Reduction of *Escherichia coli* O157:H7 and *Salmonella* using Dry Chilling in small processing plant environments

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Abstract

Contamination of beef carcasses during the slaughter process is inevitable. The objective of this study was to verify the use of dry chilling up to 28d as an effective means of reducing *E. coli* O157:H7 and Salmonella on beef lean and adipose tissues compared to conventional spray chilling/wet aging. Hot adipose and lean tissue samples were obtained before interventions from a harvest facility and transported back to TTU Pathogen Laboratory. Samples were then inoculated with a cocktail of either three strains of *E. coli* O157:H7 or three strain cocktail of Salmonella with a concentration of 107. The samples were then suspended on racks and subjected to spray chilling/wet aging or dry chilling for 28d. A Spray chilling system was developed to mimic the industry in which water was sprayed on samples for the first 15 minutes and 1 minute every 17 minutes for 17 hours. Following a 48 h samples were vacuum packaged to mimic current industry standards. Dry chilling samples were inoculated and suspend in an open air chill cooler with a temperature of 3° C, an air velocity of 0.0 to 0.25 m/s with a relative humidity of 80%.until sampling. 5 cm by 5 cm samples were aseptically excised and plated for enumeration using the thin-agar overlay method at 0d, 24h, 36h, 48h, 7d, 14d, 21d, and 28d. When enumeration was not possible, a detection process was conducted to verify the presence or absence of pathogenic bacteria. All counts were transformed into log cfu/ cm2 for statistical analyzation. Least square means were calculated using the analysis of variance in the general linear model of SAS for mean separation. Dry chilling of lean and fat tissue samples significantly decreased the amount of *E. coli* O157:H7 and Salmonella. A significant ($P< 0.05$) advantage is shown for dry chilling over spray chilling on lean and fat tissue occurring at 14 d and 7 d, respectively on *E. coli* inoculated samples. Furthermore, a significant ($P<0.05$) decrease in recovery of Salmonella was observed for dry chilling samples over spray chilling/wet aging at 14 d and continued through 28 d. Pathogen reduction on dry chilled lean and fat tissue samples was determined to be due to surface desiccation. Surfaces of dry chilled samples become increasingly drier as storage time increased resulting in much lower water activity on the surface. Spray chilled tissue displayed a significant washing effect during the initial 17 h. Nonetheless, little reduction of *E. coli* O157:H7 or Salmonella occurred throughout remaining 28 d. Vacuum packaging of the samples at 48 h demonstrated that removing oxygen had a bacteriostatic affect on pathogenic bacteria, as shown by steady populations of pathogens from 48 h to 28 d. Therefore, dry chilling is a superior method of chilling for the reduction of *E. coli* O157:H7 on fat and lean tissue samples having a range in reduction of bacteria of 4.5 to 4.95 logs cfu/cm2, respectively at 28 d. In addition, Salmonella had a reduction on fat and lean tissue of 4.79 and 4.5 logs cfu/cm2, respectively. Dry Chilling can be a cost effective means of controlling pathogens of dry chilled carcasses for small processors.

Introduction

Small processors are continually searching for avenues to meet HACCP requirements. Due to the limitation of space and resources, additional Critical Control Points are welcomed to aid in the combat against foodborne pathogens and adulterants. Preliminary data from Texas Tech University show dry chilling can reduce the pathogen load on beef surfaces (Woerner et al. 2005). Confirmation of the original determination would create an opportunity for Texas Tech to aid small processors in providing a CCP for small plants and the necessary documentation of its efficacy. Furthermore, most small producers use dry aging up to 28 d for tenderness purposes. The objective of the study was determine and quantify the reduction of growth of *E. coli* O157:H7 and *Salmonella* on beef tissue following two chilling methods (spray chilling and dry chilling) used by small processing plants, as well as determining the use of dry chilling as a CCP.

Materials and Methods

➢ **Inoculation**

- Hot lean and adipose tissue were obtained from a commercial slaughter facility prior to microbial interventions, bagged individually
- Tissue was fabricated into 20 cm X 20 cm pieces
- Samples were inoculated with cocktails of *E. coli* O157:H7 or *Salmonella* from stock culture at Texas Tech University Pathogen Laboratory
- Samples were inoculated by dipping and allowed to attached for 20 min before subjecting samples to their respective treatments

➢ **Treatments**

- Dry Chilling- Samples were hung for up to 28 days in an open air chill cooler with a temperature of 3° C, an air velocity of 0.0 to 0.25 m/s with a relative humidity of 80%.
- Spray Chilling- Samples were hung for the first 48 hrs of treatment were a 15 min spray period was started after samples were hung and then a 1 min period was sprayed every 17 minutes for 17 h. At 48 h samples were individually vacuum sealed until time to be sampled

➢ **Bacteriology**

- Lean and Fat Tissue were excised for enumeration or detection at 0, 24h, 36h, 48h, 7d, 14d, 21d, and 28d post inoculation
- 5 x 5 cm samples were aseptically excised and 25 ml of BPW was added to a stomacher bag containing sample and stomached for 2 min at 2500 rpms
- Samples were enumerated by plating *E. coli* O157:H7 on to MacConkey Agar and *Salmonella* on to XLD Agar using a thin layer agar overlay using TSA (Kang and Fung, 2000) using an Autoplate 4000 spiral plater (Spiral Biotech)
- Bacteria were enumerated following incubation at 37°C for 24 h
- All samples were enumerated by using a Q count automated counting system (Spiral Biotech)
- When enumeration was not possible, a detection process was conducted to verify the presence or absence of pathogenic bacteria.
  - *E. coli* O157:H7 samples were enriched with buffered peptone water incubated for 24 h at 37° C. 1 ml of enrichment was pipetted into 9 ml of GN Broth, incubated fror 24 h at 37° C, and streaked onto MacConkey selective media
  - *Salmonella* samples were enriched with buffered peptone water incubated for 24 h at 37° C. 1 ml of enrichment was pipetted into 9 ml of RV Broth, incubated for 24 h at 37° C, and streaked onto XLD selective media

➢ Statistical Analysis

- All counts were transformed into log CFU/ cm2
- Least square means were calculated using the analysis of variance in the general linear model of SAS

Results

- No recoverable *E. coli* O157:H7 or Salmonella were found on fat or lean tissue samples of un-inoculated (control) samples
- Higher numbers of bacteria were recovered from lean tissue samples compared to fat tissue samples for both Salmonella and *E. coli* inoculums ($P<0.05$)
  - This is caused by the difference in the higher amount of available water from lean tissue samples compared to fat tissue samples

Table 1: LS Mean comparison of recovery of *E. coli* O157:H7 for Lean and Fat Tissues Samples Inoculated *E. coli* O157:H7 and Subjected to either Dry or Spray/Wet Chilling Methods over 28d, reported in Log of cfu/cm2

Tissue	Treatment	0 d	24 h	36 h	48 h	7 d	14d	21 d	28 d
Lean	Dry	6.05^{ax}	5.14^{bx}	4.96^{bx}	4.43^{bcx}	3.66^{cdx}	2.98^{dex}	2.89^{ex}	1.10^{fx}
	Spray/Wet	6.01^{ax}	4.45^{bxy}	4.49bxy	4.23^{bxy}	4.30^{bxy}	4.07^{by}	3.74^{bx}	3.67^{by}
Fat	Dry	5.53^{ax}	4.31^{bxy}	4.24^{byz}	3.85^{by}	2.08^{cy}	2.12^{cz}	0.84^{dy}	0.95^{dx}
	Spray/Wet	5.75^{ax}	3.61^{by}	3.63^{bz}	3.81^{by}	3.37^{bz}	2.94^{bz}	3.05^{bx}	3.66^{by}

- For lean tissue samples inoculated with *E. coli* O157:H7:
  - A consistent statistical decline was seen for dry chill samples. While spray chill samples saw an initial decline and leveled not being statistically different from 24h until 28d
- A statistical difference was seen at 14d and continued until the 28d between Spray and Dry Chill treatments
- For Fat tissue samples inoculated with *E. coli* O157:H7:
  - A consistent statistical decline was seen for dry chill samples. While spray chill samples saw an initial decline and leveled not being statistically different from 24h until 28d
  - A statistical difference was seen at 7d between Spray and Dry Chill Samples, and at 21d and 28d

Table 2: LS Mean comparison of recovery of Salmonella for Lean and Fat Tissues Samples Inoculated Salmonella and Subjected to either Dry or Spray/Wet Chilling Methods over 28d, reported in Log of cfu/cm2

Tissue	Treatment	0 d	24 h	36 h	48 h	7 d	14d	21 d	28 d
Lean	Dry	6.08^{ax}	5.13^{bx}	4.82^{bx}	4.62^{bx}	3.76^{cx}	2.92^{dy}	1.93^{ey}	1.58^{ey}
	Spray/Wet	6.04^{ax}	4.95^{bx}	4.58^{bcx}	4.53^{cx}	4.06^{cdx}	3.62^{dx}	3.42^{dx}	3.76^{dx}
Fat	Dry	5.71^{ax}	4.53^{bx}	4.43^{bx}	3.94^{by}	2.94^{cy}	2.29^{cdy}	1.72^{dey}	0.92^{ey}
	Spray/Wet	5.78^{ax}	4.14^{bx}	4.16^{bx}	3.30^{cy}	3.03^{cy}	2.68^{cy}	3.26^{cx}	3.11^{cx}

- For lean tissue samples inoculated with Salmonella:
  - A consistent statistical decline was seen for dry chill samples
  - A statistical difference was seen at 14d between Spray and Dry Chill Samples, and continued until 28d
- For fat tissue samples inoculated with Salmonella:
  - A consistent statistical decline was seen for dry chill samples
  - A statistical difference was seen at 21d between Spray and Dry Chill Samples, and continued until 28d

Implications

Based upon results recommendation of a 21 d period of dry chilling under the same conditions should be used to reduce the number of pathogens on beef surface tissue. Results should be verified with an in plant study to use the Dry Chilling as a CCP step. Rapid air movement to facilitate faster desiccation of fat and lean surface tissue should be explored. Furthermore, the economic and environmental impact of water conservation by using dry chilling versus spray chilling is monumental.

Literature Cited

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