Project Title: The Risk and Thermal Susceptibility of Non-O157 and O157:H7 Shiga-toxin Producing *Escherichia coli* in Non-intact Beef Products Intended for Foodservice or Retail

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Background

Marination and blade tenderization are widely used to enhance the palatability and marketability of underutilized meat cuts. However, the risk of pathogen internalization propagated by blade tenderization and marination been previously documented (Echeverry et al., 2010; Huang & Shen, 2011; Luchansky et al., 2009). Luchansky and colleagues (2009) recovered 33% of the surface inoculum within 1 cm of the muscle surface in bladet enderized products, while Muras and others (2012) observed that when a marination solution inoculated with 105 log CFU/ml O157:H7 was used to marinate beef tri-tip roasts, the pathogen was present at counts greater than 2.0 log CFU/cm2 at 21mm below the meat surface. Furthermore, blade tenderized beef products were implicated in several outbreaks of O157:H7 in the 2000s. While research performed in our lab (Echeverry et al., 2010; Liao et al., 2012; Chancey et al., 2013) and the labs of others (Heller et al., 2007) have demonstrated intervention-based reduction of external pathogens and subsequent translocation, the survival of non-O157 STECs in the internal cores of cooked beef steaks has been documented (Liao et al., 2012; Luchansky et al., 2012).

Pathogen resistance to previously considered lethal cook temperatures is of tremendous concern--especially in non-intact meat products, which despite the recognized risk of pathogen translocation are not currently distinguishable to the consumer. While there exist several genotypic and environmental factors which can influence bacterial expression of thermotolerance, previous research also suggests that simple substrate biochemistry and structure can influence survivability. Ahmed and colleagues (2005) indicated meat composition—namely fat content—influenced the post-cooking survival of O157:H7. Other researchers have demonstrated the influence of other biochemical traits, such as water activity and pH, on the thermal tolerance of pathogenic bacteria (Doyle & Mazzotta, 2000; Carlson et al., 2005). Data regarding the influence of such biochemical traits on the onset of thermotolerance in non-O157 STECs is severely limited. Similarly, the propensity for non-O157 STEC migration and survival in marinated beef systems is not well understood.

The objectives of this study were to investigate the internalization and cooking survivability of non-O157 (“Big 6” adulterants) and O157:H7 shiga-toxin producing *Escherichia coli* in non-intact beef products directed towards retail (marination; Phase One) or food service (blade tenderization; Phase Two).

Methodology

Phase One: Beef bottom sirloin flap sections were inoculated (10^6 log CFU/ml, 10^4 log CFU/cm^2 attachment) with either *E. coli* O157:H7 one of the following non-O157 STEC

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serogroups: O145, O26, O111, O103, O45, or O121 (each one evaluated individually) STEC (7 total inoculation treatments). After inoculation, flap sections were marinated in a typical marination solution containing food-grade blue dye for 30 or 60 minutes using a vacuum tumbling marination system. At the conclusion of each tumbling interval, marination penetration and microbial translocation were evaluated. The remaining sections were stored at 0 to 4°C in vacuum packages for 14 d. After storage, sections were subjected to analysis of pathogen dispersion, marinade migration, and pathogen survival after cooking to targeted internal temperatures (55, 60, 65, or 71°C). Biochemical properties (composition-uninoculated only, pH, and water activity) were evaluated on raw and cooked samples. Surface swabs and internal core samples obtained at each tumbling and storage interval (0 or 14 d) was used to determine translocation (swabs), internalization (cores), and pathogen survival after cooking (cores).

Phase Two: Carcasses (n = 3 per criteria) meeting distinct categories of intramuscular fat and pH (marbling score; dark cutting status) were identified for strip loin collection: modest to moderate marbling and no-dark cutter; traces to slight marbling and no-dark cutter; modest to moderate marbling and dark cutter; traces to slight marbling and dark cutter. Strip loins from each side of the selected carcasses were quartered to produce eight equal sections per carcass. One section from each carcass was reserved for analysis of raw biochemical properties (composition, pH, and water activity) while the remaining seven sections per carcass were inoculated (106 log CFU/ml, 10^4 log CFU/cm^2 attachment) with one of seven STEC serogroups (O157:H7, O145, O26, O111, O103, O45, or O121). After 14 d of vacuum storage, sections were blade tenderized and portioned into five 2.54-cm steaks. Steaks were used to evaluate pathogen internalization and thermal susceptibility at targeted end-point cooking temperatures (raw, 50, 60, 71, and 85°C). Detection and enumeration of surface and core samples will accommodate quantification of pathogen concentration, internalization and cooking destruction.

Findings

Phase One:

- Marination length did not influence STEC attachment or internalization; however, 30 min of vacuum marination resulted in substantial pathogen internalization.
- O121 was not detectable in cooked core samples, regardless of cooking temperature. STEC O145 and O157:H7 were confirmed in internal cooked cores from marinated sections cooked to 65°C; STEC O145 was detected in sections cooked to 71°C.

Phase Two:

- Biochemical properties did not influence subprimal attachment; however, increased translocation was noticed on steak swabs from strip loins with a high pH.
- STEC O157:H7 exhibited generally less translocation and internalization.
- O121 and O45 were not detectable in the cooked steak cores.
- Of all serogroups, O145 was tolerant to the greatest internal temperatures (71 and 85°C).

Implications

These data reinforce the marinade and blade mediated internalization of pathogens in non-intact meat products. As such, this supports the importance of validated subprimal intervention strategies and cooking protocols aimed to reduce the risk of non-intact beef. Furthermore, these data imply that STEC O157:H7 may not be the best representative

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serogroup for all STECs. Of critical importance is the examination of each O group as individuals and the evaluation of risk based on O group. It is obvious that some strains are more heat tolerant than others, but the risk of those strains specifically in beef products should be considered.

Figure 1: Left: Internalization of marination solution in inoculated beef flap sections vacuum tumbled for 30 or 60 min. Right: Raw inoculated steak representing the dark-cutter pH category.

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Table 1. The percentage of cooked core samples with confirmed[1] shiga-toxin producing *Escherichia coli* (STEC) present in the cores of vacuum marinated sirloin flaps[2] and blade tenderized beef strip loins[3] inoculated with one of seven STEC serogroups prior to processing, storage, and cooking.

Phase One: Marination			Phase Two: Blade Tenderization		
Serogroup and Internal Temperature (°C)	Confirmed Samples (n = 12/temp)	% Confirmed	Serogroup and Internal Temperature (°C)	Confirmed Samples (n = 36/ temp)	% Confirmed
026			***026***		
55	2	16.67	50	6	16.67
60	0	0	60	0	0
65	0	0	71	1	2.78
71	0	0	85	0	0
045			***045***		
55	0	0	50	0	0
60	0	0	60	0	0
65	0	0	71	0	0
71	0	0	85	0	0
0103			***0103***		
55	1	8.33	50	7	19.44
60	0	0	60	2	5.56
65	0	0	71	0	0
71	0	0	85	0	0
0111			***0111***		
55	1	8.33	50	4	11.11
60	1	0	60	1	2.78
65	0	0	71	0	0
71	0	0	85	0	0
0121			***0121***		
55	0	0	50	0	0
60	0	0	60	0	0
65	0	0	71	0	0
71	0	0	85	0	0
0145			***0145***		
55	1	8.33	50	9	25.00
60	1	8.33	60	7	19.44
65	1	8.33	71	0	0
71	1	8.33	85	0	0
0157:H7			***0157:H7***		
55	0	0	50	6	16.67
60	2	16.67	60	3	8.33
65	1	8.33	71	0	0
71	0	0	85	0	0

[1] Cooked cores samples were subjected to the detection of *E. coli* cells using the rapid PCR-based BAX® system. Samples deemed as "positive" by initial detection were subjected to direct plating on selective media STEC (Chromagar™) prior to serogroup confirmation of morphologically representative colonies using agglutination (O157:H7) or BAX® screening panels (non-O157 STEC).

[2] Beef sirloin flaps were inoculated with individual serogroups (10^6 log CFU/cm^2) , vacuum marinated for 30 or 60 min, and stored in the dark for 14 d before cooking on clam-shell style grills. Proportions represent the pooled confirmed samples for 30 and 60 min marination times.

[3] Beef strip loins from Choice dark cutter, Choice non-dark cutter, Select dark cutter, and Select non-dark cutter carcasses were inoculated with individual serogroups (10^6 log CFU/cm^2) ,stored in the dark for 14 d before blade tenderization, steak portioning, and cooking on clam-shell style grills to targeted internal temperatures. Proportions represent the pooled confirmed samples for all carcass characteristics

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