Antimicrobial activity of lemongrass oil against *Salmonella enterica* on organic leafy greens

K. Moore-Neibel¹, C. Gerber¹, J. Patel², M. Friedman³ and S. Ravishankar¹

¹ Department of Veterinary Science and Microbiology, University of Arizona, Tucson, AZ, USA
² USDA-ARS, BARC-East, Beltsville, MD, USA
³ USDA-ARS Western Regional Research Center, Albany, CA, USA

Introduction

*Salmonella* spp. is one of the leading pathogens causing foodborne illness in the United States. Fresh produce outbreaks because of this pathogen are becoming more prevalent (Hanning *et al.* 2009). Fresh produce is traditionally prepared raw, as in the case of salads, increasing the risk of foodborne infection. *Salmonella* Newport is a multi-drug resistant serovar that has been associated with fresh produce outbreaks in the United Kingdom and the United States; it was associated with mixed bagged salads in 2001, lettuce in 2004 and with tomatoes in 2006 (Berger *et al.* 2010). *Salm.* Newport is one of the top five most commonly isolated serovars by the CDC over the last 30 years (Centers for Disease Control and Prevention 2011). Since 2006, 10 major foodborne outbreaks of *Salmonella* have been traced back to the consumption of fresh produce (CDC 2011). Based on 2006 population data, of the estimated 9.4 million cases of foodborne illnesses, nontyphoidal *Salmonella* was responsible for 11% of cases, 35% of hospitalizations and 64% of deaths (Scallan *et al.* 2011).

Over the last two decades, there has been an increase in consumption of fresh fruits and vegetables in the United States. This increase can be attributed to increased income, domestic production, worldwide availability and healthy lifestyles (Pollack 2001). There has also been an increase in organic consumption. In 2000, consumers purchased more organic food in the conventional supermarket than any other venue (Dimitri and Greene 2002).

Keywords

abuse temperature, antimicrobial activity, exposure time, lemongrass oil, organic leafy greens, *Salmonella enterica*.

Correspondence

Sadhana Ravishankar, Department of Veterinary Science and Microbiology, University of Arizona 1117, E. Lowell Street, Tucson, AZ 85721, USA.
E-mail: sadhravi@email.arizona.edu

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Abstract

**Aims:** We investigated the antimicrobial effectiveness of lemongrass essential oil on organic leafy greens, romaine and iceberg lettuces and mature and baby spinach, inoculated with *Salmonella* Newport. The influences of exposure times and abuse temperatures on bacterial survival were also investigated.

**Methods and Results:** Leaf samples were washed, inoculated with *Salm.* Newport (6-log CFU ml⁻¹) and dried. Inoculated leaves were immersed in solutions containing 0, 0.3 or 0.5% lemongrass oil in phosphate-buffered saline for 1 or 2 min and then individually incubated at 4 or 8°C. Samples were taken at day 0, 1 and 3 for the enumeration of survivors. Compared to the PBS control, romaine and iceberg lettuces, and mature and baby spinach samples showed between 0–1.5-log, 0.5–4.3-log, 0.5–2.5-log and 0.5–2.2-log CFU g⁻¹ reductions in *Salm.* Newport by day 3, respectively.

**Conclusions:** The antimicrobial activity of lemongrass oil against *Salm.* Newport was concentration and time dependent. The antimicrobial activity increased with exposure time; iceberg samples treated for 2 min generally showed greater reductions (*P* < 0.05) than those treated for 1 min (c. 1-log reduction difference for 0.3 and 0.5% treatments). Few samples showed a difference between refrigeration and abuse temperatures.

**Significance and Impact of the Study:** This study demonstrates the potential of lemongrass oil solutions to inactivate *Salm.* Newport on organic leafy greens.
Organic foods are cultivated naturally using specific pest management, composting and crop rotation practices, all following national standards supervised by the United States Department of Agriculture-National Organic Program (Dimitri and Greene 2002; USDA-NOP 2011). Organic foods are produced without the use of any chemicals, and hence, natural plant compounds may be good alternative to chemicals. Organic produce may have a higher microbial load than conventionally grown produce (Oliveira et al. 2010; Wetzel et al. 2010).

Essential oils have been used for their aroma, flavour, bactericidal, preservative and medicinal properties (Burt 2004). Carvacrol and cinnamaldehyde, the main constituents of oregano and cinnamon essential oils, showed antimicrobial activity against Campylobacter jejuni (Ravishankar et al. 2008) and antibiotic-resistant Salmo nella enterica in vitro and on celery and oysters (Ravishankar et al. 2010). The main active constituents of lemongrass oil with an aromatic taste are citral (65–86%), nerol and geraniol (Teuscher 2006). The related aromatic citrus essential oil, in which the main constituents are camphor, carvacrol, cineole, linalool, limonene, menthol, pinene and thymol (Sokovic et al. 2010), was effective against Salm. Enteritidis, Escherichia coli and Listeria monocytogenes in fruit-based salads (Belletti et al. 2008). Clove extract was active against Salm. Typhimurium and E. coli O157:H7 on fresh lettuce (Kim et al. 2011). Lemongrass oil exhibited antibacterial activity in vitro against Candida albicans, E. coli, Salm. Typhimurium, Serratia marcescens, Staphylococcus aureus and other potential pathogens (Hammer et al. 1999; Friedman et al. 2002; Maizura et al. 2007; Aiemsaarad et al. 2011) and inhibited the growth of foodborne pathogens in minced meat products and strawberry juice (Barbosa et al. 2009; Duan and Zhao 2009).

In an effort to discover new and natural antimicrobial treatments against Salm. Newport on organic produce, we previously evaluated the antimicrobial efficacy of apple, hibiscus and olive formulations against Salmonella on leafy greens (Moore et al. 2011). The objectives of the present study were to extend the cited study by evaluating the effectiveness of lemongrass essential oil on four different types of organic leafy greens inoculated with Salm. Newport as a function of concentration of the oil solutions, exposure times and storage temperatures.

Materials and methods

Bacterial culture preparation and media

The strain used for this study was a multi-drug resistant Salm. enterica serovar Newport LAJ160311, with the JJPX01.0014 PulseNet PFGE profile. This strain was isolated from an oyster and obtained from Dr Lynn Joens, University of Arizona, Tucson, Arizona. The culture was prepared by inoculating cryo-preserved cells in tryptic soy broth (TSB; Difco, Becton Dickinson, Sparks, MD, USA) and incubating overnight (18–22 h) at 37°C. A fresh overnight culture was used for each experiment. Cells were suspended in buffered peptone water (BPW; Difco, Becton Dickinson) at a concentration of 6-log CFU ml⁻¹ by dilution of the overnight culture. Leaf samples (10 g) were stomached in buffered peptone water (BPW). Enumeration of survivors following treatment was performed by means of serial dilution in 0.1% peptone water (Difco, Becton Dickinson) and surface plating on xylose-lysine-desoxycholate agar (XLD; Difco, Becton Dickinson) for Salmonella. The plates were incubated at 37°C for 24 h and counted.

Food products and antimicrobials

The organic fresh produce was obtained from a local grocery store. The following organic leafy greens were evaluated: romaine lettuce, iceberg lettuce, mature bunched spinach and baby spinach. Whole leaf samples (10 g) were used for romaine and iceberg lettuces, and multiple leaves (10 g) were used for mature and baby spinach. Lemongrass oil made from 100% pure Cymbopogon citratus leaves was obtained from Lhasa Karnak Herb Company, Berkeley, CA, USA.

Antimicrobial treatment solutions

Dip solutions of lemongrass oil at 0·1, 0·3 and 0·5% (v/v) concentrations were prepared in sterile phosphate-buffered saline (PBS, pH 7). The treatment solutions were mixed thoroughly by using a stomacher (Seward, London, UK) at normal speed (230 rev min⁻¹) for 30 s. Treatment dips were used immediately after preparation.

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Organic leafy greens were thoroughly washed in deionized water and then weighed into 10 g samples. After washing, samples were placed in a biohood under UV light (254 nm) for 30 min to reduce normal microflora. The samples were then dip inoculated with Salm. Newport (6-log CFU ml⁻¹) for 2 min and dried for 30 min under a biohood. Control samples were taken after inoculation and after the 30 min drying time to determine inoculation levels. Each sample was then submerged in one of the antimicrobial treatment solutions (200 ml) in a stomacher bag for 1 or 2 min with gentle agitation. PBS control samples were included in each experiment. Immediately following treatment, samples were placed into
individual stomacher bags and incubated at 4 or 8°C. Samples were taken at day 0, and following incubation, days 1 and 3 for the enumeration of surviving *Salmonella*. Leaf samples were processed, plated and incubated as previously mentioned. Colonies were counted after 24 h incubation. Experiments were repeated a total of three times.

**Statistical analysis**

Colony counts of *Salmonella* obtained from duplicate XLD agar plates at each sampling period were converted to log CFU g⁻¹. The experiment was performed in triplicate. Data were analysed by a three-way ANOVA using the ‘Proc Mixed’ procedure (SAS 8.2, Cary, NC, USA) for effects of lemongrass oil concentrations, sampling period, incubation temperature and their interactions. In all cases, the level of statistical significance was *P* < 0.05.

**Results**

**Antimicrobial activity of lemongrass oil against *Salm. Newport* on organic leafy greens**

Control leaf samples taken after inoculation and before and after drying for 30 min showed no loss of organisms after the drying process, as the bacterial levels were consistently around 5-log CFU g⁻¹ on all leafy greens both before and after drying (data not shown).

**Storage time-dependent reductions in *Salm. Newport* treated with lemongrass oil**

Leaves treated with lemongrass oil showed the largest reduction in bacterial populations on day 3 of sampling. As shown in Fig. 2, the greatest reduction by day 3 was seen in 2-min-treated iceberg leaves. Iceberg leaves exposed to 0.5% treatment for 2 min and stored at 4°C showed a significant 1-log CFU g⁻¹ reduction in bacterial population on day 1 compared to day 0 with a final 0.6-log reduction by day 3 (*P* < 0.05). Iceberg leaves stored at 8°C showed significant 1.2- and 2.5-log CFU g⁻¹ reductions with the 0.3 and 0.5%–2 min, respectively, treatments by day 3 (Fig. 2) (*P* < 0.05). No survivors were recovered in iceberg samples treated for 2 min in 0.5% dip stored at 8°C (detection limit > 10 cells). Similar results were seen in mature spinach samples. Mature spinach samples stored at 4 and 8°C and treated for 1 and 2 min with the 0.5% lemongrass oil showed significant reductions (1.1–1.3-log CFU g⁻¹; *P* < 0.05) by day 3 (Fig. 4). Mature spinach samples treated with the 0.3% lemongrass oil and stored at 8°C showed significant reductions (1.2-log CFU g⁻¹; *P* < 0.05) by day 3.

Effects of exposure time of lemongrass oil on *Salm. Newport* populations

Iceberg samples treated with 0.3% oil dip and stored at 4 and 8°C for 3 days showed significant 0.9-log and 1.4-log CFU g⁻¹ difference between a 1 and 2 min treatment, respectively (Fig. 2) (*P* < 0.05). Iceberg samples treated with 0.5% oil solution and stored for 4°C for 1 and 3 days showed a significant 1.9- and 1.5-log CFU g⁻¹ difference between 1 and 2 min, respectively (Fig. 2) (*P* < 0.05). Iceberg leaf samples treated with a 0.5% dip and stored at 8°C showed significant 1.2-, 3.3- and 3.8-log CFU g⁻¹ difference in treatment times (1 vs 2 min) for day 0, 1 and 3, respectively (Fig. 2) (*P* < 0.05). Romaine leaf samples treated with 0.3% lemongrass oil and stored at 8°C for 1 and 3 days showed significant 0.9- and 0.8-log CFU g⁻¹ differences in 1 and 2 min exposure times, respectively (Fig. 1) (*P* < 0.05). Mature spinach showed a significant 0.7-log CFU g⁻¹ difference in exposure time on samples treated with 0.3% lemongrass oil stored at 4°C on day 1 (Fig. 4) (*P* < 0.05). Little or no difference was seen between a 1 and 2 min exposure times in baby spinach samples (Fig. 3).

**Reductions in *Salm. Newport* populations based on storage temperature**

For iceberg lettuce treated with 0.5% lemongrass oil for 1 and 2 min and stored at 4 and 8°C, significant differences ranging from 0.5 to 1.9 log CFU g⁻¹ (*P* < 0.05) were seen on days 1 and 3 (Fig. 2). For the romaine samples treated with 0.1% lemongrass oil for 1 and 2 min and stored for 3 days, there were significant differences of 0.6- and 1.3-log CFU g⁻¹ (*P* < 0.05), respectively, at 4 and 8°C (Fig. 1). Romaine samples treated with 0.5% lemongrass dip for 1 and 2 min and stored for 3 and 1 days showed significant differences of 0.8 and 0.7 log CFU g⁻¹, respectively, between 4 and 8°C, respectively (Fig. 1) (*P* < 0.05). Mature spinach samples treated with 0.3% lemongrass oil for 2 min and stored for 1 day showed a 0.5-log difference (Fig. 4) (*P* < 0.05). Baby spinach samples treated with 0.5% lemongrass oil for 2 min and sampled on day 1 showed a significant 0.4-log CFU g⁻¹ difference in samples stored at 4 vs 8°C (Fig. 3) (*P* < 0.05).

**Reductions in *Salm. Newport* populations in lemongrass oil-treated samples as compared to the control**

Romaine lettuce showed c. 1-log reduction for samples treated with 0.3 and 0.5% concentrations for 1 min for both 4 and 8°C storage temperatures as compared to the PBS control (Fig. 1). Samples treated with 0.3 and 0.5% lemongrass oil for 2 min and stored under the same
**Figure 1** Survival of *Salmonella* Newport on organic romaine lettuce treated with PBS, 0·1, 0·3 and 0·5% lemongrass oil solution. (a) 1 min treatment stored at 4°C; (b) 2 min treatment stored at 4°C; (c) 1 min treatment stored at 8°C; (d) 2 min treatment stored at 8°C. Values plotted at each sampling point are an average of 3 replicates. Error bars represent standard deviations from the mean. Bars with different letters x, y, z show significant differences (*P* < 0·05) among sampling days (day 0, 1 and 3) for the respective control and treatment concentrations (0·1, 0·3 and 0·5%). Bars with different letters a, b, c show significant differences (*P* < 0·05) among the control and treatments for the various sampling days. (■) PBS control; (▲) 0·1%; (▲) 0·3% and (▲) 0·5%.

**Figure 2** Survival of *Salmonella* Newport on organic iceberg lettuce treated with PBS, 0·1, 0·3 and 0·5% lemongrass oil solution. (a) 1 min treatment stored at 4°C; (b) 2 min treatment stored at 4°C; (c) 1 min treatment stored at 8°C; (d) 2 min treatment stored at 8°C. Values plotted at each sampling point are an average of three replicates. Error bars represent standard deviations from the mean. Bars with different letters x, y, z show significant differences (*P* < 0·05) among sampling days (day 0, 1 and 3) for the respective control and treatment concentrations (0·1, 0·3 and 0·5%). Bars with different letters a, b, c show significant differences (*P* < 0·05) among the control and treatments for the various sampling days. (■) PBS control; (▲) 0·1%; (▲) 0·3% and (▲) 0·5%.
conditions showed up to 1·5-log CFU g⁻¹ reductions (P < 0·05) as compared to the control (Fig. 1).

Iceberg samples treated with 0·3%-lemongrass oil for 2 min and stored at 4 and 8°C showed significant reductions between 0·9 and 1·7-log CFU g⁻¹ for days 0, 1 and 3, respectively (Fig. 2) (P < 0·05). Samples treated with 0·5%-oil for 2 min and stored at 4 and 8°C showed significant reductions of 1·9–4·0 log CFU g⁻¹ on day 0, 1 and 3 (P < 0·05). No survivors were detected on day 3 for iceberg samples treated with 0·5%-lemongrass oil stored at 8°C (Fig. 2). Iceberg samples treated for 1 min with 0·3 and 0·5%-lemongrass oil and stored at 8°C showed 1·5- and 0·8-log CFU g⁻¹ reductions compared to the control at day 3 (Fig. 2) (P < 0·05).

The most significant difference (1-log CFU g⁻¹; P < 0·05) compared to the control was seen in spinach samples treated with 0·5%-lemongrass oil for 1 min and stored at 4°C for 3 days (Fig. 4). Also, samples treated with 0·5%-lemongrass oil for 1 and 2 min showed significant 1·2- and 1·7-log CFU g⁻¹ bacterial reductions, respectively, as compared to the PBS control at day 1 (Fig. 4) (P < 0·05).

Compared to the control, baby spinach samples treated with 0·5%-lemongrass oil for 1 and 2 min stored at 4°C for 0 and 1 days showed significant reductions compared to the control (1·0–2·1 log CFU g⁻¹) (Fig. 3) (P < 0·05). For baby spinach samples treated with 0·3 and 0·5%-lemongrass oil for 2 min and stored at 8°C, significant 1·4- and 2·2-log CFU g⁻¹ reductions, respectively, were seen on day 0 (P < 0·05).

**Discussion**

A dip treatment was chosen for this experiment because it was shown to have better reduction in bacterial populations than spray treatment in other studies (Gil et al. 2009; Ponce et al. 2011). Dip treating the leaves also mimics the process in which leafy greens are washed and treated prior to packaging. The cited results show that the antimicrobial activity of lemongrass oil against *Salm.* Newport was concentration and time dependent. However, differences in survivors based on incubation temperatures varied between 4 and 8°C and were seen on day 1 and 3 for each produce type.

Studies have shown that foodborne pathogenic bacteria survived on lettuce and other produce for long periods and at different temperatures. *Salm.* Typhimurium maintained bacterial levels in samples inoculated and stored at 4°C for 7 days and increased to c. 3-log levels on lettuce samples stored at 22°C for 7 days (Chang and Fang 2007). S. Baildon was found after 12 days of incubation at 4°C in shredded lettuce samples inoculated at low (0·28-log CFU g⁻¹) and high (3·28-log CFU g⁻¹) levels as well as diced tomatoes inoculated at high levels (3·4-log}
CFU g⁻¹) (Weissinger et al. 2000). Enterobacter sakazakii was recovered in inoculated apple, cantaloupe, strawberry, lettuce and tomato samples after 28 days with c. 1-log reduction in most samples (Kim et al. 2006). It seems that foodborne pathogens can survive on produce samples for long periods at different storage temperatures.

In an earlier investigation, we determined that the control samples taken after inoculation and before and after drying the leafy greens had a constant bacterial population of 5-log CFU g⁻¹ with minimal loss of organisms after the drying process (Moore et al. 2011). In the present study, when the organic leafy greens were sampled on day 0, 1 and 3, the number of bacterial survivors indicated that lemongrass oil had a time-dependent response. The greatest reductions in Salm. Newport populations were seen on day 3 as compared with the PBS control without the antimicrobial. These results are consistent with our previous findings using olive extract, apple extract and hibiscus concentrate (Moore et al. 2011). Belletti et al. (2008) also showed similar results with 2-log reductions of Salm. Enteritidis in fruit salads that were exposed to 600 ppm citron essential oil for 9 days.

The increase in activity of the lemongrass oil with exposure/contact time was best demonstrated in the organic iceberg samples. Iceberg samples that were immersed in the lemongrass solution for 2 min generally showed greater reductions than the same samples treated for 1 min (Fig. 2). By contrast, only minor differences were seen in romaine, baby spinach and mature spinach leaf samples between the 1 and 2 min treatments. This observation suggests that a shorter exposure time may be as effective as a longer time for these produces (Figs 1, 3, and 4). In a related study, Kim et al. (2011) investigated the reduction in Salm. Typhimurium on fresh lettuce leaves exposed to clove extracts for different time periods. There was increased reduction in Salm. Typhimurium on leaves treated with 10% clove extract for 10 min (4-log) compared to a 1 min treatment (1-log) (Kim et al. 2011).

Figure 4 Survival of Salmonella Newport on organic mature spinach treated with PBS, 0.1, 0.3 and 0.5% lemongrass oil solution. (a) 1 min treatment stored at 4°C; (b) 2 min treatment stored at 4°C; (c) 1 min treatment stored at 8°C; (d) 2 min treatment stored at 8°C. Values plotted at each sampling point are an average of three replicates. Error bars represent standard deviations from the mean. Bars with different letters x, y, z show significant differences (P < 0.05) among sampling days (day 0, 1 and 3) for the respective control and treatment concentrations (0.1, 0.3 and 0.5%). Bars with different letters a, b, c show significant differences (P < 0.05) among the control and treatments for the various sampling days. (■) PBS control; (□) 0.1%; (■) 0.3% and (□) 0.5%.
performed in carrot broth, antimicrobial activities of carvocrol, cinnamaldehyde and thymol were observed against different strains of Bacillus cereus at various concentrations over a temperature range of 8–16°C (Valero et al. 2003). These authors found that carvocrol increased the lag time of mesophilic strains of B. cereus, while cinnamaldehyde prevented the growth of all four strains tested after 60 days of incubation at 5, 8 and 12°C and was able to delay bacterial growth at 16°C (Valero et al. 2003). This suggests that an increased incubation temperature may not always affect the antibacterial activity of essential oils.

**Composition and antimicrobial mechanisms of lemongrass oil**

Lemongrass oil, prepared from harvested leaves by distillation at high temperature, is considered to be 100% pure. It is widely used in the culinary practice of Asian countries (Teuscher 2006). Citral and geraniol are the major bioactive components of lemongrass oil. These have been shown to inactivate foodborne pathogens in laboratory media and in apple juice (Friedman et al. 2002, 2004). The aromatic, lemon-like characteristic taste of lemongrass oil is attributed to the volatile pungent flavour of some of the constituents.

The general mechanism of antimicrobial effects of essential oils and their bioactive constituents appears to involve disruption of bacterial cell membranes followed by release of membrane components. However, Aiemsaarad et al. (2011) recently reported that the components of lemongrass oil also inhibited biofilm formation, killed preformed biofilms and have multiple targets on the bacterial cell.

Differences in antimicrobial effectiveness of lemongrass oil among species of leafy greens could be due to differences in leafy green surface morphology, roughness, hydrophobicity and/or hydrophilicity, the presence of native secondary antimicrobial metabolites on the surface as well as to leafy green composition and nutrient contents. These factors may impact the attachment of the bacteria through biofilms to food surfaces and thus antimicrobial potency. Differences in nutrient content of different food categories that the bacteria need to survive could also govern bacterial growth. These considerations imply that antimicrobial results obtained in laboratory media may not always be directly applicable to food environments.

**Conclusions**

This study demonstrated that lemongrass essential oil has the potential to be used as an antimicrobial treatment against *Salm.* Newport on organic leafy greens. The greatest (up to 4.3-log) reductions were seen on iceberg lettuce followed by baby spinach, mature spinach and romaine lettuce. The activity of lemongrass oil increased over storage time, with the greatest reductions in bacterial populations seen on day 3 of sampling. The antibacterial activity also increased with initial exposure time (1 vs 2 min). However, there was minimal difference in the activity among the samples stored under refrigeration and abuse temperatures. The results of the present study suggest that lemongrass oil could potentially be used in the organic fresh produce industry as an alternative decontaminant for fresh leafy greens. Sensory properties of treated leafy greens merit further study.

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**References**


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