Carvacrol and Cinnamaldehyde Inactivate Antibiotic-Resistant *Salmonella enterica* in Buffer and on Celery and Oysters

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**ABSTRACT**

The emergence of antibiotic-resistant *Salmonella* is of concern to food processors. The objective of this research was to identify antimicrobial activities of cinnamaldehyde and carvacrol against antibiotic-resistant *Salmonella enterica* in phosphate-buffered saline (PBS) and on celery and oysters. Twenty-three isolates were screened for resistance to seven antibiotics. Two resistant and two susceptible strains were chosen for the study. *S. enterica* cultures (10⁷ CFU/ml) were added to different concentrations of cinnamaldehyde and carvacrol (0.1, 0.2, 0.3, and 0.4% [vol/vol]) in PBS, mixed, and incubated at 37°C. Samples were taken at 0, 1, 5, and 24 h for enumeration. Celery and oysters were inoculated with *S. enterica* (10⁶–⁷ CFU/ml), treated with 1% cinnamaldehyde or 1% carvacrol, incubated at 4°C, and then sampled for enumeration on days 0 and 3. Both antimicrobials induced complete inactivation of *S. enterica* in PBS at 0.3 and 0.4% on exposure, and on 0.2% in 1 h. Exposure to cinnamaldehyde at 0.1% inactivated all pathogens at 1 h, and survivors were observed only for *Salmonella* Newport with 0.1% carvacrol at 1 h. In celery, 1% carvacrol reduced *S. enterica* populations to below detection on day 0, while 1% cinnamaldehyde reduced populations by 1 and 2.3 log on day 0 and day 3, respectively. In oysters, both antimicrobials caused about 5-log reductions on day 3. These results show the potential antimicrobial effects of carvacrol and cinnamaldehyde against antibiotic-resistant *S. enterica* in vitro and in foods.

*Salmonella enterica* is one of the leading causes of foodborne illnesses. According to the 2006 preliminary FoodNet data with estimates from the Center for Disease Control and Prevention, of the total 17,252 laboratory-confirmed cases of foodborne infections in 10 states, 6,655 (overall incidence of 14.81 per 10,000 people) were due to *S. enterica* (45, 46). There is therefore, an urgent need to devise appropriate control measures for this pathogen.

Antibiotic-resistant strains of *S. enterica* have emerged in recent years and are a concern to the food industry. Antibiotic resistance may arise from several distinct pathways: changes in permeability in the bacterial cell wall, which restricts access of antibiotics to target sites; efflux of the antibiotic from the cell; and formation of new metabolic pathways for survival to overcome those inhibited by the antibiotic. A common mechanism is the acquisition of external genes that provide resistance to an entire class of antibiotics. Bacteria also possess pumps that export antibiotics before they can reach sensitive intracellular targets. The use of antibiotics in animal husbandry and human medicines creates selective pressure that favors the development of selective resistance among microorganisms. Extensive use of antibiotics in humans, animals, and plants has resulted in formation of a pool of resistance genes in the environment (10, 39). For example, pasteurized milk from a contaminated dairy plant was implicated in an outbreak involving 180,000 cases associated with *Salmonella* Typhimurium that was resistant to five antibiotics (38). Previously, Niemira et al. (29), Rajashekara et al. (34), and Schwaiger et al. (40) reported on antibiotic-resistant *S. enterica* isolates that are relevant to the present study.

*Salmonella*-resistant strains were also found to be prevalent in a large number of imported seafood samples (32, 48). The majority of worldwide outbreaks associated with bivalve shellfish have been linked to oysters, and then to clams and mussels. Oysters, which are filter feeders, often concentrate pathogenic bacteria from marine and brackish waters (8). Rising ocean temperatures seem to contribute to observed increases in outbreaks associated with oysters (27). Studies have shown that *Salmonella* was present in 7.4% of U.S. market oysters (2, 3). These oyster isolates were susceptible to ciprofloxacin, gentamycin, and trimethoprim-sulfamethoxazole, but were largely resistant to ampicillin and tetracycline. Successful efforts to inactivate *S. enterica* on oysters include the use of high pressure (26) and ionizing radiation (22). Chitosan treatment proved ineffective over a 12-day storage period (7).

A U.S. Food and Drug Administration survey of high-volume imported fresh produce including celery showed that a significant fraction of all vegetables were contaminated with *Salmonella* and other pathogens (4). Quiroz-Santiago et al. (33) reported that *Salmonella* contamination of vegetables imported from Mexico ranged as follows (percentage of

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isolates for antibiotic resistance. O157:H7 and E. coli were molecular weight 150.2, Salmonella pure, molecular weight 132.2, Chemical Abstracts Services [CAS] no. 14371-10-9) and carvacrol (>98% pure, molecular weight 150.2, CAS no. 499-75-2).

Bacterial culture preparation and media. Each Salmonella culture was prepared by inoculating cryopreserved cells in tryptic soy broth (Difco, Becton Dickinson, Sparks, MD) and then incubating the cells overnight (18 to 20 h) at 37°C. Two transfers were done before a working culture was prepared. Cells (10⁷ CFU/ml) were harvested by centrifugation (2,000 × g for 10 min) and washed twice in sterile buffered peptone water (BPW; Difco, Becton Dickinson). Cells suspended in BPW to a concentration of about 10⁶ CFU/ml (from overnight culture) were then diluted in BPW to the required concentration, depending on the initial inoculum needed for experiments. Viable organisms after each treatment were enumerated by serially diluting in BPW and plating on xylose-lysine-deoxycholate agar (XLD; Difco, Becton Dickinson) and tryptic soy agar (TSA; Difco, Becton Dickinson), and then incubating the cells at 37°C for 24 to 48 h. For all studies involving plant antimicrobials, controls not treated with these antimicrobials were used as standards for comparison to those samples treated with antimicrobials.

Screening of S. enterica isolates for antibiotic resistance. To determine antibiotic resistance, Salmonella strains were screened with BD BBL Sensi-Disc antibiotics (BBL, Becton, Dickinson, Sparks, MD). The antibiotics used from the Sensi-Disc were amoxicillin–clavulanic acid (20–10 μg), ampicillin (10 μg), cefoxitin (30 μg), chloramphenicol (30 μg), streptomycin (10 μg), trimethoprim-sulfamethoxazole (1.25–23.75 μg), and tetracycline (30 μg). A breakpoint approach was used to determine resistance of S. enterica isolates to the various antibiotics tested. The breakpoints used for the control strain E. coli ATCC 25922 correspond to values established for Enterobacteriaceae by the Clinical Laboratory Standards Institute and approved by the U.S. Food and Drug Administration. The following observed breakpoints were used to define resistance of the bacteria to the seven antibiotics: amoxicillin–clavulanic acid (≤13 mm), ampicillin (≤13 mm), cefoxitin (≤14 mm), chloramphenicol (≤12 mm), streptomycin (≤11 mm), trimethoprim-sulfamethoxazole (≤10 mm), and tetracycline (≤14 mm).

Isolates were grown on Luria-Bertani agar (Difco, Becton Dickinson) plates, and CFUs were suspended in 0.85% NaCl to an optical density at 600 nm of 0.80 (equivalent to a McFarland standard of 1.0). The suspension was then swabbed confluently onto Mueller-Hinton agar (Difco, Becton Dickinson) plates. Then, a Sensi-Disc for each antibiotic was placed on the plates. After incubation at 37°C for 24 to 48 h, the MIC was determined as per the manufacturer’s instructions.

Antimicrobial activities of cinnamaldehyde and carvacrol against resistant and susceptible S. enterica in PBS buffer. The antimicrobial compounds (cinnamaldehyde and carvacrol) were suspended in sterile PBS. A 0.5% stock solution was prepared in PBS. The stock solution was serially diluted in microcentrifuge tubes to 0.4, 0.3, 0.2, and 0.1% to a volume of 90 μl. The test organism (10 μl, 10⁵ CFU/ml) was then added to each microcentrifuge tube, mixed thoroughly, and sampled immediately (0-min sample). The 0-min sample was spread plated on XLD. The samples were then incubated at 37°C. After incubation, the samples were evaluated at 0, 1, 5, and 24 h. At each sampling, serial dilutions were done in BPW and plating on XLD. CFUs were counted after incubation of the plates at 37°C for 24 h.

Antimicrobial activities of cinnamaldehyde and carvacrol against resistant and susceptible S. enterica on celery. Fresh celery was obtained from local retail outlets. Celery was washed thoroughly in deionized water and then cut into 10-g pieces. The cut pieces were exposed to UV light under a biohood for 30 min. Celery samples were then dip inoculated in Salmonella Newport culture (10⁶–7 CFU/ml) for 2 min, and then dried for 1 h under a biohood. The inoculated celery samples were immersed in 1% cinnamaldehyde or 1% carvacrol in PBS for 10 min. After treatment, celery samples were stored in sterile petri dishes at 4°C for 3 days. Enumerations of surviving bacteria were done on...
Antimicrobial activities of cinnamaldehyde and carvacrol against resistant and susceptible S. enterica on oysters. Oysters (in shell) were purchased from local grocery stores. They were shucked and washed 10 times in deionized water, and were then kept immersed in deionized water overnight at 4°C. The cleaned oysters were cut into 10-g pieces, dipped in hot water for 40 s, and dried under a biohood for 30 min. Oyster samples were dip inoculated in Salmonella Newport culture (10^9 CFU/ml) for 1 min, serially diluted in BPW, and then plated on XLD and TSA. Colonies were counted after 24 h of incubation at 37°C.

Statistical analysis. Each experiment was repeated at least three times. Tables 2 through 4 show means and standard deviations (SD) for the surviving bacterial populations for each sampled time.

RESULTS AND DISCUSSION

Screening of S. enterica isolates for antibiotic resistance. A total of 23 isolates were tested for susceptibility to amoxicillin–clavulanic acid, ampicillin, cefoxitin, chloramphenicol, streptomycin, trimethoprim-sulfamethoxazole, and tetracycline (Table 1). Of the 23 isolates, 9 (39.1%) were resistant to at least one antibiotic, and 14 isolates (60.9%) were susceptible to all seven antibiotics tested. Only one isolate (4.3%) was resistant to amoxicillin–clavulanic acid, 8 (34.8%) were resistant to ampicillin and tetracycline, 6 (26.1%) were resistant to cefoxitin and tetracycline, 5 (21.7%) to chloramphenicol, and 1 isolate (4.3%) to amoxicillin–clavulanic acid or to cefoxitin. None of the tested isolates was resistant to trimethoprim-sulfamethoxazole. These results suggest that the latter combination has the potential to be a drug of choice for treating salmonellosis in animals and humans.

Salmonella Newport was found to be the most resistant serotype, showing resistance to six of the seven antibiotics tested. Four strains of Salmonella Typhimurium DT104 (H3278, H3880, H2662, and H3380) were each resistant to four antibiotics. Salmonella Newport and Salmonella Typhimurium DT104 H3278, H3880, H2662, and H3380 were each resistant to four antibiotics.
Typhimurium DT104 strain H3278 exhibited salmonella, and 0.1 ng cinnamaldehyde, for 0.4 ng carvacrol, no 0.1 2.3 0.1 4.2 no completely inactivated both resistant and sensitive 0.1 NG NG NG NG NG NG. Enteritidis, the population declined by about 1 0.1 NG NG NG NG NG NG. Salmonella enterica Typhimurium 645 were each resistant to one 237 Salmonella 0.7 4.0 0.3 0.0 NG NG NG NG NG NG. CAR TSA 0.0 Salmonella 0.0 NG 3.9 result in inactivation of all Salmonella in PBS buffer. 0.2 NG 4.8 a 0.1 NG NG NG NG NG NG. Typhimurium DT104 H3278. Exposure to carvacrol, no survivors were detected at all times 0.2 NG NG NG NG NG NG. Typhimurium H3278 0.5 NG NG NG NG NG NG. Salmonella Typhimurium, slightly 0.1 2.2 0.3 Values are means ± SD (n = 3) and are expressed in log CFU per milliliter. CAR, carvacrol; CIN, cinnamaldehyde. 0.0 4.0 Salmonella Typhimurium and 0.3 NG NG NG NG NG NG. Newport, and there was no 0.0 4.0 Salmonella Newport, a 2.8-log reduction in population 24 4.5 0.5 NG NG NG NG NG NG. Antimicrobial effects of carvacrol and cinnamaldehyde against antibiotic-resistant and susceptible Salmonella enterica isolatesa

<table>
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<th>Salmonella isolate</th>
<th>Sampling time (h)</th>
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<th>0.2</th>
<th>0.3</th>
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<td></td>
<td>Control</td>
<td>CAR</td>
<td>CIN</td>
<td>CAR</td>
<td>CIN</td>
</tr>
<tr>
<td>Typhimurium</td>
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<td>3.1 ± 0.7</td>
<td>4.0 ± 0.1</td>
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<tr>
<td></td>
<td>1</td>
<td>5.1 ± 0.0</td>
<td>NG</td>
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<td>NG</td>
<td>NG</td>
<td>NG</td>
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<tr>
<td></td>
<td>24</td>
<td>4.5 ± 0.5</td>
<td>NG</td>
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</tr>
<tr>
<td>Typhimurium H3278</td>
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<td>NG</td>
<td>4.8 ± 0.0</td>
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</tr>
<tr>
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<tr>
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<td>5</td>
<td>7.0 ± 0.2</td>
<td>NG</td>
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<td></td>
<td>24</td>
<td>8.0 ± 0.2</td>
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<tr>
<td></td>
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<td>5.1 ± 0.1</td>
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<td></td>
<td>24</td>
<td>8.7 ± 0.1</td>
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<tr>
<td></td>
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<td>3.0 ± 0.3</td>
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<td>6.9 ± 0.0</td>
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<td>7.7 ± 0.6</td>
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</table>

a Values are means ± SD (n = 3) and are expressed in log CFU per gram. CAR, carvacrol; CIN, cinnamaldehyde.

Antimicrobial activities of cinnamaldehyde and carvacrol against S. enterica in PBS buffer. Cinnamaldehyde and carvacrol were tested at 0.1, 0.2, 0.3, and 0.4% concentrations in PBS against S. enterica at 0, 1, 5, and 24 h (Table 2). Both compounds at concentrations of 0.4 and 0.3% completely inactivated both resistant and sensitive organisms at all sampling points. Because no survivors were detected at all times at 0.4%, the data for this concentration are not included in Table 2. With 0.2% carvacrol, no survivors were detected at all times for all four isolates. With 0.2% cinnamaldehyde, no survivors were detected at all times for Salmonella Typhimurium and Salmonella Newport. For Salmonella Typhimurium DT104 H3278, there was very limited reduction in the population at 0 h, and no survivors were detected thereafter at ≥1 h with 0.2% cinnamaldehyde. With 0.2% cinnamaldehyde, for Salmonella Enteritidis, the population declined by about 1 log at 0 h, and no survivors were detected thereafter.

Carvacrol at 0.1% reduced the population of Salmonella Typhimurium by about 2 log, and that of Salmonella Enteritidis and Salmonella Newport by about 1 log each, immediately on exposure. No survivors were detected at 1 h or later for Salmonella Typhimurium and Salmonella Enteritidis. For Salmonella Newport, a 2.8-log reduction in population was observed at 1 h, and no survivors were detected thereafter. With 0.1% carvacrol, no survivors were detected at all times for Salmonella Typhimurium DT104 H3278. Exposure to cinnamaldehyde at 0.1% resulted in inactivation of all organisms at 1 h or longer for all four isolates tested. Immediately on exposure, the reduction was 1 log for Salmonella Typhimurium, slightly <1 log for Salmonella Enteritidis and Salmonella Newport, and there was no reduction for Salmonella Typhimurium DT104 H3278.

When comparing the two antimicrobials, carvacrol showed stronger activity than did cinnamaldehyde at low concentrations (0.1 to 0.2%) against S. enterica.

<table>
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<tr>
<th>Treatmentb</th>
<th>Agar medium</th>
<th>Day 0</th>
<th>Day 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>TSA</td>
<td>4.9 ± 0.2</td>
<td>4.6 ± 0.3</td>
</tr>
<tr>
<td></td>
<td>XLD</td>
<td>4.9 ± 0.1</td>
<td>4.4 ± 0.3</td>
</tr>
<tr>
<td>1% CAR</td>
<td>TSA</td>
<td>&lt;1.0 ± 0.0</td>
<td>&lt;1.0 ± 0.0</td>
</tr>
<tr>
<td></td>
<td>XLD</td>
<td>&lt;1.0 ± 0.0</td>
<td>&lt;1.0 ± 0.0</td>
</tr>
<tr>
<td>1% CIN</td>
<td>TSA</td>
<td>3.8 ± 0.1</td>
<td>2.3 ± 0.3</td>
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<tr>
<td></td>
<td>XLD</td>
<td>3.8 ± 0.1</td>
<td>2.2 ± 0.3</td>
</tr>
</tbody>
</table>

a Values are means ± SD and are expressed in log CFU per gram. CAR, carvacrol; CIN, cinnamaldehyde.
TABLE 4. Survival of antibiotic-resistant Salmonella Newport on oysters treated with carvacrol and cinnamaldehyde\(^a\)

<table>
<thead>
<tr>
<th>Treatment(^a)</th>
<th>Time</th>
<th>Agar</th>
<th>Day 0</th>
<th>Day 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>10 min</td>
<td>TSA</td>
<td>5.7 ± 0.1</td>
<td>5.6 ± 0.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>5.7 ± 0.1</td>
<td>5.1 ± 0.1</td>
</tr>
<tr>
<td></td>
<td>1 h</td>
<td>TSA</td>
<td>5.7 ± 0.2</td>
<td>5.5 ± 0.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>5.8 ± 1.1</td>
<td>5.0 ± 0.1</td>
</tr>
<tr>
<td>CAR</td>
<td>10 min</td>
<td>TSA</td>
<td>4.7 ± 0.2</td>
<td>4.0 ± 0.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>4.6 ± 0.2</td>
<td>3.5 ± 0.4</td>
</tr>
<tr>
<td></td>
<td>1 h</td>
<td>TSA</td>
<td>4.2 ± 0.4</td>
<td>&lt;1.0 ± 0.0</td>
</tr>
<tr>
<td></td>
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<td>3.9 ± 0.6</td>
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<tr>
<td>CIN</td>
<td>10 min</td>
<td>TSA</td>
<td>4.9 ± 0.1</td>
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<td>4.8 ± 0.0</td>
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<td>1 h</td>
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<td></td>
<td>4.3 ± 0.3</td>
<td>&lt;1.0 ± 0.6</td>
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</table>

\(^a\) Values are means ± SD and are expressed in log CFU per gram. 
\(^b\) CAR, carvacrol; CIN, cinnamaldehyde.

Antimicrobial activities of cinnamaldehyde and carvacrol against S. enterica on celery. Fresh produce including celery can be a vehicle for transmission of S. enterica and other pathogens (1, 4). With the possible exception of gamma irradiation (25), to our knowledge, no effective treatments are available against antibiotic-resistant S. enterica on celery.

The results of the present study listed in Table 3 show that dipping of celery samples in 1% carvacrol for 10 min induced complete inactivation of Salmonella Newport on day 0 and day 3, respectively. By contrast, celery samples dipped in 1% cinnamaldehyde showed only 1- and 2.3-log reductions on day 0 and day 3, respectively. In general, there was no difference between counts of surviving salmonellae on selective (XLD) and nonselective (TSA) media (Table 3), indicating there was no substantial injury caused to the bacterial cells on celery due to antimicrobial treatments.

These results show that the effectiveness of carvacrol against antibiotic-resistant Salmonella Newport on celery is greater than that of cinnamaldehyde.

Antimicrobial activities of cinnamaldehyde and carvacrol against S. enterica on oysters. Table 4 shows that dipping of oyster samples in 1% carvacrol solution for 10 min resulted in a reduced count of Salmonella by about 1 and 1.5 log on days 0 and 3, respectively. Dipping the oysters in 1% carvacrol for 1 h showed about 1.5- and 5-log reductions (no survivors detected) at day 0 and day 3, respectively. Dipping treatments in 1% cinnamaldehyde showed similar results. The counts on selective (XLD) and nonselective (TSA) media were similar (Table 4), indicating no significant injury to S. enterica cells due to antimicrobial treatments on oysters.

These results show that both carvacrol and cinnamaldehyde are effective natural antimicrobials against antibiotic-resistant Salmonella Newport on oysters.

Mechanism of antimicrobial effects. To facilitate understanding the effectiveness of plant antimicrobials against both susceptible and resistant pathogens, we briefly present published information on the mechanism of the antimicrobial effects of carvacrol and cinnamaldehyde (5, 11). Ultee et al. (43) showed that the consequences of exposing Bacillus cereus to carvacrol include depletion of the intracellular ATP pool, change in membrane potential, and increase in permeability of the cytoplasmic membrane to proteins and potassium ions. Other studies showed that carvacrol damaged the outer membrane of Pseudomonas aeruginosa, destroying the lipopolysaccharide barrier (9), and that carvacrol can form ion channels though the membrane, partitioning the fatty acid chains of the phospholipids, thereby permitting ions to leave the cytoplasm (43). Leakage of phosphate ions occurred in Staphylococcus aureus and P. aeruginosa cells treated with oregano essential oil, which has carvacrol as a main antimicrobial component (13, 24).

Friedman (11) describes the use of autofluorescence spectra to determine the effect of carvacrol on E. coli C600. The data showed significant changes at much lower concentrations of carvacrol (0.01 mM) than changes in membrane potential or release of ATP (ATP flux) after disruption of the bacterial cell membrane, occurring at higher concentrations of carvacrol (1 to 2 mM). This observation suggests that autofluorescence detects physiological responses to lower concentrations of carvacrol more efficiently than it does changes in membrane potential or release of ATP occurring at higher concentrations of carvacrol.

Disintegration of bacterial outer membrane has not been observed with cinnamaldehyde. Instead, cinnamaldehyde is thought to interfere with the activity of some enzymes. For example, the carbonyl group of cinnamaldehyde appears to bind to proteins in Enterobacter aerogenes, preventing the action of amino acid carboxylases (47).

Comparison of the antibiotic-resistant versus -sensitive isolates of S. enterica to carvacrol and cinnamaldehyde observed in the present study does not allow specific conclusions about mechanisms. It is, however, noteworthy that Salmonella Newport, which was found to be resistant to six of the seven antibiotics tested, showed different susceptibilities to carvacrol and cinnamaldehyde than the other strains demonstrated. For example, no Salmonella Newport survivors were detected even at 0 min with the 0.2% cinnamaldehyde treatment, while survivors were detected for the other resistant isolate Salmonella Typhimurium DT104 H3278 and susceptible isolate Salmonella Enteritidis. With 0.1% carvacrol, no survivors were detected for Salmonella Typhimurium DT104 H3278 soon after exposure, while survivors were detected in case of Salmonella Newport up to 1 h, and in case of the other two susceptible isolates, up to 0 h. However, at >0.2% concentration, both antimicrobials completely inactivated the antibiotic-resistant and -susceptible isolates of S. enterica. Our results suggest that the various S. enterica isolates may not be equally susceptible to inactivation, and that the antimicrobials may act by similar mechanisms against resistant and susceptible S. enterica isolates.

Related relevant studies showed that (i) the essential oil of Origanum vulgare exhibited high antimicrobial activity...
against S. enterica at <1% (31); (ii) cinnamaldehyde, carvacrol, and thymol completely inhibited growth of B. cereus in carrot broth stored at 16°C for more than 60 days (44); (iii) carvacrol prevented biofilm formation of dual-species bacteria including S. aureus and Salmonella Typhimurium (23); and (iv) drug resistance did not affect the heat resistance of Salmonella serotypes in cooked ground beef (41).

The results of the present study show that both carvacrol and cinnamaldehyde inactivated both antibiotic-resistant and -susceptible S. enterica isolates in PBS and on two foods, celery and oysters. The antibiotic-resistant isolates largely exhibited similar susceptibilities to inactivation as did the susceptible isolates. Whether these compounds are equally effective against these and other resistant pathogens (such as vibrios in seafood) in other contaminated human foods, animal feeds, and infected animals and humans merits further investigation. However, we do not know whether the bacteria would develop cross-resistance toward the evaluated plant compounds as they did with the synthetic antimicrobials chlorhexidine and quaternary ammonium compounds (37). Finally, the results of the present study suggest that adding carvacrol or cinnamaldehyde to tanks containing oysters or other seafood may inhibit foodborne pathogenic bacteria during storage.

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REFERENCES


