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

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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* spp 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

Preparation of Pathogen Cocktails. Three strains of *E. coli* O157:H7 (966, 922, and 944), and three strains of *Salmonella* spp. , (*Typhimurium, Hydelburg and Enteritidis*) all isolated from beef animals or beef products were selected for use in the study. These cultures were obtained from the stock culture collection at Texas Tech University. All strains were maintained as frozen (-80°C) stocks in TSB with 10% (vol/vol) sterile glycerol as a cryo-protectant. The three strains of *E. coli* and *Salmonella* spp. were combined into a cocktail and concentrated into frozen cultures as described by Brashears et al. (1999). A frozen stock culture of each strain was thawed to room temperature (25-27° C), placed into 200 ml of Tryptic Soy Broth and incubated at 37° C for 24 hrs. The cells were separated from the broth by centrifugation (4,000 3 g for 20 min at 18⁰C), and each pellet was re-suspended in 10 ml of a 10% solution of sterile glycerol as a cryo-protectant to obtain concentrations of approximately 10^9 cfu/ml. Re-suspended solutions of each strain were mixed in equal volumetric parts to obtain a cocktail of three strains for each pathogen. The cocktail suspensions were transferred into 2 ml cryogenic vials, quickly frozen in liquid nitrogen, and stored at -80°C.

Sample Preparation and Inoculation. Hot (25-30° C) adipose tissue, removed from the area covering the deep pectoral (brisket), and/or hot lean tissue (25-30° C), *Cutaneous omo-brachialis* (shoulder rose), were removed from carcasses following hide removal, but prior to application of any post-mortem antimicrobial washes from a commercial slaughtering facility. The tissue samples were transported to Texas Tech University Pathogen Processing Lab using unsealed vacuum bags in an ice chest to maintain temperature (25 - 30° C), until inoculation (< 3 hrs). Each sample was laid horizontally, aseptically cut with a sterile knife blade into approximately 15 cm x 10 cm rectangular portion. All samples were dipped into one of two inoculums, three strain *E. coli* cocktail or three strain *Salmonella* spp. cocktail from frozen concentrate, at a concentration of 10^7 cfu/ ml. Following inoculation, each sample was

randomly assigned to a treatment and held at room temperature (25°C to 28°C) for 20 min to allow for attachment of pathogens. Samples were suspended using sterile fishing hooks and line for application of treatments in a refrigerated room (3° C, with 4 defrost time periods of 20 min) for up to 28 d.

Definition of Treatments.

Spray Chill/Wet Ageing. A custom spray chilling system was developed to mimic industry application. The system was composed of 36 nozzles with a flow rate of 500 ml/ min with a circular pattern.   All samples were suspended 30 cm from the nozzles. Suspended tissue samples were sprayed continuously for 15 min and then sprayed for 1 min spray cycle every 17 min for the remainder of the 17 hours. After 48 hrs, all remaining samples were packaged in vacuum bags and randomly assigned to an end-point sampling time.   Samples were excised for enumeration/ detection at the following time periods 0 d, 24 h, 36 h, 48 h, 7 d, 14 d, 21 d and 28 d.

Dry Chill/Dry ageing. All samples were suspended in a cold room (3° C, with 4 defrost time periods of 20 min) with an air velocity of 0.0 m/ s to 0.25 m/ s with a relative humidity of 80 %. Samples were randomly assigned to end point time periods for enumeration/ detection at 0 d, 24 h, 36 h, 48 h, 7 d, 14 d, 21 d and 28 d.

Excision of Samples and Enumeration of Pathogens.

Surface tissue samples measuring 5 cm by 5 cm or $25cm^2$ were aseptically removed using sterile scalpels and forceps. Samples were placed into sterile stomacher bags, and 25 ml of buffered peptone water was added. Samples were then stomached at 2500 rpms for 2 m.   Samples inoculated with *Salmonella* spp. were plated on XLD agar overlaid with TSA using the thin-layer agar method to allow for recovery of injured cells (Kang and Fung 2000). Samples containing *E. coli* O157:H7 were plated onto MacConkey Agar with Sorbitol overlaid with TSA using the thin-layer agar method as well. Samples were plated using a spiral plating system and counted using a Q count automated counting system. All plates were incubated for 18-24 hrs at 37°C prior to counting. In the instance no growth was detected, samples then were subjected to the following detection processes.

Detection. Samples inoculated with *E. coli* O157 were enriched with buffered peptone water for 24 h at 37°C. Following enrichment, 1 ml of enrichment was pipetted into 9 ml of GN broth and incubated for 24 h at 37°C. Then streaked onto MacConkey Agar with Sorbitol, were typical colonies were to be observed.
   Samples inoculated with Salmonella spp. were enriched with buffered peptone water for 24 h at 37°C. Following enrichment, 1 ml of enrichment was pipetted into 9 ml of RV broth and incubated for 24 h at 37°C. Then streaked onto XLD selective media, were typical colonies were to be observed.

Statistical Design and Analysis
All counts were transformed into log CFU/ cm^2 for statistical analyzation. Least square means were calculated using the analysis of variance in the general linear model of SAS. Both pathogens were analyzed separately and blocked by tissue (lean or fat). Day and treatment were analyzed separately as well as interactively.

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 28 d, reported in Log of cfu/cm^2.

Tissue	Treatment	0 d	24 h	36 h	48 h	7 d	14d	21 d	28 d	# of samples	SE
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}	24	.309
	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}	24	.309
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}	24	.309
	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}	24	.309

[abcdef] LS Means with differing superscripts in a row are significantly different (P< 0.05)
[xyz] LS Means with differing superscripts in a column are significantly different (P< 0.05)

- For Lean tissue samples, a difference in treatments did not exists until 14 and 28 d of treatments with Spray/wet chilling having significantly more *E. coli* O157:H7 than recovered from samples from Dry Chilling.
- For Lean tissue samples, as Dry Chilling Storage time increased amount of pathogen recovered from tissue decreased with significant reductions from 0d to 24 h, 24 h to 7d, 7 d to 21 d and 21 d to 28 d. However, Spray/wet Chilling only had a significant reduction from 0d to 24 h. All other time points for Spray/wet Chilling were not significantly different.
    - A constant decrease of pathogens from Dry Chilling is caused by continued desiccation of the exterior of the samples. However, an initial decrease was seen in Spray/wet Chilling from a washing effect of the water. A numerical decrease in recovery of pathogens over time was seen for Spray/wet Chilling, however this decrease was not statistically significant from 24 h to 28 h.
- For Fat tissue samples at 7d, 21 d and 28 d significantly less *E. coli* was recovered from samples subjected to Dry Chilling than Spray/wet chilling.
- Fat tissue samples subjected to Dry chilling had significant decreases in recovery as storage time increased with 0 d being significantly different from 24 h, 24 h significantly higher in recovery of pathogens than 7 d and a significant decrease from 7 d to 21 d.

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

Tissue	Treatment	0 d	24 h	36 h	48 h	7 d	14d	21 d	28 d	# of samples	SE
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}	24	.246
	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}	24	.246
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}	24	.246
	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}	24	.246

[abcdef] LS Means with differing superscripts in a row are significantly different (P< 0.05)
[xyz] LS Means with differing superscripts in a column are significantly different (P< 0.05)

- Lean samples inoculated with *Salmonella* spp. subjected to Dry Chilling over a 28 showed significant reductions in pathogens. A significant reduction was shown with a decrease from 0 d to 24h, a significant reduction in recovery of *Salmonella* was also seen from 24 h to 7 d of Dry Chilling. Furthermore, a significant decrease was observed from 7 d to 14 d and 14 d to 21 d.
- For lean samples inoculated with *Salmonella* subjected to Spray/wet Chilling, a significant decrease was observed in recovery of *Salmonella* from 0 d to 24 h.
    - This reduction is a washing effect of Spray chilling.
- A significant decrease was seen from 24 h to 48 h of Spray/wet Chilling of lean samples. As well as 48 h to 14 d.

- o Decreases from 24h to 48 h can be explained by the drying out of the samples surfaces.
- Treatment differences were for lean tissues inoculated with *Salmonella* were observed at 14 d and continued throughout 28 d with Dry Chilled samples having significantly less recovery of *Salmonella* than Spray/wet Chilled samples.
- Fat samples inoculated with *Salmonella* subjected to Dry Chilling over 28 d period were observed to significantly decrease as time increased. A significant reduction was observed for Dry Chilling samples from 0 d to 24 h, 24 h to 7 d, 7 d to 21 d and 21d to 28 d.
- Fat samples inoculated with *Salmonella* in which were Spray/wet Chilled showed a significant decrease in the quantity of *Salmonella* recovered from 0d to 24 h. Furthermore a significant decrease was observed from 24 h to 48 h, with the remaining time period not being significantly different from the 48 h quantification.

Discussion

A decrease in recovery was seen across both pathogens, as well as both treatments. However, Dry Chilling had the most constant and significant reduction in pathogenic bacteria across the 28 d sampling period. Spray/wet Chilling was observed to have an initial drop in recovery of pathogenic bacteria, being attributed to a washing effect of the water during the first 17 h. However, *Salmonella* samples continued to slowly decline during the holding period of wet ageing. This would indicate that vacuum packing has a bactericidal effect on S*almonella* spp. The unexplained rise in recovery of pathogenic bacteria, for example 21 d of *Salmonella*, could have resulted in variation in samples and the result of the limited amount of samples that were tested per replication.

Implications

Small processors are continually striving to explore cost effective means to reduce pathogenic bacteria on beef carcasses. The results from the current study have shown that dry chilling for 21 d is an effective intervention in reducing E. coli O157:h7 and Salmonella spp. on fresh beef carcasses compared to conventional spray chilling/wet ageing. Currently, small beef or custom beef processors allow beef carcass to dry age for 28 d to improve beef palatability. This recommendation is compared to Buege and Ingram (2003), in which suggests a 6 day aging of carcasses and critical limits of cooler temperature, as well as days of aging. However, recommendation from the above results prove that at 21 d of aging beef tissue samples can achieve approximately a 4 log reduction of pathogenic bacteria by Dry Chilling. Therefore, implicating dry chilling to decrease pathogenic bacteria on the surface of beef carcass is effective and economical for small producers. Small processors should validate these results in plant setting using indicator organisms.

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