Inactivation of *Salmonella* and *Escherichia coli* O157:H7 on Sliced and Whole Tomatoes by Allyl Isothiocyanate, Carvacrol, and Cinnamaldehyde in Vapor Phase

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**ABSTRACT**

Little is known about the effectiveness of antimicrobials in the vapor phase for control of pathogens on the surface of fresh produce. We determined the activity of allyl isothiocyanate (AIT), cinnamaldehyde, and carvacrol against *Salmonella* and *Escherichia coli* O157:H7 on sliced and whole tomatoes. Samples were treated with various concentrations of antimicrobial in the vapor phase at 4, 10, and 25°C in a closed container. AIT exhibited the highest antimicrobial activity followed by cinnamaldehyde. The lowest level of AIT (8.3 µl/liter of air) inactivated *Salmonella* on sliced tomatoes by 1.0 and 3.5 log at 4 and 10°C, respectively, in 10 days and by 2.8 log at 25°C in 10 h. This level of AIT inactivated *Salmonella* on whole tomatoes to the detection limit of ~2 log CFU per tomato at 4 and 10°C in 10 days and by 1.3 log CFU per tomato at 25°C in 10 h. AIT also inactivated *E. coli* O157:H7 on sliced tomatoes by 3.0 log at 4 and 10°C in 10 days, but there was no inactivation at 25°C in 10 h. AIT reduced *E. coli* O157:H7 on whole tomatoes surface by 3.0 and 1.0 log CFU per tomato at 4 and 10°C, respectively, in 10 days and by 2.0 log CFU per tomato at 25°C in 10 h. Overall, greater inactivation occurred at 10 than at 4°C and on the tomato surface than between slices. Antimicrobials in vapor phase may be useful for controlling pathogens on fresh tomatoes marketed in packages containing enclosed headspace.

Recent foodborne outbreaks associated with tomatoes created a need for understanding the sources of contamination and the survival and/or growth of pathogens and to develop innovative control technologies. Generally, pathogens on tomatoes are controlled by preventing contamination during growth and harvesting, by using antimicrobial rinses, and by low-temperature storage. Prevention of contamination is the most effective control strategy because pathogen growth is not required to cause illness and antimicrobial rinses are not sufficiently effective at killing or removing attached pathogens. Therefore, additional control measures could be of value.

The behavior of pathogens on tomatoes is affected by pathogen location on the fruit, tomato quality, storage temperature, packaging type, and relative humidity. Tomatoes usually have sufficient acid to limit pathogen growth during storage at refrigeration temperatures. However, the natural mycoflora and fungal and yeast infections of raw tomatoes increase the pH of the pericarp tissues to a level that favors pathogen growth (21, 22). As storage temperatures increase above the refrigeration temperature, *Salmonella* will survive and/or grow more rapidly on whole tomato skin and the stem scar and in chopped tomatoes (1, 5, 21, 23, 26). *Salmonella* Montevideo produces extracellular polymers on tomato cuticles after 10 h at 22 and 30°C and high relative humidity (97%), leading to a well-defined biofilm after 4 days (12). Pathogens on the surface of tomatoes may contaminate internal tissues during slicing and may then survive or grow in the slices. *Salmonella* that had infiltrated tomatoes grew at 25°C (10). Various research findings indicate that bacterial pathogens can infiltrate into whole tomatoes (2, 3, 8, 10, 26) when there is a temperature differential between the tomato and the washing water and by hydrostatic pressure when tomatoes are submerged in the dump tank (2, 4). Bacterial infiltration increases in the presence of wounds and punctures on the tomato. Infiltrated pathogens are not removed by normal washing practices.

The main benefit of adding antimicrobial chemicals to tomato wash water is to control the spread of pathogens by killing those released from the produce, because the pathogen-reducing benefits of antimicrobial washes on tomatoes are limited. Chlorinated water, hydrogen peroxide, peroxycetic acid, and electrolyzed water have been studied for their ability to reduce pathogens on tomatoes during the washing process. These treatments have limited effectiveness, presumably because active agents do not sufficiently contact the target pathogens.

Recent research indicates that antimicrobial chemicals in vapor phase can significantly reduce pathogen populations on the tomato surface. The use of 5 mg/liter chlorine dioxide gas for 1 h was significantly more effective against *Salmonella* on the stem scar than were aqueous solutions of 200 ppm of sodium hypochlorite (2 min exposure) and 1,200 ppm of acidified sodium chlorite (2 min exposure) (25). The use of 10 mg/liter ozone completely inactivated (7 log CFU per tomato) *Salmonella Enteritidis* on the surface of cherry tomatoes after 15 min for a 1-h attachment and after 20 min for a 4-h attachment; however, this con-

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TABLE 1. Salmonella strains used in this study

<table>
<thead>
<tr>
<th>Salmonella strain</th>
<th>Outbreak-associated food</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pooni</td>
<td>Cantaloupe</td>
</tr>
<tr>
<td>Stanley H 1256</td>
<td>Alfalfa sprouts</td>
</tr>
<tr>
<td>Baildon</td>
<td>Tomato</td>
</tr>
<tr>
<td>Typhimurium DT 104</td>
<td>Resistant to multiple antibiotics</td>
</tr>
<tr>
<td>Montevideo</td>
<td>Tomato</td>
</tr>
</tbody>
</table>

* Obtained from Dr. Mark Harrison (Department of Food Science and Technology, University of Georgia, Athens).

Concentration changes the color of the tomatoes (5). Because vapor-phase antimicrobials may be effective against attached bacteria at locations on the fruit not reached by active agents in aqueous solution, their use in packaged produce or during produce processing could provide an added pathogen control benefit.

Essential oils are able to inactivate pathogens of concern in fresh produce. Of 96 tested essential oils, the 3 most effective against *E. coli O157:H7* and *Salmonella enterica* were oregano, thyme, and cinnamon. The 3 most effective oil compounds among 16 compounds tested against *Escherichia coli* O157:H7 were carvacrol, cinnamaldehyde, and thymol, and the most effective compounds against *S. enterica* were thymol, cinnamaldehyde, and carvacrol (7). This information was obtained using the oil in liquid phase. Only limited information is available on the efficacy of essential oils in vapor form.

The efficacy of antimicrobials in vapor phase is expected to depend on the temperature, time of the exposure, and concentration. The aim of this project is to elucidate the effect of these parameters on pathogen inactivation on tomato skin (using whole tomatoes) and on tomato pulp (using sliced tomatoes). Allyl isothiocyanate (AIT, from mustard and horseradish), carvacrol (from oregano), and cinnamaldehyde (from cinnamon) were the volatile antimicrobials selected for this study. These compounds are derived from natural sources and have previously been demonstrated to be effective against gram-negative pathogens in aqueous solution.

**MATERIALS AND METHODS**

**Inoculum preparation.** *Salmonella* and *E. coli* O157:H7 strains isolated from produce associated with illness outbreaks were used to inoculate the tomatoes (*Lycopersicon esculentum* Mill.). A five-strain cocktail of *Salmonella* (Table 1) and a four-strain cocktail of *E. coli* O157:H7 (Table 2) were prepared. The strains were grown to stationary phase by transferring each twice in tryptic soy broth (TSB) at 24-h intervals with incubation at 35°C. Equal volumes of each pathogen strain were mix and then centrifuged at 4,500 rpm for 25 min at 4°C, the supernatant fluid was discarded, and the pellet was suspended in 0.1% peptone water. The cell suspensions contained approximately 9 log CFU/ml.

**Inoculation of sliced tomatoes.** Fresh light red tomatoes were purchased at retail from a local grocery store in Athens, GA (Table 1). Equal volumes of each pathogen strain were mix and then centrifuged at 4,500 rpm for 25 min at 4°C, the supernatant fluid was discarded, and the pellet was suspended in 0.1% peptone water. The cell suspensions contained approximately 9 log CFU/ml.

**Inoculation of whole tomatoes.** Whole grape tomatoes were used for surface inoculation experiments because these small tomatoes allowed the desired head space. Fresh grape tomatoes were purchased from a grocery store in Athens, GA, the same day of the experiment. Tomatoes were immersed in 200 ppm of chlorine for 5 min and then rinsed under running tap water to remove chlorine residues. Working in a biosafety cabinet, a 3-cm-diameter circle was drawn on each tomato with a liquid blocker super pap pen (Daido Sangyo Co., Ltd., Tokyo, Japan). Each tomato was spot inoculated inside the circle with 100 μl of inoculum (equivalent to 6.7 log CFU/ml) placed in 10 separate spots (11). This high initial inoculum was used because preliminary work indicated that *Salmonella* and *E. coli* O157:H7 populations decreased during the drying period. Lang et al. (14) also observed that *E. coli* O157:H7 and *Salmonella* populations on tomato surface decreased by 3.2 and 2.2 log CFU, respectively, during drying at 22 ± 2°C for 24 h. The inoculated tomatoes were left under the biosafety cabinet to dry for 24 h at 22°C (14), and one grape tomato was aseptically placed in each septa jar.

**Antimicrobial application.** The inoculated sliced and whole tomatoes were exposed to the antimicrobial compounds in a similar manner. Autoclave-sterilized filter paper pieces (2 by 2 cm; Whatman Inc., Clifton, NJ) were saturated with sterile distilled water by immersion. One wet filter paper piece was placed on the top inner side of each septa jar (with caution taken not to contact the tomato sample) to create high relative humidity inside the jars. The antimicrobial compounds were allowed to equilibrate to room temperature. Then 5, 10, and 15 μl (equivalent to 41.5, 83.3, and 125 μl/liter of air, respectively) of ≥97% pure carvacrol or ≥98% pure cinnamaldehyde or 1, 2, and 4 μl (equivalent to 8.3, 16.6, and 33.3 μl/liter of air, respectively) of ≥98% pure AIT (Sigma-Aldrich, St. Louis, MO) were deposited on a second filter paper piece of the same size that had been previously attached with double-stick tape (Henkel Consumer Adhesives, Inc, Avon, OH) on the top inner side of the septa jar. The location of the filter was designed to avoid direct contact of the antimicrobial com-

**TABLE 2. Escherichia coli O157:H7 strains used in this study**

<table>
<thead>
<tr>
<th>E. coli strain</th>
<th>Outbreak-associated food</th>
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</thead>
<tbody>
<tr>
<td>C7927</td>
<td>Apple cider</td>
</tr>
<tr>
<td>F4556</td>
<td>Alfalfa sprouts</td>
</tr>
<tr>
<td>H1730</td>
<td>Lettuce</td>
</tr>
<tr>
<td>SEA 13B88</td>
<td>Unpasteurized apple juice</td>
</tr>
</tbody>
</table>

* Obtained from Dr. Larry Beuchat (Center for Food Safety, University of Georgia, Griffin).
ound with the tomato sample. Samples in the sealed jars were stored at 4, 10, and 25°C.

Microbiological analysis. Samples held at 4 and 10°C were analyzed after 0, 4, 7, and 10 days of incubation. Samples held at 25°C were analyzed after 0, 4, 7, and 10 h. The entire tomato sample was removed aseptically from the jar and placed in a filter bag (Nasco, Inc., Ft. Atkinson, WI). For the sliced tomato samples, the jars also were rinsed with peptone water to include any released juice in the analysis. The weight of each bag was brought to 100 g, and the bag was stomached for 1 min at high speed with a stomacher (Stomacher 400 Laboratory Blender, Seward, Worthington, UK). Numbers of Salmonella cells were determined by spiral plating (Spiral Biotech, Inc., Norwood, MA) of appropriate dilutions on MacConkey agar (Difco, Becton Dickinson) and incubated at 35°C for 18 h. Numbers of E. coli O157:H7 cells were determined by spiral plating on sorbitol MacConkey agar (SMAC; Difco, Becton Dickinson) and incubated at 37°C for 18 h. Only typical colonies were counted. Data were calculated as CFU per inoculated site because the entire inoculated sample was analyzed.

Statistical analysis. The study employed a factorial random block design. The experimental design consisted of three antimicrobials (AIT, carvacrol, and cinnamaldehyde), four concentrations (0, 8.3, 16.6, and 33.3 μl/liter AIT and 0, 41.5, 83.3, and 125 μl/liter carvacrol and cinnamaldehyde), three temperatures (4, 10, and 25°C), four sampling times (0, 4, 7, and 10 days for 4°C samples and 0, 4, 7, and 10 h for 25°C samples), two inoculation methods (on whole tomato and between slices), and two pathogens (Salmonella and E. coli O157:H7). Each experiment was replicated four times. Data were analyzed using SAS 9.1.3 (SAS Institute, Inc., Cary, NC). Multiple comparisons were evaluated by analysis of variance using a general linear model. A 5% significance level was employed for all analyses.

RESULTS

In the preliminary work, carvacrol and cinnamaldehyde inactivated Salmonella on sliced tomatoes at a concentration of 150 μl/liter of air with no adverse effect on aroma, as determined from an informal evaluation by four or five untrained panelists. Equivalent or lower concentrations (41.5, 83.3, and 125 μl/liter of air) of carvacrol and cinnamaldehyde were used in this study. Concentrations of AIT similar to those evaluated in preliminary research produced a detectable odor when the containers were opened. Therefore, lower concentrations of AIT of 8.3, 16.6, and 33.3 μl/liter of air were used in this study.

Inactivation of Salmonella in the presence of vapor-phase antimicrobials at 4°C. In the absence of the antimicrobials (control samples), the population of Salmonella decreased by ca. 1.0 log CFU on sliced tomatoes in 10 days at 4°C (Fig. 1). The presence of the antimicrobials caused a significantly greater reduction. AIT produced the greatest inactivation followed by carvacrol and then cinnamaldehyde. Reduction of Salmonella at 4°C by all antimicrobials increased with time of incubation, but only AIT exhibited concentration-dependent inactivation. The reduction caused by AIT and carvacrol was observed after 4 days of incubation and continued until day 10, but the inactivation by cinnamaldehyde was not observed until day 10.

In the absence of the antimicrobials (control samples), the population of Salmonella on whole tomato decreased significantly (>3 log CFU) in 10 days at 4°C (Table 3). The lowest concentration of each antimicrobial inactivated Salmonella to below the detection limit (4 log CFU less than the control) within 4 days.

Inactivation of Salmonella in the presence of vapor-phase antimicrobials at 10°C. In the absence of the antimicrobials, the population of Salmonella increased on the tomato slices by approximately 2.4 log CFU between the days 4 and 7 of incubation at 10°C (Fig. 2). All antimicro-
TABLE 3. Behavior of Salmonella on whole tomatoes stored at 4°C for 10 days in the presence of vapor-phase antimicrobials

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Day 0</th>
<th>Day 4</th>
<th>Day 7</th>
<th>Day 10</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>7.09 ± 0.17</td>
<td>5.39 ± 0.81</td>
<td>4.04 ± 0.11</td>
<td>3.59 ± 0.57</td>
</tr>
<tr>
<td>Carvacrol (41.5 µl/liter)</td>
<td>7.09 ± 0.17</td>
<td>&lt;3</td>
<td>&lt;2</td>
<td>&lt;2</td>
</tr>
<tr>
<td>Cinnamaldehyde (41.5 µl/liter)</td>
<td>7.09 ± 0.17</td>
<td>&lt;3</td>
<td>&lt;2</td>
<td>&lt;2</td>
</tr>
<tr>
<td>AIT (8.3 µl/liter)</td>
<td>7.09 ± 0.17</td>
<td>&lt;3</td>
<td>&lt;2</td>
<td>&lt;2</td>
</tr>
</tbody>
</table>

Inactivation of Salmonella in the presence of vapor-phase antimicrobials at 25°C. In the absence of the antimicrobials, the population of Salmonella on sliced tomatoes increased significantly by about 2.9 log CFU during 10 h at 25°C (Fig. 4) after a 4-h lag phase. The presence of the antimicrobials did not cause any significant reduction in the population at any concentration when samples were incubated for more than 10 h. Carvacrol and cinnamaldehyde had no effect on the growth of Salmonella on sliced tomatoes during the first 7 h of incubation and no significant inhibitory effect after 10 h, producing a total increase of ca. 2.4 log CFU. However, AIT prevented growth of Salmonella at the lowest concentration used.

The population of Salmonella on whole tomato increased by 0.8 log CFU/g after 10 h at 25°C (Fig. 5) when no antimicrobial was used. However, the presence of any of the antimicrobials caused significant inactivation of Salmonella, and the amount of inactivation increased with an increase in antimicrobial concentration. Cinnamaldehyde and AIT produced the most inactivation of Salmonella, resulting in a significant 2.8-log reduction at the highest concentrations used (125 and 33.3 µl/liter, respectively) during the 10-h incubation period, whereas carvacrol produced a significant 2.0-log reduction at its highest concentration (125 µl/liter) compared with the control for the same incubation period.

Inactivation of E. coli O157:H7 in the presence of vapor-phase antimicrobials at 4°C. No significant change in the population of E. coli O157:H7 occurred on sliced tomatoes after 7 days, but a significant increase was observed after 10 days of incubation at 4°C. The presence of carvacrol and cinnamaldehyde caused a significant reduction in population between 7 and 10 days of storage ($P < 0.05$) (Fig. 6A and 6B). AIT (Fig. 6C) produced the greatest inactivation ($P < 0.05$) of all the antimicrobials at all concentrations.
centrations used. After 7 days, AIT concentrations of 16.6 and 33.3 µl/liter inactivated the pathogen by more than 2 log CFU, and at a concentration of 8.3 µl/liter AIT reduced the pathogen population by almost 1 log CFU compared with the day 0 control.

In the absence of the antimicrobials, the population of E. coli O157:H7 on whole tomato decreased by 1.6 log CFU at 4°C, with most of the decrease occurring during the first 7 days (Table 4). All of the antimicrobials inactivated E. coli O157:H7 on the tomato surface to below the detection limit (3 log CFU less than the control) by day 4.

Inhibition of E. coli O157:H7 in the presence of vapor-phase antimicrobials at 10°C. In the absence of the antimicrobials, E. coli O157:H7 grew on the sliced tomatoes and achieved a 4-log increase in 10 days at 10°C (Fig. 7). The presence of the antimicrobials did not reduce the population but slowed or prevented growth in a concentration dependent manner, with AIT producing the greatest inhibition and carvacrol able to inhibit growth when used at 125 µl/liter. Carvacrol was effective at preventing growth of E. coli O157:H7 during the first 4 days of incubation,
FIGURE 5. Behavior of Salmonella on whole tomatoes stored at 25°C for 10 days in the presence of different concentrations of vapor-phase carvacrol (A), cinnamaldehyde (B), and allyl isothiocyanate (AIT) (C). Control; ■ 41.5 μl/liter carvacrol and cinnamaldehyde and 8.3 μl/liter AIT; ▲ 83.3 μl/liter carvacrol and cinnamaldehyde and 16.6 μl/liter AIT; □ 125 μl/liter carvacrol and cinnamaldehyde and 33.3 μl/liter AIT. Detection limit was 100 CFU per inoculation site.

FIGURE 6. Behavior of E. coli O157:H7 on sliced tomatoes stored at 4°C for 10 days in the presence of different concentrations of vapor-phase carvacrol (A), cinnamaldehyde (B), and allyl isothiocyanate (AIT) (C). Control; ■ 41.5 μl/liter carvacrol and cinnamaldehyde and 8.3 μl/liter AIT; ▲ 83.3 μl/liter carvacrol and cinnamaldehyde and 16.6 μl/liter AIT; □ 125 μl/liter carvacrol and cinnamaldehyde and 33.3 μl/liter AIT. Detection limit was 100 CFU per inoculation site.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Day 0</th>
<th>Day 4</th>
<th>Day 7</th>
<th>Day 10</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>7.84 ± 0.15</td>
<td>5.73 ± 0.1</td>
<td>5.24 ± 0.07</td>
<td>5.17 ± 0.1</td>
</tr>
<tr>
<td>Carvacrol (41.5 μl/liter)</td>
<td>7.84 ± 0.15</td>
<td>&lt;3</td>
<td>&lt;2</td>
<td>&lt;2</td>
</tr>
<tr>
<td>Cinnamaldehyde (41.5 μl/liter)</td>
<td>7.84 ± 0.15</td>
<td>3.16</td>
<td>&lt;2</td>
<td>&lt;2</td>
</tr>
<tr>
<td>AIT (8.3 μl/liter)</td>
<td>7.84 ± 0.15</td>
<td>&lt;3</td>
<td>&lt;2</td>
<td>&lt;2</td>
</tr>
</tbody>
</table>
but the lower two concentrations lost effectiveness after that time. In contrast, the lowest two concentrations of AIT allowed about 1 log CFU of growth during the first 4 days, after which growth was completed inhibited. AIT was the most effective growth inhibitor for *E. coli* O157:H7 on the tomato slices at 10°C; it allowed a slower increase in the population compared with the control, resulting in a 3.0- to 4.7-log lower population than that of the control after 10 days of treatment.

*E. coli* O157:H7 maintained a stable population on tomato surfaces at 10°C during the 10 days of incubation (Fig. 8). Addition of antimicrobials to the headspace inactivated the pathogen. AIT produced the greatest inactivation followed by cinnamaldehyde and then carvacrol. Inactivation increased with increasing concentration of the antimicrobial. For example, the presence of 8.3, 16.6, and 33.3 μl/liter AIT inactivated *E. coli* O157:H7 by 1.1, 2.8, and 4.4 log CFU, respectively, after 10 days when compared with the control. The presence of 41.5, 83.3, and 125 μl/liter cinnamaldehyde inactivated *E. coli* O157:H7 by 1.5, 2.0, and 2.7 log CFU, respectively, compared with the control by day 10. However, the presence of 41.5, 83.3, and
125 μl/liter carvacrol inactivated *E. coli* O157:H7 by 0.7, 1.4, and 2.5 log CFU, respectively, compared with the control by day 10.

**Inactivation of *E. coli* O157:H7 in the presence of vapor-phase antimicrobials at 25°C.** In the absence of the antimicrobials, the population of *E. coli* O157:H7 on sliced tomatoes increased about 1.0 log CFU at 25°C by 10 h after a 4- to 7-h lag phase (Fig. 9). The presence of 33.3 μl/liter AIT decreased *E. coli* O157:H7 by 0.7 log CFU compared with the control after 10 h. No other antimicrobial treatments significantly reduced levels of *E. coli* O157:H7 on sliced tomato during the 10-h treatment period.

In the absence of the antimicrobials, the population of *E. coli* O157:H7 on whole tomatoes remained stable at 25°C over the 10-h incubation period (Fig. 10). All of the antimicrobial treatments achieved inactivation of the pathogen, and inactivation increased with increasing antimicrobial concentration. AIT and cinnamaldehyde produced significant inactivation after 4 h at all concentrations applied. Inactivation on whole tomatoes diminished after 4 h when AIT was applied at 8.3 and 16.6 μl/liter, but AIT main-
tained activity when applied at a 33.3 μl/liter. Inactivation by cinnamaldehyde and carvacrol also diminished after 4 h when applied at 41.5 and 83.3 μl/liter, but these antimicrobials maintained activity when applied at 125 μl/liter.

**DISCUSSION**

In the absence of antimicrobials, *Salmonella* and *E. coli* O157:H7 grew more on tomato slices than on tomato surfaces as temperature increased. The pathogens also survived better on the whole tomatoes than on tomato slices. For example, the pathogens decreased ca. 1.0 log CFU on sliced tomatoes but decreased by more than 3 log CFU on the tomato surface at 4°C after 10 days. Lin et al. (15) found that the survival of *Salmonella* Montevideo on tomato skin was lower than that on tomato stem scar at 4°C after 2 days, and a nearly 1-log reduction occurred on the tomato surface after 2 days at 4°C. Zhuang and Beuchat (26) found that *Salmonella* Montevideo populations did not change in chopped tomato at 5°C after 9 days although a decrease of about 0.3 log CFU was observed, whereas we observed a 0.8-log decrease in the *Salmonella* multistrain population during storage at 4°C for 10 days. Survival on tomatoes is serovar dependent; *Salmonella* Hadar, Montevideo, and Newport are the most well adapted to growth and survival in or on the fruit (17). *Salmonella* and *E. coli* O157:H7 grew on the sliced tomatoes at 10°C, but their populations remained stable on the tomato surface, a result similar to that of Zhuang and Beuchat (26), who evaluated the behavior of *Salmonella* Montevideo. We also found that the two pathogens increased in population on sliced tomato at 25°C after 10 h, but no significant change in numbers was observed on the tomato surface at this temperature. This result also is similar to that of Zhuang and Beuchat (26), who observed that *Salmonella* Montevideo on the tomato surface increased within 7 days and 1 day at 20 and 30°C, respectively. Differences in pathogen behavior at different locations on tomatoes may be attributed to differences in moisture and nutrient availability. Previous research indicated that growth of *Salmonella* Montevideo on the tomato surface increases at higher relative humidity (12). Stine et al. (19) also observed that survival of several pathogens and viruses on produce surfaces (lettuce, cantaloupe, and bell pepper) is influenced by relative humidity.

AIT, carvacrol, and cinnamaldehyde in vapor form inactivated and/or inhibited growth of *Salmonella* and *E. coli* O157:H7 on whole and sliced tomato at 4 and 10°C. Carvacrol and cinnamaldehyde produced little inhibition at 25°C after 10 h, but AIT prevented growth of the pathogens in sliced tomatoes, and all the antimicrobials inactivated the pathogens on tomato surfaces at 25°C. Although AIT was used at a concentration five times lower than those of carvacrol and cinnamaldehyde, it produced the greatest inactivation at all temperatures and exposure times.

The antimicrobials in vapor phase were more effective for inactivating pathogens on the tomato surface than on the tomato flesh (slices). These findings are consistent with those of Lin et al. (15), who reported that AIT vapor at 132 μl/liter of air reduced *Salmonella* Montevideo by 8 log CFU on the tomato surface but by only 5 log CFU on tomato stem scars at 4°C after 2 days. However, in the present study, lower levels of AIT were evaluated, and 8.3 μl/liter produced 1.3- and 2.0-log reductions of *Salmonella* on tomato slices and surfaces, respectively, after 10 days. The present study was conducted with a cocktail of *Salmonella* strains, whereas Lin et al. (15) used a single strain of *Salmonella* Montevideo. The cocktail was used in our study to account for strain differences in persistence and growth on tomatoes and in possible antimicrobial susceptibility (17). Another gaseous antimicrobial, chlorine dioxide, also produced greater inactivation at a fruit surface as compared with the tissue (blueberry surface compared with stem scar) (20). The use of 4.1 mg/liter chlorine dioxide for 25 min reduced *Salmonella* on tomato surface by 4.33 log CFU per tomato; however, this level of chlorine dioxide resulted in adverse sensory effects (20). Chlorine dioxide also produced greater inactivation of *Salmonella* on apple skin than on calyx and stem cavities (6).

Generally, extrinsic factors such as antimicrobial concentration, time, and temperature of exposure affect the efficacy of an antimicrobial treatment. The inactivation or inhibition activity of AIT, carvacrol, and cinnamaldehyde against *Salmonella* and *E. coli* O157:H7 on sliced tomato increased with concentration and time. However, data would need to be collected at shorter time intervals than those used in this study to draw an overall conclusion about the effect of time. Other researchers have reported that the ability of chlorine dioxide gas to inactivate *E. coli* O157: H7 on apple surface increased with gas concentration and exposure time (6). Treatment with 12.0 mg/liter for 10 min, 4.8 mg/liter for 20 min, and 3.3 mg/liter were the optimum concentration and exposure time combinations to inactivate *E. coli* O157: H7 on apple surfaces and reduced the pathogen by 5 log CFU on the skin and by 3.0 to 3.7 log CFU on the calyx and in the stem cavities (6). Chlorine dioxide gas also was used to inactivate *E. coli* O157:H7 on green peppers; the gas concentration, treatment time, relative humidity, and temperature significantly affected the inactivation ability (9). The gas concentration was the most important factor; temperature was the least important, and the gas concentration and relative humidity had a synergistic effect on inactivation (9).

Intrinsic characteristics of the food, of which pH may be the most important in fresh produce, also affect the antimicrobial activity against pathogens. Applying AIT at 1,000 and 2,000 μg/liter did not reduce *Salmonella* on sprouts but adversely affected the sensory quality, after 11 days of exposure at 10°C (24). However, in our study a concentration of 8.3 μl/liter reduced the population on sliced tomato by 1.2 log CFU after 10 days at 10°C. The difference between the findings of Weissinger et al. (24) and those in this study result from the different food matrices; the acidity of the tomatoes may enhance inactivation, and sprouts may provide additional physical protection for pathogens. Previous research indicates that the inherent acidity of fruits enhances the antimicrobial activity of lemon grass, cinnamon, and geraniol against *Salmonella* Enteritidis, *E. coli*, and *Listeria innocua* (higher in apple and pear juice than in melon juice and trypsic soy broth) (16).
Lemongrass, cinnamon, or geraniol essential oil at a concentration of 2 μl/ml was needed to inactivate Salmonella Enteritidis, E. coli, and L. innocua in apple and pear juices. However, 8 and 10 μl/ml cinnamon was required to inactivate these pathogens in melon juice and tryptic soy broth, respectively, and 6 μl/ml geraniol or 5 μl/ml lemongrass was needed to inactivate these pathogens. The activity of oregano essential oil against E. coli O157:H7 in eggplant salad and against Salmonella Enteritidis in taramasalad (cod roe, bread, and olive oil mixture) was enhanced at lower pH (addition of citric acid) (13, 18). The enhanced activity of essential oils at acidic pH values could be the result of the essential oil becoming more hydrophobic at low pH and therefore exhibiting increased solubility at the lipid layer of the bacterial membrane (18).

The results of this study indicate the potential of AIT, carvacrol, and cinnamaldehyde, individually or in combination, for use as part of a kill or growth prevention step in packaged sliced and grape tomatoes. These antimicrobials could be used to control Salmonella and E. coli O157: H7 on packaged tomatoes stored at refrigeration temperatures. If there were a break in the cold chain, the antimicrobial activity would increase and reduce the potential for pathogen growth. Additional research on the influence of these treatments on sensory quality is needed before their practical use. In addition, data validating the application of these treatments in industrial systems are required.

ACKNOWLEDGMENTS

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REFERENCES