Internalization of Bacterial Pathogens in Tomatoes and Their Control by Selected Chemicals

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ABSTRACT

The effect of different washing or sanitizing agents was compared for preventing or reducing surface and internal contamination of tomatoes by Salmonella Typhimurium and Escherichia coli O157:H7. The tomatoes were inoculated by dipping them in a bacterial suspension containing approximately 6.0 log CFU/ml of each pathogen and then rinsing them with tap water, hypochlorite solution (250 mg/liter), or lactic acid solution (2%, wt/vol). All treatments were applied by dipping or spraying, and solutions were applied at 5, 25, 35, and 55°C. With the exception of the lactic acid dip at 5°C, all treatments reduced both pathogens on the surfaces of the tomatoes by at least 2.9 cycles. No significantly different results were obtained (P > 0.05) with the dipping and spraying techniques. For internalized pathogens, the mean counts for tomatoes treated with water alone or with chlorine ranged from 0.8 to 2.1 log CFU/g. In contrast, after lactic acid spray treatment, all core samples of tomatoes tested negative for Salmonella Typhimurium and, except for one sample with a low but detectable count, all samples tested negative for E. coli O157:H7 with a plate count method. When the absence of pathogens was verified by an enrichment method, Salmonella was not recovered from any samples, whereas two of four samples tested positive for E. coli O157:H7 even though the counts were negative. Few cells of internalized pathogens were able to survive in the center of the tomato during storage at room temperature (25 to 28°C). The average superficial pH of tomatoes treated with tap water, chlorine, or lactic acid was 4.9 to 5.2, 4.1 to 4.3, and 2.5, respectively (P < 0.05), whereas no differences were observed in the internal pH (3.6 to 3.7) of the tomatoes treated with different sanitizers. The general practice in the tomato industry is to wash the tomatoes in chlorinated water. However, chlorine is rapidly degraded by organic matter usually present in produce. Therefore, lactic acid sprays may be a more effective alternative for decontaminating tomato surfaces. The use of warm (55°C) sprays could reduce pathogen internalization during washing.

Current research indicates that daily ingestion of at least five servings of fruits and vegetables is correlated with decreased risk of cardiovascular disease, stroke, and high blood pressure, which are all important factors associated with heart attacks (5, 15, 20, 25, 29). This information has prompted increased sales of fresh produce and an increased consumption of minimally processed fruits and vegetables in the United States. Although increased consumption of fruits and vegetables can result in better heart health, this same eating behavior may result in acquisition of foodborne diseases. Outbreaks of different diseases have been associated with consumption of fresh fruits and vegetables (27).

Tomatoes have been involved with four multistate outbreaks of Salmonella infection (9, 11, 18). In some of these outbreaks, contamination has been traced to the packing house, where tomatoes are normally dumped in a common water bath for washing (18). Dip washing tomatoes may result in the diffusion of water to the interior of the fruit. If the wash water or the surface of the tomatoes is contaminated, the microorganisms may also contaminate the interior tissues of the product, causing faster product decay or a public health hazard, depending on the type of microorganisms involved (2, 4). Even if pathogens are located on the tomato surface only, these pathogens can be transferred to the flesh during further handling or cutting (22) and can survive or even grow, further increasing the risk for the consumer (1). Disinfecting treatments can be applied after washing to reduce bacterial pathogens (31), and these treatments may also have a positive impact on quality and shelf life by reducing spoilage organisms. However, these treatments are intended to reduce microorganisms on the surface. If the microorganisms are located in the interior of the product, even just underneath the skin, they may not be reached by the disinfectant or the disinfectant may be inactivated if it is sensitive to organic matter (28, 30).

Pathogen internalization has been reported to occur in nonbruised tomatoes (32), apples (6), and oranges (14). This internalization is often due to a noncontrolled wash or precleaning treatment applied to warm produce in cold water. This treatment creates a transient differential in pressure that can result in the diffusion of the wash water and bacteria into the product. Bruises or postharvest diseases in tomatoes increase the potential for water internalization during washing, and therefore packers recommend chlorinating the wash water. Bartz (2) reported a reduction in postharvest diseases in tomatoes by adding 50 to 1,000 ppm chlorine to the water used for dip washing.

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The objective of this study was to determine the potential for bacterial pathogens located on the surface of tomatoes to move to the interior of the fruit and the effectiveness of chlorine or lactic acid washes in reducing internalized pathogens.

MATERIALS AND METHODS

Source of tomatoes. Ripe field tomatoes (Lycopersicon esculentum) were provided by a nearby grower. The fruits were harvested, immediately placed in cardboard boxes without applying any washing or waxing, and transported to the laboratory at room temperature. In the laboratory, the tomatoes were stored overnight at room temperature so that treatments were applied no later than 24 h after harvesting.

Bacterial cultures. Test organisms were rifampicin-resistant mutants derived from parent strains of Salmonella serovar Typhimurium (ATCC 13311) and Escherichia coli O157:H7 (provided by Gary Acuff, Texas A&M University) and were maintained on tryptic soy agar (TSA; Becton Dickinson, Mexico City, Mexico) slants at 4°C until use. Three days before use, each microorganism was resuscitated by two subsequent transfers to fresh TSA slants and incubated at 35°C for 24 h. Rifampicin resistance was then confirmed by streaking each culture onto a separate plate of lactose sulfite phenol red rifampicin (LSPR) agar, which was previously developed for differential enumeration of rifampicin-resistant Salmonella and E. coli (8), and incubated at 35°C for 24 h. The genus of the rifampicin-resistant colonies was then confirmed by biochemical tests (13), and cultures were transferred to individual flasks with 1 liter of sterile tryptic soy broth (Becton Dickinson) and incubated at 35°C for 18 h.

Inoculum preparation and inoculation of tomatoes. On the day of the experiment, the contents of the flasks containing 18-h cultures of rifampicin-resistant Salmonella Typhimurium and E. coli O157:H7 were poured into a sanitized tub, and 8 liters of sterile 0.1% peptone water at room temperature (26 to 28°C) was added. Mean plate counts of the resulting 10-liter suspension were 7.1 and 7.9 log CFU/ml for added. Mean plate counts of the resulting 10-liter suspension were 7.1 and 7.9 log CFU/ml for

Surface decontamination of tomatoes. Inoculated tomatoes were separated into groups of four units. The control group was not subjected to any treatment, and the counts obtained in this group were used to calculate log reductions resulting from the treatments. The other groups were subjected to one of the following treatments: water wash applied with a hand-held noncorrosive polyethylene compressed-air sprayer (Champ model RB 202, Chapin Co., Batavia, N.Y.) for 1.5 min; water wash followed by lactic acid dip at 5, 20, 35, or 55°C; water wash followed by lactic acid spray at 5, 20, 35, or 55°C; water wash followed by chlorine dip at 5, 20, 35, or 55°C; water wash followed by chlorine spray at 5, 20, 35, or 55°C. For lactic acid treatments, a 2% (wt/vol) solution was prepared by mixing 88% L-lactic acid (Purac, Inc., Arlington Heights, Ill.) with enough distilled water to achieve a 2% lactic acid concentration. The water had been previously adjusted to the desired temperature. For chlorine treatments, 6.25% hypochlorite (Clorox Co., Mexico City, Mexico) was mixed in previously tempered water to obtain a concentration of 250 mg/liter. For dip treatments, the solutions at each temperature, either 2% L-lactic acid or 250 mg/liter hypochlorite, were immediately placed in a sanitized Nalgene tub (Nalge Nunc International, Rochester, N.Y.), where the corresponding groups of tomatoes were immediately submerged and gently agitated for 15 s. For spray treatments, 250 ml of each solution was sprayed onto the corresponding group of tomatoes, hand rotating the tomatoes during the spraying to ensure an even distribution of the solution over the surface of the fruits. All spray treatments were applied with a hand-held compressed-air sprayer (Champ model RB 202) at 69 kPa for 15 s, the time necessary under the spraying conditions to deliver 250 ml of solution over each four-tomato group.

Surface sampling. Surface samples for microbiological analysis were obtained by cutting a 10-cm² outline area using a sterile borer (approximately 2 to 3 mm deep), followed by excising the surface sample at a depth of ≤2 mm using a sterile scalpel and forceps. This approach was used instead of sampling the whole tomato to prevent any possible underestimation of counts due to attached cells or any effect of uneven bacterial accumulation in the stem scar, which might have interfered with the treatment comparison. Therefore, all surface samples were collected from the smooth skin and no attempt was made to sample from different anatomic regions of the fruit. From every tomato subjected to each treatment, three surface samples were excised and blended together for 1 min in a stomacher bag (Stomacher 400, Tekmar Co., Cincinnati, Ohio) to which 100 ml of sterile 0.1% peptone water had been added. The resulting suspension was used to prepare decimal dilutions for microbial counts.

Pathogen internalization. Inoculated tomatoes were separated into five groups, which were assigned to one of the following treatments: control, water wash at 5°C, water wash at 55°C, water wash (room temperature) followed by lactic acid spray at 5 or 55°C, and water wash (room temperature) followed by chlorine spray at 5 or 55°C. To ensure that tomatoes in the control group became contaminated internally, 0.1 ml of a suspension containing ca. 6.0 log CFU/ml of each pathogen was injected with a sterile insulin syringe through the stem scar into all control tomatoes. After applying the treatments, tomatoes were sampled for enumeration of internalized pathogens. Prior to sampling, all tomatoes were dipped in 95% ethanol and flamed three times to reduce superficial pathogens and reduce the chances for cross contamination from organisms located on the surface. After flaming, no contamination was detected in a set of superficial samples collected as a control. To enumerate internalized bacteria, a core sample was obtained from each tomato using a sterile borer with a sharp end to cut through the tomato from the stem scar. The core sample was extracted from the rest of the fruit and cut three times on both ends with a sterile scalpel. A new sterile scalpel was used each time the ends were cut. The resulting core sample, weighing approximately 10 g, was placed in a stomacher bag and weighed, and 90 ml of sterile 0.1% peptone water was added. Serial dilutions then were prepared for pathogen enumeration. After preparing the dilutions, the remaining contents of the stomacher bag were transferred to a sterile Nalgene bottle (Nalge containing lactose broth, incubated at 35°C for 18 to 24 h, and plated onto LSPR plates to determine whether pathogens were still detectable in samples where the enumeration method did not yield a detectable count.

Survival and growth of internalized Salmonella Typhimurium and E. coli O157:H7 in tomatoes sprayed with lactic acid. A set of tomatoes was subjected to dip inoculation and separated into two groups. One group was left untreated (control) and...
the other group was treated with a 55°C 2% t-lactic acid spray. All of these tomatoes were then stored at room temperature. At 0, 12, 24, 36, 48, 60, and 72 h of storage, a core sample was obtained from four tomatoes within each treatment group. The core sample was placed in a stomacher bag with sterile 0.1% peptone water for pathogen enumeration.

Analysis of samples. Counts of rifampicin-resistant Salmonella Typhimurium and E. coli O157:H7 were determined by plating appropriate dilutions of each sample onto plates of LSPR agar. The plates were incubated at 35°C for 26 h, and differential counts of colonies were then determined for each pathogen. The identity of a representative number of colonies characteristic of each pathogen was confirmed by standard biochemical tests (13). The pH was tested using a portable pH meter (Model 612, Markson Science, Inc., Phoenix, Ariz.).

Data analysis. All experiments were conducted with four replicates. Bacterial counts for each pathogen were converted to log values and then entered into an analysis of variance (ANOVA) using the PROC GLM of SAS 6.10 (SAS Institute, Inc., Cary, N.C.). When the ANOVA indicated a significant difference between treatments, mean separation was achieved using Duncan’s multiple range test.

The populations of Salmonella Typhimurium and E. coli O157:H7 on the surface of tomatoes after inoculation were 3.1 and 3.7 log CFU/cm², respectively. These populations were significantly reduced (P < 0.05) by either lactic acid or chlorine washes to levels below or close to the detection limit of the plating method, except for lactic acid dip at 5°C (Fig. 1). A water wash alone reduced the populations of both pathogens by 0.6 to 0.9 log cycles. Additional reductions of approximately 1 log cycle were obtained by further dipping in 2% lactic acid at 5°C. All other treatments produced between 3 and >3.1 log reductions in pathogen populations on the tomato surface. All chlorine treatments reduced the pathogen populations to undetectable levels, but a few tomatoes treated with lactic acid still contained detectable pathogens at <1.0 log CFU/cm² (Fig. 1). Although these results seem to indicate that chlorine was more effective than lactic acid at reducing pathogens on the smooth skin of tomatoes, a more accurate conclusion is that the effectiveness of chlorine and lactic acid was comparable. With the exception of the lactic acid dip at 5°C, all

RESULTS AND DISCUSSION

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other lactic acid treatments reduced the populations of both pathogens to undetectable levels or levels close to the detection limit of the counting method and were not significantly different from the reduction obtained with chlorine treatments ($P > 0.05$). Reduced antimicrobial activity of organic acids at low temperatures has been reported (10, 26) and is consistent with the results of this study. Therefore, a cold lactic acid treatment is not recommended.

In general, *E. coli* showed greater resistance to lactic acid than did *Salmonella* Typhimurium, a finding consistent with those of various reports on the acid tolerance of this organism (16, 19, 23). However, the method of treatment seemed to have some influence on bacterial reduction by lactic acid treatment. At 5°C, spraying with lactic acid reduced the numbers of both pathogens to undetectable or close to undetectable levels, whereas the counts obtained after dipping were 1.0 and 1.9 log CFU/cm² for *Salmonella* Typhimurium and *E. coli* O157:H7, respectively. When lactic acid was applied at 55°C, the mean count of *E. coli* O157:H7 on dip-treated tomatoes was 0.8 log CFU/cm², whereas this pathogen was not detectable on sprayed tomatoes. Although the antimicrobial effect of lactic acid should be the same regardless of the treatment method, the spray apparently produced the double effect of both killing microorganisms on the surface and washing them off, which could have resulted in a greater reduction of bacterial counts on sprayed tomatoes compared with dipped tomatoes. Figure 2 shows the populations of *Salmonella* Typhimurium and *E. coli* O157:H7 detected inside tomatoes after the application of water wash, lactic acid spray, or chlorine rinse at 5 or 55°C. After the lactic acid sprays, all core samples of tomatoes tested negative by a plate count method for *Salmonella* Typhimurium, and except for one sample with low but detectable count, all samples also tested negative for *E. coli* O157:H7. When the enrichment method was used for qualitative testing, *Salmonella* Typhimurium was not recovered from any sample, whereas two of four samples tested positive for *E. coli* O157:H7 by this method even though the counts were negative (data not shown). In contrast to the results for tomatoes treated with lactic acid, pathogens were consistently found in the core samples of tomatoes treated with water wash or chlorine, by either quantitative or qualitative methods. The data in Figure 2 indicate the potential for bacteria to diffuse into susceptible produce such as tomatoes while the product is immersed in contaminated water, even though the water temperature is similar to the temperature of the product. Initially, we hypothesized that the internal pH of the tomato is affected by the lactic acid, adding a continued antimicrobial effect against internalized bacteria, whereas chlorine does not lower the internal pH of the product and is inactivated by the organic matter inside the tomatoes. However, the pH of tomato core samples did not differ between treatment groups (Table 1), demonstrating that internal pH was not changed by the spray solution. The only instance where a significant difference was observed was after 60 h of storage for the core pH of tomatoes subjected to water wash and those subjected to lactic acid spray. The pH difference in these samples was 0.19 pH units. Although statistically significant, this slight difference does not seem to be of biological significance and is inconsistent with the general trend in pH for all other samples. In contrast, Figure 3 shows large differences in the surface pH of tomatoes treated with a plain water wash, chlorine, or a lactic acid wash. From the data in Table 1 and in Figure 3, we concluded that even if some lactic acid diffuses into the tomatoes during spraying, it does not seem to be enough to reduce the pH. The lack of recovery of internalized pathogens in tomatoes sprayed with lactic acid may be due to fast bacterial reduction on the surface accelerated by the reduction of the superficial pH to values close to the $pK_a$ of lactic acid, which might have prevented live bacteria from diffusing into the tomatoes, whereas free chlorine might have been rapidly reduced by contact with inside and outside tomato structures. Although a pressure differential has been reported to promote bacterial diffusion in tomatoes when they are warmer than the washing solution (3, 32), it is unlikely that pressure played a role in increasing the exposure of the pathogens to the lactic acid, because similar results were observed with lactic acid spray at 5 and 55°C. The antibacterial capacities of chlorine and lactic acid seem to be similar, as determined by Martínez-Cárdenas (24),

<table>
<thead>
<tr>
<th>Storage (h)</th>
<th>Control$^b$</th>
<th>Water wash$^c$</th>
<th>Water wash + lactic acid spray$^d$</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>3.71 ± 0.15 A</td>
<td>3.64 ± 0.06 A</td>
<td>3.68 ± 0.07 A</td>
</tr>
<tr>
<td>12</td>
<td>3.70 ± 0.10 A</td>
<td>3.72 ± 0.10 A</td>
<td>3.76 ± 0.07 A</td>
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<tr>
<td>24</td>
<td>4.24 ± 0.95 A</td>
<td>4.26 ± 0.08 A</td>
<td>4.23 ± 0.08 A</td>
</tr>
<tr>
<td>36</td>
<td>4.12 ± 0.06 A</td>
<td>4.19 ± 0.06 A</td>
<td>4.28 ± 0.16 A</td>
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<tr>
<td>48</td>
<td>4.07 ± 0.05 A</td>
<td>4.17 ± 0.09 A</td>
<td>4.18 ± 0.10 A</td>
</tr>
<tr>
<td>60</td>
<td>4.12 ± 0.08 AB</td>
<td>4.07 ± 0.05 A</td>
<td>4.26 ± 0.11 B</td>
</tr>
<tr>
<td>72</td>
<td>4.25 ± 0.11 A</td>
<td>4.13 ± 0.11 A</td>
<td>4.24 ± 0.13 A</td>
</tr>
</tbody>
</table>

$^a$ Values with same letter within rows are not significantly different ($P > 0.05$).

$^b$ No treatment applied to tomatoes.

$^c$ Tap water sprayed for 90 s over each four-tomato group.

$^d$ Two hundred fifty milliliters of 2% L-lactic acid solution at 55°C sprayed for 15 s over each four-tomato group.
who reported no differences in the reduction of *Salmonella* Typhimurium and *E. coli* O157:H7 on the smooth epidermis of tomatoes or bell peppers after spraying with 2% lactic acid solution or 200 mg/liter hypochlorite solution. Further research should allow formulation of a more complete explanation for the almost total absence of internalized pathogens in tomatoes subjected to lactic acid sprays, in contrast to the consistent detection of internalized bacteria in tomatoes treated with chlorine or tap water.

Data in Table 2 document the survival of *Salmonella* Typhimurium and *E. coli* O157:H7 internalized in tomatoes during storage at room temperature. Counts of *Salmonella* Typhimurium and *E. coli* O157:H7 seemed to increase inside nonsanitized tomatoes up to 60 h of storage at room temperature. For tomatoes that were treated with lactic acid, internalized pathogens were only occasionally detected. However, despite the strong antibacterial effect of lactic acid, a few cells may diffuse into the tomatoes and survive for relatively long periods of time.

The results of this study indicate the potential for pathogenic bacteria to diffuse into the interior of tomatoes that have been dipped in a bacterial suspension and for these bacteria to survive inside the tomatoes for at least 72 h at room temperature. Survival and growth of *Salmonella* inside or on the surface of tomatoes has been previously reported (17, 32). Lactic acid sprays, although commonly used in the meat industry for reducing pathogens on carcasses (7), have not been fully studied as an alternative to chlorinated water for sanitizing tomatoes or other types of produce. A few studies have indicated that organic acids such as acetic or lactic acid can be used to reduce bacterial populations on fresh or fresh-cut produce (12, 21, 30).

In this study, pathogen populations were consistently reduced in and on tomatoes by applying a warm (55°C) lactic acid spray. Because of the lower sensitivity to organic acid degradation, lactic acid sprays may be an effective alternative to the use of chlorinated water for improving the safety of tomatoes at the packing level.

**ACKNOWLEDGMENT**

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**REFERENCES**


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**TABLE 2. Counts of internalized Salmonella Typhimurium and Escherichia coli O157:H7 during 25 to 28°C storage of tomatoes with or without lactic acid treatment**

<table>
<thead>
<tr>
<th>Storage (h)</th>
<th><em>Salmonella</em> Typhimurium</th>
<th><em>E. coli</em> O157:H7</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control(^a)</td>
<td>Lactic acid(^b)</td>
</tr>
<tr>
<td>0</td>
<td>1.8 ± 0.3</td>
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<tr>
<td>12</td>
<td>3.5 ± 0.9</td>
<td>&lt;1.0 ± 0.0</td>
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<tr>
<td>24</td>
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<td>0.7 ± 0.3</td>
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<tr>
<td>36</td>
<td>2.6 ± 2.2</td>
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<td>4.4 ± 0.3</td>
<td>&lt;1.0 ± 0.0</td>
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<td>60</td>
<td>3.2 ± 1.1</td>
<td>&lt;1.0 ± 0.0</td>
</tr>
<tr>
<td>72</td>
<td>&lt;1.0 ± 0.0</td>
<td>&lt;1.0 ± 0.0</td>
</tr>
</tbody>
</table>

\(^a\) No treatment applied to tomatoes. Pathogens were inoculated internally by injecting 0.1 ml of a composite bacterial suspension containing ca. 6.0 log CFU/ml of each marker organism.

\(^b\) Two hundred fifty milliliters of 2% l-lactic acid solution at 55°C sprayed for 15 s over every four-tomato group after washing with water (tap water sprayed for 90 s at room temperature).