ml and 10^6 CFU/ml inocula at 6 °C and 18 °C.

Inoculum level	Inoculum temp.	Full slip					
		Western (42 °C)			Eastern (42 °C)		
		Stem	Equator	Calyx	Stem	Equator	Calyx
10^6 CFU/ml	6 °C	a 3.96 (0.26)	b 2.86 (0.38)	b 2.13 (0.41)	a 3.99 (0.03)	b 2.61 (0.48)	b 1.69 (0.25)
	18 °C	a 4.16 (0.09)	b 2.91 (0.41)	c 1.39 (0.15)	a 3.30 (0.33)	b 1.83 (0.32)	b 1.34 (0.16)
10^4 CFU/ml	6 °C	a 3.22 (0.35)	b 2.06 (0.28)	c 1.18 (0.02)	a 2.59 (0.33)	b 1.40 (0.30)	b 1.15 (0.06)
	18 °C	a 2.16 (0.21)	ab 1.70 (0.20)	b 1.31 (0.11)	a 1.36 (0.10)	a 1.26 (0.08)	a 1.28 (0.14)
		Western (18 °C)			Eastern (18 °C)		
10^6 CFU/ml	18 °C	a 3.31 (0.22)	b 0.59 (0.10)	c 1.35 (0.15)	NA	NA	NA
		Clipped					
		Western (42 °C)			Eastern (42 °C)		
		Stem	Equator	Calyx	Stem	Equator	Calyx
10^6 CFU/ml	6 °C	a 2.67 (0.26)	a 1.33 (0.06)	a 2.75 (0.24)	a 1.76 (0.28)	b 1.32 (0.05)	b 1.25 (0.01)
		Western (18 °C)			Eastern (18 °C)		
10^6 CFU/ml	18 °C	a 1.66 (0.29)	a 1.13 (0.13)	a 1.84 (0.30)	NA	NA	NA

[a] Values represent average (3 mesocarp samples of each area in 3 full slip cantaloupes (n = 9) and 3 mesocarp samples of each area in 6 clipped cantaloupes (n = 18)) and numbers in parenthesis indicate standard error. Average values in the same row and within the same cantaloupe type (Western or Eastern) that are preceded by a different letter are significantly ($p < 0.0167$) different from each other.

to 18 °C and immersed in inocula at 18 °C average *L. monocytogenes* populations were 1.35 log CFU/g (Table 1). All mesocarp samples not permeated by dye were devoid of *L. monocytogenes*.

3.3.5. Full slip Western cantaloupes dump tank washed (18 °C) and hydrocooled (6 °C) in 10^4 CFU/ml inocula

Average *L. monocytogenes* populations in stem areas were 2.16 and 3.22 log CFU/g in Western cantaloupes one week after immersion in 10^4 CFU/ml inocula at 18 °C and at 6 °C, respectively and they were significantly higher ($p < 0.0167$) than those from the calyx areas of the mesocarp (Table 1). In hydrocooled cantaloupes *L. monocytogenes* populations were also significantly different between stem and equator areas of the fruits (Table 1). All mesocarp samples not permeated by dye were devoid of *L. monocytogenes*.

3.4. Detection and enumeration of L. monocytogenes in various areas of the mesocarp in clipped cantaloupes hydrocooled (6 °C) in 10^6 CFU/ml inocula

Hydrocooling of clipped cantaloupes in 10^6 CFU/ml inocula at 6 °C led to *L. monocytogenes* internalization in all three areas of the mesocarp (Table 1). In both Western and Eastern cantaloupes, the highest levels of *L. monocytogenes* were generally recovered from stem areas of the mesocarp (data not shown). However, the difference in average *L. monocytogenes* populations recovered from stem, equator, and calyx areas was not always statistically significant (Table 1). Average *L. monocytogenes* populations were significantly ($p < 0.0167$) higher in stem (2.67 log CFU/g) than in equator (1.33 CFU/g) areas of the mesocarp in pre-warmed Western cantaloupes (Table 1). The differences in average *L. monocytogenes* populations among the 3 mesocarp areas of Eastern cantaloupes were not statistically significant (Table 1). All mesocarp samples not permeated by dye were devoid of internalized *L. monocytogenes*.

3.5. Effect of cultivar, inoculum temperature (6 °C and 18 °C), inoculum level, and harvesting technique on L. monocytogenes internalization in cantaloupes

In a number of mesocarp samples from cantaloupes immersed in 10^4 CFU/ml inocula, the levels of *L. monocytogenes* were close or below the limit of detection by the direct plating (LOD, 25 CFU/g). Therefore, only data on the *L. monocytogenes* enumeration in mesocarp of cantaloupes immersed in 10^6 CFU/ml inocula at 18 °C and at 6 °C were analyzed using two-factor ANOVA to determine the effect of inoculum temperature, cultivar, and harvesting method on levels of *L. monocytogenes* internalized in cantaloupes. There was no statistically significant difference between the levels of *L. monocytogenes* internalized in Western and Eastern full slip cantaloupes (Table 2). There was no statistically significant difference between the levels of *L. monocytogenes* internalized in dump tank washed (18 °C) and hydrocooled (6 °C) pre-warmed cantaloupes (Table 2). The difference in *L. monocytogenes* levels in stem and equator, but not calyx areas between Western cantaloupes pre-warmed to 42 °C and those equilibrated to 18 °C were significantly different one week after immersion in 18 °C inoculum (Table 2). Average population levels of *L. monocytogenes* recovered from the stem areas from full slip Eastern cantaloupes were significantly ($p < 0.0167$) higher than those from clipped Eastern cantaloupes hydrocooled in 10^6 CFU/m inoculum. Average *L. monocytogenes* populations recovered from the stem and equator, but not calyx, areas of full slip Western cantaloupes were significantly ($p < 0.0167$) higher than those from the corresponding mesocarp areas of clipped Western cantaloupes hydrocooled in 10^6 CFU/ml inoculum (Table 2) *L. monocytogenes* populations internalized in stem areas of Western cantaloupes after isothermal immersion were significantly higher in full slip than in clipped cantaloupes.

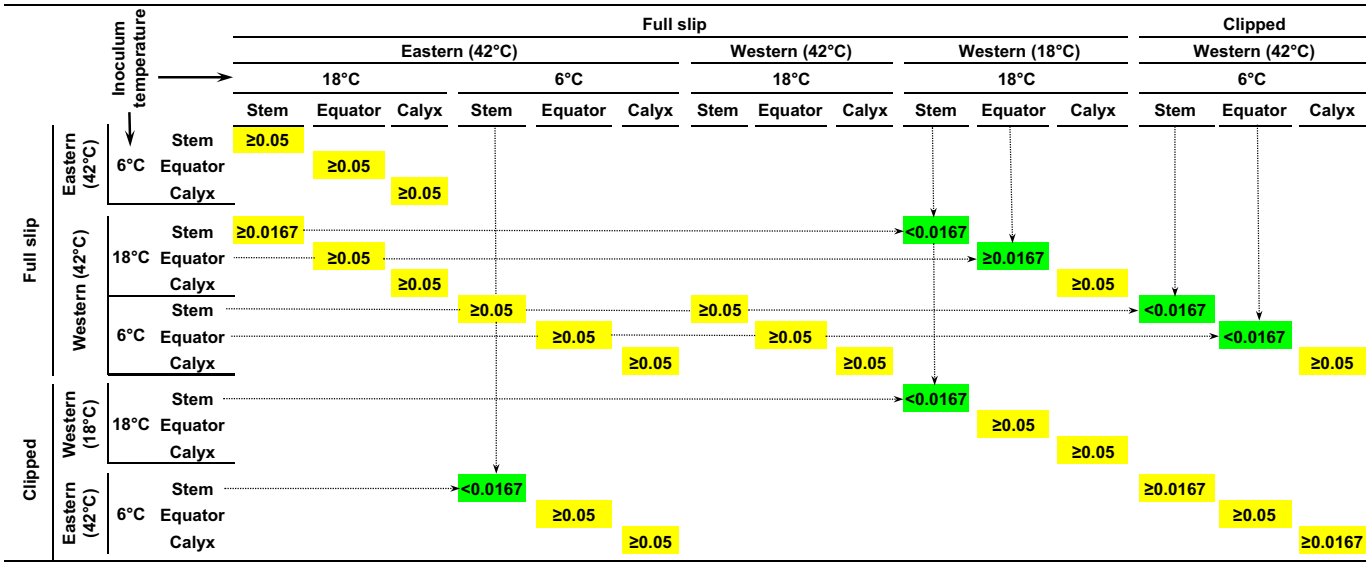
Table 3 illustrates the percentage of *L. monocytogenes* internalization in stem, equator, and calyx areas of the mesocarp as affected by the inoculum level, inoculum temperature, cantaloupe cultivar, and harvesting technique. In pre-warmed full slip Western cantaloupes *L. monocytogenes* was detected in all three areas: stem, equator, and calyx of all mesocarp samples. In pre-warmed full slip Eastern cantaloupes, *L. monocytogenes* was not detected in some of the calyx and equator areas of the mesocarp, the difference in internalization incidence between cultivars was not statistically significant. Similarly, in some of the stem, equator and calyx portions of the mesocarp of Western cantaloupes *L. monocytogenes* was not detected after isothermal immersion in *L. monocytogenes* inoculum. The difference in internalization incidence between cantaloupes pre-warmed to 42 °C and those equilibrated to 18 °C was not statistically significant in stem areas, but significant in equator and calyx areas. Internalization incidence of *L. monocytogenes* in edible portions of the fruits was not significantly ($p < 0.05$) different between pre-warmed cantaloupes immersed in *L. monocytogenes* suspensions at 18 °C and 6 °C (Table 3). Inoculum level had no statistically significant effect on the *L. monocytogenes* internalization incidence in cantaloupes. Internalization incidence of *L. monocytogenes* in clipped cantaloupes was lower ($p < 0.05$) than in full slip cantaloupes (Table 3) both in the presence and absence of temperature differential.

4. Discussion

The domestic sources of cantaloupes implicated in outbreaks were mostly from Texas, Colorado, and Indiana, and imported cantaloupes were harvested in Mexico, Guatemala, and Honduras (Walsh et al., 2014). Notably, the handling of cantaloupes in all implicated geographical regions involved the transportation of the harvested fruits to a facility for dump tank or spray washing and sometimes hydrocooling prior to packing (FDA, 2011, 2013; Figueroa-Aguilar et al., 2005; Fresh QuesT, 2015; Gagliardi et al., 2003). Commonly, there is not a drying stage after washing or hydrocooling, and wet cantaloupes are packed in carton boxes with or without polyethylene lining, which could increase water-activity and potentially facilitate survival and proliferation of microorganisms. For example, investigations of the 2011 multistate outbreak of listeriosis and the 2012 multistate outbreak of salmonellosis both pointed to free residual moisture left on the fruits from washing procedures as a possible contributor to contamination of fresh whole cantaloupes by these pathogens (FDA, 2011, 2013). Overall, cantaloupes implicated in multistate outbreaks underwent either washing or washing and hydrocooling at the packing facilities, and, in some instances, in-process contamination was traced back to a primary wash tank or hydrocooler (Figueroa-Aguilar et al., 2005; Gagliardi et al., 2003; FDA, 2011, 2013). The current study aimed to investigate potential *L. monocytogenes* internalization into cantaloupes after exposure to contaminated water in the packinghouse. Specifically, this study examined the role of dump tank washing and immersion-type hydrocooling on *L. monocytogenes* internalization in whole cantaloupes.

A number of studies showed that fruits can reach the temperatures well above 40 °C in a wide range of crops even in temperate climates without high air temperatures (Hopp, 1947; Kliewer and Lider, 1968; Kotze et al., 1988; Schroeder, 1965; Thorpe, 1974; Woolf et al., 1999). Depending on the degree of exposure to direct sunlight fruit flesh temperature where shown to reach up to 15 °C above ambient. For instance, at air temperatures between 23 and 26 °C avocado flesh temperatures reached from 43 to 45 °C (Woolf et al., 1999; Woolf and Ferguson, 2000). Tomato fruit flesh was reported to reach 41 °C at the ambient temperature of 28 °C (Hopp, 1947). Apple fruit temperatures of 41 and 44 °C were recorded at the 27 °C ambient temperature (Kotze et al., 1988; Thorpe, 1974). At ambient temperatures between 33 and 36 °C fruit flesh temperatures in cantaloupes, watermelons and honeydews ranged from 42 to 45 °C (Schroeder, 1965). After harvest, depending on the duration of cantaloupe exposure to the direct sunlight, an additional buildup of heat in the fruit might take place. Therefore, rapid removal of field heat (precooling) from melons prior to packing is crucial for extended shelf life. Cantaloupes were pre-warmed to 42 °C in

Table 2
Statistical significance [a] (p values) for two-way ANOVA with replication testing of the effect of harvesting technique [b], cultivar [c], and water temperature [d] on *L. monocytogenes* populations internalized in the corresponding areas of the mesocarp.



[a]Bonferroni correction for the significance threshold set at $p < 0.0167$. For p values < 0.0167 (highlighted in green) arrows were traced from the pairs of data sets compared. p values ≥ 0.0167 and those ≥ 0.05 are highlighted in yellow.
[b]The effect of harvesting technique was evaluated by comparing *L. monocytogenes* populations internalized in the corresponding areas of the mesocarp in clipped and full slip cantaloupes.
[c]The effect of cultivar was evaluated by comparing *L. monocytogenes* populations internalized in the corresponding areas of the mesocarp between Western and Eastern cantaloupes.
[d]The effect of temperature differential was evaluated by comparing *L. monocytogenes* populations internalized in the corresponding areas of the mesocarp between pre-warmed and equilibrated to 18 °C cantaloupes immersed in inoculum at 18 °C and 6 °C.

current study to imitate peak high field temperatures of freshly harvested cantaloupe. Precooling of fresh cantaloupes is often accomplished by forced-air cooling, hydrocooling or ice packing to reduce internal fruit temperature to ≤ 4 °C (Cantwell and Kasmire, 2002). For large fruits such as melons, a typical hydrocooling time ranges from 30 to 60 min, depending on the fruit size, with the use of either an immersion or a shower system (Thompson et al., 2002). For instance, the cantaloupe growers on the East Coast of the U.S. and those from Central America commonly employ dump tank washing technique (Fresh QuesT, 2015; Lewis Taylor Farms, 2013). Likewise, in Georgia (South Atlantic region of the U.S.), to facilitate rapid cantaloupe unloading, some growers back up trailers directly into large in-ground dumping pools (Lewis Taylor Farms, 2013). Average groundwater temperature in Georgia range from to 14 to 20 °C (United States Geological Survey Agency, https://www.usgs.gov/data). According to Webb et al. (2015) dump tank washing of cantaloupes in Georgia is conducted in water at 20 to 22 °C. The dump tank washing stage is usually followed by hydrocooling (Lewis Taylor Farms, 2013). The melon packing facility from Indiana implicated in the 2012 multistate outbreak of salmonellosis used well water in their cantaloupe dump tank (FDA, 2013). Average groundwater temperature in the latter geographic area is 12.5 °C (United States Geological Survey Agency, https://www.usgs.gov/data) and there is no record if water was continuously supplied to the dump tank from the well or was recirculated. In the current study, 18 °C water was chosen for experimental dump washing of the cantaloupes. Hydrocooling (6 °C) and dump washing (18 °C) in contaminated water were conducted to evaluate the potential of *L. monocytogenes* internalization into cantaloupes. Usually, cantaloupes leave the dump tank within 5 to 10 minutes after entry. To be able to compare the levels of *L. monocytogenes* internalized in whole cantaloupes after dump

Table 3
The incidence of *L. monocytogenes* internalization in cantaloupe mesocarp as affected by cultivar, harvesting technique, inoculum temperature, and inoculum level one week after immersion in the inocula at 18 °C and 6 °C.

Cantaloupe cultivar	Mesocarp area	Full slip cantaloupes (42 °C)				Full slip cantaloupes (18 °C)	Clipped cantaloupes (42 °C)	Clipped cantaloupes (18 °C)
		Inoculum temperature 6 °C		Inoculum temperature 18 °C		Inoculum temperature 18 °C	Inoculum temperature 6 °C	Inoculum temperature 18 °C
		Inoculum level		Inoculum level		Inoculum level	Inoculum level	Inoculum level
		10^4 CFU/ml	10^6 CFU/ml	10^4 CFU/ml	10^6 CFU/ml	10^6 CFU/ml	10^6 CFU/ml	10^6 CFU/ml
Eastern	Stem	a 100.0 (0.0)	a 100.0 (0.0)	a 100.0 (0.0)	a 100.0 (0.0)	NA	b 38.3 (15.3)	NA
	Equator	a 88.7 (11.3)	a 100.0 (0.0)	a 66.6 (33.3)	a 88.7 (11.3)	NA	b 28.6 (11.3)	NA
	Calyx	a 88.7 (11.3)	a 100.0 (0.0)	a 77.3 (11.3)	a 100.0 (0.0)	NA	b 14.3 (6.7)	NA
Western	Stem	a 100.0 (0.0)	a 100.0 (0.0)	a 100.0 (0.0)	a 100.0 (0.0)	a 83.3 (16.7)	ab 55.6 (20.5)	b 27.8 (13.4)
	Equator	a 100.0 (0.0)	a 100.0 (0.0)	a 100.0 (0.0)	a 100.0 (0.0)	b 50.0 (9.7)	b 33.3 (12.2)	b 27.7 (18.8)
	Calyx	a 100.0 (0.0)	a 100.0 (0.0)	a 100.0 (0.0)	a 100.0 (0.0)	b 41.7 (15.9)	b 16.7 (16.7)	b 11.1 (17.2)

Values represent average (n = 9 for full slip and n = 18 for clipped cantaloupes) and numbers in parenthesis indicate standard error. Average values in the same row that are preceded by a different letter are significantly ($p < 0.05$) different from each other.

washing and hydrocooling, herein, both immersions (at 6 °C and 18 °C) were conducted for 30 min. The extension of dump washing to 30 min was also necessary to annihilate the effect of the immersion time (30 min versus 5 to 10 min) in order to evaluate if the magnitude of the temperature differential would have a significant effect on the *L. monocytogenes* internalization into cantaloupes.

Dye infiltration into full slip cantaloupes primarily took place through the stems and stem scars as evidenced by the strongest intensity of vessel staining observed in the stem areas of the fruits (Fig. 3A and B). As the vessels progressed away from the stem (towards the calyx) intensity of their staining gradually decreased. Staining of the much thinner (secondary) vessels carrying the dye into the middle-mesocarp was observed in all 3 areas of the fruit (Fig. 3B and D). Dye infiltration was observed in both hydrocooled and dump tank washed cantaloupes with and without temperature differential. Dye uptake and permeation up to the calyx area was also observed after the immersion of clipped cantaloupes. The pattern of dye infiltration in clipped cantaloupes was identical to that observed in full slip cantaloupes; however, the dye staining intensity of vascular bundles was visually lower than in clipped cantaloupes (Fig. 4C and D). This implies less water infiltrated in clipped compared to full slip cantaloupes during hydrocooling. It is important to mention that all negative control samples (mesocarp portions free of dye) were devoid of *L. monocytogenes* that demonstrates the lack of the inadvertent transfer of *L. monocytogenes* from cantaloupe rind to the mesocarp during dissection of the fruit.

The pathway of dye infiltration was consistent with bacterial populations recovered from various portions of the fruits. Thus, the highest numbers of *L. monocytogenes* cells were predominantly recovered from the stem areas (Table 1), which were most strongly permeated by the dye (Fig. 3). The equator and calyx areas had less dye staining and correspondingly had lower bacterial populations. This gradient in the concentration of *L. monocytogenes* in the mesocarp, high at the stem and low at calyx, was consistently observed in Eastern and Western full slip cantaloupes immersed in 10^6 CFU/ml inoculum at 18 °C and at 6 °C (Table 1). It important to point out that total sugar content in melon mesocarp is highest in the calyx and lowest in the stem area (Zhang and Li, 2005), demonstrating that the observed gradient in *L. monocytogenes* populations across the fruit is not conditioned by the nutrient availability. The absence of *L. monocytogenes* in mesocarp samples which were free of dye and the consistency of the bacterial gradient occurrence across analyzed cantaloupes, together with the fact that average *L. monocytogenes* populations were significantly ($p < 0.0167$) different among mesocarp areas, strongly suggest that bacteria infiltration followed the dye permeation trajectory via or along vascular system of the fruit. It remains unclear if *L. monocytogenes* penetrated the fruit from stem to calyx areas through the water conducting vessels or via the intercellular spaces surrounding the vascular bundles. The ability of human enteric pathogens to internalize the plant and disperse within the vegetal matrix along the vascular system was previously reported (Bartz et al., 2015; Bernstein et al., 2007; Chen et al., 2016; Gu et al., 2011; Macarisin et al., 2014; Solomon et al., 2002). The hypothesis on the formation of a continuous water column along the xylem vessel bundles, as a potential corridor for enteric pathogen migration via vascular system of the vegetal tissue, was proposed (Bartz et al., 2015); nevertheless, the actual mechanism of enteric pathogen migration within vegetal matrix remains to be elucidated. The same trend of a gradual decline in *L. monocytogenes* numbers from stem to calyx areas of the mesocarp was observed in cantaloupes immersed in 10^4 CFU/ml inocula (Table 1).

The internalization of microorganisms was suggested to be affected by the temperature differential during the hydrocooling of whole fruits (Bartz and Showalter, 1981; Buchanan et al., 1999; Eblen et al., 2004; Zhuang et al., 1995) that is consistent with the current study, showing that *L. monocytogenes* populations internalized in stem and equator areas of the mesocap were higher in cantaloupes pre-warmed to 42 °C than in those equilibrated to 18 °C after immersion in 18 °C inoculum (Table 2). However, the similar incidence and prevalence of *L. monocytogenes* in pre-warmed cantaloupes after hydrocooling (at 6 °C) and dump washing (at 18 °C) indicated that the magnitude of temperature differential did not significantly affect *L. monocytogenes* internalization. Likewise, an earlier study showed that *Salmonella* levels infiltrating cantaloupe stem scar tissue were unaffected by differences in temperatures between cantaloupes and inocula (Richards and Beuchat, 2004).

Western cultivars of cantaloupes have denser netting than Eastern cultivars, which is associated with a greater rind roughness and deeper disruption of the cuticle (Boyhan et al., 2014). These morphological differences between Western and Eastern cantaloupes could affect the infiltration of pathogenic microorganisms into cantaloupe tissues. Current study showed that the incidence of *L. monocytogenes* internalization into different areas of the mesocarp was generally lower in Eastern compared to Western cantaloupes; the difference, however, was not statistically significant (Table 3). Integral to that, comparison of average *L. monocytogenes* populations recovered from stem, equator, and calyx areas of the mesocarp did not reveal significant differences between Western and Eastern cantaloupes immersed in 10^6 CFU/ml *L. monocytogenes* suspension at either 18 °C or 6 °C (Table 2). Similar to our results, the populations of *Salmonella* Poona recovered from the stem scar tissue of Western and Eastern cantaloupes, after immersion in 10^7 CFU/ml *Salmonella* Poona suspension, were not significantly different (Richards and Beuchat, 2004). The current data on *L. monocytogenes* internalization together with an earlier study on *Salmonella* Poona internalization suggest that the cultivar is unlikely to play a significant role in the internalization of the human enteric pathogens into cantaloupes.

Because of a less intensive dye staining of the vascular bundles in clipped cantaloupes comparing to full slip cantaloupes, it was inferred that less water infiltrated the fruit with stem than without stem. Integral to that, the incidences of *L. monocytogenes* internalization in stem, equator, and calyx areas of the mesocarp were significantly ($p < 0.05$) lower in clipped than in full slip cantaloupes, with the exception of the stem areas in Western cantaloupes, for which the difference was not statistically significant (Table 3). Furthermore, average *L. monocytogenes* populations in stem and equator areas of the mesocarp from clipped cantaloupes were significantly ($p < 0.0167$) lower than in those from full slip cantaloupes (Table 2). Although, this observation should be interpreted with caution as field-harvested clipped cantaloupes were compared to store-purchased full slip cantaloupes, taken together, these data demonstrated that the presence of a stem appeared to be an important factor affecting water ingress in the internal tissues of the cantaloupes during fruit immersion in water.

Our data, along with data from previous surveillance and outbreak investigations and from previous internalization studies on other pathogens or other fruits, established that contamination during postharvest processing, especially during hydrocooling or washing, was an important source of contamination (Sivapalasingam et al., 2003; Castillo et al., 2004, 2014; Gagliardi et al., 2003; Penteado et al., 2004; Bordini et al., 2007; Materon et al., 2007; Akins et al., 2008; Munnoch et al., 2009; CDC, 2015). However, other factors could also play very important roles. Food processing techniques, such as scrub brushing, could contribute to the spread of *Salmonella* over different areas of cantaloupe rind (Parnell et al., 2005). The transfer of *L. monocytogenes* and *Salmonella* from contaminated cantaloupe rind to the mesocarp, as a route of flesh contamination during preparation of fresh-cut fruits, was well documented (Ukuku and Sapers, 2001; Ukuku and Fett, 2002; Ukuku et al., 2004). Native microflora and postharvest storage condition can affect the growth of *L. monocytogenes* on whole cantaloupes (Ukuku et al., 2004). Post-preparation time (before the ready to eat product is refrigerated) also played critical role on pathogen proliferation in fresh-cut cantaloupes (Ukuku and Sapers, 2007; Ukuku et al., 2012). All these studies could contribute to the establishment of preventive control strategies at various step of cantaloupe production.

The current study showed that cantaloupes with and without stems uptake water during dump tank washing and immersion-type hydrocooling. In line with previous reports on bacteria infiltration in cantaloupes; the current study also revealed that, when immersed in water, water ingress into cantaloupes is not entirely affected by temperature differential. Water infiltrated the fruits through the stems/stem scars and then was distributed within the fruit through the vascular system in hypodermal mesocarp. In both cantaloupe varieties used in the current study, water influx spread through the middle-mesocarp via secondary vascular bundles and reached the calyx areas of the fruit. It is important to note that *L. monocytogenes* inoculation levels used in the current study (4 and 6 log CFU/ml) did not necessarily emulate the levels of *L. monocytogenes* naturally occurring in washing/hydrocooling tanks in cantaloupe processing facilities. Had this study focused on the precise determination of the risk associated with *L. monocytogenes* internalization in the function of inoculum level, this factor might have warranted further investigation. The objective of this study was met through the demonstration that, under experimental conditions, *L. monocytogenes* can internalize into cantaloupes during dump tank washing and immersion-type hydrocooling if it is present in water. In addition, before the results of the current study are used to make harvesting recommendations to the industry, large-scale trials would be necessary.

Conflict of interest statement

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the U.S. FDA.

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