Survival of *Salmonella* spp. on Lime Slices

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Additional index words. *Salmonella, Citrus latifolia, food safety*

Lime slices are commonly added to beverages in the food service industry; little is known about microbial risks associated with this practice. The objective of this study was to determine survival of *Salmonella* spp. on lime slices stored on ice for 8 h then transferred to 4 °C or at room temperature. A five-strain cocktail of *Salmonella* [5 log colony-forming units (cfu) per slice)] was spot inoculated onto the flesh, flavedo, or albedo of sliced limes and held on ice or at room temperature for up to 24 h. *Salmonella* were enumerated by plating on selective agar during the 24-h storage. *Salmonella* inoculated onto the albedo and held at room temperature first decreased by 1 log cfu/slice within the first hour then grew back to 6 log cfu/slice. When inoculated onto either the flesh or flavedo and held at room temperature no significant difference was observed in the counts of *Salmonella* over the 24-h period; counts remained at ≈5 log cfu/slice. When stored on ice the initial *Salmonella* load (at time 0 h) was ca. 4.5 log cfu/slice and remained with no significant difference over the 24-h storage time. The demonstrated ability of *Salmonella* to survive on lime slices suggests a potential food safety risk associated with improperly handled sliced limes.

Lime (*Citrus latifolia*) slices are commonly added to beverages in the food service industry; however, little is known about the microbial safety of this practice. The fruit is typically prepared at the beginning of a day and held for use through the serving day. This task can be performed by kitchen staff who may or may not have adequate food safety training.

*Salmonella* causes salmonellosis; symptoms include diarrhea, fever, vomiting, and abdominal cramps for 5–7 d. Coliforms and fecal coliforms have been shown survive in commercially produced iced tea at levels greater than 1100 MPN/mL (Zhoa et al., 1997), thus suggesting *Salmonella* would also be capable of survival although research has not yet been performed to demonstrate this. Therefore, once introduced into the beverage it is unlikely the *Salmonella* would be inactivated by the matrix itself. It is important for the garnish being added to the beverage to be free of pathogens.

It is well understood that oranges, another citrus variety, although with a higher pH [≈3.8 (Pao et al., 1998)], are able to support *Salmonella* survival and have lead to numerous salmonellosis outbreaks from juice consumption (Jain et al., 2009). Lemon slices (pH 2.3) have previously been shown to support the survival of a variety of bacteria on both the flavedo and flesh (Loving and Prez, 2007). The survival of *Salmonella* on fresh-cut limes has not yet been established in other research, but is suspected due to the demonstrated survival on other similar citrus fruits. The objective of the current study was to determine the survival of *Salmonella* on fresh cut lime slices stored at room temperature (RT) for 24 h or on ice for 8 h then transferred to refrigerated storage for 16 h.

Materials and Methods

*Salmonella* serovars were obtained from the culture collection of Dr. Michelle Danyluk (University of Florida, Citrus Research and Education Center). Serovars used included: Gaminara, Rubislaw, Typhimurium, Hardford, and Meunchen. All serovars were originally isolated from orange juice outbreaks. Before use, all serovars were made nalidixic acid resistant. *Salmonella* serovars were individually cultured overnight in tryptic soy broth with nalidixic acid at 35 °C. Equal volumes of each serovar were combined to form a *Salmonella* cocktail. The cocktail was diluted to ≈6 log cfu/mL using peptone water.

Limes were purchased from a local grocery retailer. Before slicing limes were rinsed in running water and dried with paper towel. Limes were sliced into 1/6th sections. To inoculate the lime slices, 20 mL of *Salmonella* cocktail was spot inoculated onto either the flesh, albedo, or flavedo of each lime slice. Lime slices were then stored in open whirlpak bags either at room temperature (23 °C) or on ice. The slices stored on ice were transferred to refrigerated storage after 8 h.

At 0, 1, 2, 4, 6, 8, and 24 h into storage one lime slice from each inoculate zone was enumerated for *Salmonella*. To enumerate the *Salmonella* 15 mL of Dey/Engley neutralizing broth was added to the whirlpak bag. A shake-rub-shake protocol for 30 s was used to dislodge *Salmonella* from the surface of the fruit. Appropriate serial dilutions of the Dey/Engley neutralizing broth were made and spread plated onto bismuth sulfate naladixic acid agar and tryptic soy nalidixic acid agar. Plates were incubated at 35 °C for 24 h.

Experiments were repeated three times. Statistical analysis was done using single tailed ANOVA and the *t*-test to determine significant differences overtime or among treatments.

Results and Discussion

Storage at room temperature after inoculation did not cause a significant difference (*P > 0.05*) for peel or flesh inoculated lime.
slices over the 24-h storage period. While there was no growth at room temperature storage, it is also important to note that there was no significant death of the Salmonella either, which could have been expected because of the harsh acidic environment along with peel oils that are known to have antibiotic properties. Albedo inoculated lime slices experienced a slight decrease in Salmonella then a ≈ 1 log increase between 4 and 24 h. While this growth may be statistically significant, it is likely not biologically significant in that this increase in numbers may be either an artifact of the enumeration method or that the Salmonella became less bound to the albedo over time and thus more Salmonella was dislodged into the broth during the rub-shake-rub. Previous work by Pao et al. (1998) showed that Salmonella on peeled orange segments stored at room temperature were also capable of growth; however, on the peeled orange segments significant growth was observed as early as 12 h after inoculation. The same work showed Salmonella on peeled orange segments steadily decreased in number when stored at 4 °C (Pao et al., 1998) although this decrease was noted after days of storage rather than hours as was the time framed used in the current study.

Storage on ice after inoculation did not cause a significant difference over time for any of the inoculated locations on the lime slices (P > 0.05). Thus, no growth, and no death of the Salmonella was observed when the lime slices were stored on ice.

When comparing the two storage methods over the 24-h sampling period, the flesh inoculated lime slices showed no significant difference in Salmonella survival when stored on ice as compared to room temperature storage (P > 0.05). The albedo inoculated lime slices showed significantly (P > 0.05) lower Salmonella survival at 24 h of storage on ice and refrigeration, as compared with room temperature storage; no significant difference between ice and room temperature storage was observed within the beginning 8-h period for the albedo inoculated lime slices. Peel inoculated lime slices had significantly (P > 0.05) lower Salmonella survival when stored on ice then refrigerated, than when stored at room temperature starting at 1 h of storage and continuing throughout the sampling period. Storage on ice cannot be relied on as a means to reduce risk of salmonellosis associated with fresh cut limes. Although a lower survival resulted for peel contaminated lime slices stored on ice, this reduction was not enough to ensure safety of a previously contaminated product.

Increased food safety awareness is an important measure to decrease the risk of salmonellosis from the use of fresh sliced limes as beverage garnishes. The rate of transfer into various beverages from contaminated lime slices needs to be determined. Further investigation should also be done to examine the survival of Salmonella on other beverage garnishes, such as lemon slices (pH 2.3) or maraschino cherries, whose pH values are not as harsh as lime (pH 1.8–2.0).

**Literature Cited**


