Escherichia coli O157:H7 and *Salmonella* Typhimurium Penetration During Vacuum Tumbling and Survivability During Storage in Marinated Beef

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Abstract— **To determine the survival of *Escherichia coli* O157:H7 and *Salmonella* Typhimurium in marinades used to vacuum tumble beef inside skirts and tri-tip roasts, and to measure depth of pathogen penetration in these products. Two commercial marinades were inoculated with *E. coli* O157:H7 and *Salmonella* Typhimurium. Eighteen beef inside skirts and 18 tri-tip roasts were vacuum tumbled with the inoculated marinade for 1 h. Samples were taken immediately following tumbling (day 0), or after product was vacuum packaged and stored (~ 2°C) for 7 or 14 days. Spent marinade was sampled immediately following tumbling (day 0) and after 3 and 7 days of storage (~ 2°C).**

***S.* Typhimurium and *E. coli* O157:H7 penetrated throughout the skirt steak, whereas penetration in the tri-tip roasts varied with thickness. After storing the meat for 7 days, the log value of both *S.* Typhimurium and *E. coli* O157:H7 decreased. The log value of both *S.* Typhimurium and *E. coli* O157:H7 continued to decrease during the 14-day storage; however, both pathogens were still detectable. The spent marinade sampled on days 0, 3, and 7 showed that the pathogens survived in the refrigerated marinade; however, there was no increase in log value.**

Pathogens may penetrate into the interior of beef skirt steaks and tri-tip roasts during vacuum tumbling with contaminated marinade, and they may survive during refrigerated storage of both enhanced beef products and spent marinade.

Keywords— **Non-intact beef, *Escherichia coli* O157:H7, *Salmonella* Typhimurium.**

I. INTRODUCTION

Following the foodborne outbreak in late 1992 and early 1993 caused by *E. coli* O157:H7 associated with ground beef, the safety of beef products has been highly scrutinized. In 1994, USDA's Food Safety and Inspection Service (FSIS) declared *E. coli* O157:H7 an adulterant in raw ground beef. In 1999, FSIS clarified that the public health risk by raw beef products contaminated with *E. coli* O157:H7 was not limited to ground beef, but also included non-intact beef products *[1]*. These non-intact beef products include beef that has been injected, mechanically tenderized, or reconstructed. This definition of non-intact is consistent with the Food and Drug Administration's (FDA) food code, which defines whole muscle, intact beef, as beef that is not injected, mechanically tenderized, reconstructed, or scored and marinated *[2]*.

To provide additional information to strengthen establishment's food safety programs, this project evaluated the effect of inoculated marinade used in vacuum tumbling on pathogen penetration into two different commonly marinated beef cuts. The marinade was inoculated with *S.* Typhimurium and *E. coli* O157:H7 before the product was enhanced by vacuum tumbling.

II. MATERIALS AND METHODS

Vacuum packaged, beef inside skirt steaks and tri-tip roasts were obtained for this project. Two different marinade formulations, and associated processing parameters were obtained from commercial processing facilities in Texas. Rifampicin-resistant microorganisms derived from parent strains of *Salmonella* Typhimurium and *E. coli* O157:H7 were used to inoculate the marinades used in this study.

The dry marinade was mixed according to manufacturer's instructions. In order to inoculate the marinade, 120 ml was taken of each organism (*E. coli* O157:H7 and *S.* Typhimurium) and BPW and was added to 960 ml of marinade, resulting a total volume of 1200 ml of marinade and inoculum.

The inoculated marinade was added to the BIRO vacuum tumbler. Three tri-tip roasts or three inside skirt steaks were added to the tumbler on each sampling day. Following vacuum tumbling for one

hour, the 3 pieces of product were removed and placed on sterile foil. One of these pieces was sampled immediately to represent 0 day. The 2 remaining pieces of product were individually vacuum packaged and held at 4˚C to be sampled at 7 days and 14 days.

Samples of marinade were plated as controls. Samples of "spent" marinade were sampled at days 0, 3, and 7. Product and "spent" marinade samples were analyzed for pathogen growth and survival. The project was repeated in triplicate.

Before inoculation, negative control surface samples of tri-tip roasts and inside skirt steaks were collected to measure possible natural presence of rifampicin-resistant microorganisms. Samples were plated on lactose-sulfite phenol red rifampicin (LSPR) agar plates. LSPR plates were incubated for 24 h at 35˚C before counting and reporting the number of rifampicin-resistant *E. coli* O157:H7 and *S.* Typhimurium/cm^2.

The inoculum cocktail was plated on LSPR each sampling day in order to determine the initial bacterial population of the individual microorganisms. Three samples of the spent marinade were taken immediately following tumbling of the inoculated marinade and product. One sample was plated immediately to determine the log value of the microorganism after being vacuum tumbled with meat product. The other two samples were stored at 4˚C and plated on day 3 and day 7.

The product was removed from the tumbling drum and placed on foil, using a flamed scalpel blade, and forceps, two square plugs were removed from the center of each roast and inside skirt steak. The square plugs were placed individually on foil.

Using flamed forceps, the plug was removed from the foil and was dipped into 95% ethanol. The four plug sides and bottom were further flamed. Again, using a flamed scalpel blade and forceps, a cross-sectional cut was made. This cross sectional sample was placed in a sterile Petri dish. Consecutive 3 mm samples were taken. This process was repeated on all tri-tip roast samples until the geometric center of the plug was reached. There were 2 samples taken from the inside skirt plug, exterior and interior. The cross sectional samples in the Petri dishes were sampled by excising a 10-cm^2 sample using a flamed borer. These samples were pummeled and plated on LSPR.

Colonies were counted, recorded and reported as log CFU/cm^2.

III. RESULTS AND DISCUSSION

The initial log value used to inoculate the marinades used in this experiment is higher than what would be expected in contaminated marinade found in a plant. However, this is an initial inoculum level commonly used in laboratory experiments in order to determine log reductions.

After tumbling in the inoculated Marinade A, penetration of the microorganisms was throughout the inside skirt steak. Counts of both microorganisms on the exterior inside skirt steaks treated with Marinade A was the same as the level on the interior inside skirt steaks, both statistically and microbiologically (at least 1 log), as shown in Table 1.

Table 1 Least squares means for interior and exterior layer effect on counts ($\log_{10}$ CFU/cm^2) of *S.* Typhimurium and *E. coli* O157:H7 for marinated inside skirts

[a] Least squares means within a row lacking a common letter significantly differ ($P < 0.05$).

[1]SEM = is the standard error of the least squares means.

	Layer	
	Interior	Exterior
Marinade A		
S. Typhimurium	4.1[a] (0.1)[1]	4.2[a] (0.2)[1]
E. coli O157:H7	3.5[a] (0.2)[1]	3.6[a] (0.2)[1]
Marinade B		
S. Typhimurium	3.9[a] (0.1)[1]	3.9[a] (0.1)[1]
E. coli O157:H7	3.5[a] (0.2)[1]	3.4[a] (0.2)[1]

After the product was tumbled in inoculated Marinade A, and stored for 7 days, there was a similar log count, both statistically and microbiologically, of *S.* Typhimurium compared to that of day 0, as shown in Table 2.

Table 2 Least squares means for day effect on counts (log_{10} CFU/cm^2) of *S.* Typhimurium and *E. coli* O157:H7 for marinated inside skirt steaks

[a-c] Least squares means within a row lacking a common letter significantly differ ($P < 0.05$).
[1]SEM = is the standard error of the least squares means.

	Day		
	0	7	14
Marinade A			
S. Typhimurium	4.4[a] (0.1)[1]	4.2[a] (0.1)[1]	3.8[b] (0.1)[1]
E. coli O157:H7	4.2[a] (0.1)[1]	3.5[b] (0.1)[1]	3.0[c] (0.1)[1]
Marinade B			
S. Typhimurium	4.3[a] (0.1)[1]	3.9[b] (0.1)[1]	3.6[c] (0.1)[1]
E. coli O157:H7	4.4[a] (0.1)[1]	3.2[b] (0.1)[1]	2.9[c] (0.1)[1]

The inside skirt steaks had a significant decrease in *E. coli* O157:H7 log value on 7 day compared to the log value found at 0 day. The difference ($P < 0.05$) of a decrease in log count in *E. coli* O157:H7 occurred on the product after being stored for 7 days, and again after the product was stored for 14 days. After processing, the microbiological counts of both microorganisms were similar in exterior and interior samples of inside skirt steaks treated with Marinade B, as show in Table 1. There was a difference ($P < 0.05$) in log values of both microorganisms when comparing samples from 0 day to samples taken on 7 day. Although there was not a microbiological difference when comparing day 0 to day 7 of *S.* Typhimurium, there was a microbiological difference in *E. coli* O157:H7 on those days, as shown in Table 2. Comparing day 0 to day 14 there was a microbiological difference in presence of *S.* Typhimurium.

There was a difference ($P < 0.05$) in log values of both organisms as the depth increases and samples are taken towards the geometric center of tri-tip roasts treated with Marinade A. There was a dilution effect seen in *S.* Typhimurium and *E. coli* O157:H7 as samples were taken from depths further towards the center of the tri-tip roast, as shown in Table 3.

There were statistical differences from depth 0-3 mm to 6-9 mm; however, there were similarities between samples taken from depths of 0-3 mm and 3-6 mm, as would be expected from neighboring samples. There was microbiological difference between samples depths of 0-3 mm and 9-12 mm. There were unexpected results as the product was stored over time and sampled on day 0, 7, and 14, as shown in Table 4.

Table 4 Least squares means for day effect on counts (log_{10} CFU/cm^2) of *S.* Typhimurium and *E. coli* O157:H7 for marinated tri-tip roasts

[a,b] Least squares means within a row lacking a common letter significantly differ ($P < 0.05$).
[1]SEM = is the standard error of the least squares means.

	Day		
	0	7	14
Marinade A			
S. Typhimurium	2.7[b] (0.2)[1]	3.8[a] (0.2)[1]	2.8[ab] (0.4)[1]
E. coli O157:H7	2.8[b] (0.2)[1]	3.6[a] (0.2)[1]	2.8[ab] (0.3)[1]
Marinade B			
S. Typhimurium	3.0[a] (0.2)[1]	2.5[b] (0.2)[1]	2.7[ab] (0.2)[1]
E. coli O157:H7	3.0[a] (0.1)[1]	2.2[b] (0.1)[1]	2.2[b] (0.2)[1]

There was a difference ($P < 0.05$) and a microbiological difference from day 0 to day 7, and again from day 7 to day 14 when looking at results of both microorganisms.

There was a difference ($P < 0.05$) in log values of both organisms as the depth increases and samples were taken towards the geometric center of tri-tip roasts treated with Marinade B, as shown in Table 3. There was a microbiological difference seen between sample depths of 0-3 mm to 12-15 mm, due to dilution of microorganisms as depth increased. The first three layers had similar *E. coli* O157:H7 counts. There were differences ($P < 0.05$) seen in tri-tip roasts sampled on day 0 compared to those stored and sampled on day 7 for both microorganisms; however, at day 14, there were similarities seen of *S.* Typhimurium compared to both day 0 and day 7, and there were similarities of *E. coli* O157:H7 seen from day 7 to day 14, as shown in Table 4.

IV. CONCLUSIONS

E. coli O157:H7 and *S.* Typhimurium are major food safety concerns for the beef industry. Although the *E. coli* O157:H7 prevalence is typically low on beef subprimals, processors must still be concerned about contaminating marinade. If processors reuse spent marinade, they need to be aware of the potential risk of contaminating products during subsequent marination. The survival of pathogens in spent marinade, and the transfer of pathogens into the interior during vacuum tumbling may both contribute to potential food safety concerns.

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REFERENCES

1. U.S. Department of Agriculture, Food Safety and Inspection Service (1999) FSIS policy on non-intact raw beef products contaminated with *E. coli* O157:H7 at http://www.fsis.usda.gov/OA/background/O157policy.htm
2. U.S. Department of Health and Human Services, Food and Drug Administration (2009) Food Code at http://www.fda.gov/downloads/Food/FoodSafety/RetailFoodProtection/FoodCode/FoodCode2009/UCM189448.pdf

Table 3 Least squares means for depth (mm) effect on counts (log_{10} CFU/cm^2) of *S.* Typhimurium and *E. coli* O157:H7 for marinated tri-tip roasts

[a-d] Least squares means within a row lacking a common letter significantly differ ($P < 0.05$).

[1]SEM = is the standard error of the least squares means.

	Depth (mm)						
	0-3	3-6	6-9	9-12	12-15	15-18	18-21
Marinade A							
S. Typhimurium	4.1^{a} $(0.2)^{1}$	3.5^{ab} $(0.2)^{1}$	3.2^{bc} $(0.2)^{1}$	2.7^{cd} $(0.2)^{1}$	2.4^{d} $(0.2)^{1}$	2.3^{d} $(0.3)^{1}$	2.5^{cd} $(0.4)^{1}$
E. coli O157:H7	3.9^{a} $(0.2)^{1}$	3.4^{ab} $(0.2)^{1}$	3.1^{bc} $(0.2)^{1}$	2.7^{bc} $(0.2)^{1}$	2.5^{c} $(0.2)^{1}$	2.4^{c} $(0.3)^{1}$	2.3^{bc} $(0.5)^{1}$
Marinade B							
S. Typhimurium	3.3^{ab} $(0.2)^{1}$	3.5^{a} $(0.2)^{1}$	2.9^{b} $(0.2)^{1}$	2.4^{c} $(0.2)^{1}$	2.1^{c} $(0.2)^{1}$	1.9^{c} $(0.3)^{1}$	1.6^{c} $(0.4)^{1}$
E. coli O157:H7	2.9^{a} $(0.2)^{1}$	3.2^{a} $(0.2)^{1}$	2.7^{a} $(0.2)^{1}$	2.2^{b} $(0.2)^{1}$	2.0^{b} $(0.2)^{1}$	1.9^{b} $(0.3)^{1}$	1.4^{b} $(0.4)^{1}$