Food Safety Systems, LLC

Research Report

Treatment of Beef Carcass Tissue in Simulated Cold Storage for Control of *Escherichia coli* O157:H7 and *Salmonella* using AirROS[tm] Surface and Air Sanitation Technology

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Introduction:

The control of contamination of beef carcasses with *Escherichia coli* O157:H7 is a primary food safety objective for the beef industry. In 1994, USDA's Food Safety and Inspection Service established a zero tolerance policy for this organism in raw ground beef. Since contamination of beef products results from carcass contamination, a great deal of emphasis has been placed on slaughter related interventions. Contamination of carcass surfaces by other pathogenic organisms such as *Salmonella* spp. also has regulatory and public health consequences. Hide removal and evisceration defects that occur during the slaughter process can result in pathogenic bacterial contamination that may be carried through the remaining slaughter, fabrication, and processing operations.

Several antimicrobial intervention technologies are available, and widely used, to effectively decontaminate beef carcasses (i.e. steam pasteurization, steam vacuuming, acidified rinses, etc.). However, contamination of carcasses still occurs during the slaughter process. Even very low levels of contamination may increase to high levels during carcass chilling, especially if carcasses are not adequately spaced.

In this study, a technology designed to create an antimicrobial environment through the production of reactive oxygen species (ROS), including ozone and vaporized hydrogen peroxide was evaluated as a means for treating the surface of chilled, inoculated beef carcass tissue. The technology is termed AirROS[tm] by SAGE Industrial and is marketed by SAGE Industrial Corporation.

The objective of this study was to evaluate of the efficacy of the AirROS^{tm} treatment for the decontamination of carcass surfaces with emphasis on control of *E. coli* O157:H7 and *Salmonella*. This technology is considered a processing aid and does not require labeling. It is also a dry process and does not involve the addition of water in the form of chemical sprays. It is designed to be scalable and easily implemented into a post-chill slaughter operation.

Materials and Methods

Bacterial Culture Preparation: The following strains from the Kansas State University culture collection were used to prepare the inoculum:

- *Escherichia coli* O157:H7: ATCC 43890 and ATCC 43889, obtained from Jackie Staats at KSU Veterinary School; USDA-FSIS 380-94, KSU 01, CDC (Patient outbreak), and KSU 03, CDC (Meat outbreak).
- *Salmonella* spp.: *Salmonella choleraesuis* subsp. *cholerasuis (S. enteriditis) (ATCC 4931,* and USDA-FSIS 15060*), S. seftenburg* subsp. *cholerasuis* (ATCC 43485), *S. newport* (Dr. Phebus, KSU), and *S. montevideo* (Dr. L. Beuchat, UGA).

To prepare the inoculum, stock cultures were cultivated by placing one impregnated bead into a 5 ml solution of Difco® Tryptic Soy Broth (TSB) and incubating for 24 h at 35^{o}C. Next, a 0.05 ml loop of the respective culture was inoculated into a 10 ml solution of TSB and incubated for 24 h at 35^{o}C. All five samples from each culture were mixed together to create a 50 ml cocktail containing 10^{9} to 10^{10}CFU/ml of *E. coli* O157:H7 or *Salmonella* spp. The cell density of this suspensions was determined by plating appropriate dilutions on MSA (MacConkey Sorbitol Agar, Difco, Detroit, MI) for *E. coli* O157:H7 and XLD (Xylose Lysine Desoxycholate Agar, Difco, Detroit, MI) for *Salmonella* spp., and placed in the incubator for 48 hours at 35^{o}C. Cultures were confirmed by cultivation on selective and differential media, and biochemical analysis of presumptive colonies using API 20E kits.

Sample Preparation: The carcass surface tissue samples were provided by the Kansas State University Meat Laboratory. Samples were frozen until time of use. They were allowed 72 hours to thaw at 45°F. Samples were inoculated with *Escherichia coli* O157:H7 or *Salmonella* spp. inside a "bio-containment" chamber by "misting" the surface of the carcass surface tissue with approximately 10 ml of the inoculum. This was done ensuring that all sides of each sample of meat received the same exposure to the inoculum. Samples were held for 30 min at room temperature to allow proper bacterial attachment to the surface of the meat. Immediately prior to treatment applications, the surfaces of the inoculated products were sampled and analyzed to establish the actual inoculum level of the attached organisms.

Sampling Method: The tissue was excised (ca. 3 mm depth) from the outside (15.9 cm^{2}) surface of each sample and put into a stomacher bag separately. There were two core samples

collected for the top and two from the bottom of each carcass tissue sample... The tissue samples were diluted with 90 ml of 0.1% sterile peptone water (PW) and homogenized in a stomacher for one minute. Samples were serially diluted in sterile PW and plated onto corresponding media for each pathogen tested. The plates were incubated at 37 °C for 48 hrs. The colony forming units were enumerated and calculated as the difference in log recovery. Non-inoculated beef carcass surface samples were also treated for 0, 10 minutes, 20 minutes and 60 minutes and tested for residues of ozone and hydrogen peroxide. Microbiological analyses were conducted using ECC (*E. coli*/Coliform) Petrifilm

Application of AirROS^{tm} Treatment: Carcass surface tissue samples inoculated with a 5-strain cocktail of *E. coli O157:H7 or Salmonella* spp. were treated in the controlled environmental chamber equipped with an 4030CS Surface and Air Sanitation System for periods of 0, 10 minute, 20 minutes and 60 minutes. The temperature in the controlled environmental chamber was 73° F and the relative humidity was 55%. The target surface inoculation was 6.0 Log CFU/cm^2. After each treatment, the beef carcass tissue samples were tested to determine levels of each pathogen tested.

A negative control was conducted in which inoculated samples were placed in a controlled environmental chamber without the AirROS system. Samples were removed from the chamber at 0, 10, 20 and 60 minutes.

Non-inoculated beef carcass tissue samples were treated using the 4040CS Surface and Air Sanitation System for periods of 0, 10, 20 and 60 minutes and tested for residual levels of ozone and hydrogen peroxide using gas spectrometry. TBA values were also measured (TBAR assay) on treated carcass tissue.

Results and Discussion:

Results from this preliminary study can be found in Table 1. Log CFU/cm^2 reductions were calculated as the difference in log recoveries from the inoculated products prior to treatment and the log recovery after treatment.

Table 1. Average recoveries (Log CFU/cm^2) of *Salmonella* spp and *E. coli* O157:H7 on beef carcass surface tissue treated in the Controlled Environmental Chamber equipped with AirROS Surface and Air Sanitation Technology for periods of 0, 10, 20 and 60 minutes.

Sample	*Salmonella*	E. coli *O157:H7*
0 time	**6.3**	**6.5**
10 Minute	**4.6**	**4.9**
20 Minutes	**4.1**	**4.4**
60 Minutes	**3.5**	**3.9**

Table 2. . Average recoveries (Log CFU/cm^2) of *Salmonella* spp and *E. coli* O157:H7 on beef carcass surface tissue treated in the Controlled Environmental Chamber without AirROS Surface and Air Sanitation Technology for periods of 0, 10, 20 and 60 minutes (Control)

Sample	*Salmonella*	E. coli *O157:H7*
0 time	**6.30**	**6.50**
10 Minute	**6.25**	**6.40**
20 Minutes	**6.25**	**6.40**
60 Minutes	**6.10**	**6.30**

This study demonstrates the efficacy of the AirROS Surface and Air Sanitation Technology for the surface decontamination of beef carcass tissue inoculated with *Salmonella* and *E. coli* O157:H7. Reductions greater than 1.5 log CFU/cm^2 were observed after the 10 minute treatment for both pathogens. Reductions of 2.2 log CFU/ cm^2 and 2.1 log CFU/cm^2 were observed for *Salmonella* and *E. coli* O157:H7, respectively after a 20 minute treatment. The 60 minute treatment resulted in a 2.8 log CFU/cm^2 reduction for *Salmonella* and a 2.6 log CFU/cm^2 reduction for *E. coli* O157:H7. The reductions in the negative control were < 0.1 log CFU/cm^2 for the 0, 10 and 20 minute sampling times for both pathogens tested. The reduction was 0.2 log CFU/cm^2 at the 60 minute sampling time for both pathogens tested.

The ozone and hydrogen peroxide residue testing showed no detectible residual in beef carcass tissue samples. TBA (thiobarbituric acid) values were similar between the treated and untreated carcass tissue samples, 0.32 and 0.30, respectively.

During the course of the study ambient ozone levels were measured using a Model 500 Aeroqual (New Zealand) monitoring instrument. Ozone levels were recorded at 0.085 PPM inside the controlled environmental chamber. Hydrogen peroxide levels were measured using Dragger tubes. The levels for hydrogen peroxide ranged from 0.095 – 0.110 ppm.
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