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Effects of Postharvest Handling Conditions on Internalization and Growth of *Salmonella enterica* in Tomatoes†

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ABSTRACT

Salmonella internalization in tomatoes during postharvest handling is a major food safety concern. This study was conducted to determine the effect of immersion time, immersion depth, and temperature differential between bacterial suspension and tomato pulp on the internalization of *Salmonella enterica* in tomato fruits. The effect of storage temperature and duration on the survival and growth of internalized *Salmonella* cells was also evaluated. Overall, immersion time significantly affected the incidence and extent of *S. enterica* internalization ($P < 0.0001$), with a linear correlation between immersion time and *Salmonella* internalization. The depth of *Salmonella* internalization in tomato tissues also increased with increasing immersion time. Immersion time also significantly influenced the degree to which the temperature differential affected *Salmonella* internalization. With an immersion time of 2 min, the temperature differential had no significant effect on *Salmonella* internalization ($P = 0.2536$). However, with an immersion time of 15 min, a significantly larger *Salmonella* population became internalized in tomatoes immersed in solutions with a −30°F (−16.7°C) temperature differential. Internalized *S. enterica* cells persisted in the core tissues during 14 days of storage. Strain type and storage duration significantly affected ($P < 0.05$) both the frequency detected and the population of internalized *Salmonella* recovered, but storage temperatures of 55 to 70°F (12.8 to 21.1°C) did not ($P > 0.05$). These findings indicate the importance of preventing pathogen internalization during postharvest handling.

Salmonella is a major causal agent of foodborne illness outbreaks and has been ranked as the most burdensome foodborne pathogen for public health in the United States *(19)*. Salmonellosis outbreaks associated with fresh fruits and vegetables have been reported more frequently in recent years *(11)*. From 1990 to 2011, tomatoes were confirmed as vehicles of transmission in at least 14 salmonellosis outbreaks and were suspected in another 20 outbreaks in the United States *(13, 24),* leading to growing concerns about the safety of fresh tomatoes.

Salmonella contamination of tomato fruits may occur during field production or postharvest handling. In packing houses, tomatoes are typically washed in a dump tank or a flume, and wash water often is reused throughout the entire day of operation. Although no direct evidence has linked the internalization of *Salmonella* to illness outbreaks, internalization of plant pathogens during postharvest handling has been a significant problem for the tomato industry. Hedberg et al. *(14)* and Segall et al. *(20)* found that the water used in these wash systems was the most critical factor contributing to microbial contamination of fresh market tomatoes. Bartz *(5–7)* reported that the negative temperature differential between wash water and tomato pulp, i.e., wash water that was colder than tomatoes, had a significant effect on water uptake by tomatoes and the development of soft rot. To mitigate this problem, packing house operators usually heat up the dump tank water to at least 10°F (5.6°C) higher than the incoming tomato pulp temperature. Because water uptake that causes internalization of phytopathogens could also result in internalization of human pathogens, this same temperature differential requirement has been included in the tomato food safety standards and the associated commodity-specific audit checklists *(23)*. However, as stated in the 2009 Center for Produce Safety request for proposals *(10),* during development of these control point metrics and limits, a number of important data gaps were identified, including tomato dump tank water management conditions. Scientific information is needed determine the limits of the temperature differential that may cause internalization and to determine whether there is a ''safe'' residence time frame for dump tanks that would obviate the need for the strict 10°F temperature differential. Xia et al. *(26)* investigated the distribution of bacteria cells inside tomatoes and the effect of temperature differential on *Salmonella* internalization. These authors found that internalized cells were predominantly located under the tomato stem scars and that temperature differentials of −10, 0, and 10°F had no impact on pathogen internalization. They also found that tomato variety had a large influence on

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Salmonella internalization and that extension of poststem removal time significantly reduced pathogen internalization. However, Xia et al. evaluated the effect of temperature differential and immersion within only a limited range, and the impacts of broader temperature differentials and longer immersion time were not addressed.

Pathogen proliferation during postharvest handling is another major concern for tomato food safety. Tomatoes are temperature sensitive and are usually stored at 55 to 81°F (13 to 27°C), depending on their maturity stage *(16, 18)*. Many researchers have found that internalized *Salmonella* cells can persist and even grow in tomato fruits within this temperature range *(15, 21, 27)*. Zhuang et al. *(27)* found that *Salmonella enterica* Montevideo grew at 68°F (20°C) but not at 50°F (10°C), and Shi et al. *(21)* found that the growth of internalized *Salmonella* in tomatoes was serovar dependent. However, these studies either employed much more aggressive inoculation methods (direct injection using syringe or repeated vacuum release cycle plus negative temperature differential) than would occur in commercial practice or included external stem scar tissues with the core tissues. The growth potential of *Salmonella* cells that infiltrate tomatoes without mechanical damage is not clear. The main objectives of this study were to (i) examine the effects of tomato washing conditions, including temperature differential, immersion depth, and immersion time, on the internalization of *S. enterica* into round tomato fruits and (ii) evaluate the effect of storage temperature and duration on the survival and growth of the internalized cells of three *Salmonella* serovars.

MATERIALS AND METHODS

Bacterial culture and inoculum preparation. *S. enterica* Newport strain FDA2757 (tomato isolate) transformed with plasmid pGT-KAN (Dr. P. Millner, U.S. Department of Agriculture [USDA], Agricultural Research Service [ARS], Beltsville, MD) was used throughout this study. The two other strains used, *Salmonella* Typhimurium SL1344 (calf isolate) and *Salmonella* Thompson RM1987 (isolate from patient during a cilantro-associated salmonellosis outbreak), also were transformed with pGT-KAN (Dr. M. Brandl, USDA, ARS, Western Regional Research Center [WRRC], Albany, CA). All three strains were used to determine the survival and proliferation of *Salmonella* in tomato internal tissues. Before experimentation, the bacterial stock was streaked on tryptic soy agar plates (BD, Sparks, MD) and incubated at 35°C overnight. A single colony of this culture was then inoculated into tryptic soy broth (TSB; BD) containing appropriate antibiotics and incubated overnight at 35°C with shaking. Bacterial cells were harvested by centrifugation, washed once in phosphate-buffered saline (PBS; Fisher Bioreagents, Fair Lawn, NJ), and then suspended in PBS. The cells were directly diluted in distilled water to approximately 10^5 CFU/ml for inoculation.

Tomato harvesting and preparation. Mature green tomatoes (*Lycopersicon esculentum* Mill. cv. Sun Bright) at the breaker stage were harvested from a local farm in Beltsville, MD, and used within 24 h. Fruits were harvested by clipping the petiole about 3 cm from the calyx so that part of the stem remained on the fruit until testing. Tomatoes were visually inspected, and those with visible defects or damage were excluded. Efforts were made to maximize the uniformity of the size and shape of tomato samples. All tomatoes were incubated overnight at 90°F (32.2°C) to ensure uniform tomato pulp temperature before inoculation.

Tomato inoculation. The effect of temperature differential between *Salmonella* suspension and tomato pulp was assessed by immersing tomato fruits in distilled water containing the *Salmonella* strains at approximately 10^5 CFU/ml for inoculation at designated temperatures while maintaining constant immersion depth and time. Water temperature was maintained at 60, 80, 90, and 100°F (15.6, 26.7, 32.2, and 37.8°C) before inoculation to attain the desired temperature differentials of −30, −10, 0, and 10°F (−16.7, −5.6, 0, and 5.6°C) between inoculum solution and tomato pulp. The effect of immersion time was examined by immersing tomatoes in the inoculum solution of *Salmonella* Newport for 2, 5, 12, and 15 min at a temperature differential of −10°F. The effect of immersion depth was assessed by immersing tomatoes in the *Salmonella* Newport inoculum solution at depths of 6, 12, and 24 cm for 2 min at a temperature differential of −10°F. The effect of immersion time on depth of *Salmonella* internalization in tomato tissue was determined by immersing tomatoes in the *Salmonella* Newport inoculum solution at an immersion depth of 12 cm for 2, 5, 10, and 15 min at a temperature differential of −10°F. The survival and growth of the internalized cells of the three *Salmonella* strains was evaluated by immersing tomatoes in respective inoculum solutions at a depth of 12 cm for 15 min with a temperature differential of −10°F.

Postinoculation storage of tomatoes. After immersion in the inoculum solutions for 2 min at a temperature differential of −10°F, the tomatoes were surface cleaned by spraying with 75% ethanol (stem scar pointing down) and wiped with ethanol-saturated towelettes. Tomatoes were individually stored in Ziploc bags (S.C. Johnson & Sons, Inc., Racine, WI) at 55 and 70°F (12.8 and 21.1°C). Tomato samples were removed on days 0, 7, and 14 for microbiological analyses.

Sampling of tomato internal tissues. *Salmonella* cells from internal tomato tissues were sampled and enumerated according to the procedure described by Xia et al. *(26)*. Each tomato (stem scar pointing down) was first sprayed with 75% ethanol and wiped with ethanol-saturated towelettes to ensure that the tomato skins were free of *Salmonella*. A cylindrical plug of tomato tissue approximately 2 to 32 mm beneath the stem scar was then excised with a cork borer (11 mm in diameter). To examine the depth of *Salmonella* internalization in tomato tissue, the excised tomato tissue was cut into 5-mm sections. Five sections proximal to the stem scar were collected as subsamples. Positive and negative controls were included in each trial.

Microbiological analysis. The internal tissue samples were placed in a filtered stomacher bag and mixed with TSB supplemented with 50 μg/ml kanamycin (*Salmonella* Newport and *Salmonella* Typhimurium) or 50 μg/ml gentamicin (for *Salmonella* Thompson). The samples were macerated for 1 min with a stomacher blender (Seward 80 Biomaster, Brinkmann Seward, Mississauga, Ontario, Canada). *Salmonella* populations in the filtrate were enumerated with a microplate-based eight-well most-probable-number (MPN) method as previously described *(26)*. The MPN for each sample or subsample was determined with MPN calculator software *(12)*.

Experimental design and statistical analysis. Randomized factorial designs were employed for all experiments with three to

TABLE 1. *Effect of temperature differential on the internalization of* Salmonella enterica *Newport into tomatoes immersed in inoculant for 2 or 15 min at a depth of 12 cm*[a]

Temp differential[b]		Incidence (%)		Population (log MPN/g)	
°F	°C	2 min	15 min	2 min	15 min
−30	−16.7	40.00 a A	93.33 b A	0.78 a A	3.11 b A
−10	−5.6	26.67 a A	86.67 b A	0.06 a A	2.59 b AB
0	0	33.33 a A	60.00 b A	0.44 a A	1.22 b C
10	5.6	26.67 a A	73.33 b A	0.06 a A	1.69 b BC

[a] Within each row and immersion time, values with different lowercase letters are significantly different at $\alpha = 0.05$ using Sidak-adjusted P values ($n = 15$). Within each column, values with different uppercase letters are different at $\alpha = 0.05$ using Sidak-adjusted P values ($n = 15$).

[b] Temperature difference between the *Salmonella* suspension and tomatoes.

six replications, except for the immersion depth study. Because of the large variation among replications, the immersion depth study experiment was conducted 10 times to improve the statistical power under such conditions. The incidence (frequency) data were arcsine transformed and the MPN data were log transformed to meet the requirement for normality and variance homogeneity. Statistical analyses for all experiments were performed using the Proc Mixed model (GLM) of SAS 9.3 (SAS Institute Inc., Cary, NC). When effect(s) were significant, mean comparisons were performed with Sidak-adjusted P values to maintain the experimentwise error (α) at 0.05.

RESULTS

Effect of temperature differential and immersion time on the internalization of *S. enterica* into tomatoes. The effect of the temperature differential between wash water and tomatoes on the incidence of *Salmonella* internalization and on the populations of the internalized bacteria was examined using *Salmonella* Newport with immersion times of 2 and 15 min and temperature differentials of −30, −10, 0, and 10°F (−16.7, −5.6, 0, and 5.6°C). Immersion time significantly affected ($P < 0.0001$) the incidence of internalization and the populations of the internalized bacteria, and there was a significant interaction ($P = 0.0179$) between immersion time and temperature differential for the populations of internalized bacteria but not for the incidence of internalization ($P = 0.2536$) (Table 1). Tomatoes immersed for 15 min had consistently higher internalization incidence (60 to 93%) and larger internalized population (1.69 to 3.11 log CFU/g) than did tomatoes immersed for 2 min (27 to 40% and 0.06 to 0.78 log CFU/g) regardless of the temperature differential. However, the effect of temperature differential on *Salmonella* internalization was significantly affected by immersion time. When immersed for 2 min, no difference was observed in either internalization incidence or bacterial population for temperature differentials of −10, 0, and 10°F. This observation was consistent with the findings of Xia et al. *(26)*. Although a noticeable increase in internalization incidence and internalized bacteria population occurred when the temperature differential was increased to −30°F, the difference did not reach the significant level ($P = 0.1160$) for the 2-min immersion time. However, when the immersion time was increased to 15 min, the effect of temperature differential on *Salmonella* internalization and the population of the internalized bacteria was more pronounced. The highest incidence of internalization (93.3%) and highest population of internalized *Salmonella* (3.11 log CFU/g) were observed with the combination of the widest temperature differential (−30°F) and longest immersion time (15 min).

Given the significant effect of immersion time observed in this study, additional experiments were conducted to evaluate the effect of immersion time at 2, 5, 10, and 15 min. *Salmonella* internalization incidence was significantly affected by immersion time ($P = 0.0228$), with a positive linear correlation between immersion time and internalization incidence (Fig. 1A). A similar correlation also was found between immersion time and internalized population (Fig. 1B).

Immersion time also significantly affected ($P < 0.0001$) the distribution of internalized *Salmonella* cells (Fig. 2). With immersion times of 2 to 5 min, the internalization was localized within the core tissues immediately underneath the stem scars (up to 7 mm), with a population density of less than 0.5 log CFU/g. When immersion time was extended to 10 and 15 min, *Salmonella* cells infiltrated further into the internal tissues to depths of up to 22 and 27 mm, respectively, beneath the stem scar, and the internalized population significantly increased, with cell density exceeding 3.0 log CFU/g after an immersion time of 15 min.

Effect of immersion depth on the internalization of *S. enterica* into tomatoes. The incidence of S*almonella* internalization and the S*almonella* populations were similar at immersion depths of 6 and 12 cm (Fig. 3). Although increasing the immersion depth to 24 cm increased both the incidence of internalization and populations of the internalized cells, the difference was not significant ($P = 0.3098$) compared with the numbers for immersion depths of 6 and 12 cm.

Fate of internalized *S. enterica* cells in tomatoes. The effect of storage temperature and duration on pathogen survival and growth differed significantly among the *Salmonella* strains (Table 2). In general, the detection of *Salmonella* in tomato tissue was not significantly affected by the storage temperature ($P > 0.05$) or storage duration ($P > 0.05$), with the exception of *Salmonella* Typhimurium stored at 55°F (12.8°C). However, the populations of *Salmonella* inside tomatoes were significantly affected by storage duration ($P < 0.05$) but not significantly affected by the storage temperature ($P > 0.05$), with the exception of *Salmonella* Thompson on day 7. The cell populations of *Salmonella* Newport decreased at both 55 and 70°F (21.1°C) by 1.52 and 1.03 log CFU/g, respectively, by the end of the 14-day storage period, whereas those of *Salmonella* Thompson significantly increased during the same storage time, from 2.11 to 5.68 log CFU/g at 55°F and

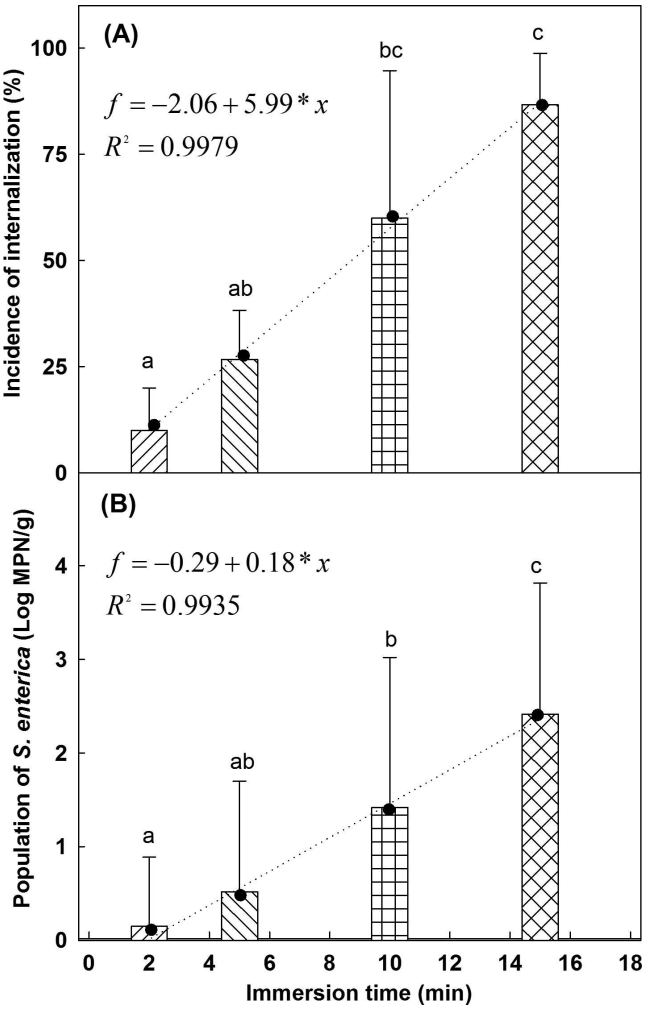

FIGURE 1. *Effect of immersion time on the internalization of* S. enterica *Newport FDA2757 into tomato tissues with a temperature differential of −10°F (5.6°C) and an immersion depth of 12 cm. Data are the average of the replicates with five tomatoes per treatment per replicate. Different lowercase letters above bars indicate significant differences. (A) Incidence of internalization (%); (B) internalized* Salmonella *population (log MPN per gram). The trend line indicates the linear relationship between the incidence of internalization or the population and immersion time.*

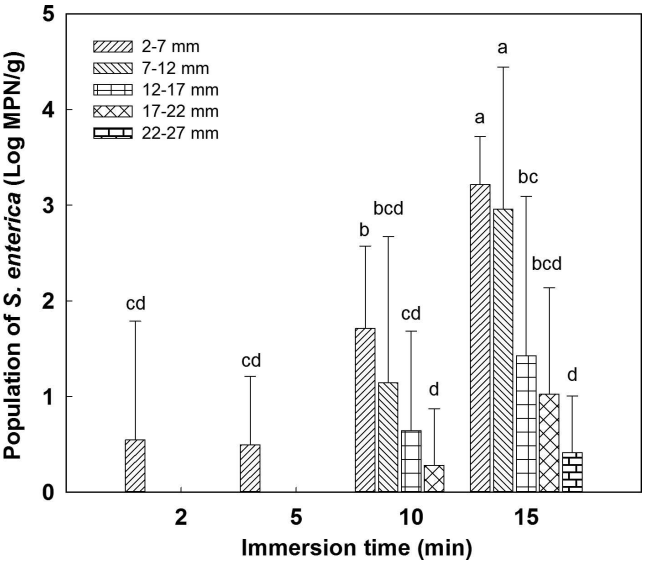

FIGURE 2. *Effect of immersion time on the internalization depth and population size of* S. enterica *Newport FDA2757 into tomato tissues with a temperature differential of −10°F (5.6°C) and an immersion depth of 12 cm. Each bar represents the average of six replicates with five tomatoes per replicate. Different lowercase letters above bars indicate significant differences.*

to 4.02 CFU/g at 70°F ($P < 0.05$). However, no significant difference was observed for *Salmonella* Typhimurium populations during the entire storage period at either 55 or 70°F, although a general increase in pathogen populations was observed at 55°F.

DISCUSSION

Tomatoes are usually washed in a dump tank or flume after harvesting, before shipping or long-term storage. When submerged, tomato fruits are vulnerable to the uptake of wash water and foreign material, including microorganisms, through the stem scars *(2–6, 8)* by hydrostatic pressure, vacuum induced by a temperature differential, or capillary action. This uptake process is affected by the inherent structure of the tomato vascular tissues and environmental or operational conditions *(22, 26)*.

Previous studies regarding the development of tomato soft rot revealed that the temperature differential between the dump tank water and the tomato pulp was a critical factor affecting pathogen internalization during packing-house operations *(5)*. Bartz and Showalter *(8)* reported that soft rot decay caused by *Erwinia carotovora* occurred at higher rates in fruits immersed in a bacterial suspension under a negative temperature differential. However, most such studies linked temperature differential to water uptake and soft rot development, and no studies available that specifically examined the effect of temperature differential on *Erwinia carotovora* internalization incidence and bacterial populations. Xia et al. *(26)* found a high incidence of internalization and low cell populations in tomatoes regardless of whether the temperature differential was −10 or 10°F (5.6°C). In the current study, the range of temperature differentials was expanded to include −30°F (−16.7°C). However, the temperature differential did not have a significant impact on pathogen internalization at the current recommended immersion time of 2 min or less, and *Salmonella* internalization was observed in all treatments, including those with a 10°F positive temperature differential. These observations suggest that raising the dump tank wash water temperature to 10°F above that of the incoming tomatoes would not be an effective approach to preventing *Salmonella* internalization. Therefore, maintaining adequate sanitizer concentrations in the dump tank wash water is critically important for minimizing pathogen survival and preventing internalization of pathogens in tomatoes *(27)*.

Tomato immersion depth has been considered an important factor for development of soft rot *(5, 6)*. Bartz *(6)* found that the amount of water uptake by tomatoes was a function of the immersion depth. However, no information has been available regarding the effect of immersion depth

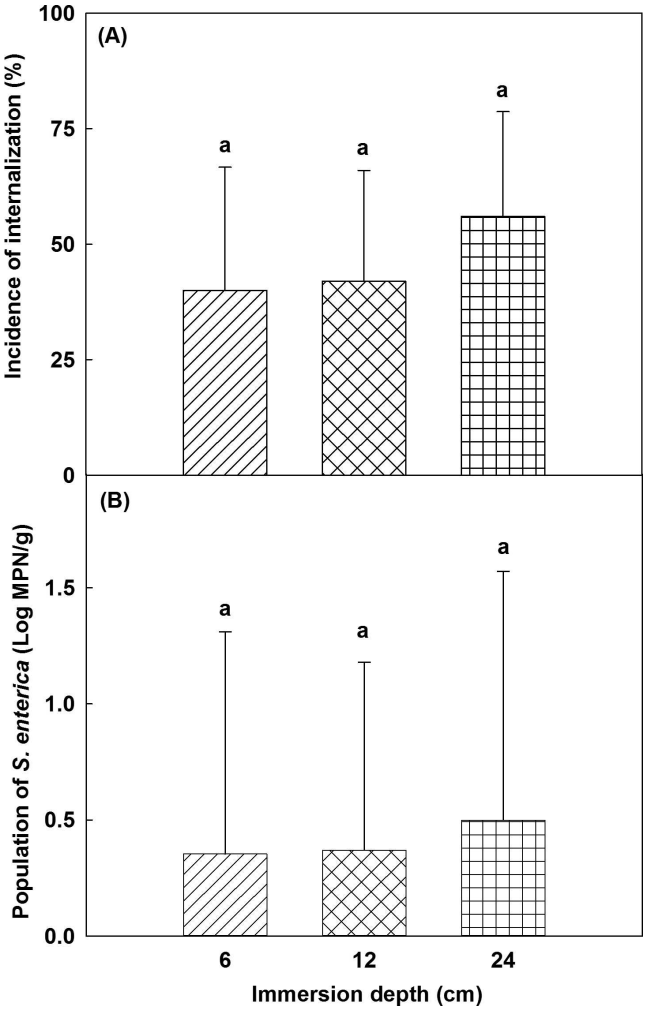

FIGURE 3. *Effect of immersion depth on the internalization of* S. enterica *Newport FDA2757 into tomato tissues with a temperature differential of −10°F (5.6°C) and an immersion time 2 min. Data are the average of 10 replicates with five tomatoes per replicate. Different lowercase letters above bars indicate significant differences. (A) Incidence of internalization (%); (B) internalized* Salmonella *population (log MPN per gram).*

on internalization of *Salmonella*. The present findings indicate that although the average incidence of *Salmonella* internalization and the size of the internalized population increased with increasing immersion depth, the effect of immersion depth on either the incidence of *Salmonella* internalization or the population of the internalized bacteria was not significant. The range of immersion depths in this study was chosen to simulate that found in commercial dump tank operations. Depths beyond this range could have a greater effect on *Salmonella* internalization but are not relevant to commercial practices.

Immersion time is another important factor to be considered for reducing the development of soft rot. However, to our knowledge no studies have been conducted to examine the effect of immersion time on the internalization of human pathogens in tomatoes. In the present study, we found that immersion time had a significant effect on *Salmonella* internalization, and a linear correlation was found between immersion time and *Salmonella* internalization. Immersion time also affected the depth of *Salmonella* penetration. Reducing the immersion time reduced the infiltration incidence and the population of the internalized bacteria and limited the distribution of the internalized bacterial cells to the tissue immediately beneath the stem scars rather than deep inside the tomatoes. These result and those of Xia et al. *(26)* indicate that immersion time could be the most important factor that influences *Salmonella* internalization in tomatoes. Although immersion times shorter than 2 min were not tested in this study, the linear trend observed between immersion time and *Salmonella* internalization indicates that *Salmonella* internalization problems could be further reduced by shortening the immersion time. Personal communication with tomato industry personnel and on-site survey at the tomato packing facilities suggest that tomato washes with a 30-s residence time can be accomplished in most of the existing dump tank or flume systems. Therefore, reducing the immersion time could be a cost-effective measure for minimizing *Salmonella* infiltration. Additional studies in this field are warranted.

TABLE 2. *Fate of internalized* Salmonella enterica *in tomatoes stored at 55 and 70°F*[a]

		Incidence (%)		Population (log MPN/g)	
Salmonella strain[b]	Expt day	55°F (12.8°C)	70°F (21.1°C)	55°F (12.8°C)	70°F (21.1°C)
Newport FDA 2797	0	85.00 a A	85.00 a A	2.68 a A	2.68 a A
	7	70.00 a A	65.00 a A	1.57 a AB	1.51 a B
	14	55.00 a A	70.00 a A	1.16 a B	1.65 a AB
Thompson RM1987	0	80.00 a A	80.00 a A	2.11 a B	2.11 a B
	7	85.00 a A	100.00 a A	3.51 b A	4.64 a A
	14	85.00 a A	70.00 a A	5.68 a A	4.02 a AB
Typhimurium SL1344	0	85.00 a AB	85.00 a A	2.55 a A	2.55 a A
	7	100.00 a A	85.00 a A	3.79 a A	2.49 a A
	14	75.00 a B	70.00 a A	3.90 a A	2.83 a A

[a] Within each row and temperature, values with different lowercase letters are significantly different at $\alpha = 0.05$ using Sidak-adjusted P values ($n = 20$). Within each column, values with different uppercase letters are different at $\alpha = 0.05$ using Sidak-adjusted P values ($n = 20$).

[b] Samples were analyzed for each strain separately.

The effect of storage temperature and duration on *Salmonella* growth on the surface of tomatoes has been well studied *(9, 17, 25, 27)*, but information on the growth of *Salmonella* inside tomatoes is limited. *Salmonella* growth patterns were serovar dependent in our study, in agreement with the results of Shi et al. *(21)*. Strain differences in adaptation to the environment may explain the differential growth inside tomatoes. In our study, the growth of *Salmonella* was much more moderate than that reported by Shi et al. *(21)*. Differences in the inoculation conditions in these two studies may have contributed to the observed differences. Shi et al. *(21)* introduced *Salmonella* cells into tomatoes via a strong negative temperature differential and three vacuum-release cycles. The tissue injuries sustained during that process and the accompanying nutrient release may have provided additional support for *Salmonella* growth. The depth of *Salmonella* internalization also may have affected *Salmonella* growth. The distribution of internalized *Salmonella* cells is highly dependent on immersion time. With shorter immersion times, internalized cells predominately were found proximal to the stem scars, where the tissue mainly consists of corky vascular bundles with low nutrient availability, whereas longer immersion times resulted in *Salmonella* internalization into deeper, more nutrient-rich tomato tissues despite the relatively low pH *(1, 9)*. The results of the present study suggest that *Salmonella* cells internalized into tomatoes under common commercial processing conditions are capable of long-term survival and moderate growth. These observations highlight the importance of preventing pathogen internalization during postharvest processing.

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