Project Title: Decontamination of Beef Cuts Intended for Blade/Needle or Moisture-Enhancement Tenderization by Surface Trimming versus Rinsing with Solutions of Hot (82°C) Water, Warm (55°C) Lactic Acid or Activated Lactoferrin Plus Warm (55°C) Lactic Acid

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Background

Research has shown that the incidence of *E. coli* O157:H7 on surfaces of beef cuts intended for blade or needle tenderization is extremely rare. One study showed a 0.2 percent occurrence of *E. coli* O157:H7 on 1,014 cuts from six processing facilities throughout the United States. The fact that *E. coli* O157:H7 rarely occurs on beef primal and subprimal cuts makes the risk of its internalization when blade, needle or moisture enhancement tenderization technologies are used very low. Even though the risks are low, the probability of its occurrence is dramatically increased if improper cleaning and sanitizing of equipment is practiced. Past research has also shown that blade tenderization can transfer from 1 to 7 percent of surface contamination to the interior of the muscle. Needle injection during moisture enhancement can result in a 4 to 8 percent translocation of surface contamination to the center of the cut. While cooking needle tenderized or moisture enhanced product to sufficient internal temperatures (140°F or higher) has been shown to destroy *E. coli* O157:H7, intervention methods applied prior to tenderization should help prevent the transfer of bacteria to internal surfaces, thus reducing any risks of foodborne pathogens being passed to consumers.

Methodology

Outside rounds were obtained from a commercial packing company and cut into equal halves. The rounds were inoculated with *E. coli* O157:H7 and were individually vacuum packaged and stored for 10 to 18 days at 2 to 4°C.

Samples were suspended from a sterilized meat hook and one of five pathogen interventions was applied to each outside round piece:

1) No treatment
2) Surface trim using good manufacturing practices
3) 20 second hot (82°C) water spray application
4) 20 second warm (55°C) 5 percent lactic acid spray application
5) Sequential application of activated lactoferrin (20 seconds) followed by warm (55°C) lactic acid (20 second spray)

After the intervention treatment, the outside round pieces were allowed to sit for five minutes before undergoing blade tenderization or moisture enhancement to simulate in-plant line speed.

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Study A
Outside round pieces (n = 120) were inoculated with a target of 102 CFU/cm2 of E. coli O157:H7. Following storage, individual round pieces were subjected to one of the five bacterial interventions (n = 24 per intervention) and were subjected to either blade tenderization (n = 60; n= 12 per intervention per process) or needle-injected enhancement (n = 60; n = 12 per intervention per process). Samples were analyzed for E. coli O157:H7 using polymerase chain reaction (PCR).

Study B
Outside round pieces (n = 160) were inoculated and interventions were applied as described above (n = 32 per intervention) and subjected to either blade tenderization (n = 80; n = 16 per intervention per process) or needle enhancement (n = 80; n = 16 per intervention per process).

Following processing, a five centimeter slice was removed from each piece, which was subsequently split into two, 2.5 centimeter thick slices. A surface sponge sample was then collected from a newly exposed internal surface of one slice. Surface samples were analyzed to quantitatively determine the surface populations of *E. coli* O157:H7 by direct plating and counting morphologically typical colonies. All results were reported in log colony forming units per cm^2 (CFU/cm^2).

Uninoculated (n = 40) outside round pieces were also subjected to the interventions and processing treatments as previously described, so that there was a total of four samples per intervention and per process.

Findings

Study A
The prevalence of *E. coli* O157:H7 found within outside round pieces was 99.2 percent (119 out of 120 samples). Only one sample, sanitized with hot (82° C) water and needle-injected did not return a positive result. The high rates can be attributed to the fact that the inoculation levels used in this study are far higher than levels of *E. coli* O157:H7 one would expect to find on uninoculated beef surfaces.

Table 1. Number of positive samples/number of samples tested (percent positive) for prevalence of *E. coli* O157:H7, as determined by PCR-BAX, after inoculated outside round pieces were subjected to one of four antimicrobial interventions and were blade-tenderized (BT) or moisture-enhanced (ME). Inoculated, untreated outside-rounds served as positive controls.

	Positive Control[a]	Trimming[b]	Hot Water (82°C)[c]	Warm 5% Lactic Acid (55°C)[d]	Activated Lactoferrin +5% Lactic Acid (55°C)[e]
BT	12/12 (100)	12/12 (100)	12/12 (100)	12/12 (100)	12/12 (100)
ME	12/12 (100)	12/12 (100)	11/12 (91.7)	12/12 (100)	12/12 (100)

[a]Positive controls were inoculated and not subjected to an antimicrobial intervention.
[b]Samples were carefully trimmed to remove all external surfaces prior to further processing.
[c]Hot water (82°C) was sprayed on the surface of each cut for 20 seconds.
[d]Five percent lactic acid (55°C) was sprayed on the surface of each cut for 20 seconds.
[e]Activated lactoferrin was sprayed on the surface of each cut for 20 seconds, followed by a spray application of 5 percent (55°C) lactic acid for 20 seconds.

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Study B

Product Storage

Outside rounds were vacuum packaged and stored (2°C) for 10 to 18 days after inoculation. At the time of inoculation, the samples had surface levels of *E. coli* O157:H7 of 2.17 log CFU/cm^2. Following vacuum packaging and refrigerated storage, surface samples were collected prior to application of any antimicrobial treatments or further processing. During storage, inoculated populations increased from an average of 2.17 log CFU/cm^2 to 3.4 to 3.7 log CFU/cm^2. These results indicate that *E. coli* O157:H7, when present in high levels can survive and grow in vacuum packages at refrigerated temperatures.

Antimicrobial Interventions

Intervention treatments resulted in a 0.9 to 1.1 log CFU/cm^2 reduction compared to pre- intervention inoculated inside round surface samples. Survival of *E. coli* O157:H7 ranged from 12.3 to 17.3 percent of pre-intervention surface levels and all interventions equally reduced the presence of pathogens on the surface level.

Table 2. Least square means ± standard errors for *E. coli* O157:H7 (log CFU/cm^2) recovered from the external surface (minimum detection level = 1.0 CFU/cm^2) of inoculated outside round pieces prior to application of an antimicrobial intervention (PRE) and following an antimicrobial intervention (POST), and internal surface levels of *E. coli* O157:H7 (CFU/cm^2) following moisture enhancement (ME). Inoculated, untreated outside- rounds served as positive controls (n = 16 for each intervention treatment).

	Positive Control[a]	Trimming[b]	Hot Water (82°C)[c]	Warm 5% Lactic Acid (55°C)[d]	Activated Lactoferrin +5% Lactic Acid (55°C)[e]
PRE	-	3.7±0.08^x	3.6±0.08^x	3.6±0.08^x	3.6±0.08^x
POST	3.6±0.08^x	2.6±0.08^y	2.5±0.08^y	2.4±0.08^y	2.7±0.08^y
ME	2.1±0.08^y	1.3±0.08^z	1.2±0.08^z	1.2±0.08^z	1.4±0.08^z

[a]Positive controls were inoculated and not subjected to an antimicrobial intervention.
[b]Samples were carefully trimmed to remove all external surfaces prior to further processing.
[c]Hot water (82°C) was sprayed on the surface of each cut for 20 seconds.
[d]Five percent lactic acid (55°C) was sprayed on the surface of each cut for 20 seconds.
[e]Activated lactoferrin was sprayed on the surface of each cut for 20 seconds, followed by a spray application of 5 percent (55°C) lactic acid for 20 seconds.
[xyz]Least squares means, within columns, lacking common superscript letters, differ ($p<0.05$)

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Table 3. Least square means ± standard errors for *E. coli* O157:H7 (log CFU/cm^2) recovered from the external surface (minimum detection level = 1.0 CFU/cm^2) of inoculated outside round pieces prior to application of an antimicrobial intervention (PRE) and following an antimicrobial intervention (POST), and internal surface levels of *E. coli* O157:H7 (CFU/cm^2) following blade tenderization (ME). Inoculated, untreated outside- rounds served as positive controls (n = 16 for each intervention treatment).

	Positive Control[a]	Trimming[b]	Hot Water (82°C)[c]	Warm 5% Lactic Acid (55°C)[d]	Activated Lactoferrin +5% Lactic Acid (55°C)[e]
PRE	-	1.0±0.06	0.9±0.06	1.0±0.06	1.2±0.06
POST	0.9±0.06	0.9±0.06	0.9±0.06	0.9±0.06	0.9±0.06
ME	0.9±0.06	0.9±0.06	0.9±0.06	0.9±0.06	0.9±0.06

[a]Positive controls were inoculated and not subjected to an antimicrobial intervention.
[b]Samples were carefully trimmed to remove all external surfaces prior to further processing.
[c]Hot water (82°C) was sprayed on the surface of each cut for 20 seconds.
[d]Five percent lactic acid (55°C) was sprayed on the surface of each cut for 20 seconds.
[e]Activated lactoferrin was sprayed on the surface of each cut for 20 seconds, followed by a spray application of 5 percent (55°C) lactic acid for 20 seconds.

Implications

Results of this study indicate that even when surface levels of *E. coli* O157:H7 are several hundred fold higher than those reported in national surveys, application of antimicrobial interventions of surface trimming, hot water (82°C), 5 percent lactic acid (55°C) or activated lactoferrin plus 5 percent lactic acid (55°C), can reduce pathogen loads on the surface of subprimal cuts. For those subprimals subjected to further processing, the interventions can subsequently reduce internalization of surface pathogens. Both blade tenderization and moisture enhancement resulted in pathogen transmission into internal surfaces of inoculated subprimals. Moisture enhancement resulted in the greatest transmission rates compared to blade tenderization. Implementation of a surface intervention to beef subprimals, prior to further processing, would reduce the risk of pathogenic organisms being internalized, as well as reducing the risk of encountering foodborne illness from non-intact, blade tenderized or moisture enhanced beef products.

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