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The influence of beef quality characteristics on the internalization and thermal susceptibility of Shiga toxin-producing *Escherichia coli* (STEC) in blade-tenderized beef steaks

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ABSTRACT

The risk of Shiga toxin-producing *Escherichia coli* (STEC) survival in blade-tenderized beef is a concern for beef processors. This study evaluated the internalization and post-cooking survival of individual STEC serogroups (O157:H7, O26, O45, O103, O111, O121, and O145) in blade-tenderized beef steaks with different quality traits. Strip loins representing four combinations of USDA Quality Grade (Choice or Select) and pH category (High pH or Normal pH) were inoculated (10^6 log CFU/cm^2 attachment) with individual STEC serogroups before storage (14 days), blade tenderization, and cooking (50, 60, 71, or 85 °C). Serogroup populations on raw steak surfaces and internal cores were determined. Rapid-based methods were used to detect the internal presence of STEC in cooked steaks. Internalization and post-cooking survival varied among STECs. All serogroups, except O45 and O121, were detected in the internal cores of steaks cooked to 50 °C, while O103, O111, and O145 STEC were detected in steaks cooked to 50, 60, and 71 °C.

1. Introduction

Mechanical tenderization, which relies on the physical manipulation of muscle fibers, is a method commonly used to add value and improve consistency of beef cuts (Huang & Sheen, 2011). In fact, the National Cattlemen's Beef Association reports that approximately 95% of beef processors utilize mechanical tenderization (Luchansky, Phebus, Thippareddi, & Call, 2008).

Blade tenderization involves a surface-initiated disruption of muscle fibers and connective tissue, which results in a more palatable eating experience (Jeremiah, Gibson, & Cunningham, 1999); however, prior research supports the potential for translocation of surface bacteria into the previously sterile internal muscle (Johnston, Harris, & Moran, 1978; Luchansky et al., 2008) The food safety risk associated with non-intact beef products as posed by the potential internalization of surface pathogens has been documented (Echeverry et al., 2010; Huang & Sheen, 2011; Luchansky et al., 2009). Additionally Gill et al. (2005) found *Escherichia coli*, internalized in blade-tenderized beef in a retail style research setting; another study (Gill, 2009) found *E. coli* present in cooked beef (<70 °C) previously injected with *E. coli* O157:H7 contaminated brine. Sporing (1999) found that approximately 3 to 4% of surface inoculated *E. coli* O157:H7 was translocated to internal tissues during blade tenderization. Similarly, Luchansky et al. (2009) demonstrated that although the greatest concentration of translocated *E. coli* O157:H7 was restricted to the outermost 1 cm of beef steaks, the pathogen was also present in more internal tissues of beef subprimals.

Of particular concern with the internalization of surface pathogens is the potential for internalized pathogens to survive during cooking, particularly when beef products are not cooked to the minimum recommended temperature. Luchansky et al. (2011) observed the survival of *E. coli* O157:H7 cells in brine-injected beef steaks cooked to 71.1 °C. Likewise, Chancey et al. (2013) documented the survival of O157:H7 at 71.1 °C in surface-inoculated blade tenderized beef steaks. Further, Luchansky et al. (2012) observed the survival of O157:H7 and non-O157:H7 serogroups in blade tenderized steaks cooked to 71.1 °C. As a result, in recent years, consumer groups and food safety advocates have filed petitions asking the United States Department of Agriculture Food Safety and Inspection Service (USDA-FSIS) to "promulgate regulations requiring… these products to identify that they have been pinned, bladed, or otherwise mechanically manipulated…" so that "consumers know they must cook the product differently," (CFP, 2009). In response, the USDA-FSIS recently proposed a final rule, which will require on-package labeling and validated cooking methods for non-intact meat products (USDA FSIS, 2012).

Although fresh meat contamination with STEC has been a preeminent concern of the U.S. meat industry for the past three decades, until recently, the primary focus has centered on *E. coli* O157:H7. However, concern for a group of six non-O157 STEC serogroups has risen

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significantly within the past decade. These non-O157 STEC serogroups, specifically STEC O26, O45, O103, O121, O111, and O145 in the U.S., can produce illnesses that are equally as severe and impactful as their STEC O157:H7 counterparts. Furthermore, despite the advances in O157:H7 mitigation, management and understanding, a report published by Scallan et al. (2011), evidenced that illnesses associated with non-O157 STEC serogroups were more than four times greater than previously published estimates (Mead et al., 1999). It should be noted that the discrepancies in illness could be due to improved detection methods, or the 2002 declaration of STEC illnesses as reportable diseases by the Centers for Disease Control. Regardless, although the studies utilized varying methods, both provide evidence that these STEC serogroups are a threat to public health.

Given the public health risk and current regulatory status of these STEC serogroups, a more comprehensive understanding of not only the pathogen's ability to migrate during mechanical tenderization, but also the susceptibility of each individual serogroup to temperatures that are considered lethal to STEC O157:H7 is necessary. Of the available data, it is suggested that non-O157 STECs can exhibit patterns of survivability at internal temperatures representing a medium degree of doneness (71.1 °C; Luchansky et al., 2012). However, investigations of specific parameters and characteristics that might influence internalization and thermal tolerance are sparse, yet needed. For example, prior research suggests meat composition (including but not limited to, fat, moisture, protein, and collagen) and biochemical traits (i.e. pH, water activity, etc.) can influence pathogen attachment, internalization, and thermal susceptibility (Ahmed, Conner, & Huffman, 1995; Carlson, Marks, Booren, Ryser, & Orta-Ramirez, 2005; Doyle & Mazzotta, 2000). However, investigations regarding the influence of meat characteristics on STEC internalization and thermal susceptibility have yet to be performed. Furthermore, previous investigations have not determined the influence of carcass and meat quality variables, particularly USDA Quality Grade (QG) and pH, on the extent of pathogen internalization and thermal tolerance. Given the variable nature of the U.S. beef supply, we believe this data is imperative.

Thus, this project was performed with two primary objectives regarding the internalization and thermal susceptibility of STEC O157:H7 and non-O157 STEC in mechanically tenderized beef. First, we aimed to determine the influence of intramuscular fat (represented by QG) and pH (represented by High pH/Normal pH) on the attachment of pathogens to subprimal surfaces. Secondly, we evaluated the influence of QG and pH on the internalization and thermal susceptibility of individual STEC serogroups in steaks cooked to common end-point cooking temperatures.

2. Materials and methods

2.1. Beef subprimal procurement and processing

The objectives of this project include the evaluation of internalization and thermal tolerance in beef with targeted biochemical characteristics. As such, utilization of beef cuts from carcasses expressing distinct biochemical traits was imperative. The USDA Quality Grade system is a well-proven indicator of intramuscular fat (American Meat Science Association, 1995). Similarly, Page, Wulf, and Schwotzer (2001) indicated that a dark carcass lean color correlates strongly with a high intramuscular pH. Therefore, in order to meet the objective of selecting distinct biochemical parameters, USDA Quality Grade (QG) was used as an indicator of intramuscular fat and carcass lean color (dark lean indicating high pH or non-dark lean indicating normal pH) as an indicator of intramuscular pH.

Trained university personnel were used to identify carcasses representing the following (n = 3 carcasses or 6 carcass sides per combination): USDA Choice and non-dark lean color representing normal-pH (**CH-NpH**); USDA Choice and dark lean color representing high-pH (**CH-HpH**); USDA Select and non-dark lean color representing normal pH (**SEL-NpH**); USDA Select and dark lean color representing high pH (**SEL-HpH**). Marbling score, used to determine USDA Quality Grade, and lean color, used as an indicator of muscle pH, were evaluated on the loin surface of the 12th rib of each carcass. Carcasses were selected so that postmortem age would be no more than 7 days at the time of inoculation and processing. Paired strip loins (Institutional Meat Purchase Specifications (IMPS) #180; USDA-AMS, 2014) were procured from each side of the carcasses meeting the four criteria combinations (n = 6 pieces per treatment combination; 24 total pieces per replication; replication = 3). The selected strip loins were immediately transported to the Gordon W. Davis Meat Laboratory at TTU (Lubbock, TX) and stored under refrigeration.

Upon arrival, paired strip loins from each carcass were fabricated using the Beef Alternative Merchandising method (Smith et al., 2014) to longitudinally divide the *Longissimus dorsi* into two equal halves. Each longitudinal half was halved again to produce eight equal sections per carcass. One raw, un-inoculated section was vacuum packaged for later biochemical analysis (described below). The remaining seven sections per carcass were assigned to inoculation with one of seven STEC serogroups (*E. coli* O157:H7, O145, O26, O111, O103, O45, or O121). Subprimal sections for each serogroup (n = 3 sections per treatment combination per serogroup; 84 total sections) were vacuum packaged and transported to the Texas Tech University BSL-II pathogen processing facility (Lubbock, TX) for inoculation within 24 h.

2.2. Raw and cooked meat biochemical properties

Samples from each subprimal reserved for evaluation of un-inoculated samples were processed at the Texas Tech University Gordon W. Davis Meat Laboratory (Lubbock, TX). Subsequent evaluation of inoculated, raw and cooked samples was performed on the sample remaining after removal of the core for evaluation of pathogen internalization. All inoculated raw and cooked samples were processed in the BSL-II Food Microbiology Laboratory at Texas Tech University (Lubbock, TX).

2.2.1. Compositional analysis (raw, un-inoculated only)

Compositional variables (% fat, moisture, protein, and collagen) were measured on raw samples using an AOAC-approved near infrared spectrophotometer (FOSS FoodScan, Hilleroed, Denmark; Anderson, 2007). Methods for sample preparation were the same as those described by Martin et al. (2013).

2.2.2. pH analysis (raw and cooked)

The pH of raw and cooked steaks (for each cooking temperature) were evaluated using the procedures described by Luque et al. (2011) Samples were evaluated in duplicate and results averaged prior to statistical analysis.

2.2.3. Water activity analysis (raw and cooked)

Water activity was measured on raw and cooked steaks (for each cooking temperature) using a Hygrolab 3 bench-top water activity meter (Rotronic Inc., Huntington, NY).

2.3. Inoculum preparation

Cocktail mixtures of inoculation solutions for each STEC serogroup were prepared using strains from the TTU Food Microbiology Laboratory Stock Collection (Lubbock, TX). Three strains (Table 1) from each serogroup were utilized to form an inoculation cocktail for each serogroup. The frozen cultures were taken out of frozen storage (−80 °C), and placed in an ice bath to thaw. Seven, 1 l bottles of buffered peptone water (BPW, EMD Chemicals Inc. Gibbstown, NJ) were used and one inoculum loop of each culture were put into each bottle. Prior to formulation of each serogroup cocktail, frozen stock cultures of individual strains were added to 50 ml tryptic soy agar (TSA) and