Validation of the use of Citrus Essential Oils as a Post Harvest Intervention against *Escherichia coli* O157:H7 and *Salmonella* spp. on Beef Carcasses

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Executive Summary

**Background**

Gastrointestinal illness remains one of the main causes of morbidity and mortality in the U.S., of which a large proportion is caused by *Escherichia coli* O157:H7 and *Salmonella* spp. Cattle and other ruminants have been established as natural reservoirs for both of these pathogens and food production animals play a significant role in the epidemiology of human infections. The intestines or hide of beef cattle, the principal reservoirs of *E. coli* O157:H7, may cross contaminate the beef carcass if care is not taken during slaughter. *E. coli* O157:H7 transmission to humans is also favored by poor hygienic procedures in the handling of dairy and beef cattle. Despite the best current production practices, both *Salmonella* spp. and *E. coli* O157:H7 are still a risk in fresh meat production. Current interventions cannot provide 100% assurance of the safety of ground beef. Additional hurdles that provide consistent 1-2 log reductions of these two pathogens would be a significant improvement in reducing the risk of pathogenic microorganisms contaminating our meat supply. Current interventions include thermal pasteurization using hot water or steam and the use of organic acid rinses. Steam vacuums are also an effective tool for localized decontamination. These interventions have become part of a science-based HACCP (Hazard Analysis and Critical Control Point) system that seeks to prevent microbial contamination before it occurs. Nevertheless, despite the food safety advances made in beef production, it is clear that meat products continue to become contaminated with *E. coli* O157:H7 and *Salmonella* spp. Therefore, newer interventions should be developed that could be employed as additional hurdles to support current HACCP systems. The verification of antimicrobials that are effective at chilling temperatures would be particularly useful, since these antimicrobials could be used to decrease the concentrations of *E. coli* O157 and *Salmonella* spp. on beef carcasses immediately before fabrication.

**Objectives**

The objective of this project was to validate the use of all natural, citrus essential oils as an additional processing aid to reduce *Escherichia coli* O157:H7 and *Salmonella* spp. on beef carcasses prior to fabrication.

**Methods**

Determination of Minimum Inhibitory Concentration (MIC) of Citrus essential Oils against *E. coli* O157:H7 and *Salmonella* spp.

One hundred microliters of citrus essential oils was mixed with One hundred microliters of each bacterial isolate to be tested in a well of a microtiter plate. The plates were incubated at 4oC, for 24 hours. After incubation, a sample from each well was streaked onto Trypticase Soy Agar (TSA). The TSA plates were incubated for 24 hrs at 37°C, after which time growth observations were recorded. For each bacterial isolate, experiments were repeated three times, and the MICS from each experiment were averaged.
Briskets were used during the contaminated meat trials, to investigate the ability of the citrus essential oils to reduce or eliminate the E. coli surrogates from the meat (carcass) surface. The brisket site of the carcass was chosen because it represents a portion of the beef carcass most often contaminated with the indicator organism E. coli. 120A beef brisket flats were spot inoculated with approximately 6 log of an E. coli cocktail. Inoculated meat sections were placed in a single layer on a fiberglass tray, covered with aluminum foil, and stored at 4°C for 60 min to allow bacteria to attach to the meat surface. After drying, citrus essential oils were applied in two concentrations to the surface of different pieces of meat. Treatments included citrus essential oils applied at concentrations of 3.0% and 6.0%, deionized water (water spray), and no spray. Treatments were applied using a custom built spray cabinet (Chad Co, Olathe, KS) at 2.07 bar and applied at a rate of 3.79 liters per minute to replicate commercial practices.

Following treatment, samples were vacuum packaged and stored at 4°C until microbial sampling. Microbial samples were analyzed at Day 0, 20 minutes after spraying and packaging, Day 1, Day 2, Day 3, Day 4, and Day 5, and every five days from Day 5 to Day 90. All samples were analyzed to determine the concentrations of E. coli, total aerobic bacteria, and psychrotrophic bacteria.

**Important Results**

Nineteen multidrug resistant (MDR) Salmonella Newport isolates were inhibited at an average citrus essential oil concentration of 0.4% or below. One of the two non MDR Salmonella isolates was inhibited at an average concentration of 0.2%. All E. coli isolates were inhibited at a citrus essential oil concentration of 0.4% or below. Both non O157:H7 isolates were inhibited at 0.2%. When concentrations of 3% and 6% citrus essential oils were sprayed onto pieces of brisket used to simulate beef carcasses, the oils significantly reduced the concentration of E. coli that was artificially inoculated onto the beef, and total aerobic bacterial counts, in comparison to inoculated, no spray or water sprayed controls, over a period of 90 days (Figure 4). The citrus essential oils were also capable of reducing the presence of naturally occurring microflora on the surface of uninoculated pieces of brisket, in comparison to no spray or water spray controls, for various periods of time. These results show that citrus essential oils could be used as a cold active antimicrobial, in an additional step to inhibit foodborne bacterial pathogens on beef carcasses during fabrication.
Figure 4. Reduction of *Escherichia coli* counts in artificially inoculated and uninoculated brisket samples treated with 3% and 6% citrus essential oil over 90 days.

**Impact on the Beef Industry**

*E. coli* O157:H7 and *Salmonella* spp. are bacterial pathogens with significant public health concern. It has been estimated that beef recalls due to safety concerns have cost the beef industry as much as $2.7 billion in lost demand. Since cattle are natural reservoirs for *E. coli* O157:H7 and *Salmonella* spp., a carcass intervention strategy that is optimized to control *E. coli* O157:H7 and *Salmonella* spp. at chilling temperatures immediately before fabrication would provide an additional control step for the *E. coli* O157:H7 and *Salmonella* HACCP system. The implementation of novel control methods should lead to a decrease in the number of *E. coli* O157:H7 and *Salmonella* contaminated carcasses, thereby improving the microbial quality and safety of ground beef. In the beef industry, carcasses are chilled to temperatures close to or at 4°C within 24 hours. The results of this study indicate that a solution of 1% cold pressed terpeneless Valencia orange oil could be used as an additional intervention against *E. coli* O157:H7 and *Salmonella* spp. at the chilling stage of processing, and should remain effective if the carcasses are subjected to temperature abuse. The oils could also be used as a processing aid on cuts of beef, as the results of this study show the ability of the citrus essential oils to control growth of bacteria on vacuum packaged meats for various periods of time.
Lay Summary

*Escherichia coli* O157:H7 and *Salmonella* spp. are two bacteria associated with beef which cause many cases of foodborne illness each year in the United States. During beef slaughter and processing these bacteria may spread from the hide or intestines to the meat. The beef industry is continuously working to eliminate this contamination from the beef before it reaches the consumer. The objective of this research was to investigate the use of naturally occurring compounds (citrus essential oils) from orange peel to reduce or eliminate these two pathogens from beef before it reaches the consumer. The citrus essential oils in this study were effective at stopping growth of *E. coli* O157:H7 and Salmonella in pure culture experiments at concentrations of 0.2 to 0.4%. When concentrations of 3% and 6% citrus essential oils were sprayed onto pieces of brisket used to simulate beef carcasses, the oils significantly reduced the concentration of *E. coli* that was artificially inoculated onto the beef, and total aerobic bacterial counts, in comparison to inoculated, no spray or water sprayed controls, over a period of 90 days.

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