Inactivation of *Salmonella enterica* serovar Typhimurium and Quality Maintenance of Cherry Tomatoes Treated with Gaseous Essential Oils

Juan Yun, Xuetong Fan, and Xihong Li

Abstract: The antimicrobial activity of the essential oils (EOs) from cinnamon bark, oregano, mustard, and of their major components cinnamaldehyde, carvacrol, and allyl isothiocyanate (AIT) was evaluated as a gaseous treatment to reduce *Salmonella enterica* serovar Typhimurium *in vitro* and on tomatoes. In *vitro* tests showed that mustard EO and AIT had the greatest inhibition of *Salmonella*, followed by cinnamon EO and cinnamaldehyde, while oregano and carvacrol showed the least inhibition. Scanning electron microscopy images of *S. Typhimurium* on tomatoes suggest that the EOs and their major components damaged the bacteria, and the damage was more obvious after posttreatment storage at 10°C for 4 and 7 d. *Salmonella* on inoculated tomatoes was reduced by more than 5 log colony forming units (CFU)/g by mustard EO and AIT, by 4.56 and 3.79 log CFU/g following cinnamon EO and cinnamaldehyde treatments, respectively, and 1.54 and 3.37 log CFU/g after oregano EO and carvacrol treatments, respectively. Mustard EO and AIT induced discoloration, softening, and loss of the vitamin C and lycopene during 21 d of storage at 10°C, while treatment with cinnamon EO and cinnamaldehyde did not result in significant changes in tomato quality. Tomatoes treated with oregano EO had better quality than nontreated samples after storage. Therefore, treatment with cinnamon and oregano EO and their major components appeared to be feasible for inactivation of *Salmonella* on tomatoes and maintaining quality.

Keywords: essential oil, quality, SEM, *Salmonella*, tomato

Introduction

Tomatoes are rich in health-promoting components including vitamins A, C, E, lycopene, flavonoids, and β-carotene (Mangels and others 1993; Beecher 1998; Leonardi and others 2000). However, in recent years, fresh tomatoes have attracted public attention due to more than 15 *Salmonella* outbreaks since 1998 in the United States (CSPI 2010). Tomatoes can be contaminated through irrigation water, use of manure fertilizers during growing, with packing house wash water, by human handlers, and by contact with contaminated surfaces during food processing (Tauxe and others 1997; Hanning and others 2009). Therefore, intervention technologies and techniques to inactivate pathogens on tomatoes are needed.

Essential oils (Eos) and purified compounds have been found to possess antimicrobial activity *in vitro* against a broad spectrum of bacteria including *Salmonella* (Burt 2004). Cinnamon, mustard, and oregano oils are all natural antimicrobial and flavoring substances. They are classified as generally recognized as safe (GRAS) by the U.S. Food and Drug Administration (2009). Cinnamon oil is rich in antimicrobial agents such as cinnamaldehyde (Ojagh and others 2010). Lopez and others (2005) studied cinnamon, clove, basil, rosemary, dill, and ginger EOs in solid and vapor diffusion tests *in vitro* and found that cinnamon and clove oils exhibited significant antimicrobial activity. Goni and others (2009) reported the antimicrobial activity of vapor generated by a combination of cinnamon and clove EOs against a large number of pathogens. Lu and Wu (2010) evaluated the antimicrobial activities of aqueous thymol, carvacrol, and thyme EOs against *Salmonella* on cherry tomatoes. Allyl isothiocyanate (AIT) has been shown to have a particularly strong antimicrobial effect against *Escherichia coli O157: H7, Salmonella enterica* serovar Typhimurium, *Listeria monocytogenes*, and other pathogenic bacteria (Muthukumarasamy and others 2003; Obaidat and Frank 2009). More studies have been conducted on the antimicrobial activity of EOs against microorganisms *in vitro* than on food. Limited studies were concerned with the antibacterial activities of EOs in the vapor phase (Burt and others 2007; Du and others 2009; Obaidat and Frank 2009). In addition, little research is available on bactericidal effects of gaseous EOs and their effect on the quality of fresh fruits and vegetables such as tomatoes.

Gaseous antimicrobials offer some advantages over currently used aqueous sanitizers such as chlorine (Herdt and Feng 2009). First of all, gaseous antimicrobials can be more effective in reaching locations (such as cracks and stem ends) that pathogens hide compared to aqueous chemical sanitizers (Obaidat and Frank 2009). Furthermore, gaseous antimicrobials tend to dissolve in wound sites on fruits and vegetables, and microbes in those areas are likely to be inactivated, making gaseous application of antimicrobials attractive.

In this study, we evaluated the antibacterial activities of oregano, cinnamon, and mustard EOs and major active components in the EOs against *S. Typhimurium* *in vitro* and on cherry tomatoes. Quality (color, firmness, vitamin C, and lycopene) of treated
tomato fruits and bacterial morphology on cherry tomatoes, following treatments, were also investigated during storage at 10 °C.

Materials and Methods

S. Typhimurium strains

S. Typhimurium LT2 and 3 attenuated strains of S. Typhimurium (α3985, α4096, and α8089) were used in the study. S. Typhimurium LT2 (ATCC 700720) was obtained from American Type Culture Collection (Manassas, Va., U.S.A.). The 3 attenuated strains of S. Typhimurium, selected from the culture collection maintained at the USDA Eastern Regional Research Center (ERRC), Wyndmoor, Pa., U.S.A., were originally obtained from Roy Curtiss III, Center for Diseases and Vaccinology, Arizona State University, Tempe, Ariz., U.S.A. S. Typhimurium α3985 is a Δαα Δαα derivative of wild-type S. Typhimurium strain UK-1 (3761 (Hassan and Curtiss 1990)). The strain carries deletion mutations that impair the ability of the bacterium to synthesize adenylate cyclase (ψαα) and the cyclic AMP receptor protein (rψαα). α4096 is also a Δαα Δαα attenuated strain derived from S. Typhimurium SR-11. The strain is a nalidixic acid-resistant (gγλ1816) and cured of the virulence plasmid, pSTV (Gulig and Curtiss 1987). α8089 is a ΔδαδΔQ2 attenuated strain in which PhoPQ genes are inactivated (Zhang and others 1997). The S. Typhimurium PhoP-PhoQ 2-component regulatory system controls the expression of several genes that are necessary for virulence (Miller and others 1989).

The Salmonella strains used in the present study have been used in previous studies. For example, the S. Typhimurium LT2 strain has been used to study responses to chlorine wash on cantaloupe and honeydew melons (Parnella and others 2005), to ionizing radiation on tomato, cantaloupe, romaine lettuce, and baby spinach (Moreira and others 2012), and to EOs (Olasupo and others 2003; Chaieb and others 2007). The attenuated strains have been used to assess the persistence of Salmonella in soils, irrigation water, and on carrots and radishes (Islam and others 2012), the survival of Salmonella spp. in orange juice after pulsed electric field (PEF) treatment (Gurtler and others 2010), and effectiveness of in-package ozonation treatment on tomatoes (Fan and others 2012).

Preparation of Salmonella

To suppress the growth of native microorganisms from tomato, nalidixic-acid-resistant Salmonella was used in the study. Earlier studies demonstrated that nalidixic-acid-resistant strains had similar growth rates and response to antimicrobials as nalidixic-acid-sensitive parent strains (Blackburn and Davies 1994; Taormina and Beuchat 1999). Individual Salmonella strains were made nalidixic acid resistant by successive transfers into Tryptic Soy Broth (TSB) with increasing concentrations of nalidixic acid to a final concentration 100 μg/mL over 10 d (Fan and others 2012). The bacteria were grown individually in 150 mL TSB with 100 μg/mL nalidixic acid for 18 h. Cultures were centrifuged at 10,000 × g for 10 min. The pellets were then suspended in 25 mL of 0.1% peptone water, and all 4 strains were combined for a final population of 10⁸ to 10⁹ colony forming units (CFU)/mL.

EOs and their extracts

Cinnamon bark oil, cinnamaldehyde (purity ≥ 93%), and carvacrol (≥98%) were purchased from SAFC Supply Solutions (St. Louis, Mo., U.S.A.). AIT was purchased from Sigma-Aldrich (St. Louis). Mustard EO and oregano EO were purchased from Naturex (South Hackensack, N.J., U.S.A.).

Antimicrobial activity test

In vitro test.

The inhibition of S. Typhimurium by the EOs was determined similarly as described by Burt and others (2007). Solidified tryptic soy agar (TSA) medium was inoculated with 100 μL composite S. Typhimurium suspension containing 6 log CFU/mL of Salmonella. Each EO and their major components were diluted in ethanol to obtain serial dilutions down to 1% (v/v), and 10 μL of each dilution was added to 10-mm-diameter sterile filter discs and placed in the center of the lid of the Petri dish. The Petri dishes were then sealed using parafilm (Pechiney, Chicago, Ill., U.S.A.). Blanks (controls) were prepared by adding 10 μL of ethanol to the filter discs, which was demonstrated to have no effect on the viability of the S. Typhimurium. Analyses were carried out in triplicate. The concentration of EO was expressed as μL/L air. The diameter (in mm) of inhibition zone was measured using a ruler after 48 hr incubation at 37 °C.

Treatment of cherry tomatoes.

Cherry tomatoes were purchased from a local supermarket and immediately transported to ERRC and stored at 4 °C. Tomatoes were sanitized with a 290 ppm chlorine solution for 2 min before being rinsed in sterilized deionized water and dried as a single layer in a biohood for 30 min, and then dip inoculated with the S. Typhimurium suspension (10⁹ to 10⁷ CFU/mL). After drying in a biohood for 2 h, the tomatoes were placed with EOs. Four tomatoes were put into 250-mL glass jars (Quilted Crystal Inc., Bohemia, N.Y., U.S.A.), which contained small glass bottles (Glass Vials Inc., Boston, Mass., U.S.A.). A filter disk (55 mm) (Whatman™, Buckinghamshire, U.K.) with the EO solution (EO: ethanol = 1:1, v/v) was added to the bottles. The amounts of oregano EO, carvacrol, cinnamon EO, and cinnamaldehyde on the filter paper were 250 μL, while the amounts of mustard EO and AIT were 10 μL. Tomatoes were kept away from the filter disk. Glass jars were sealed for 18 h at 22 °C. Tomatoes were then removed from the jars and placed into sample bags with side mesh filter, and pummeled in an equal weight of peptone water (0.1%) using a Stomacher (260 RPM, 2 min) (Seward Laboratory Systems Inc., Bohemia). Filtrates were serially diluted, and 100 μL were spread plated on XLT-4 with 100 μg/mL nalidixic acid. Plates were inoculated at 37 °C for 48 h. The number of colonies of S. Typhimurium was recorded. When no colonies were observed on plates after 48 hr incubation, 500 μL of the same filtrates stored at 4 °C were replated on TSA to increase detection sensitivity.

Analysis of headspace concentration of EOs

Noninoculated fruits were treated in jars with EOs and their major components as described above. The headspace concentration of the major components, namely, AIT, cinnamaldehyde, and carvacrol in the jars was measured during the 18 h treatments. A gas chromatograph (GC)—mass spectrometer (MS) (6890N GC, 5973N MS, Agilent Technologies, Santa Clara, Calif., U.S.A.) equipped with a DB-5 column (30 m × 0.32 mm × 0.5 μm from J&W Scientific, Folsom, Calif.) was used for analysis of the concentration of the EOs in the glass jars. Five microliter of headspace was withdrawn using a gas-tight syringe from the jar lid with a septum and was injected into the GC. The column oven
temperature was programmed from 50 to 110 °C at 5 °C/min and then raised at 25 °C/min to 250 °C with a 1 min hold. Injector and detector temperature was 250 °C. Mass spectrometry conditions were as follows: transfer line temperature at 250 °C, mass range of 30 to 300 amu, scan rate of 5.10 scan/s, and ionization energy of 70 eV. Helium was used as the carrier gas at a flow rate of 1 mL/min. Compounds were identified by comparison of spectra of the sample compounds with those of standards and with those contained in the NIST 2002 mass spectra library as well as by comparing retention times of sample compounds with those of the standards.

Scanning electron microscopy (SEM) Tomatoes were inoculated with S. Typhimurium and treated with vaporous EOs and major components as described previously. At 1, 4, and 7 d of storage, tomato skins (ca. 1 cm in diameter and 1 mm in thickness) were excised from tomatoes with a stainless steel razor blade and immersed in a 20 mL of a 2.5% glutaraldehyde—0.1M imidazole buffer solution (pH 7.2). Samples were then prepared as described earlier (Ukuka and others 2008) and imaged using a model Quanta 200 FEG scanning electron microscope (FEI Co., Inc., Hillsboro, Oreg., U.S.A.).

Quality analysis Noninoculated tomatoes were treated with EOs as described previously and stored in clamshell containers (2.0 cm × 16.5 cm × 13.5 cm) (Dart Container Corporation, Mason, Mich., U.S.A.) at 10 ± 2 °C for 3 wk. The clamshell is a one-piece lidded plastic container, often used by the produce industry to pack cherry tomatoes and other berries. Quality (color, firmness, vitamin C, and lycopene) was determined weekly on 3 containers (12 fruits/each container) for each treatment.

Color and firmness measurements The color of cherry tomato surfaces was measured with a Hunter UltraScan® VIS colorimeter (Hunter Associates Lab, Reston, Va.) and firmness was evaluated with a TA-XT2i Texture Analyzer (Texture Technologies Corp., Scarsdale, N.Y.) as described earlier (Fan and others 2012).

Vitamin C analysis Ascorbic acid (AA) was analyzed as described previously (Fan and others 2003).

Lycopene. Lycopene was determined according to the method developed by Fish and others (2002).

Statistical analysis Each experiment was repeated 3 times with 2 measurements for each replicate for color and firmness. Data were analyzed using the analysis of variance (ANOVA) procedure (SPSS 13.0, SPSS Inc., Chicago). The least significant difference test was used to determine the significance of the treatment effects. Only significant (P < 0.05) results were discussed unless stated otherwise.

Results and Discussion

Antimicrobial activity in vitro The diameters of inhibition zone on TSA plates treated with the mustard EO and AIT were similar and the highest among EOs, followed by cinnamon EO and cinnamaldehyde, while oregano EO and carvacrol had the lowest inhibition (Figure 1). Inhibition of Salmonella increased concomitantly with increasing concentrations of EOs and their components. No visible growth of Salmonella was found on plates treated with mustard EO and AIT at 16.7 and 33.3 μL/L, respectively.

Treatment of tomatoes The concentrations of oregano, cinnamon, and mustard oils were represented and expressed by their respective major components, carvacrol, cinnamaldehyde, and AIT, respectively. The headspace concentration of carvacrol in the jars containing oregano EO and carvacrol reached maximums within 1 h at 22 °C, while cinnamaldehyde level in jars with cinnamon EO and cinnamaldehyde reached maximums at 4 h (Figure 2). The level of AIT in the jars reached the maximums immediately after sealing of the jars. It seems that the times to reach the maximums corresponded to the volatility of the compounds. AIT is a smaller molecule and more volatile than the other 2 chemicals. The headspace concentrations were relatively stable during the treatment period after reaching equilibrium even though the times to reach the equilibrium varied among the EOs and major components. The concentration of carvacrol in the jars containing the pure compound was higher than that containing oregano EO. Table 1 shows the reduction in S. Typhimurium populations on tomatoes treated with EOs and their major components. The trend of the antibacterial activities of the EOs and the components on the inoculated tomatoes was similar to that in vitro. Approximately 1.5, 3.4, 4.6, and 3.8 log CFU/g reductions of S. Typhimurium were achieved after treating with oregano EO, carvacrol, cinnamon EO, and cinnamaldehyde for 24 h, respectively. Treatments with mustard EO and AIT reduced S. Typhimurium to undetectable levels (<20 CFU/g), achieving more than 5 log CFU/g reduction. Despite being applied at the lowest concentrations, mustard EO and AIT demonstrated the highest antibacterial
activities, followed by cinnamon EO, cinnamaldehyde, carvacrol, and oregano. Obaidat and Frank (2009) found that treating tomatoes with 8.3 μL/L AIT for 10 hr at 25 °C inactivated Salmonella by 1.3 logs CFU/tomato.

In this study, we also found that mustard and cinnamon EOs were more effective against S. Typhimurium than their major components, AIT and cinnamaldehyde in vitro. This agrees with previous research by Kanemaru and Miyamoto (1990), who found that 0.1% mustard EO containing 9.4 ppm of AIT inhibited the growth of E. coli in culture medium, while 12.3 ppm of purified AIT was required to achieve the same inhibitory activity. This phenomenon was probably due to presence of other antimicrobials in mustard EO and synergistic effects between AIT and other minor components (Burt 2004). However, carvacrol was more effective than oregano EO in terms of its antimicrobial ability. Cinnamaldehyde and cinnamon had similar antimicrobial proper-

<table>
<thead>
<tr>
<th>EO</th>
<th>Inactivation (log CFU/g tissue)</th>
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<tbody>
<tr>
<td>Oregano</td>
<td>1.54 ± 0.32ab</td>
</tr>
<tr>
<td>Carvacrol</td>
<td>3.37 ± 0.85b</td>
</tr>
<tr>
<td>Cinnamon</td>
<td>4.56 ± 0.43b</td>
</tr>
<tr>
<td>Cinnamaldehyde</td>
<td>3.79 ± 0.49b</td>
</tr>
<tr>
<td>Mustard</td>
<td>6.18 ± 0.31c</td>
</tr>
<tr>
<td>AIT</td>
<td>5.52 ± 0.38c</td>
</tr>
</tbody>
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*Initial counts of S. Typhimurium on the cherry tomatoes (calculated according to water treatment) were 6.32 ± 0.12 log CFU/g tissue (means ± standard errors) (n = 3).

Microbial cell surface morphology

Images of S. Typhimurium after 1 d exhibited a typical rod-shaped bacterial appearance with homogeneous and smooth surfaces after treatment (Figure 3). Salmonella cells treated with cinnamaldehyde exhibited many wrinkles and a rough surface. Cells treated with mustard EO and AIT exhibited broken cell walls. After 4 d, the surface of S. Typhimurium treated with carvacrol had wrinkles and clefts. Cells exposed to cinnamaldehyde had a few irregularly shaped extruded spots on the surface of the bacterial body. Large holes appeared on the bacterial cells treated with mustard EO and AIT. On day 7, the S. Typhimurium on control

![Figure 2](image1.png)

**Figure 2**—Changes in concentrations of the EOs and their major components in the sealed glass jar during 18 h treatment at 22 °C. Vertical bars represent standard error (n = 3).

![Figure 3](image2.png)

**Figure 3**—Surface structure of S. Typhimurium on the cherry tomatoes treated with EOs and major components at 1, 4, and 7 d of storage as observed by SEM. Three representative cells from each treatment were selected from SEM images and lined up parallel.
Inactivation of *Salmonella* on tomatoes...

fruit displayed an intact, plump cell structure with light cracks. Modification of the bacteria by oregano EO was the least severe among all EOs tested, while some cells treated with cinnamon EO had cracks and folds. Deformation of *Salmonella* cells by carvacrol, cinnamaldehyde, mustard EO, and AIT were visible, in which cells showed a loss of the original shape and a marked bumpy surface. In particular, mustard EO- and AIT-treated cells were severely damaged and shriveled with many holes.

Changes in color and firmness of cherry tomatoes during storage

$L^*$ values indicated the darkness of the tomato surface color. $L^*$ values of all samples did not significantly change during storage (Figure 4A and 4B). The $a^*$ and $b^*$ values represent redness and yellowness of tomatoes, respectively. The higher the $a^*$ values, the redder the tomatoes were. After 14 d of storage, the $a^*/b^*$ values of the samples did not change significantly except for samples treated with mustard EO and AIT (Figure 4C and 4D), which showed a decrease in $a^*/b^*$ values. After 21 d of storage, the $a^*/b^*$ values of both mustard EO- and AIT-treated tomatoes were significantly lower, suggesting that the tomatoes were less red when compared with the control and other treatments.

The firmness of all samples decreased during storage at 10 ± 2 °C (Figure 5). Cinnamon EO, cinnamaldehyde, and carvacrol had no significant effect on firmness of tomatoes. Firmness of tomatoes treated with oregano was higher than the control sample during the storage. However, firmness was reduced by the mustard EO and AIT treatments, with final values of 0.53 and 0.61 kg, respectively, representing decreases of 32.9% and 22.8%, respectively, compared to the control. SEM analysis of the cross

![Figure 4](image-url) Changes in $L^*$ (A, B) and $a^*/b^*$ (C, D) of cherry tomatoes during storage at 10 ± 2 °C. Vertical bars represent standard error ($n = 3$).

![Figure 5](image-url) Effect of the EOs (A) and major components (B) on the firmness of cherry tomatoes stored at 10 ± 2 °C. Vertical bars represent standard error ($n = 3$).
sections of tomato skin showed that several layers of cells under the surface collapsed after treatment with mustard EO and AIT (data not shown). Our results suggest that mustard EO and AIT damaged tomatoes even though the 2 treatments achieved the highest reduction of *Salmonella* on tomatoes. To reduce the injury of tomatoes caused by mustard EO and AIT, microencapsulation of EOs with slow release mechanisms during storage may be used as demonstrated by Chacon and other (2006) on chopped beef.

**Changes in the concentration of ascorbic acid and lycopene during storage**

Ascorbic acid was not significantly affected by any of the treatments during first 7 d (Figure 6A and 6B). At day 14, tomatoes treated with AIT and mustard EO had lower vitamin C content than the control. The vitamin C content of the samples treated with mustard EO and AIT decreased by 11.98% and 17.36%, respectively, compared to the control at the end of the storage. The ascorbic acid content of samples treated with cinnamon EO and cinnamaldehyde was similar to that of controls during storage. However, the oregano EO-treated tomatoes had slower rate of decrease in vitamin C content than the controls during storage. After 21 d of storage, the vitamin C content of oregano EO-treated samples was 7.65% higher than that of the control.

The effects of EOs on the lycopene concentrations in the tomato are presented in Figure 6C and 6D. Compared to the control, lycopene content was not significantly affected by EOs and their extract treatments during the first 14 d of storage, except that lycopene in the AIT-treated samples decreased by 12.32% compared to the control. At 21 d of storage, the lycopene content in the AIT-treated sample was still the lowest among all the samples, while oregano EO had 13.13% higher lycopene than the control.

These results indicate that the mustard EO and AIT may serve as pro-oxidants, and AIT may induce the formation of hydroxyl radicals (·OH) (Wang and others 2010). A possible explanation for the decrease in ascorbic acid and lycopene, as well as the discoloration and softening of fruits is the reaction of these compounds with -OH radicals. Our investigations determined that oregano EO and carvacrol had improved retention of ascorbic acid and lycopene. Aeschbach and others (1994) indicated that carvacrol inhibited peroxidation. The intrinsic mechanism for the improved retention of the ascorbic acid and lycopene with oregano treatment is still unclear.

**Conclusions**

Our results revealed that *Salmonella* can be inactivated by EOs and their components when used as a vapor. Mustard EO and AIT achieved more than 5 log CFU/g reduction of *S. Typhimurium* on tomatoes, and were more effective than oregano and cinnamon EO and their components in reducing the pathogens. However, mustard EO and AIT decreased the levels of vitamin C and lycopene, and softened and discolored the fruit. Carvacrol, cinnamon EO, and cinnamaldehyde achieved 3.37, 4.56, and 3.79 log CFU/g reductions of *S. Typhimurium*, respectively, and did not affect the color, texture, level of ascorbic acid, or lycopene content. Although oregano EO reduced only 1.54 log CFU/g of bacteria, it exhibited an ability to enhance tomato quality retention. In summary, cinnamon EO, cinnamaldehyde, carvacrol, and oregano EO may potentially be used as effective natural alternatives to aqueous chemical sanitizers to enhance the microbial safety of tomatoes while maintaining or improving quality.
Inactivation of Salmonella on tomatoes...

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