Inactivation of *Salmonella* on Tomato Stem Scars by Edible Chitosan and Organic Acid Coatings

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ABSTRACT

This study was conducted to investigate the efficacy of antimicrobial coatings for inactivation of *Salmonella* on the surface of tomato stem scars. Scars were inoculated with a four-strain cocktail of *Salmonella* (serovars Montevideo, Newport, Saintpaul, and Typhimurium) and coated with acid-chitosan solutions. The chitosan coating with three acids (3A plus chitosan), the chitosan coating with one acid, and the three-acid solution without chitosan reduced the populations of *Salmonella* by 6.0, 3.6, and 5.3 log CFU per stem scar, respectively. Addition of allyl isothiocyanate (10 μl/ml) to the 3A plus chitosan coating did not significantly increase (P > 0.05) the antimicrobial efficacy. Although the populations of *Salmonella* in the controls (ca. 7.5 log CFU per stem scar) did not change significantly throughout the 14-day storage period at 10°C, *Salmonella* cells were reduced to undetectable levels (<0.7 log CFU per stem scar) in the samples treated with 3A plus chitosan coating after two days of storage, and no growth was observed for the remaining storage period. Results from this study demonstrate that coatings of acid plus chitosan provide an alternative antimicrobial intervention for decontamination of tomatoes.

The number of documented outbreaks of foodborne diseases associated with the consumption of raw fruits and vegetables has increased in recent years. The most frequently identified and reported bacterial pathogen was *Salmonella*, which was associated with 18% of all outbreaks in the United States between 1998 and 2007. Among all salmonellosis outbreaks, 17 have been attributed to the consumption of contaminated tomatoes, which caused 1,684 illnesses (7).

Epidemiological investigations have revealed that tomatoes can become contaminated with *Salmonella* from a variety of sources, including irrigation water, wash water, food preparation environments, and animals (16). Once the tomatoes are contaminated, *Salmonella* can survive during postharvest storage (27) and is capable of growing to populations exceeding 10^7 CFU/g with adequate time and appropriate conditions (31, 34). Beuchat and Mann (6) determined that populations of *Salmonella* increased significantly in stem scar and pulp tissues of Roma tomatoes stored at 12 and 21°C for 14 and 27 days, respectively.

The stem scar region of the tomato has been identified as an important potential source of enteric pathogen contamination because the scar area is highly porous but sanitizers cannot effectively penetrate and inactivate pathogens harbored there (15). Therefore, a method that is effective for killing *Salmonella* on the stem scar also might be applied to whole tomatoes with equivalent or more efficacious results. However, only limited information is available regarding inactivation of *Salmonella* on tomato stem scars. In most of the available studies regarding the use of sanitizers, washing with water or with disinfectant solutions reduced microbial populations on the surface of the produce by fewer than three log units (1, 5, 11, 12, 17, 28, 30). *Salmonella* inoculated into tomato wounds, growth cracks, or stem scars generally survives and/or grows better than do cultures inoculated onto the surface of fruits. When present on the cracks or stem scars, *Salmonella* is more difficult to inactivate without causing adverse effects on sensory quality (31, 32). Biofilm formation within the stem scar also complicates sanitation efforts. Biofilms have been found on tomato cuticles after 10 days of storage at 22 and 30°C (18, 19).

In our previous study (8), we found that chitosan coatings significantly reduced *Salmonella* on cantaloupes, which have rough surfaces similar to tomato stem scars. The objective of the present study was to investigate the efficacy of acid-chitosan coatings for inactivation of *Salmonella* (serovars Montevideo, Newport, Saintpaul, and Typhimurium) on the surface of tomato stem scars.

**MATERIALS AND METHODS**

**Preparation of inocula.** Four serovars of *Salmonella enterica* were used in this study: *Salmonella* Montevideo (Salmonella group C, ATCC 8387), *Salmonella* Newport (group C, Eastern Regional Research Center [ERRC] culture collection),
Salmonella Saintpaul (group B, isolate 02-517-1 from a cantaloupe outbreak via Bassam Annous, ERRC), and Salmonella Typhimurium (group B, ATCC 14028). All serovars were selected for resistance to 100 ppm of nalidixic acid. Cultures were incubated for 24 h at 37°C in tryptic soy broth plus 100 ppm of nalidixic acid (Difco, BD, Sparks, MD), centrifuged for 10 min at 1,800 × g, concentrated 10-fold by resuspension in 10% of the original volume with sterile 0.1% peptone water, and composited in a single test tube. The inoculum was maintained at 22 to 24°C and applied to tomatoes within 1 h of preparation.

Procedure for inoculation. Red round tomatoes that were not treated with either oil or wax were purchased at local grocery stores and stored at 10°C until used. Tomatoes were held at 21°C for 16 to 18 h immediately preceding inoculation with Salmonella. One hundred microliters of the four-strain inoculum was spotted on the surface of each stem scar. The inoculum was applied in approximately equal volumes at 10 locations across the tomato stem scars to facilitate drying. Inoculated tomatoes were dried for 2 h at 22°C in a biosafety hood before coating treatments were applied.

Coating treatments. Two hundred milligrams of chitosan (low molecular weight, 75 to 85% deacetylation; Sigma Aldrich, St. Louis, MO) was added to 10 ml of an acid solution containing 2% acetic, 2% lactic, and 2% levulinic acids and stirred with a magnetic stir bar on a stir plate until the polymer was completely dissolved. Modifications of this formula with chitosan in 2% acetic acid alone (1A plus chitosan), chitosan in all three acids with 10 ppm of allyl isothiocyanate (3A plus chitosan plus 10AIT), and a three-acid solution without chitosan (3A) were also evaluated (Table 1). Tomatoes were placed stem end up in a biosafety hood, and 1 ml of the coating solution was evenly pipetted and distributed over the entire surface of each stem scar. The coated and noncoated (control) tomatoes were placed in a biosafety hood at room temperature (22°C) for 24 h before microbiological analyses. For storage tests, tomatoes were held in a temperature-controlled chamber at 10°C for up to 14 days.

Microbiological analysis. Each tomato stem scar (ca. 2 g each) was excised by hand in a conical manner from the fruit and added to 5 ml of Dey Engley neutralizing broth (Difco) in a Whirl-Pak filter bag. The bag contents were carefully macerated with a small mallet on the laboratory bench and then pummeled in a stomacher for 2 min. Sample filtrates were surface plated in duplicate onto tryptic soy agar (Difco) plus 100 ppm of nalidixic acid plus 0.1% sodium pyruvate (Sigma Aldrich) to assist in the recovery of injured cells. All plates were incubated at 37°C for 24 h before colonies were counted.

Statistical analysis. All experiments were conducted two or three times, and four samples per treatment were analyzed. Data were subjected to an analysis of variance and Duncan’s multiple range test (Statistical Analysis Systems Institute, Cary, NC). Differences between mean values were considered significant at P < 0.05.

RESULTS

The populations of Salmonella inoculated on tomato stem scars after 2 and 24 h of drying were ca. 7.5 and 7.3 log CFU per stem scar, respectively, which was not a significant difference (P > 0.05). Bacterial populations were significantly reduced (P < 0.05) by either acid solution or acid-chitosan coating treatments (Fig. 1). A combination of acids alone (3A solution) reduced the populations of Salmonella by 5.3 log CFU per stem scar. Additional reductions of approximately 0.7 CFU per stem scar were obtained with 3A plus chitosan coatings. When only one acid (acetic acid) was used in the chitosan coating (1A plus chitosan), an approximately 3.7-log reduction was achieved, less than that obtained with 3A plus chitosan. The bacterial reductions obtained with the combination of acid plus chitosan coating with 10 μl/ml AIT (3A plus chitosan plus AIT) were not significantly different from those obtained with 3A plus chitosan alone. Therefore, 3A plus chitosan coatings were selected for further investigation.

Figure 2 shows the effect of coating solution contact time with tomato stem scars on the survival of Salmonella. Tomatoes treated with 1 ml of coating solution were held for 2, 10, 60, and 240 min and then inverted to allow any unsolidified coating solutions on stem scars to drain. The survival of Salmonella on tomato stem scars was then analyzed. Populations of Salmonella were reduced from 7.5 to 3.2 log CFU per stem scar at 2 min, 2.5 log CFU per stem scar at 10 min, and 2.3 log CFU per stem scar at both 60 and 240 min. No significant differences in bacterial reductions were noted among tomatoes treated for 10, 60, and 240 min or for tomatoes treated and held upright for 24 h (Fig. 2). After 10 min of drying, the coating solution solidified to

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<th>TABLE 1. Compositions of antimicrobial coating solutions</th>
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a AIT, 10 ppm of allyl isothiocyanate.
b 3A, 2% solution of lactic acid, acetic acid, and levulinic acid. c 1A, 2% solution of acetic acid.

FIGURE 1. Survival of Salmonella on tomato stem scars as affected by the application of various coatings. Treated tomatoes were stored in a biohood at room temperature; samples were analyzed 24 h after each treatment. The coating compositions are listed in Table 1. Error bars represent the standard deviation of the mean. Means with the same letter are not significantly different (P > 0.05). The minimum detection limit was 0.7 log CFU per stem scar.
form a thin and transparent film on the stem scars (Fig. 3C through 3F); therefore, inverting tomatoes to drain did not reduce the contact time or affect the antimicrobial functionality. In contrast, 2 min of drying time was not enough for the chitosan solution to form a solid film on stem scars (Fig. 3B) before tomato inversion. Hence, with no film formed on the surface, the antimicrobial activity decreased with decreasing contact time.

Figure 4 shows the survival of Salmonella on tomato stem scars during storage at 10°C. The populations of Salmonella on the tomato surface without acid-chitosan coating treatments did not change significantly during a 14-day period. The coating treatment reduced the populations of Salmonella to 2.2 log CFU per stem scar after 1 day and then reduced the pathogen populations to undetectable levels (<0.7 log CFU per stem scar) at all storage times longer than 1 day.

DISCUSSION

Many chemical sanitizers and physical treatments have been utilized with varying degrees of success for inactivating Salmonella on tomatoes (4, 20). Plant essential oils such as sumac, oregano (14), and myrtle (13) also have been used for inactivating Salmonella on tomatoes. These antimicrobials caused only minimal reductions in the pathogen population. Although chlorine is the most common sanitizer used in the food industry to decontaminate fresh produce, tomatoes washed with chlorine (40 to 60 ppm) have been associated with foodborne illness outbreaks (31). Zhuang et al. (34) reported that complete inactivation of Salmonella on tomatoes was not achieved even with 320 ppm of chlorine. Formation of chlorinated organic compounds, such as trihalomethanes, from chlorine treatments have raised safety concerns because of their potential impact on humans and the environment, thus triggering the search for alternatives to chlorine (23, 24).

In a few studies, organic acids such as acetic or lactic acid have been used to reduce bacterial populations on fresh or fresh-cut produce (9, 22, 33). In our previous study (unpublished), the efficacy of acid washing for reducing Salmonella on tomato stem scars was dependent on the combination and concentration of various acids. In general, higher acid concentrations or a multiplicity of acids in a washing solution resulted in greater reduction. In the present study, a 2% solution of three acids (acetic, lactic, and levulinic acids) reduced Salmonella populations by 5.5 log CFU per stem scar, and the addition of chitosan (3A plus chitosan) achieved an additional 0.7-log reduction.
(Fig. 1). In our previous study (8), treatments with the chitosan coating reduced *Salmonella* by ca. 1.5 log units on the surface of cantaloupe. Chitosan is a renewable, nontoxic polymer that is well known for its excellent film-forming properties, broad antimicrobial activity, and high compatibility with other incorporated compounds due to the presence of high-density amino and hydroxyl groups in the chitosan polymer structure (21, 29). Antimicrobials included in chitosan-based films could be released in a controlled manner (25).

In addition to ineffectual decontamination, current washing and sanitizing practices have economic and environmental implications, mainly because of the large amount of water needed to assure that water quality is adequate at both the beginning and end of all washing processes. One challenge for the food industry is minimizing water consumption and wastewater discharge (23). Compared with washing with water, an acid solution, or other antimicrobials, the acid-chitosan coatings both inactivated pathogens and reduced the amounts of water and acids used. The acid-chitosan solution can be sprayed on tomatoes as needed. This coating process has less of an impact on the environment and, thus, is more economically feasible.

For practical application, the food industry needs the shortest washing and drying times possible for efficient production. Several researchers used 2-min washing times to investigate the antimicrobial efficacy of washing methods (31, 32, 34). In the present study (Fig. 2), the antimicrobial efficacy of the acid-chitosan coating was dependent on contact or drying times; approximately 10 min of drying time was required for forming an antimicrobial film under laboratory conditions (laminar flow in a biosafety cabinet). The use of a forced air drying tunnel would dramatically reducing drying time, but this approach must be confirmed in a scale-up study.

Allende et al. (2) observed that despite the initial differences, the total bacterial counts after storage were similar when the produce was washed with tap water or when a sanitizing solution was used. Other authors have suggested that washing produce with antimicrobial solutions initially reduces inoculated strains and the initial total mesophilic bacterial population; however, during extended storage the bacterial levels on this produce can increase more rapidly and even exceed the levels on water-washed counterparts (3, 10–12, 26). In the present study, acid-chitosan coatings completely inactivated *Salmonella* on stem scars after 2 days of treatment, and no regrowth occurred, even after 14 days of storage at 10°C (Fig. 3). In contrast, populations of *Salmonella* on the tomato surface, without the acid-chitosan coating treatment, did not change significantly during the 14-day storage period (Fig. 3). This finding is in agreement with those reported elsewhere (6, 34). The risk of survival of *Salmonella* in control samples highlights the potential value of the results presented herein and suggests that the antimicrobial coatings evaluated in this study could be used to enhance the safety of tomatoes.

No differences were detected by visual observation among all samples because only the stem scars were treated. However, the coating on the surface area around the stem scars formed a thin and “wrinkle-like” film, causing a slight change in color in this area (Fig. 3). Consumer acceptance of this type of change should be studied.

In summary, the combination of organic acids and chitosan was effective for reducing the population of *Salmonella* on tomato stem scars. Chitosan, which is edible, soluble in acidic solutions, and acquired from a renewable biosource and has film formation capability plus broad antimicrobial functionality, is in a unique position to be used in antimicrobial packaging. Organic acids have been used for food preservatives for centuries. Both chitosan and organic acids are generally recognized as safe by food regulating agencies. Acid-chitosan coatings represent a significant advancement over current washing and sanitizing practices in terms of economics, impact on the environment, and efficacy for decontaminating *Salmonella* on tomato stem scars. The acid-chitosan coatings evaluated in this study could be equally or more effective for decontaminating whole tomatoes, as will be determined in future studies. The acid-chitosan coatings are an effective intervention technique for enhancing the safety of tomatoes. Future studies might also investigate antimicrobial film drying times from 2 and 10 min to determine the minimal drying time needed to produce maximal pathogen inactivation.

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