A Novel Continuous Intervention System for the Reduction of *Escherichia coli* O157:H7 and *Salmonella typhimurium* in Ground Beef

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INTRODUCTION

Escherichia coli O157:H7 and *Salmonella typhimurium* are two leading pathogenic organisms of concern in the meat industry. Costly outbreaks associated with these pathogens, particularly in ground beef have led to the passage of the 1996 Pathogen Reduction and HACCP rule. The regulations associated with this rule have led to research, development and application of intervention strategies that can be used to control the growth of *Salmonella* and *E. coli* O157:H7 in ground beef thus providing the consumer with a microbiologically cleaner and safer product. Although specific interventions in beef slaughter are designed to reduce bacterial contamination on the carcasses, research on the effect of antimicrobial interventions on ground beef have only begun to be studied.

It would be advantageous to develop antimicrobial application methods that would not only decontaminate the tissue surfaces, but also provide a lethal function for sub-surface microorganisms.

The Rinse & Chill™ Technology is an enhanced bleeding technique that involves vascular transfer of a chilled isotonic solution containing dilute concentrations of approved common substrates (sugars and salts) through the cardiovascular system of cattle during the slaughtering process. The purpose of the Rinse & Chill™ Technology is to lower pH and temperature earlier and more rapidly, and to more thoroughly remove blood from the animal. In previous research, we demonstrated that the Rinse & Chill™ Technology provides a novel intervention for improving microbial control of contamination on bovine carcasses. The objective of this study was to demonstrate that the Rinse & Chill™ Technology could also provide a novel intervention system for the continued reduction of *E. coli* O157:H7 and *Salmonella typhimurium* in ground beef.

MATERIALS AND METHODS

Slaughter. Cattle were slaughtered humanely and assigned randomly to two groups, control or rinsed. For this study, animals were slaughtered and rinsed over a two-day period. Cattle, to be rinsed through the vascular transfer of chilled solution in the arterial/venous system, were bled by severing both jugular veins. When the bleeding was nearly completed, an incision was made in the left carotid artery, and a catheter was inserted into the artery for the rinsing process. The rinsing solution (MPSC, Inc., St. Paul, MN) consisted of a dilute mixture of sugars and salts in water. Control groups were bled using the traditional method. A total of 20 cattle were randomly selected and designated control (10) or rinsed (10).

Preparation of the ground beef. The *Triceps brachii* (TB) muscle was collected from the 20 cattle slaughtered (10 Controls; 10 Rinsed). The TB muscle from the control and rinsed cattle were passed through a 4-mm plate and then through a 3-mm plate (finely ground). The grinder head was cleaned and sanitized between the grinding of control and rinsed muscle.

Inoculation, packaging and storage of the ground beef. One thousand gram portions each of ground beef representing the control, rinsed and 50/50 mixture were separately placed in sterile containers. The ground beef samples were inoculated with 10 ml of peptone water containing both overnight cultures of *Escherichia coli* O157:H7 and *Salmonella typhimurium* at a final concentration of 4 log10 CFU/g of each. Each container was hand mixed for 2 min to homogeneously distribute the two pathogens in the sample. Thirty-gram samples of the inoculated ground beef from each treatment were placed into tray package containers or vacuum-sealed containers. All samples were placed in a cooler at 4°C.

Microbiological Analysis. Sampling for the presence of microbial growth was done in triplicate at day 0, 3, 9, 16, 22, 28, 32, 38, and 42 days. Twenty-five grams from three randomly selected refrigerated inoculated samples from each of the treatments were diluted in peptone water and homogenized in a stomacher bag for 1 min. Serial 10-fold dilutions were prepared in peptone water and plated onto 3M™ Petrifilm™ Aerobic Count Plates, 3M™ Petrifilm™ *E. coli* Count Plates, Rainbow ™ Agar (Difco, Detroit, MI) and Salmonella media (Tatini, University of Minnesota). The plates were incubated at 37ºC for 48h. Each data collection point represented an average of three 25-gram samples.

Table 1. Change in log10 of *E coli* O157:H7 in vacuum packaged inoculated ground TB beef stored at‰€ over 42 days.

Days	Control	Change in Log_{10}	R&C	Change in Log_{10}	50/50	Change in Log_{10}
0	4.76		4.76		4.76	
3	4.67	-0.09	4.08	-0.68	4.21	-0.55
9	4.42	-0.34	3.35	**-1.41**	4.34	-0.42
16	3.79	-0.97	2.73	**-2.03**	3.68	**-1.08**
22	3.52	**-1.24**	2.03	-2.73	3.27	-1.49
28	3.22	-1.54	1.23	**-3.53**	2.84	**-1.92**
32	2.89	-1.87	0.53	**-4.23**	2.35	-2.41
38	2.64	**-2.12**	0	-4.76	1.88	-2.88
42	2.44	-2.32	0	-4.76	1.81	**-2.95**

Table 2. Change in log10 of *Salmonella typhimurium* in vacuum packaged inoculated ground TB beef stored at 4°C over 42 days.

Days	Control	Change in Log_{10}	R&C	Change in Log_{10}	50/50	Change in Log_{10}
0	4.75		4.75		4.75	
3	4.72	-0.03	4.26	-0.49	4.59	-0.16
9	4.80	+0.05	3.51	**-1.24**	4.42	-0.33
16	3.46	**-1.29**	0	**-4.75**	2.74	**-2.00**
22	3.37	-1.38	0	-4.75	2.51	-2.24
28	2.93	-1.82	0	-4.75	2.18	-2.57
32	2.88	-1.87	0	-4.75	1.30	**-3.45**
38	2.67	**-2.08**	0	-4.75	0	**-4.75**
42	2.49	-2.26	0	-4.75	0	-4.75

Table 3: Change in log10 of *E. coli* O157:H7 in tray packed inoculated ground TB beef stored at 4°C over 42 days.

Days	Control	Change in Log_{10}	R&C	Change in Log_{10}	50/50	Change in Log_{10}
0	4.58		4.57		4.56	
3	4.56	-0.02	3.41	**-1.16**	4.29	-0.27
9	4.51	-0.07	3.08	-1.49	3.68	-0.88
16	3.80	-0.78	2.00	**-2.57**	3.14	**-1.42**
22	3.74	-0.84	1.67	-2.93	2.87	-1.69
28	3.71	-0.87	0	**-4.57**	2.64	-1.92
32	3.73	-0.85	0	-4.57	2.37	**-2.19**
38	3.71	-0.87	0	-4.57	2.30	-2.26
42	3.64	**-0.94**	0	-4.57	2.10	-2.46

Table 4: Change in log10 of *Salmonella typhimurium* in tray packed inoculated ground TB beef stored at 4°C over 42 days.

Days	Control	Change in Log_{10}	R&C	Change in Log_{10}	50/50	Change in Log_{10}
0	4.61		4.60		4.62	
3	4.59	-0.02	4.45	-0.15	4.37	-0.25
9	4.64	+0.03	4.22	-0.38	4.21	-0.41
16	4.11	-0.50	3.85	-0.75	3.81	-0.81
22	4.01	-0.60	3.19	**-1.41**	3.69	-0.93
28	4.00	-0.61	2.95	-1.65	3.37	**-1.25**
32	3.95	-0.66	2.39	**-2.21**	3.08	-1.54
38	3.91	-0.70	2.02	-2.58	2.87	-1.75
42	3.89	-0.72	1.63	**-2.97**	2.62	**-2.00**

RESULTS

Vacuum Packaged Inoculated TB Ground Beef. A progressive reduction of both *E. coli* 0157:H7 and *Salmonella typhimurium* occurred over a 42-day period when rinsed and 50/50 blend ground TB beef were compared to the control. A 4.76, 2.95 and 2.32 log reduction was observed for *E. coli* 0157:H7 in rinsed, 50/50 blend and control TB ground beef, respectively (Table 1). For *Salmonella typhimurium*, a 4.75 log reduction in rinsed and 50/50 blend compared to a 2.26 log reduction in the control ground beef (Table 2).

A 1-log10 reduction of *E. coli* O157:H7 occurred at day 9 with the rinsed beef, day 16 with the 50/50 blend and day 22 with the control beef. A 2-log occurred at day 16 with the rinsed beef, compared to 28 days with the 50/50 blend and 38 days with the control beef. No *E. coli* O157:H7 was detected at 38 days in the rinsed beef, whereas by day 42 the 50/50 blend and the control beef had a log reduction of 2.95 and 2.32, respectively (Table 1).

The *Salmonella typhimurium* vacuum-packaged rinsed beef demonstrated a 1.24 log reduction at 9 days and a 4.75 log reduction with no detectable *Salmonella* at 16 days. The 50/50 blend had 2.01, 3.45 and 4.75 log reduction at 16, 32 and 38 days, respectively. In comparison, the control vacuum-packaged TB ground beef achieved a 1.29, 2.08 and 2.26 log reduction at 16, 38 and 42 days, respectively (Table 2).

In an oxygen-depleted environment (vacuum-packaging), the rinsed ground beef demonstrated a 4.75 log reduction in 16 days with *Salmonella typhimurium* when compared to *E. coli* O157:H7, which took 38 days to demonstrate a 4.76 log reduction. In the 50/50 blend, *S. typhimurium* was not detectable after 38 days, whereas, at 42 days, a 2.95 log reduction was achieved with *E. coli* O157:H7. The control ground beef only achieved a 2.44 log reduction of both *E. coli* O157:H7 and *S. typhimurium* by day 42.

Tray Pack Inoculated Ground TB Beef. As with the vacuum packaged ground beef samples, a progressive reduction of both *E. coli* O157:H7 and *S. typhimurium* occurred in tray pack, but the reduction was not as substantial. The log10 reduction for *E. coli* O157:H7 was 4.57 over 28 days in rinsed product, 2.46 and 0.94 over 42 days in the 50/50 blend and control product, respectively (Table 3). For *Salmonella typhimurium*, the log10 reduction was 2.97, 2.0 and 0.72 for rinsed, 50/50 blend and control product over a 42-day shelf life at 4ºC (Table 4).

A 1 log10 reduction of *E. coli* O157:H7 occurred at day 3 with the rinsed beef, day 16 with the 50/50 blend and day 42 with the control beef. A 2-log10 reduction occurred at day 16 with the rinsed beef, compared to 32 days with the 50/50 blend. The control beef never reached a 2-log10reduction in the 42 day shelf-life (Table 3).

The *Salmonella typhimurium* tray-packaged rinsed beef demonstrated a 1.41 log10 reduction at 22 days and a 2.97 log10 reduction by 42 days. The 50/50 blend had 1.25, and 2.00 log10 reduction at 28 and 42 days, respectively. In comparison, the control tray-packaged TB ground beef only achieved a 0.72 log10 reduction over the 42 day study (Table 4).

DISCUSSION

Ground beef was prepared from the *Triceps brachii* (TB) muscle collected from 20 (10 controls; 10 R&C) cattle slaughtered over two days. Control, rinsed and 50/50 blend of control and rinsed ground beef were inoculated with *E. coli* O157:H7 and *Salmonella typhimurium*, placed in both vacuum-packaging and tray packaging environments then stored at 4°C until sampled intermittently for the presence of both pathogens over 42 days. A progressive reduction of *E. coli* O157:H7 and *Salmonella typhimurium* occurred over the 42 day sampling period in both tray and vacuum packaged inoculated ground beef when rinsed and 50/50 blend were compared to control.

Several researchers have demonstrated when antimicrobial treatments or compounds have been applied to beef carcasses (hot water, organic acids, alkaline phosphates and other novel compounds) or beef trimmings, a reduction in microbial numbers has occurred in the ground beef. But in addition to the effectiveness of antimicrobial treatments, another concern is the impact of these treatments on meat quality attributes such as color and odor. It has been shown that treatments, such as hot water, organic acids in high concentrations, or other decontaminants can have an adverse affect on the color and/or odor of beef tissues.

Scientific studies have documented improvements in tenderness, palatability, flavor and color as well as cholesterol being lower in meat that has been processed using the Rinse and Chill Technology. The data presented in the this study demonstrated that the Rinse and Chill Technology also provides a novel intervention system for the continued reduction of *E. coli* O157:H7 and *Salmonella typhimurium* in ground beef.

The mechanism(s) involved in Rinse and Chill Technology that contribute to its effectiveness as a novel intervention technology for reduction of bacteria on both beef carcasses and in further processed ground beef is a combination of factors. The reduction in pH and internal temperature of the carcasses, in addition to removal of blood with the vascular transfer of the chilled solution, may provide an unfavorable environment for growth and survival of bacteria. The vascular rinse allows the muscle structure to open up, thus allowing the solution of salts and sugars to penetrate throughout the muscle. Previous studies have demonstrated that the detrimental effects of polyphosphates on gram-negative bacteria, including *E. coli* O157:H7 and *Salmonella typhimurium*. Polyphosphates sequester/chelate cations, such as copper, calcium, iron, zinc and magnesium, making them unavailable for microbial growth or survival. The chelation of iron or other essential cations, as a result of the Rinse and Chill Technology, may in part explain the continued reduction of pathogens such as *E. coli* O157:H7 and *Salmonella typhimurium* over time as demonstrated in this study.

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