Determination of Antimicrobial Mechanisms of Hot Water and L-lactic Acid Carcass Interventions Against *Escherichia coli* O157:H7

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Project Summary

**Background**

*Escherichia coli* O157:H7 remains a challenge for the beef industry. Research has shown carcass interventions consisting of hot water sprays paired with organic acid rinses to be highly effective at reducing pathogen loads on carcasses. Nevertheless, the mechanisms by which hot water and organic acid interventions interact to inhibit foodborne pathogens remain unclear. Hot water is thought to function primarily by physically dislodging loosely attached pathogens, thermal inactivation, or some combination thereof. A widely accepted theory of organic acid action states that at reduced environmental pH, the protonated acid penetrates the bacterium’s interior. This consequently induces a cascade of microbial responses designed to ultimately restore pH balance to the pathogen interior. At sufficient acid levels, the cell will be forced to repeatedly expel protons at the cost of energy reserves. This model has been criticized as too simplistic in its assertion that antimicrobial activity results only by acidification of the bacterium interior. Accumulation of deprotonated acid in the cellular interior is thought to play a major role in the inhibition of cells by alkalinizing the cell and inhibiting synthesis of macromolecules. The purpose of this research study was to determine the extent to which exposure to hot water would result in degradation to the outer membrane of *E. coli* O157:H7 and ultimately influence L-lactic acid inhibition of the pathogen.

**Methodology**

*Escherichia coli* O157:H7 strains were grown in nutritious medium and cocktailed prior to experimentation. Cocktails were then serially diluted to 8.0 ± 0.2 log10 CFU/ml, prepared in sterile glass tubes, and incubated at 25, 65, 75, or 85°C for 0, 5, 15, 30, or 60 sec. Surviving *E. coli* O157:H7 were enumerated by plating on non-selective microbiological medium and incubating plates under conditions optimal for bacterial growth. Aliquots of hot water-treated bacteria were then treated to extract membrane lipids and formation of lipid hydroperoxides was determined spectrophotometrically. Finally, the survival of hot water and 5% L-lactic acid treated cells was assayed by plating on microbiological medium and accumulation of L-lactate by *E. coli* O157:H7 was determined spectrophotometrically.

**Findings**

Exposure of *E. coli* O157:H7 to increasing temperatures for increasing intervals resulted in statistically greater inactivation of the pathogen via hot water. Log reductions ranged from approximately 3.0-7.0 log cycles, dependent upon exposure temperature and duration of 3 exposure. Increased exposure to heat produced significant increases in hydroperoxides from *E. coli* O157:H7 membrane lipids. No detectable surviving *E. coli* O157:H7 were recovered on microbiological medium following exposure to any experimental heating protocol and organic acid. Hot water-treated cells did not accumulate significant amounts of organic acid anion, presumably due to significant degradation to outer membrane lipids.

**Implications**

Research results provide increased validation to use of hot water and organic acid interventions applied to beef carcasses for the inactivation of *E. coli* O157:H7. The enhancement of organic acid efficacy by use of increasing water temperature demonstrates the need for consistent control and validation of intervention parameters for the control of carcass-contaminating pathogens by
processors. Observed increases in hydroperoxides indicate that hot water inactivation of *E. coli* O157:H7 may result in part from degradation of membrane lipid. Nevertheless, further research should be conducted to determine the contributions of hot water to physically dislodge, inactivate via heat transfer, or sensitize bacterial pathogens located on carcass surfaces to organic acid rinses. Additionally, studies quantifying the accumulation of organic acids in pathogen cytoplasmic spaces and the inhibitory effects of such accumulation should be investigated using carcass-attached pathogenic microbes.

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