

ESCHERICHIA COLI O157:H7 RISK ASSESSMENT FOR PRODUCTION AND
COOKING OF BLADE TENDERIZED BEEF STEAKS

by

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ABSTRACT

Blade tenderization (BT) is the most effective mechanical technology used by the beef industry to ensure tenderness. It has been shown that the BT process can translocate surface bacteria, including pathogens, to the interior of muscle. Research was conducted to quantify bacterial relocation and define safe cooking schedules for resultant products.

In Study 1A, beef top sirloin subprimals were inoculated with 10^6 cfu/cm² of *Escherichia coli* O157:H7 and passed through a blade tenderizer. Core samples were removed and cut into cross-sections. Results indicated that the BT process transferred 3-4% of surface contamination to the center of the muscle. Bacterial translocation was confirmed using confocal microscopy in Study 1B.

Studies 2-4 evaluated thermal destruction of *E. coli* O157:H7 in BT steaks by different cooking methods. Six top sirloin subprimals were surface inoculated with a 5-strain cocktail to a level of ca. 10^7 cfu/cm². Three were tenderized and three served as non-tenderized controls. Steaks of varying thicknesses (1.3, 1.9 and 3.2 cm) were cut and cooked to one of 6 internal temperatures (48.9, 54.4, 60.0, 65.6, 71.1, and 76.7°C) on a gas grill (Study 2), electric skillet (Study 3), or oven broiler (Study 4). *E. coli* O157:H7 levels were assayed on MacConkey Sorbitol Agar (MSA) and Phenol Red Sorbitol Agar (PRSA).

Results indicated that there was no difference ($p>0.05$) between tenderized (T) and non-tenderized (NT) steaks, except at 48.9°C, wherein T steaks showed lower reductions ($p\#0.05$) than NT steaks. The oven broiler was the most effective method, producing a 5 log cycle reduction at 60.0°C. The commercial gas grill was less effective, requiring an internal temperature of at least 65.6°C to achieve a 5 log reduction. This level of reduction was not consistently achieved by the skillet, even at 76.7°C. High variability in cooking performance may reduce the margin of safety associated with these methods of cookery. Recovery of *E. coli* O157:H7 was consistently higher on PRSA than MSA, indicating that a sub-population of the pathogen was sublethally injured by the thermal treatments.

Evaluation of cooking parameters indicated that the oven broiler method produced the shortest cook times. Temperature increase after removal from the grill was quite similar among all methods. This post-cook temperature increase was higher in steaks cooked to low target temperatures than those cooked to high target temperatures.

TABLE OF CONTENTS

List of Figures	iv
List of Tables	vi
Acknowledgments	vii
Introduction	1
Literature Review	3
History of Meat Tenderization	3
The Blade Tenderization Process	4
Sensory Aspects of Blade Tenderization	8
Industry Use of Blade Tenderization	9
Microbiological Aspects of Blade Tenderization	10
Introduction to <i>Escherichia coli</i> O157:H7	13
Categories of Pathogenic <i>E. coli</i>	13
Outbreaks Associated with <i>E. coli</i> O157:H7	16
Pathogenicity of <i>E. coli</i> O157:H7	18
Reservoirs for <i>E. coli</i> O157:H7	19
Detection/Destruction of <i>E. coli</i> O157:H7	20
Regulatory Issues Related to Blade Tenderization	24
Materials and Methods	27
Study One A: Characterization of <i>Escherichia coli</i> O157:H7 Translocation Due to Blade Tenderization	27
Bacterial Cultures	27
Inoculation of Subprimals	27
Blade Tenderization of Subprimals	28
Sampling Procedure	28
Bacterial Enumeration	32
Statistical Analysis	36
Study One B: Visualization of Generic <i>Escherichia coli</i> Translocation in Beef Steaks Via the Blade Tenderization Process	36

Sample Preparation	36
Confocal Microscopy	36
Study Two: Efficiency of Cooking by Gas Grill Method for <i>Escherichia coli</i> O157:H7	
Elimination from Blade Tenderized Steaks	37
Bacterial Cultures	37
Inoculation of Subprimals	38
Blade Tenderization of Subprimals	38
Cooking Procedure	38
Sample Collection	39
Enumeration of <i>E. coli</i> O157:H7 Populations	39
Statistical Analysis	41
Study Three: Efficiency of Cooking by the Electric Skillet Method for <i>Escherichia coli</i>	
O157:H7 Elimination from Blade Tenderized Steaks	41
Study Four: Efficiency of Oven Broiling for <i>Escherichia coli</i> O157:H7 Elimination from	
Blade Tenderized Steaks	43
Results and Discussion	45
Study One A: Characterization of <i>Escherichia coli</i> O157:H7 Translocation Due to Blade	
Tenderization	45
Study One B: Visualization of Generic <i>Escherichia coli</i> Translocation in Beef Steaks Via	
the Blade Tenderization Process	47
Study Two: Efficiency of Cooking by Gas Grill Method for <i>Escherichia coli</i> O157:H7	
Elimination from Blade Tenderized Steaks	47
Bacterial Reduction Analysis	47
Evaluation of Heat Injury	53
Cooking Profile	55
Study Three: Efficiency of Cooking by the Electric Skillet Method for <i>Escherichia coli</i>	
O157:H7 Elimination from Blade Tenderized Steaks	59
Bacterial Reduction Analysis	59
Evaluation of Heat Injury	61

Cooking Profile	61
Study Four: Efficiency of Oven Broiling for <i>Escherichia coli</i> O157:H7 Elimination from Blade Tenderized Steaks	65
Bacterial Reduction Analysis	65
Evaluation of Heat Injury	68
Cooking Profile	71
F-Value Comparisons for Cooking Methods Evaluated	71
Conclusion	84
References	86
Appendices	94

LIST OF FIGURES

Figure	Page
1. The Ross TC700M Blade Tenderizer	5
2. Ross TC700M blade head	6
3. Perpendicular pattern of double edged blades used for most commercial blade tenderizers	7
4. Description of mist inoculation procedure	29
5a. Diagram of blade tenderization process	30
5b. Diagram of blade tenderization process, cont	31
6a. Sample collection method using a sterile coring device	33
6b. Sample collection method using a sterile coring device, cont	34
7. Sampling method used in Study 1A to profile <i>Escherichia coli</i> O157:H7 contamination in blade tenderized subprimals	35
8. Diagram of steak sampling procedure used for Studies 2-4	40
9. Contamination profile of tenderized beef subprimals, with low (ca. 10 ³ cfu/g) and high (ca. 10 ⁶ cfu/g) levels of <i>Escherichia coli</i> O157:H7 on the surface	46
10. Autofluorescent generic <i>E. coli</i> within one blade tract of a tenderized beef subprimal at a depth of ca.0.5 cm below the inoculated surface, visualized by confocal microscopy	48
11. Autofluorescent generic <i>E. coli</i> within one blade tract of a tenderized beef subprimal at a depth of ca. 6.0 cm below the inoculated surface, visualized by confocal microscopy	49
12a,b,c. Average <i>E. coli</i> O157:H7 log reductions for steaks cooked on a commercial gas grill, enumerated on PRSA	51
13a,b,c. Time required for 1.3, 1.9, and 3.2 cm steaks to reach target endpoint temperatures on a commercial gas grill (standardized from 10°C)	56
14a,b,c. Post-cooking temperature increases observed in 1.3, 1.9, and 3.2 cm steaks after removal from a commercial gas grill	58
15a,b,c. Average <i>E. coli</i> O157:H7 log reductions for 1.3, 1.9, and 3.2 cm steaks cooked on an electric skillet, enumerated on PRSA	60

16a,b,c.	Time required for 1.3, 1.9, and 3.2 cm steaks to reach target endpoint temperatures on an electric skillet (standardized from 10°C)	63
17a,b,c.	Post-cooking temperature increases observed in 1.3, 1.9, and 3.2 cm steaks after removal from an electric skillet	64
18a,b,c.	Average <i>E. coli</i> O157:H7 log reductions for 1.3, 1.9, and 3.2 cm steaks cooked under a broiler oven, enumerated on PRSA	67
19a,b,c.	Time required for 1.3, 1.9, and 3.2 cm steaks to reach target endpoint temperatures under a broiler oven (standardized from 10°C)	72
20a,b,c.	Post-cooking temperature increases observed in 1.3, 1.9, and 3.2 cm steaks after removal from a broiler oven	73
21.	F-value regression lines obtained for 3 cooking methods, using PRSA as a recovery medium for <i>E. coli</i> O157:H7	75
22.	Comparison of F-values obtained for the grill method, using PRSA as a recovery medium for <i>E. coli</i> O157:H7	76
23.	Comparison of F-values obtained for the skillet method, using PRSA as a recovery medium for <i>E. coli</i> O157:H7	77
24.	Comparison of F-values obtained for the broiler method, using PRSA as a recovery medium for <i>E. coli</i> O157:H7	78
25.	F-value regression lines obtained for 3 cooking methods, using MSA as a recovery medium for <i>E. coli</i> O157:H7	79
26.	Comparison of F-values obtained for the grill method, using MSA as a recovery medium for <i>E. coli</i> O157:H7	81
27.	Comparison of F-values obtained for the skillet method, using MSA as a recovery method for <i>E. coli</i> O157:H7	82
28.	Comparison of F-values for the broiler method, using MSA as a recovery method for <i>E. coli</i> O157:H7	83

LIST OF TABLES

Table	Page
1. Percent of <i>E. coli</i> O157:H7 population injured by cooking on a gas grill	54
2. Percent of <i>E. coli</i> O157:H7 population injured by cooking on an electric skillet	62
3. Percent of <i>E. coli</i> O157:H7 population injured by cooking in a broiler oven . . .	69

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Sincerely,

Sarah Sporing

INTRODUCTION

Beef has long been considered a staple of the American diet, due to its availability and versatility. However, the beef industry must provide consistent quality and uniformity in its products to remain competitive. The characteristic used most by consumers to judge quality and overall acceptability is tenderness (Carpenter, 1975). One of the most effective and efficient technologies used to ensure tenderness is blade tenderization. This process adds value to lower-cost muscle pieces, especially those high in connective tissue, and improves product uniformity.

While sensory characteristics of blade tenderization have been researched extensively, microbiological aspects of the process have not been investigated to the same degree. Although the generic microbiological quality of blade tenderized muscle was equivalent to non-tenderized controls on a per gram basis (Boyd et al., 1978), bacteria were translocated into the interior of the muscle (Johnston et al., 1979). Therefore, research is required to quantify this bacterial relocation, identify critical control points to minimize the risk for pathogenic contamination, and define effective cooking schedules for blade tenderized products.

In November 1997, the National Advisory Committee on Microbiological Criteria for Foods (Acuff, 1999), Meat and Poultry Subcommittee, stated that “*Due to the low probability of pathogenic organisms being present in or migrating from the external surface to the interior of beef muscle, cuts of intact muscle (steaks) should be safe if the external surfaces are exposed to temperatures sufficient to effect a cooked color change*”. However, if the surface of an intact muscle or muscle system is violated by mechanical tenderization (blade tenderization), contamination may be carried from the surface to the interior of the cut. The

NACMCF Meat and Poultry Subcommittee also stated that there was a lack of scientific data to address the hazards associated with those processes that may cause translocation of pathogens. Because of the widespread commercial use of the blade tenderization technology and the potential food safety risks it may pose, the recently formed Beef Industry Food Safety Council (Holmes, 1999) identified this research as a priority for the beef industry. This industry group facilitates input from producers, packers, processors, distributors, restaurateurs, and food retailers in an effort to identify and implement workable *Escherichia coli* O157:H7 controls in the beef industry.

Blade tenderization technology requires research to determine processing and handling protocols that will ensure the safety of tenderized products. Unlike ground beef, which is now almost exclusively cooked to 71EC by food service establishments as a result of Food and Drug Administration (FDA) and state health department recommendations, mechanically tenderized steaks are often perceived as whole muscle cuts and are, therefore, prepared to customer specifications of rare to medium doneness, which may pose a health risk to consumers. The objectives of this study were to: 1) evaluate the potential health risks associated with beef steaks derived from blade tenderized top sirloin subprimals, as compared to non-tenderized subprimals, artificially surface-inoculated with *E. coli* O157:H7; and 2) determine adequate cooking protocols for tenderized beef steaks to assure consumer safety. These studies should allow regulators, suppliers, and food service operators to effectively establish standard cooking recommendations for preparation of blade tenderized beef steaks.

LITERATURE REVIEW

History of Meat Tenderization

Beef is a versatile product that can be fabricated and prepared in a variety of ways, from ground beef to standing rib roasts. For whole muscle cuts, tenderness is perhaps the most important factor used by consumers to judge palatability, quality and overall acceptance (Carpenter, 1975).

Methods to increase the tenderness of beef cuts have been researched for many years. The simplest and most effective method is grinding, which has been and continues to be a widely accepted process (Johnston, 1979). However, for popular cuts such as steaks and roasts, for which grinding is not applicable, several other methods have been developed. Prior to 1960, the most common practice was to hang carcasses in refrigerated coolers for prolonged periods of time, known as aging. While effective, this method resulted in excessive carcass moisture loss and demanded great space and energy requirements, which in this day of mass production, are no longer economically feasible. Another economic concern with aging was the occurrence of surface spoilage, which resulted in the costly tankage of trimmings (Johnston, 1979).

In the 1970's, scientists and nutritionists also considered the effect of various feeding practices on the tenderness of beef. Grain-fed cattle produced well-marbled and more tender beef than that of grass-fed cattle (Kropf et al., 1975). Prior to 1974, American grain prices were relatively low and this information was put to good use. However, a grain shortage in 1974 greatly increased the cost of grain and ultimately, the feeding costs of market cattle. Not only did it

decrease the amount of grain fed per head, but it also increased the number of grass-fed cattle being sent to market (Johnston, 1979). During this time, researchers had been investigating newly developed mechanical methods of tenderization and the economic pressures created by the grain shortage spurred these concepts into consideration for commercial application.

Several reports throughout the mid to late 1970's concluded that mechanical tenderization, commonly referred to as “needling”, could improve the tenderness of some types of meat (Davis et al., 1977; Glover et al., 1977; Schwartz and Mandigo, 1974, 1977). Schwartz and Mandigo (1974) reported that this process could make low grade and cheaper cuts of beef more palatable. Since Americans are consuming an excess of animal fats in their diets, the fact that mechanical tenderization is generally performed on lean cuts of beef may be considered advantageous to the consumer, as well (Johnston, 1979).

The Blade Tenderization Process

The concept of blade tenderization involves a series of very sharp, double-edged blades (Figs. 1 and 2), which are capable of penetrating meat by cutting through muscle tissues and fibers, rather than tearing the tissue or “punching holes” (Ross Industries, 1998). The product to be tenderized, typically in subprimal form, is placed fat-side-down on a conveyor system, which moves in tandem with the blade heads, advancing when the heads are retracted, and stopping as the blade heads drop to allow penetration. The blades are usually arranged in a perpendicular (Fig. 3) configuration to prevent a sliced or sheared appearance.



Figure 1. *The Ross TC700M Blade Tenderizer*

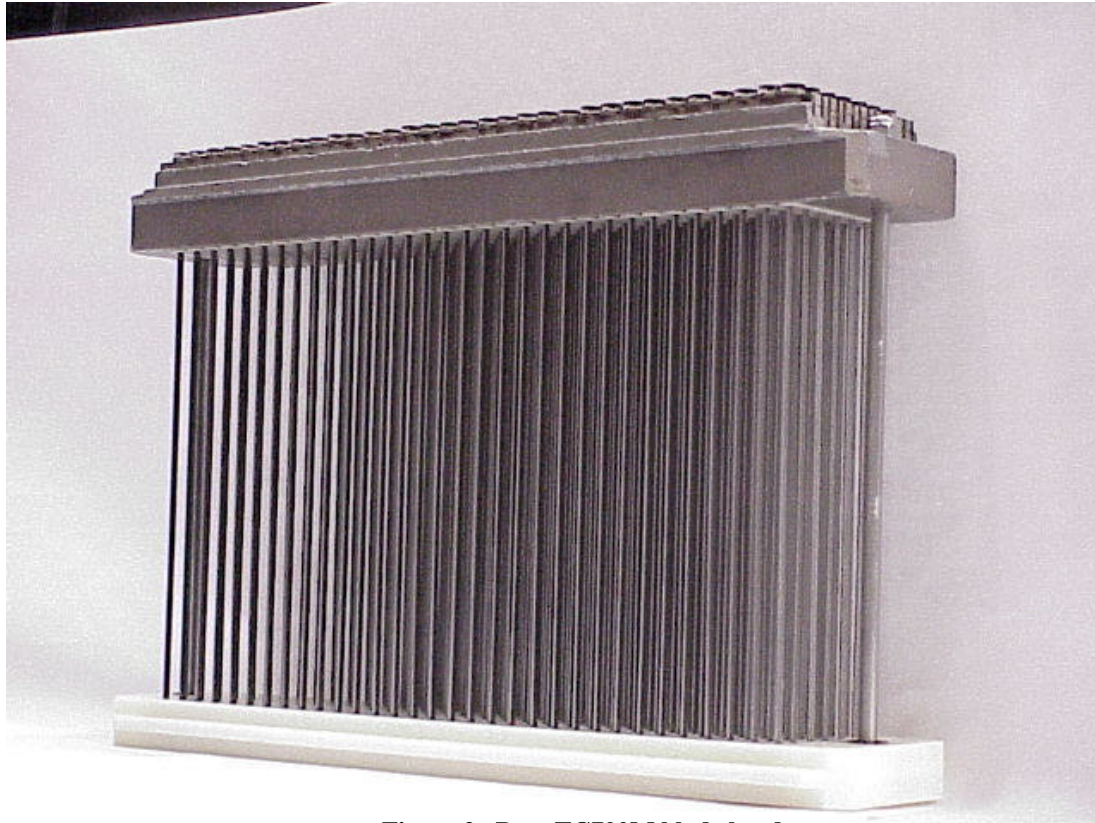


Figure 2. Ross TC700M blade head

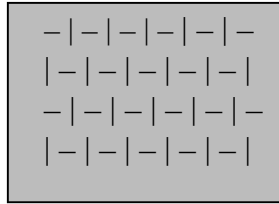


Figure 3. *Perpendicular pattern of double edged blades used for most commercial blade tenderizers.*

The number of blades per square cm varies with equipment manufacturer and/or processor specifications, depending on degree of tenderness and acceptability of product appearance. Effect of blade size on tenderness was evaluated by Mandigo and Olson in 1982. They found that larger blades (3.2 mm) produced significantly higher initial and overall tenderness scores than small blades (1.9 mm), but did not show significant differences in thaw and cook losses, Warner Bratzler shear forces, juiciness, or flavor. The researchers recommended the larger blades, suggesting that along with improved tenderness, they may also provide additional strength and durability.

While early tenderizing equipment was limited to boneless cuts, obviously due to potential breakage of the stainless steel blade tips, today's tenderizers have incorporated a new technology which utilizes magnetic force to allow for the tenderization of bone-in products. The metal blades are controlled by magnetic head plates, which consist of an array of rare-earth magnets and electrical steel pole pieces (Ross Industries, 1998). The magnets provide enough force to drive the blades through muscle tissue, but as soon as anything tougher is encountered, such as a bone, the magnetic force is overcome and the blade stops, unharmed. Each blade moves independently of the others, so those out of the way of bone continue to penetrate. Additionally, the tenderizer may be equipped with a conveyor system that not only moves

forward, but also from side to side. This allows for blade contact with all surface area of the meat, maximizing tenderization (Ross Industries, 1998). Conveyor speed may also be adjusted to fit desired output parameters.

Sensory Aspects of Blade Tenderization

As the popularity of mechanical tenderization increased, many studies were performed to objectively evaluate its effect on various muscle types and cuts. Results of these studies were somewhat mixed. While virtually all studies agreed that blade tenderization improved tenderness, some inconsistencies existed regarding degree of improvement, optimal number of passes through the tenderizer, and the effect of tenderization on cooking loss, cooking time, and juiciness of cooked products.

Sensory panel and Warner-Bratzler shear evaluations have suggested that mechanical tenderization does not have as great an effect on high grade cuts as it does on Utility grade cuts (Davis et al., 1975; Petersohn et al., 1979). Higher grade (Choice) steaks were significantly more tender than Utility grade steaks prior to tenderization, which likely accounted for the greater effects of blade tenderization on the lower grade beef (Petersohn et al., 1979). Therefore, from an economic standpoint, if the cut is already of acceptable tenderness, blade tenderization may be unnecessary and undesirable.

Another factor influencing the degree of tenderness achieved is the number of passes through the blade tenderizer. In general, tenderness increased as the number of passes increased (Miller, 1975). However, as mentioned previously, the magnitude of improved tenderness from repeated passes is not as great in higher grade cuts as in Utility grade cuts (Miller, 1975). Some studies also suggested that single and multiple passes through the

tenderizer contributed to significantly increased purge and drip cook loss (Davis et al., 1975; Glover et al., 1975). However, conflicting research by Goldner et al. (1974), Schwartz and Mandigo (1974), and Bowling et al. (1975, 1976) indicated that blade tenderization did not have effects on cooking losses in beef, pork, and lamb cuts. The researchers agreed that blade tenderization did not significantly influence sensory scores for juiciness, flavor, or overall palatability. In fact, Bowling et al. (1976) suggested that shear tests for mechanically tenderized products tended to overestimate the degree of tenderization when compared to sensory evaluation. Boyd et al. (1978) evaluated the effectiveness of multiple passes through the mechanical tenderizer in regard to cooking parameters, sensory testing, and microbial characteristics, concluding that one pass was as effective as two, three, or four passes for improving tenderness. It should be noted that tenderization of bone-in cuts requires two passes: a primary pass, followed by a second pass after the product has been turned over.

Industry Use of the Blade Tenderization Process

By 1975, it was estimated that over 90% of hotel, restaurant, and institutional (HRI) operations utilized blade tenderization (Miller, 1975). Initially, there was some resistance due to concern over the changes in cooking parameters previously mentioned, as well as the consumer perception that blade tenderized cuts were less desirable. However, based on informal and unpublished research conducted by equipment manufacturers, food service operators, and HRI purveyors, the HRI trade embraced the process for three reasons (Miller, 1975). The first was the assurance of acceptable tenderness when using regular table-grade cuts. The second benefit was more uniform tenderness in items that contained two or more muscles. Examples of this type of product are T-bone or porterhouse steaks, in which the

longissimus dorsi may be significantly less tender than the psoas major, and the sirloin top butt, which contains the gluteus medius and the somewhat less tender biceps femoris. Thirdly, the HRI trade saw an opportunity to upgrade cuts that had not previously been used without some type of enzymatic or chemical tenderization. Blade tenderization was found to be a more efficient method than enzyme dripping, and produced more uniform results (Miller, 1975).

Current data regarding industry usage of blade tenderization is not readily available, in part due to the negative connotations still associated with the process (Lindeman, 1999). An unpublished survey of North American Meat Processors (NAMP) members, conducted by Kansas State University in conjunction with NAMP, revealed that 84% of respondents used mechanical tenderization in their facilities (George et al., 1998). The process is used to a large degree on Select grade products, which account for over one-third of all graded beef, most of which is sold in retail stores (Holmes, 1999).

Microbiological Aspects of Blade Tenderization

While sensory characteristics of mechanical tenderization have been extensively researched, microbiological aspects of the process, especially regarding pathogens, have not been investigated to the same degree. A study by Boyd et al. (1978) determined that one pass through the tenderizer resulted in lower aerobic and anaerobic bacterial counts than two, three, or four passes during a four week shelf-life evaluation. This study also showed that microbial quality of blade tenderized muscle was equivalent to non-tenderized controls on a per gram basis. However, this research did not address the issue of translocation of bacteria, including pathogens, to the interior of the muscle.

A study by Raccach and Henderson (1979) focused on the importance of a good

sanitation program when using blade tenderization. They inferred that unsanitary conditions could turn the tenderizer into an “inoculating machine,” resulting in a product with a shorter shelf-life, and in the presence of pathogens, a public health hazard. However, the study concluded that proper sanitation, using an iodine-based sanitizer on both the blades of the tenderizer and the conveyor system, was adequate for controlling contamination of the tenderized product.

Petersohn et al. (1979) evaluated surface and interior muscle samples of tenderized steaks and non-tenderized controls for aerobic, anaerobic, and psychrotrophic counts over a ten day storage period. In general, surface counts for all bacterial groups did not differ significantly between tenderized and non-tenderized steaks, but overall, counts were slightly higher in the tenderized samples. This trend also held true for the interior muscle samples.

Although to date, no outbreaks of foodborne illness have been linked solely to blade tenderized meat products, concerns regarding its safety were addressed in 1977 by the Community Nutrition Institute (CNI), a non-profit consumer advocacy group (Johnston, 1978). This organization submitted a petition to the USDA Food Safety and Quality Service (FSQS), suggesting that Blade tenderization be banned for meat products, based on the principle of translocation of pathogens into the interior muscle. The petition stemmed from two similar outbreaks of salmonellosis, the first occurring in 1969, wherein beef roasts were needle injected with phosphate solution, reinjected with recycled solution, then grossly undercooked, to temperatures documented as low as 45.5°C (114°F) (Anon, 1969). After this outbreak, regulation was passed that required injected roast beef to be cooked to an internal temperature of 62.8°C (145°F) (USDA/FSIS, 1993a). However, in 1975, a second outbreak occurred, linked to the same processor, who had apparently ignored the regulation and was operating

under virtually identical conditions as those that caused the previous outbreak (Johnston, 1978). While the CNI petition was ultimately rejected, the FSQS did research the issue and found very little information regarding translocation of bacteria from the tenderization process or other similar processes. Understanding that the concerns noted in the petition were valid, the FSQS proposed that pathogen studies be conducted to gather more information. Johnston et al. (1979) conducted an inoculation study of blade tenderized beef rounds, in which surface and core samples were analyzed for the presence and quantity of *Salmonella newport*. Interestingly, *Salmonella* was found inside the cores of both tenderized and non-tenderized rounds, although levels were higher in the tenderized samples. After cooking the rounds to a 54.4°C endpoint temperature, *Salmonella* was still detected in some, but not all core samples, suggesting that cooking guidelines might be beneficial to ward off potential public health hazards involving the blade tenderization process.

While it seems that this study would have spurred further investigation of bacterial, and especially pathogen translocation into muscle tissue, very little research has been published regarding this issue since 1979. However, work has been done to characterize penetration of microorganisms into muscle tissue by means other than blade tenderization. Reports by Gill and Penney (1977; 1982), Maxcy (1981), and Sikes and Maxcy (1980) indicated that microorganisms penetrate the surface of post-rigor muscle, to depths of 20-40 mm, during storage at elevated temperatures (up to 37°C), depending on the type of organism (and its ability to produce proteolytic enzymes), and the type and structure of meat. Sikes and Maxcy (1980) reported that the orientation of the muscle fibers affects the bacterial penetration to the interior of the muscle. Thus, in the event of pathogen contamination on the surface of a subprimal, and

assuming cross contamination during the fabrication process, it is possible that the pathogens could be found in the interior of a steak. However, the extent and depth of penetration would depend on type of organism, temperature and duration of storage, and orientation of the muscle fiber at the point of contamination.

DeZuniga et al. (1991) and Anderson et al. (1992) reported that spray washing beef carcasses with water under pressure resulted in the penetration of a blue lake (water insoluble dye) to a depth of 1.44 mm. The authors hypothesized that the bacteria present on the surface of the carcass just after slaughter would also penetrate the muscle tissue. They used blue lake to simulate bacteria to facilitate visualization of the depth of penetration, as the blue lake particles (0.6 μm) are only slightly smaller than the size of most bacteria (2-6 μm).

Introduction to Pathogenic *Escherichia coli*

Categories of Pathogenic Escherichia coli

Pathogenic *Escherichia coli*, specifically *E. coli* O157:H7, has emerged as a foodborne pathogen of great concern in beef products. While generic *E. coli* is considered a part of the normal microflora in the intestinal tract of most warm-blooded animals, including humans, many pathogenic strains can cause diarrheal disease and have been associated with food-borne illness (Doyle, 1990). These pathogenic strains have been classified into four subgroups.

Enteropathogenic *E. coli* (EPEC) historically have been linked to outbreaks of infantile diarrhea (Doyle, 1990; Reed, 1994). These outbreaks typically occur in hospital nurseries, especially those in developing countries, where the importance of sanitation and hygiene is not clearly understood. Symptoms of EPEC infections include fever, malaise, vomiting and diarrhea

(Levine, 1987). The pathogenic mechanisms for this subgroup of *E. coli* are thought to be attachment and effacing adherence to the intestinal tract, rather than toxin production (Doyle, 1990; Levine, 1987).

Enteroinvasive *E. coli* (EIEC) produce symptoms similar to *Shigella dysenteriae*, including fever, abdominal cramps, malaise, watery diarrhea, and toxemia (Doyle, 1990; Levine, 1987; Reed, 1994). These organisms also resemble *Shigella*, in that they are non-motile and unable to ferment lactose. Serologically, EIEC and *Shigella* often produce cross reactions (Levine, 1987). Pathogenicity of EIEC is caused primarily by invasion of the epithelial tissue of the colon, which leads to inflammation and ulceration of the mucosa as the bacteria replicate (Doyle, 1990; Kornacki and Marth, 1982). Most outbreaks are thought to be caused by person-to-person transmission and are more common in underdeveloped countries (Doyle, 1990; Levine, 1987).

Enterotoxigenic *E. coli* (ETEC) are commonly associated with a disease known as traveler's diarrhea, which frequently strikes individuals traveling from areas of good hygiene to areas of poor hygiene (Doyle and Padhye, 1989; Kornacki and Marth, 1982; Reed, 1994). In tropical areas and developing countries, ETEC may be a cause of diarrhea in all age groups, particularly infants and children (Doyle, 1990; Levine, 1987; Reed, 1994). While man is believed to be the primary reservoir for ETEC, many outbreaks have been foodborne, presumably due to poor sanitation practices. Enterotoxigenic *E. coli* cause disease by penetrating the mucosal layer of the small intestine, after surviving the harsh stomach environment. After adherence, the bacteria produce heat-labile (LT) enterotoxins and/or heat-stable (ST) enterotoxins (Doyle, 1990; Kornacki and Marth, 1982; Levine, 1987; Reed, 1994).

Symptoms of ETEC infection include watery diarrhea, nausea, abdominal cramping, and fever (Levine, 1987).

Enterohemorrhagic *E. coli* (EHEC) contain the most virulent strains of pathogenic *E. coli*, including *E. coli* O157:H7. These strains are associated with hemorrhagic colitis (HC), a clinical syndrome characterized by abdominal cramps and watery diarrhea, followed by a hemorrhagic discharge resembling gastrointestinal bleeding (Dorn, 1993). This discharge eventually leads to edema, erosion, and hemorrhage of the mucous layer of the intestinal tract (Riley, 1987). Complications associated with HC may result in a condition known as hemolytic uremic syndrome (HUS), in which kidney tissue is severely damaged, often resulting in microangiopathic hemolytic anemia, thrombocytopenia, and acute renal failure, sometimes requiring dialysis (Dorn, 1993). A further complication stemming from EHEC infections is thrombotic thrombocytopenic purpura (TTP), wherein the central nervous system becomes involved, resulting in fever, neurological abnormalities, seizures and prolonged coma (Doyle and Padhye, 1989).

Although over 100 *E. coli* serotypes have been classified as EHEC, serotype O157:H7 has received the most widespread attention, since it appears to be the most virulent and most common cause of major outbreaks. This particular organism can be differentiated from other *E. coli* in that it is unable to ferment sorbitol rapidly (March and Ratnam, 1986) and does not produce β -glucuronidase (Thompson et al., 1990). While approximately 95% of *E. coli* have an IMViC pattern of +++-, *E. coli* O157:H7 belongs to the 5% that produces a -+-- pattern (Doyle and Padhye, 1989). Most strains of O157:H7 are susceptible to a variety of antibiotics, including ampicillin, trimethoprim-sulfamethoxazole, tetracycline, and quinolones. It has however, been found to be resistant to erythromycin, metronidazole, and vancomycin (Ratnam

et al., 1988; Pai et al., 1984).

Outbreaks Associated with E. coli O157:H7

E. coli O157:H7 was first identified as an emerging pathogen in 1982, when it was isolated in two U.S. outbreaks of bloody diarrhea linked to the same fast food restaurant chain (Riley et al., 1983). That same year, *E. coli* O157:H7 was isolated from nursing home patients in Canada suffering from bloody diarrhea (Stewart, 1983). Since 1982, outbreaks have been traced to this organism on every continent (Bettelheim, 1996) and in a wide variety of foods, including beef (primarily ground beef), unpasteurized apple cider, unpasteurized milk, raw potatoes, mayonnaise (Marks and Roberts, 1993), apple juice (USDA/FSIS, 1996), dry-cured salami (Anonymous, 1995), and cheese curds (Powell, 1998a). Non-food sources of outbreaks include lake water (Marks and Roberts, 1993) and most recently, swimming pool water (Powell, 1998b).

The landmark incident which brought *E. coli* O157:H7 national attention occurred in early December 1992 through February 1993, in the Pacific Northwest. This outbreak was linked to ground beef patties prepared in Jack-in-the-Box fast food chain restaurants. Although Washington state law required that ground beef patties be processed to 68.3°C (155°F), Jack-in-the-Box protocol for patty processing was designed to achieve only the 60°C (140°F) internal temperature recommended at that time (FDA, 1991). The epidemiological investigation regarding the outbreak revealed that in some test patties, even the 60°C temperature was not achieved at all of four internal test points (Dorn, 1993). This investigation also determined that although suspect patties were available in two weights, 45 g and 114 g, illnesses were routinely traced to the small patties. This indicates that the lethality associated with the larger patties,

even at 60°C, was sufficient to control the pathogen, likely due to longer cooking times (Marsden, 1999). Robert Nugent, CEO of Jack-in-the-Box, claimed during the Senate Agricultural Committee hearings following the outbreak, that he was never informed of the 68.3°C state requirement.

Another highly publicized outbreak occurred in Japan in 1996. This outbreak is perhaps the largest on record, thought to have infected over 9,000 people and killed at least eight children. While the source of the outbreak was originally believed to have been ground beef imported from the U.S., the investigation ultimately concluded that radish sprouts in school lunches were probably responsible (Hara-kudo et al., 1997).

The most recent major U.S. outbreak of *E. coli* O157:H7 occurred during the summer of 1998, near Atlanta, GA. The epidemiological study found the reservoir for the organism to be a chlorinated public wading pool, which had been contaminated by human feces. The particular strain isolated from the pool was genetically matched to a strain of *E. coli* O157:H7 found in ground beef served a few months before in at least one Madison County school. The meat company that supplied the beef to the school discontinued business soon after its connection to the outbreak. At least nine children were hospitalized, with one death, in this outbreak (Powell, 1998b).

Bacterial outbreaks associated with *E. coli* O157:H7 are complicated by the fact that the infectious dose is extremely low, especially for susceptible individuals such as the very young, the elderly, and those with weak immune systems. Doyle (1993) reported an infectious dose of <15 *E. coli* O157:H7/g from frozen beef patties. In a dry fermented salami outbreak, infectious dose was found to be as low as 2 and as high as 45 *E. coli* O157:H7 cells (Tilden et

al, 1996). This low infectious dose underscores the importance of proper decontamination procedures, since very low levels of injured cells could possibly recuperate and cause illness.

Pathogenicity of E. coli O157:H7

Although the mechanism of pathogenicity associated with HC, HUS, and TTP caused by *E. coli* O157:H7 is not yet fully understood, there are important factors thought to influence the virulence of the EHEC serotypes. One is the intestinal attachment and effacing adherence mechanism, encoded by the *eaeA* gene, which is homologous to the *eaeA* gene of EPEC (Knutton et al., 1989). The product of this gene, a 97-kDa protein known as intimin, is necessary for the production of attaching and effacing (AE) lesions, characterized by localized adherence to and destruction of microvilli, rearrangement of cytoskeletal elements, and “pedestal” formation, in which filamentous actin accumulates at the site of intimate bacterial attachment to the intestinal wall (Donnenberg and Kaper, 1992). Formation of these AE lesions allows a subset of *E. coli* O157:H7 organisms to further invade the epithelial cells, resulting in bowel inflammation, perforation, and necrosis, manifested as bloody diarrhea (Griffin, 1995).

The other key virulence factor is the production of one or more verotoxins (VT) that are cytotoxic to tissue cells (Bettelheim, 1996). Konowalchuck et al. (1977) reported that culture supernatants from some strains of *E. coli* were toxic to Vero cells obtained from an African green monkey kidney. O’Brien et al. (1982) reported that one *E. coli* O157:H7 VT was immunologically indistinguishable from Shiga toxin. Biological activities, such as mouse lethality and enterotoxicity of the two toxins, also were virtually identical. Thus, the term Shiga-like toxin (SLT) was coined to describe the toxin. O’Brien et al. (1983) and Johnson et al.

(1983) found that *E. coli* O157:H7 produced high levels of this verotoxin (VT).

Another *E. coli* O157:H7 VT was identified by Scotland et al. (1985) and Strockbine et al. (1986). This toxin was not neutralized by antisera against Shiga toxin. To differentiate the two, the first toxin, indistinguishable from Shiga toxin, was deemed SLT-1 or VT-1, while the other toxin was referred to as SLT-2 or VT-2. A third cytotoxin was described by Padhye et al. (1986), which also was not neutralized by Shiga antisera. Other variants of the *E. coli* VT have been identified, as well (Marques et al., 1987; Oku et al., 1989). A study by Ogasawara et al. (1987) determined that the pathogenic mechanism of both VT-1 and Shiga toxin was the blockage of protein synthesis by inhibiting elongation factor-1 (EF-1)-dependent aminoacyl binding of t-RNA to 60S ribosomal subunits. Additional studies determined that VT-2 acted in the same manner (Ogasawara et al. 1987). Data collected from human cases of *E. coli* O157:H7 infection suggest that VT-2 is a more important virulence factor than VT-1 for progression of the infection into HUS (Thomas et al., 1993; Scotland et al., 1987).

Reservoirs for E. coli O157:H7

E. coli O157:H7 enters the food chain at the farm level by inhabiting the gastrointestinal tract of dairy cattle and other animals, particularly ruminants. Although beef is considered the main reservoir, a retail meat study conducted by Doyle and Schoeni (1987) isolated *E. coli* O157:H7 from 3.7% of beef, 1.5% pork, 1.5% poultry, and 2.0% of lamb samples tested, indicating that the organism may be associated with foods of any animal origin. Beery et al. (1985) supported this finding by demonstrating that *E. coli* O157:H7 can colonize in the cecae of chickens and be shed in the feces for several months following colonization.

Numerous steps in the slaughter process may contribute to carcass contamination. The

dehiding process may transport fecal matter and debris from the hides and hooves of the animal to the carcass. Evisceration also may contribute contamination when rumen or intestinal contents are dispelled. While research is underway to develop methods for eliminating *E. coli* O157:H7 from the intestinal tract prior to slaughter, no validated technology is available for commercial use. Therefore, many slaughter facilities have implemented intervention strategies to eliminate contamination prior to fabrication, such as carcass trimming, organic acids, hot water wash cabinets, and steam pasteurization. While these methods have been proven effective at reducing bacterial populations, especially when used cooperatively (Phebus et al., 1997), the potential exists for pathogens to survive the slaughter process. Should contamination be present on the carcass during fabrication, organisms may be spread further via improperly sanitized equipment and personnel. Following fabrication, meat cuts are generally stored at refrigerated or frozen temperatures for some time prior to distribution for further processing or consumption.

Detection/Destruction of E. coli O157:H7

E. coli O157:H7 grows best within a temperature range of 30° to 42°C, the optimal temperature being 37°C (Doyle and Schoeni, 1984). The organism does not, however, grow well at 44° to 45.5°C (Doyle and Schoeni, 1984; Raghubeer and Matches, 1990), which is noteworthy because most standard detection procedures for fecal coliforms are conducted in this higher range, and would thus fail to detect the pathogen.

E. coli O157:H7 is capable of withstanding very low storage temperatures and, in fact, has been shown to increase in heat resistance following frozen storage. Doyle and Schoeni (1984) reported that *E. coli* O157:H7 in ground beef was held at -20°C for nine months with very little reduction in number. A study by Jackson et al. (1995) indicated that thermal

resistance of *E. coli* O157:H7 increased as time of storage at -18°C increased. In this study, a D_{55°C} value of 9.2 minutes was reported for *in vitro* cultures stored at -18°C for one day, while those stored for 8 and 15 days produced D₅₅ values of 14.1 and 12.0 minutes, respectively. They also reported that by holding frozen cultures at 23° or 30°C for several hours prior to thermal treatment, heat resistance was greatly decreased. This study showed the same resistance trends for *E. coli* O157:H7 inoculated into ground beef patties, which were grilled to simulate commercial application. Similar results regarding the survival of microorganisms, including generic *E. coli*, were found by Kotula et al. (1977).

Thermal death time studies by Line et al. (1991), using lean and fatty ground beef inoculated with *E. coli* O157:H7, show that higher fat content produces higher D values. Lean ground beef (2.0% fat) was found to have a D₅₅ value of 4.1 minutes, while fatty ground beef (30.5% fat) had a D₅₅ value of 5.3 minutes. At 145°C, the respective D values were 0.3 and 0.5 minutes. This conclusion is supported by findings of Ahmed et al. (1995), wherein D values for *E. coli* O157:H7 were lower in lean tissue than in fatty tissue of pork sausage, chicken, and turkey, as well as ground beef. The study by Line et al. determined z values for lean and fatty ground beef to be 8.3 and 8.4, respectively. When compared to a similar study using *Salmonella* in ground beef (Goodfellow and Brown, 1978), these results suggest that *E. coli* O157:H7 is less resistant to heat than *Salmonella*.

With regard to commercial application, several studies have been conducted to evaluate thermal destruction of *E. coli* O157:H7 by grilling or skillet cooking. Juneja et al. (1997) suggested that internal temperatures of ground beef patties differ greatly depending on the temperature of the heating surface. This study found that by cooking to an internal

temperature of 68.3°C, a four log cycle reduction can be obtained, which supports findings by Jackson et al. (1995). Kotula et al. (1977) observed a five log reduction in viable coliforms when patties were cooked at a griddle surface temperature of 177°C for 1.5 minutes on each side. Buck et al. (1975) reported that thawed hamburger patties should be cooked at 163°C for six minutes to eliminate hazards associated with pathogenic microorganisms. Juneja et al. (1997) and Buck et al. (1975) reported that *E. coli* O157:H7 were destroyed at essentially the same rate as normal spoilage flora in ground beef, which may provide an effective means for validating commercial cooking practices in retail food operations, without introducing pathogens into the environment.

E. coli O157:H7 does, however, appear to become more heat resistant following heat shock. Murano and Pierson (1992) reported higher D values for heat-shocked cells than non-heat-shocked cells. These researchers also reported that *E. coli* O157:H7 cultures grown anaerobically had higher D values than those cultures grown aerobically, for both heat-shocked and non-heat-shocked samples. In a later study (1993), Murano and Pierson found that heat-shock may enable cells to become more tolerant to toxic substances, in addition to heat. Results of this study suggested that heat-shock response, paired with anaerobic growing conditions, may actually enhance the cell's ability to recover during storage.

E. coli O157:H7 can survive a wide pH range. Miller and Kaspar (1994) reported survival of the organism at pH levels as low as 2.0 and as high as 11.0. Zhao et al. (1993) showed that the organism could survive and proliferate in apple cider (pH 3.6 to 4.0). Brackett et al. (1994) indicated that 55°C sprays of organic acids (acetic, citric, and lactic) did not inhibit survival of *E. coli* O157:H7 on beef tissue. Cutter and Siragusa (1994) suggested that organic acid sprays did not completely inactivate the pathogen on beef carcasses. Conner and

Kotrola (1995) suggested that acetic acid was inhibitory at pH levels of 4.5 to 5.0, but only at relatively high concentrations of 0.007 M (undissociated).

Many of the previously mentioned studies rely on the ability to detect heat or acid-injured cells. It should be noted that MacConkey Sorbitol Agar (MSA), a recommended method for recovering sublethally injured *E. coli* O157:H7 cells (March and Ratnam, 1986; Okrend et al., 1990a, 1990b) has been shown to be inadequate by several researchers. Ahmed

and Conner (1995) reported that recovery of heat-injured cells was consistently higher with Phenol Red Sorbitol Agar (PRSA) than on MSA. Abdoul-Raouf et al. (1993) observed that MSA supplemented with 4-methylumbelliferyl- β -D-glucuronide (MSMA) recovered significantly smaller populations of heat-stressed *E. coli* O157:H7 than Tryptic Soy Agar (TSA). A study conducted by Conner (1992) indicated that MSA was unable to resuscitate NaCl or cold-stressed cultures.

Regulatory Issues Related to Blade Tenderization

The low infectious dose of *E. coli* O157:H7 associated with outbreaks of foodborne disease, along with the serious health consequences related to *E. coli* O157:H7 infection, persuaded the United States Department of Agriculture Food Safety and Inspection Service (USDA-FSIS) to implement a policy deeming *E. coli* O157:H7 an adulterant in raw ground beef (Nowlin, 1994). USDA-FSIS also revised their ruling on heat processing temperature and time requirements for uncured meat patties so as to require a 5-D process (USDA/FSIS, 1993b). This reduction level was based on the heat resistance of the pathogens of concern, specifically *E. coli* O157:H7, *Salmonella*, and *Listeria monocytogenes*, the expected numbers of these organisms in meat products, and an appropriate margin of safety. This

requirement was supported by the FDA and the NACMCF, and was recommended to the FSIS by its staff of microbiologists (USDA/FSIS, 1993b). The FDA followed suit in 1993 by providing recommendations in the Food Code that ground beef patties be cooked to an internal temperature of 68.3°C for 15 seconds to ensure the destruction of pathogens which have been introduced to the interior of the product through the grinding process (Tisler, 1997).

While these original regulatory acts were limited to raw ground beef products, the agencies involved concluded that the potential for contamination by *E. coli* O157:H7 was not limited only to ground beef. However, under the Federal Meat Inspection Act (FMIA) (21 U.S.C. 601 *et seq.*), FSIS believes that the status of contaminated beef products “must depend on whether there is adequate assurance that subsequent handling of the product will result in food that is not contaminated when consumed.” (USDA/FSIS, 1999). In November of 1997, the National Advisory Committee on Microbiological Criteria for Foods (NACMCF), Meat and Poultry Subcommittee, stated that “*Due to the low probability of pathogenic organisms being present in or migrating from the external surface to the interior of beef muscle, cuts of intact muscle (steaks) should be safe if the external surfaces are exposed to temperatures sufficient to effect a cooked color change*” (Acuff, 1999). The Committee defined “intact beef steak” as “[a] cut of whole muscle(s) that has not been injected, mechanically tenderized, or reconstructed”, and limited the application of its statement to products meeting this definition (USDA/FSIS, 1999). Under FDA’s current Food Code (1997, Subpart 1-201.10(B)(41)), “injected” means “manipulating a meat so that infectious or toxigenic microorganisms may be introduced from its surface to its interior through tenderizing with deep penetration or injecting the meat such as with juices, which may be referred to as “injecting”, “pinning”, or “stitch pumping” (FDA, 1997).

Based on these definitions, FSIS stated in the Federal Register on January 19, 1999 that the agency believes there should be a distinction between intact cuts of muscle and non-intact products, including those that have been tenderized, injected, frenched, pounded, or restructured via chopping, grinding, flaking or mincing. It also stated that intact muscle destined for further processing into non-intact products prior to distribution, such as beef trimmings, would be classified with other non-intact items, and treated accordingly. In light of the potential public health risks associated with these products, FSIS expanded its definition of *E. coli* O157:H7 as an adulterant to all non-intact products, stating that “*with the exception of ...intact cuts of muscle that are to be distributed for consumption as intact cuts, an E. coli O157:H7-contaminated beef product must not be distributed until it has been processed into a ready-to-eat product—i.e., a food product that may be consumed safely without any further cooking or other preparation. Otherwise, such products... must be deemed adulterated.*”

Because of the widespread production of these non-intact products, the expansion of the *E. coli* O157:H7 adulteration policy by FSIS is of great concern to the beef industry. The NACMCF, Meat and Poultry Subcommittee, concluded in its 1997 report that there is a lack of scientific data to address the hazards associated with those processes that may cause translocation of pathogens. Therefore, the recently formed Beef Industry Food Safety Council (Holmes, 1999), composed of producers, packers, processors, distributors, and food retailers, identified this research as a priority for the beef industry. The following studies were designed to provide information which may be useful in identifying the extent of regulatory control required to eliminate potential food safety risks associated with the blade tenderization process.

MATERIALS AND METHODS

Study 1A: Characterization of *Escherichia coli* O157:H7 Translocation Due to Blade Tenderization

Bacterial Cultures

One strain of rifampicin-resistant *Escherichia coli* O157:H7 (USDA-FSIS 011-82) was grown at 35°C for 24 hours in Tryptic Soy Broth (TSB, Difco, Detroit, MI). The culture was then streaked onto Tryptic Soy Agar with 0.1% rifampicin (TSA-rif) and incubated for another 24 hours to confirm antibiotic resistance. An isolated colony was transferred into six centrifuge bottles, each containing 100 mL TSB, and incubated for 12 hours. The cultures were centrifuged at 15,300 X g for 10 minutes at 4°C (Beckman J2-21M/E centrifuge, JA-14 rotor, Palo Alto, CA). Each of the resulting pellets were resuspended in 50 mL of 0.1% peptone water (PW) and combined for a total volume of 300 mL. This initial volume was deemed the high-level inoculum. To prepare a low level inoculum, one mL of the high level was used to make a 1:10 dilution. This dilution was then used to make three 1:100 dilutions, which were combined to create 300 mL of the low-level inoculum. Serial dilutions of each were spiral plated onto TSA-rif and incubated for 24 hours to determine the final inoculum concentrations, which were 10⁹ and 10⁶ CFU/mL, respectively. The inocula were placed into separate HDPE plastic spray bottles (Sprayco®, Detroit, MI) for use in the mist inoculation procedure.

Inoculation of Subprimals

Eight vacuum-packaged top sirloin subprimals (NAMP #184; cap removed) were

obtained from a commercial slaughter facility (Excel Corp., Wichita, KS) and stored at 4°C. Each of four subprimals was inoculated with the low-level inoculum in a specially designed plexi-glass inoculation chamber (Nutsch, 1998) by misting ca. 7 mL (20 “pumps”) of the culture suspension over the top (lean) surface (Fig. 4). Paper towels were placed around the subprimal inside the chamber to prevent the liquid inoculum from contaminating the bottom surface. The entire process was repeated, using the high-level inoculum on the other 4 subprimals. Excise surface samples indicated that this procedure resulted in levels of 10^3 cfu/cm² and 10^6 cfu/cm² on the low and high-level subprimals, respectively. The subprimals were stored at 4°C for 30 minutes to allow for bacterial attachment. Each subprimal was considered a replication.

Blade Tenderization of Subprimals

After bacterial attachment, each of the 4 low-level inoculated subprimals was passed once through a blade tenderizer (Ross TC700M, Midland, VA; Figs. 5a, 5b), followed by the 4 high-level subprimals. The subprimals were placed on the conveyor belt with the inoculated surface facing up, allowing the blades to penetrate that surface upon initial contact. The tenderizer produced 5 blade penetrations/cm² (blade width = 3 mm). The unit was disassembled, cleaned and sanitized between passage of each subprimal to prevent cross-contamination. The subprimals were then stored at 0°C for three hours to facilitate excision core sampling for microbial analyses.

Sampling Procedure

Each subprimal was aseptically transferred onto a fresh sheet of butcher paper and inverted so that the inoculated surface faced down. A sterile coring device (stainless steel;

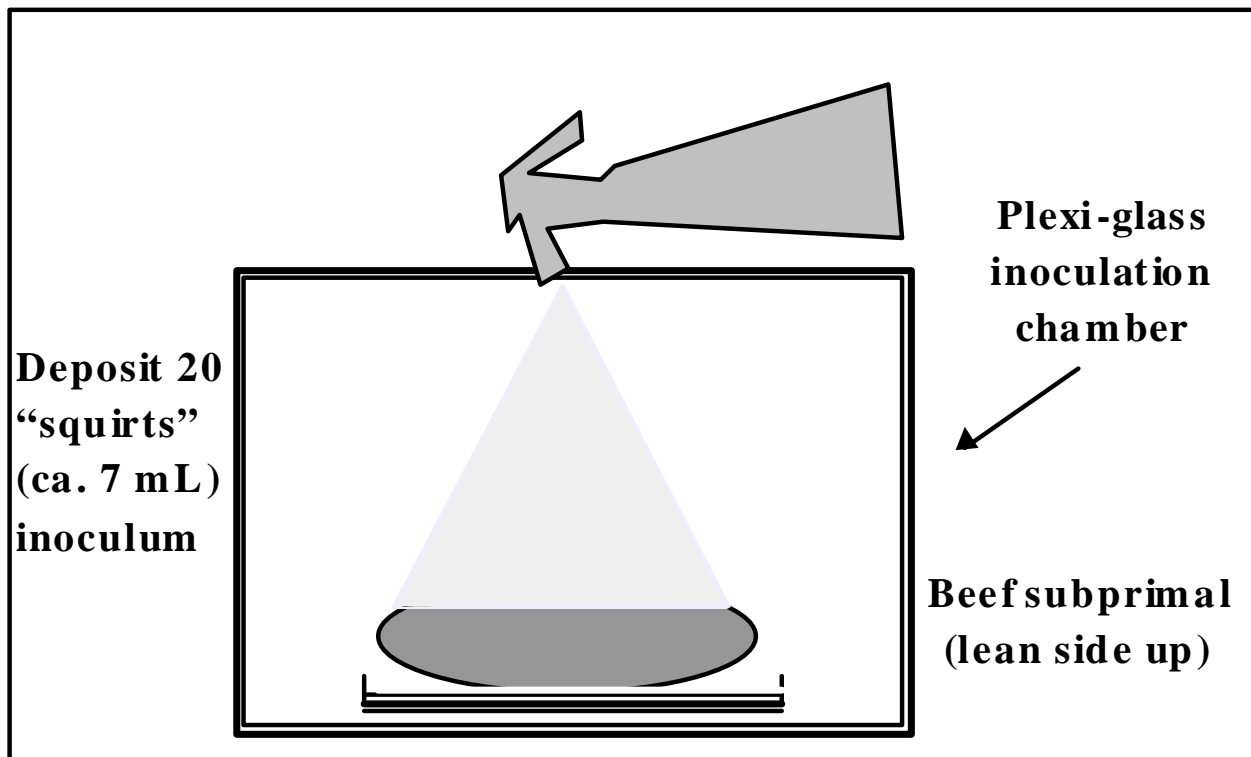


Figure 4. Description of the mist inoculation procedure

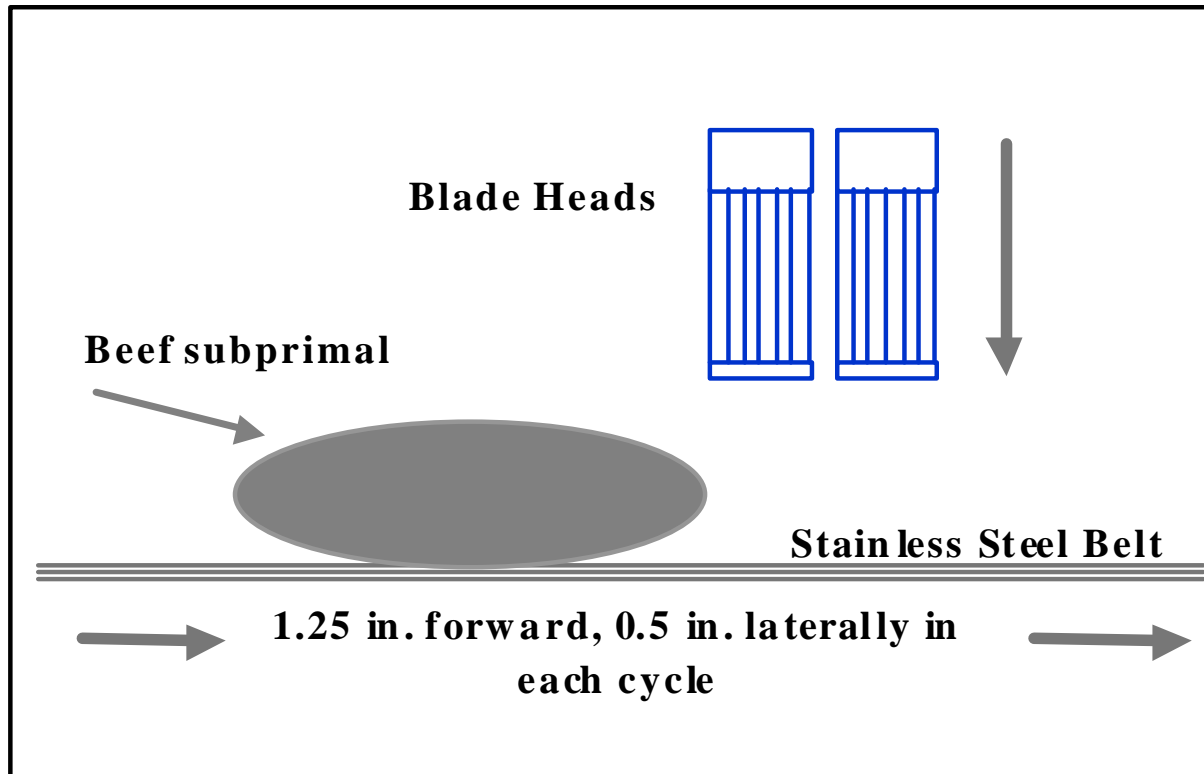


Figure 5a. Diagram of the blade tenderization process

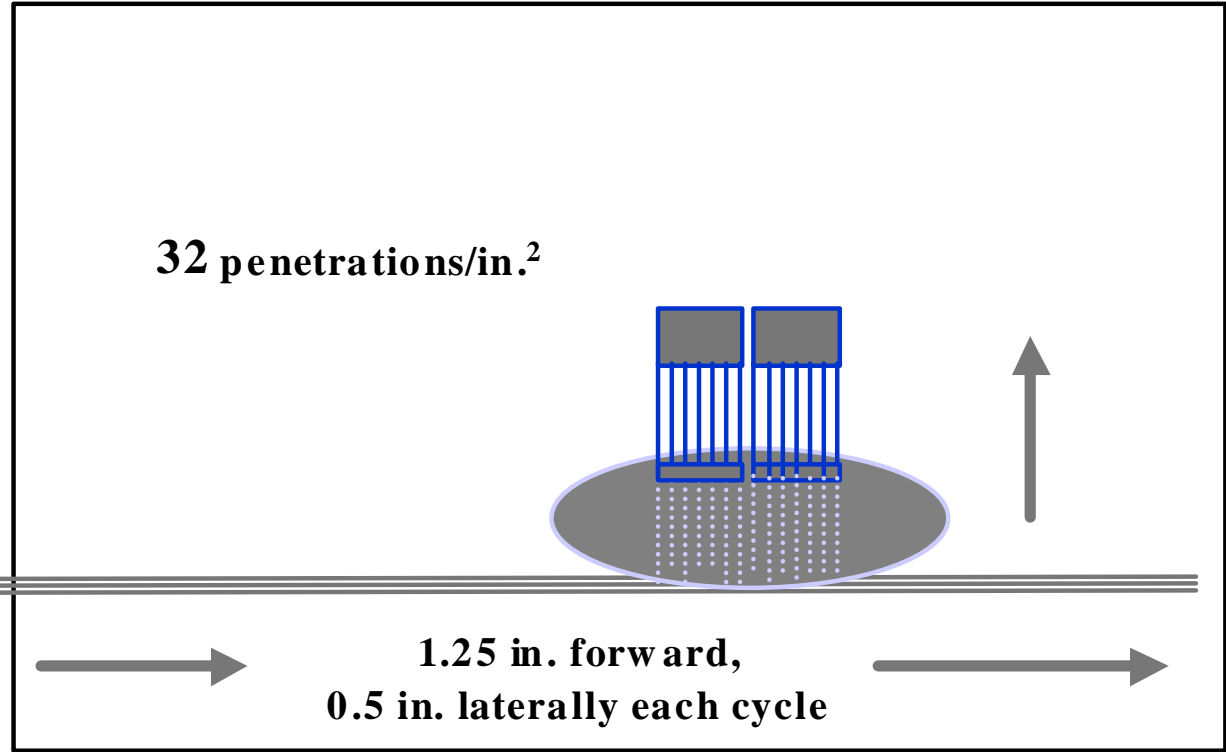


Figure 5b. Description of the blade tenderization process, cont

10 cm long X 5 cm diam.) was inserted through the non-inoculated surface to cut a core through the entire depth of the blade tenderized muscle (Fig. 6a). This sampling method prevented translocation of the inoculum to a greater depth in the muscle via the coring device. Three cores were cut along the midline of the subprimal. Each core was pushed upward through the bottom of the coring device, so as to prevent it from passing across the contaminated leading edge during its removal from the subprimal (Fig. 6b). The meat cores were then placed in a cutting guide and aseptically sliced into cross-sectional strips as shown in Fig. 7, (2 cm, 2 cm, 1 cm, and 1 cm from the non-inoculated surface). Approximately 2 mm of the bottom non-inoculated surface were removed from the samples to eliminate any contamination that may have occurred from seepage of the inoculum during the mist inoculation procedure. If a core measured less than 8 cm in length, the last cross-sectional strip may have been less than two cm thick. To account for differences in thickness, bacterial enumeration from each strip was reported on a per gram basis. Three cores were taken from each subprimal, representing triplicate samples. Four replications of the each inoculum level were performed.

Bacterial Enumeration

Cross-sectional strips from the cores were weighed and aseptically transferred to sterilized blender jars (Oster®, Schaumburg, IL). Peptone water was added to produce 1:4 (W:V) dilutions and the samples were blended for 30 seconds. The resulting slurries were then poured into sterile filter stomacher bags, serially diluted and plated on TSA-rif. The plates were incubated at 35°C for 24 hours, enumerated, and colonies confirmed serologically with a latex agglutination assay (RIM *E. coli* O157:H7, Remel, Lenexa, KS).

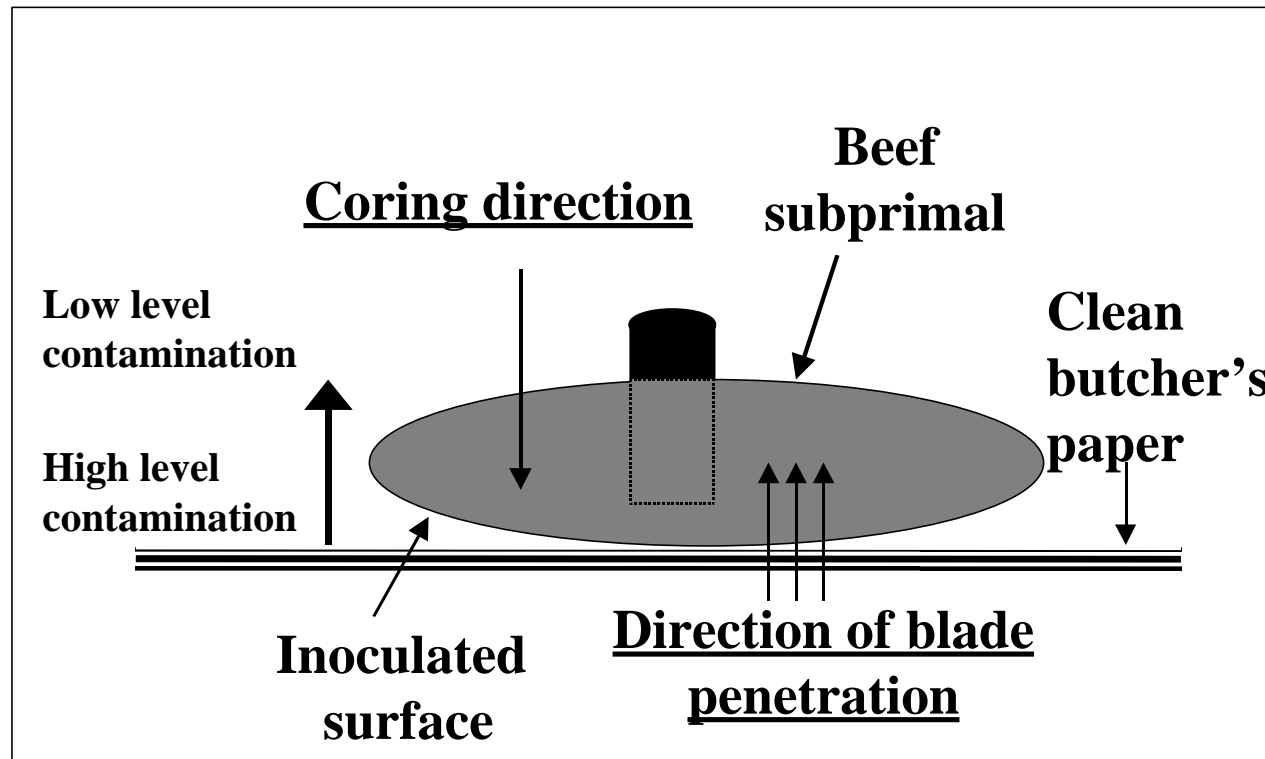


Figure 6a. *Sample Collection Method Using a Sterile Coring Device*

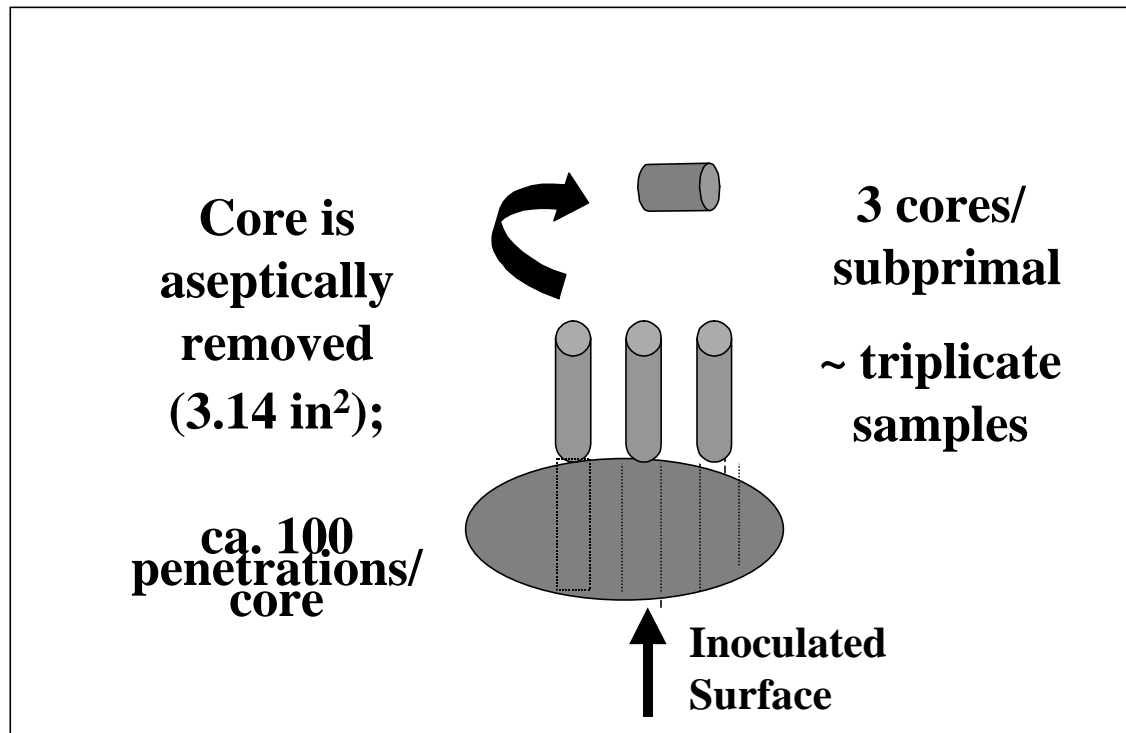


Figure 6b. *Sample Collection method using a sterile coring device, cont*

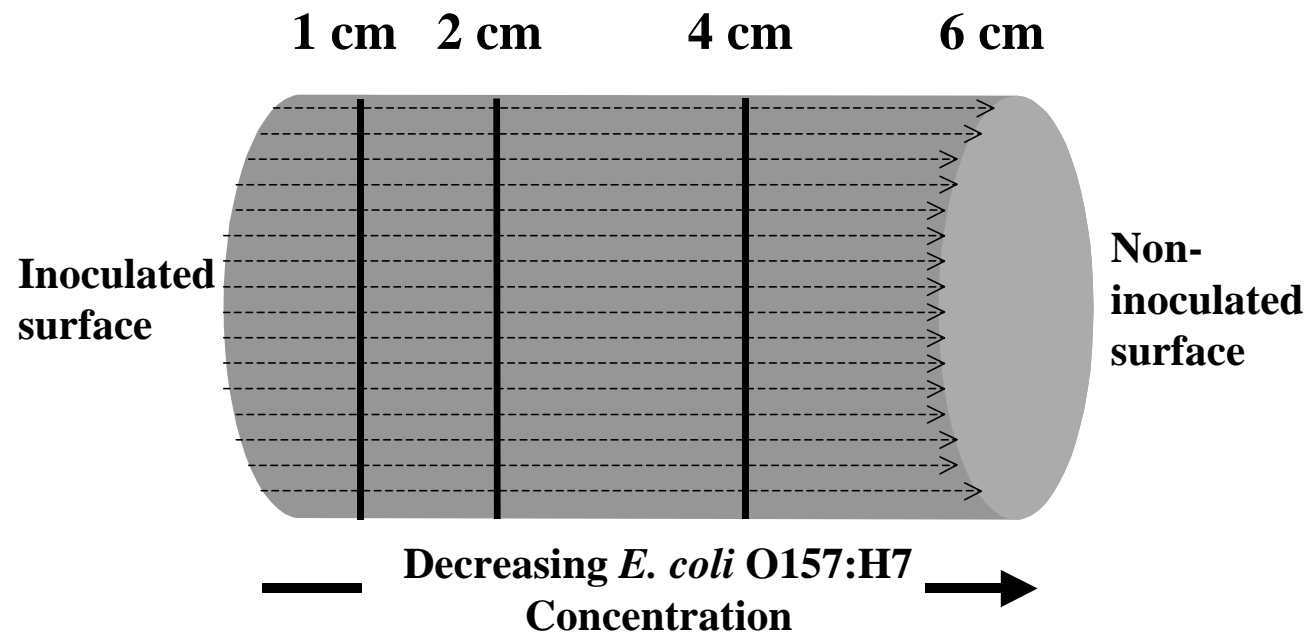


Figure 7. Sampling method used in Study 1A to profile *Escherichia coli* O157:H7 contamination in blade tenderized subprimals

Statistical Analysis

Bacterial enumeration data from the four replications were analyzed by analysis of variance using the General Linear Model procedure of Statistical Analysis System (SAS Institute, Inc., 1990). LSD was used to separate means of the log CFU/g of the cross-sectional strips.

Study 1B: Visualization of Generic *Escherichia coli* Translocation in Beef Steaks Via the Blade Tenderization Process

Sample Preparation

One strain of autofluorescent generic *Escherichia coli* (University of Guelph, Ontario, Canada) was grown in TSB at 35EC for 24 hours and confirmed for fluorescence under UV light. This culture was prepared as was the low-level inoculum in Study 1A. One top sirloin subprimal was mist-inoculated to achieve a surface inoculum level of ca. 10^3 cfu/g and passed through the tenderizer as previously described. After tenderization, the subprimal was held at 0EC for three hours. Twelve cores measuring 7 mm in diameter were removed from the subprimal as described in Study 1A, and immediately stored at 0EC until submission to the Kansas State University Department of Biology for evaluation via confocal microscopy.

Confocal Microscopy

Frozen meat cores were cut with a single edged 0.009-inch razor blade (American Safety Razor Company, Veron, VA) perpendicular to the blade tenderization tract into 3 cm lengths. Each frozen 3 cm core, representing various depths into the meat block from the inoculated surface, was serial sectioned into 1 mm sections or cut parallel to the blade tract using a razor blade. Sections were immediately placed in a CoverWell imaging chamber gasket (32mm x 19mm x 2.5 mm;

Molecular Probes, Eugene, OR) and viewed by confocal microscopy.

Sections were viewed on a Zeiss laser scanning confocal microscope model LSM 410 equipped with an Axiovert 100 invert microscope, an argon-krypton 488/568/647 laser, a Plan-Neofluar x40 numerical aperture 1.3 oil-immersion objective, an Ft 488/568 dichroic beam splitter, a BP 485-520 line selection filter, a BP 515-565 emission filter, and the software package LSM version 3.993 (Zeiss, Thornwood, NY). Individual optical sections and z-series were taken with the pinhole set at 11 (full width at half maximum = 0.745 μm). The z-step was equal to the x and y pixel dimension for rendered volumes. Digital images were captured and store in tagged-image file format (TIFF).

Study 2: Efficiency of Cooking by Gas Grill Method for *E. coli* O157:H7 Elimination from Blade Tenderized Steaks

Bacterial Cultures

Five strains of *Escherichia coli* O157:H7 (USDA-FSIS 011-82, ATCC 43888, ATCC 43889, ATCC 43890, and USDA-FSIS 45756) were grown at 35°C for 24 hours in Tryptic Soy Broth (TSB). The cultures were transferred to twelve bottles containing 100 mL of TSB and incubated for 18 hours at 35°C. The cultures were centrifuged at 15,300 X g for 10 minutes at 4°C. Each of the resulting cell pellets was resuspended in 50 mL 0.1 % PW, to produce a mixed strain inoculum (600 mL). Serial dilutions of the mixed inoculum were spiral plated onto MSA and incubated at 35°C for 24 hours to establish precise inoculum concentration. The inoculum was placed in a HDPE plastic spray bottle (Sprayco®, Detroit, MI) for use in the mist inoculation procedure.

Inoculation of Subprimals

Six vacuum-packaged top butt subprimals, with caps removed, were received from Excel, Corp (Wichita, KS) and stored at 4°C. Each subprimal was mist inoculated as described in Study One, using the 5-strain cocktail of *E. coli* O157:H7. Surface excised samples showed an inoculum level of 10⁶ cfu/cm². The subprimals were then stored aerobically at 4°C for 1 hour to allow bacterial attachment.

Blade Tenderization of Subprimals

After the attachment period, three randomly selected subprimals were passed once through a blade tenderizer (Ross TC700M, Midland, VA). The remaining subprimals were left non-tenderized (controls). The blade tenderization unit was completely disassembled, cleaned and sanitized with alcohol between passage of individual subprimals. Each subprimal was placed in a sanitized cutting guide and randomly cut into steaks of three different thicknesses: 1.3, 1.9 and 3.2 cm. The cutting guide was sanitized between each subprimal. The steaks were trimmed from the uninoculated edge to corresponding weights of 142, 226, and 340 g (5, 8, and 12 oz.), respectively, to simulate standard industry specifications. Knife blades were flame sterilized between each steak.

Cooking Procedure

A type T thermocouple (Omega Engineering, Stamford, CT) was inserted into the geometric center of each steak by passing the wire through a sterile syringe needle, which was threaded through an uninoculated edge of each steak in a direction perpendicular to that of the blade tracks, in order to prevent false carriage of *E. coli* O157:H7 into the center of the steak. This location was estimated visually and fat seams and loose-structured lean tissue were

avoided. The steaks were then placed in groups of four onto a propane grill (Model XX4, Baker's Pride, New Rochelle, N.Y.), which was divided into four quadrants. The grill's surface temperature in each quadrant was verified by allowing beakers of cooking oil to equilibrate and taking the temperature of the oil, using thermocouples. Because quadrant temperatures varied an average of 20-25°C (average range: 188-210°C), placement of each treatment was randomized across the quadrants.

Internal steak temperature was monitored using a LabView Virtual Bench Data Logging System (National Instruments, Austin, TX) which was set to record at 5 second intervals. The temperature at which to turn the steaks ("flip temperature") was determined as the midpoint between the initial steak temperature and the target endpoint temperature. To simulate typical food service preparation, target endpoint temperatures were identified as 54.4°C (130°F; rare), 60.0°C (140°F; medium rare), 65.6°C (150°F; medium), 71.1°C (160°F; medium well), and 76.7°C (170°F; well done). An uncooked steak from each treatment was also prepared to serve as a control. When steaks reached their target endpoint temperature, they were immediately removed from the grill and held on a tray covered with butcher's paper at room temperature for 5 minutes, during which temperature monitoring continued, to simulate restaurant hold times and to further characterize post-cooking temperature rise differences due to steak thicknesses and tenderization.

Sample Collection

After the 5 minute hold time, samples were taken by aseptically removing cross-sectional strips from the centers of the steaks, which represented both the inoculated surface and the interior through which the blades passed (Fig. 8). To obtain a more uniform sample

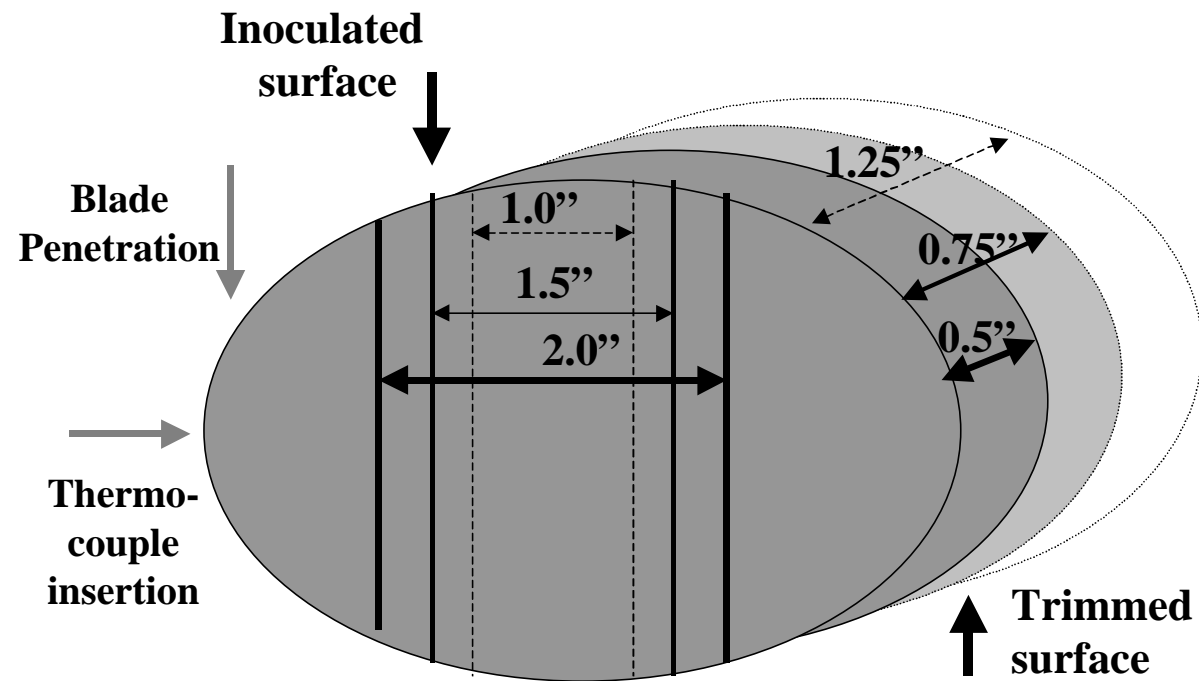


Figure 8. *Diagram of steak sampling procedure used for Studies 2-4*

size, the width of the cross-sectional strip varied, depending on the thickness of the steak: a 5 cm strip was taken from the thin steaks, a 4 cm strip from medium steaks, and a 2.5 cm strip from the thickest steaks. Sample weights were recorded and the samples were plunged immediately into cold PW to halt residual thermal lethality. PW was then added to produce a 1:5 dilution based on sample weight. The samples were blended for 30 sec. in a sterile blender (Waring, New York, N.Y.) and microbiologically analyzed.

Enumeration of E. coli O157:H7 Populations

Samples were serially diluted in PW and plated on MSA and PRSA by spiral and spread plating methods. PRSA was chosen as a plating method to better estimate the degree of microbial injury from sublethal temperature exposure (McCauley, 1997; Ahmed and Conner, 1995). All plates were incubated at 37°C, with MSA removed after 24 hours and PRSA after 48 hours. Presumptive *E. coli* O157:H7 colonies were confirmed biochemically (API 20E, bioMerieux Vitek, Inc., Hazelwood, MO) and serologically using a latex agglutination assay (RIM *E. coli* O157:H7; Remel, Lenexa, KS). Samples testing negative by direct plating methods were evaluated qualitatively for *E. coli* O157:H7 by enrichment in modified EC broth (mEC) (Johnson et al., 1995). These enriched samples were analyzed culturally on MSA and Tryptic Soy Agar, and by using the immunologically based Mini-VIDAS System (bioMerieux Vitek, Inc., Hazelwood, MO). The difference in *E. coli* O157:H7 counts between the control, non-cooked steak and the cooked steak of the same treatment and weight were calculated and reported as a log cfu/g reduction.

Statistical Analysis

The experimental design of the study was a randomized complete block design. Thirty

treatments were analyzed per replication, based on a 2 (tenderized vs. non-tenderized) X 3 (steak thicknesses) X 5 (target temperatures) treatment structure, with the blocking factor defined as replication. Bacterial reductions for each treatment were calculated, based on the initial inoculum level of the raw control steaks (ca. 10^6 cfu/g). Six replications were completed, resulting in a sample size (n) of 180. Temperature and *E. coli* O157:H7 reduction data were compiled from the 180 samples and submitted to Kansas State University's Department of Statistics for evaluation. Analysis of variance was conducted with the General Linear Model procedure of Statistical Analysis System (SAS Institute, Inc., 1990). Fisher's LSD Lines was used to separate mean log reductions of *E. coli* O157:H7 for each of the cooking temperatures. All possible interactions of tenderization, steak thickness, and target cooking temperatures were evaluated. Statistical differences were based on an alpha level of 0.05. For discussion purposes, data for variables which were not statistically significant were pooled. Graphically, data were represented as main effects. Significant differences were indicated by different letters.

Study Three: Efficiency of Electric Skillet Cooking for Elimination of *E. coli* O157:H7 from Blade Tenderized Steaks

For Study Three, the researchers followed procedures outlined in the grill study with regard to culture preparation, inoculation procedure, sampling and detection. However, in order to reduce variability in the cooking procedure, an electric skillet (Model 876, Toastmaster, Inc., Booneville, MO) with a griddle-type surface was used in place of the propane grill. Skillet temperature was set at 149°C (300°F) and was monitored throughout the cooking study using cooking oil temperature as a reference. Oil temperature was

maintained at ca. 141°C (285°F) throughout the cooking process. As in the grill study, the cooking surface of the skillet was divided into four quadrants and cooking order was randomized across them to control the variation in temperature, (ca. 8°C) across the cooking surface. To control cross-contamination during the cooking process, steaks were turned onto a clean section of the skillet within the same quadrant. Post-cooking temperature rise was also more precisely controlled in this study, by placing the steaks into sterile plastic bags and submerging into an ice bath immediately after removal from the grill. Internal temperatures were monitored until they fell below 38°C (100°F). Sampling, enumeration and statistical analysis were performed as described previously, with three replications completed (n = 90).

Study Four: Efficiency of Oven Broiling for Elimination of *E. coli* O157:H7 from Blade Tenderized Steaks

A third cooking method, oven broiling, was evaluated, using the experimental design followed for the grill and skillet evaluations. In this study, steaks were placed on an oven broiler pan approximately 10 cm from the broiling element of a standard kitchen oven (Model RF315PXDN, Whirlpool Corp., Benton Harbor, MI). The oven was set for broil, at 260°C (500°F). In place of oil, the ambient air temperature surrounding the steaks during the cooking process was measured to be ca. 149°C (300°F). To avoid cross-contamination of the cooked top surface of the steaks after turning, two steaks were cooked in adjacent quadrants and turned over onto the opposite clean quadrants of the broiler pan. A sixth internal temperature of 49°C (120°F) was added to this experiment to simulate an undercooked finished product. When target internal temperatures were reached, the steaks were removed from the oven and immediately placed in an ice bath for rapid cool-down. The sampling and

enumeration procedures continued as described for the previous section. Three replications of the 2 (tenderized vs non-tenderized) X 3 (steak thicknesses) X 6 (endpoint temperatures) treatment structure were completed (n = 108).

RESULTS AND DISCUSSION

Study 1A: Characterization of *Escherichia coli* O157:H7 Translocation Due to Blade Tenderization

E. coli O157:H7 contamination on the surface of the subprimals was translocated throughout the interior of the muscle. In the high-level subprimals, 6 logs cfu/g were recovered from the top 1 cm of the core sample, which included the inoculated surface, while approximately 3 logs of *E. coli* O157:H7/g were recovered at the bottom depth of ca. 6 cm. The geometric center of the core, which in regard to steak cooking, would be the slowest to reach a target temperature, harbored 4 logs of *E. coli* O157:H7. The low-level subprimals produced a similar trend, showing the relocation of approximately 1.8 logs of *E. coli* O157:H7 to the steak's geometric center (Fig. 9). Overall, the process of blade tenderization carried 3-4% of the surface organisms to the center of the core, regardless of surface inoculation level. Because the infectious dose of *E. coli* O157:H7 is as low as a few cells in susceptible individuals (Doyle, 1993; Tilden et al., 1996), this level of contamination should be a concern, and suggests that proper handling and cooking are necessary to control the risks associated with the blade tenderization process. These data support the findings of Raccach and Henderson (1978), who demonstrated translocation, and emphasized the importance of a proper sanitation program associated with the blade tenderizer, dubbing it a potential "inoculating machine". Petersohn et al. (1979) also reported higher aerobic, anaerobic, and psychrotrophic counts in interior muscle samples of tenderized (T) vs non-tenderized (NT) steaks.

Study 1B: Visualization of *Escherichia coli* O157:H7 Penetration in Beef Steaks Via the Blade Tenderization Process

Confocal microscopy confirmed the translocation of organisms throughout the entire depth of blade tenderized beef subprimals. Fig. 10 represents a 46 cross-sectional view of one blade tract, wherein 10 to 12 autofluorescent generic *E. coli*, located at a depth of ca. 0.5 cm beneath the inoculated surface, were visualized. Fig. 11 is a similar cross-section, observed at a depth of ca. 6 cm below the inoculated surface. Although fewer autofluorescent cells appeared to be clustered in this location, their presence further indicated that the blade tenderization process does relocate surface contamination throughout the muscle tissue. It should be noted that autofluorescent *E. coli* were not observed within every cross-sectional sample viewed under confocal microscopy, indicating some inconsistency in the deposition of cells during the tenderization process.

Study 2: Efficiency of Cooking by Gas Grill Method for *Escherichia coli* O157:H7 Elimination from Blade Tenderized Steaks

Bacterial Reduction Analysis

Initial statistical analysis of main effects (treatment, thickness, and temperature) and all possible interactions, with bacterial reduction as the dependent variable, indicated that there were significant differences ($p \leq 0.05$) between tenderized (T) and non-tenderized (NT) steaks and that interactions were significant between thickness and temperature. To eliminate interaction effects, the data were analyzed again, first by temperature, then by thickness. Within each temperature group, there were no significant differences ($p > 0.05$) between T and NT steaks. However, when reductions were grouped and analyzed by

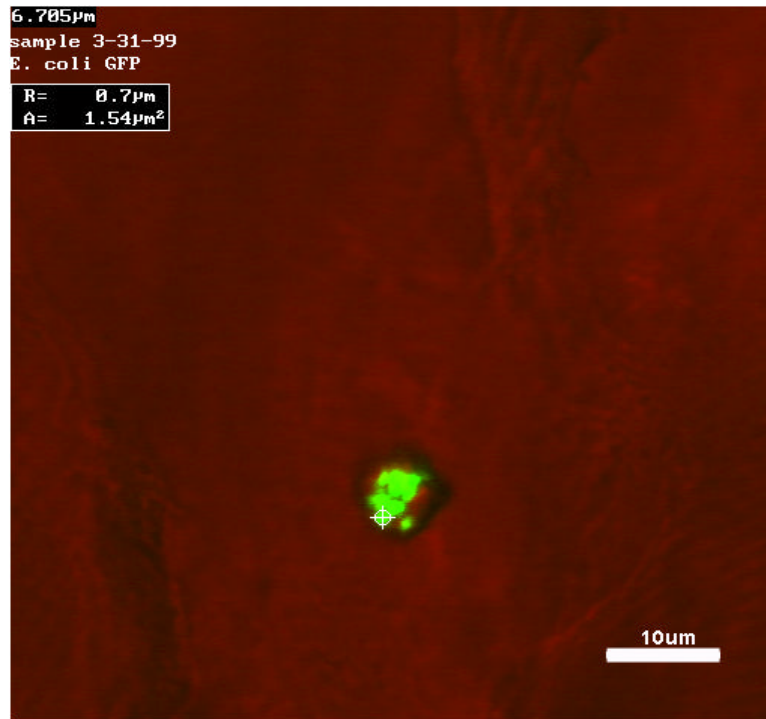


Figure 10. Autofluorescent generic *E. coli* within one blade tract of a tenderized beef subprimal at a depth of ca. 0.5 cm below the inoculated surface, visualized by confocal microscopy

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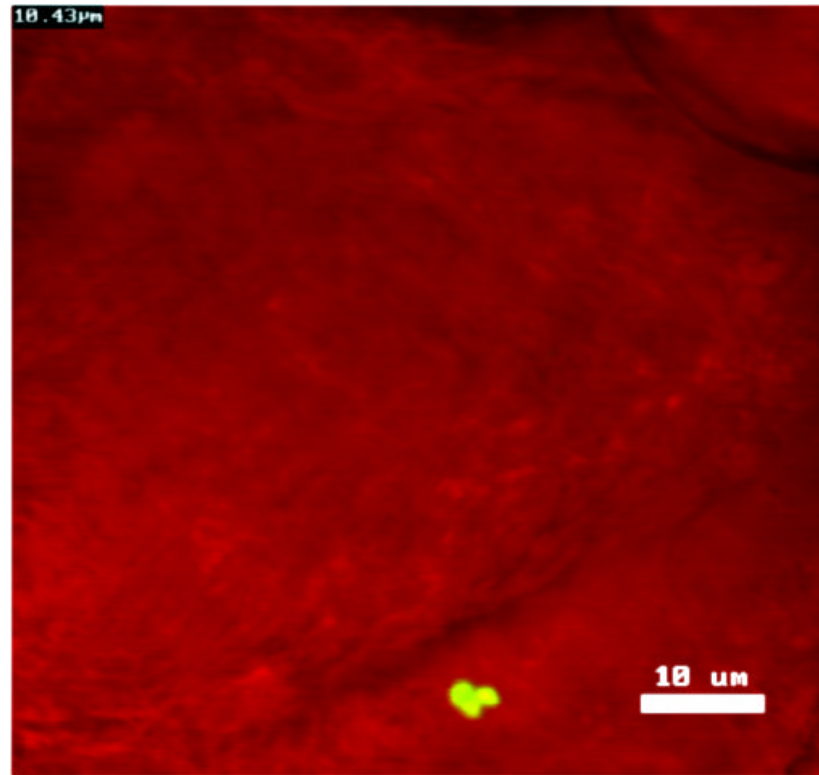


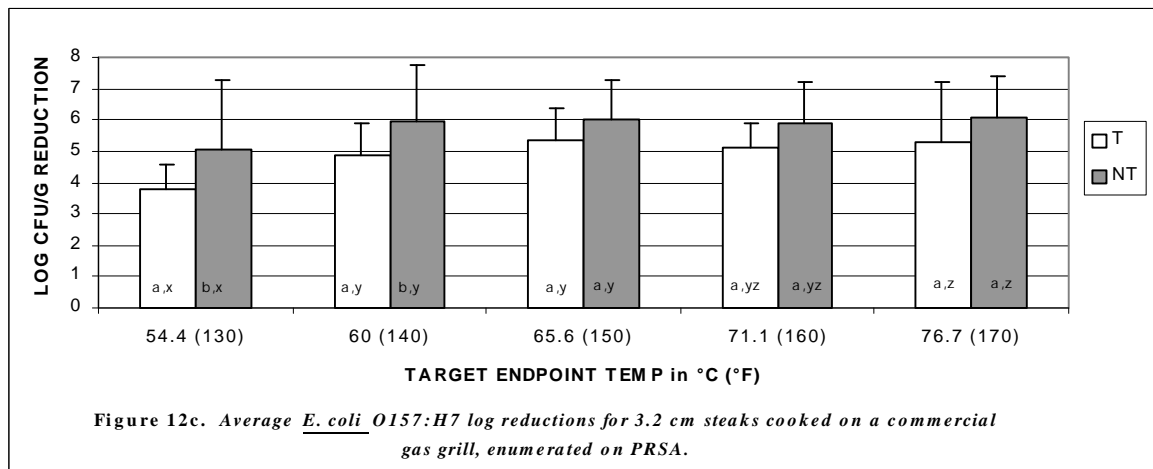
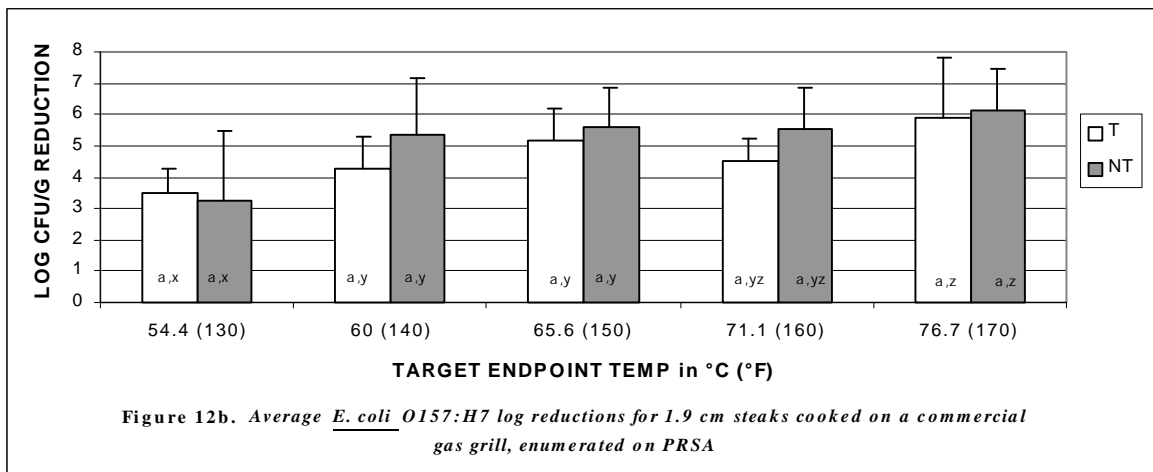
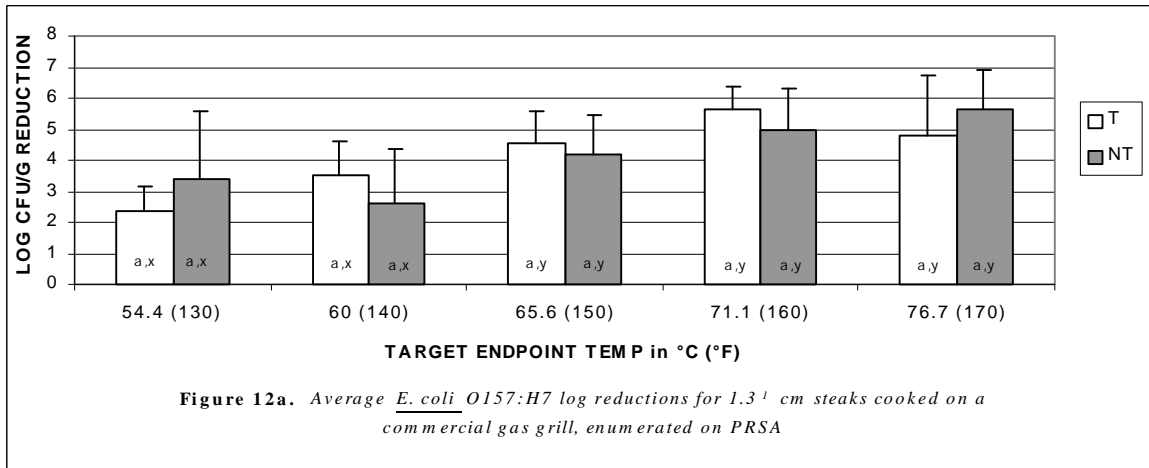
Figure 11. *Autofluorescent generic E. coli within one blade tract of a tenderized beef subprimal at a depth of ca. 6 cm below the inoculated Surface*

ation had an effect, and within each thickness, endpoint temperatures produced significantly different bacterial reductions.

In 1.3 cm steaks (Fig. 12a), target internal temperatures of 54.4°C and 60.0°C produced similar ($p>0.05$) bacterial reductions of 2.4 and 3.5 logs, respectively, in T steaks, and 3.4 and 2.6 logs, respectively, in NT steaks. Standard deviations in log reductions were quite high at these temperatures, ranging from 0.8 to 2.2 logs, which may account for the inability to detect statistical differences.

Johnston et al. (1978) reported that *Salmonella newport* survived on both the surface and in the core of mechanically tenderized roasts which were oven cooked to an internal temperature of 54.4°C. The authors hypothesized that the presence of viable cells on the surface could be due to purging of the cells from the interior or from actual survival at the surface. The study concluded that tenderized roasts cooked to 54.4°C could pose a public health risk. The study did not, however, include an evaluation of inoculated, non-tenderized roasts cooked to 54.4°C. Further, Johnston's study analyzed 5 pound beef roasts, whereas the current study evaluated the microbial reductions that could be achieved in steaks processed from tenderized roasts (subprimals) to more accurately describe restaurant operations.

Target temperatures of 65.6°, 71.1°, and 76.7°C produced greater ($p\#0.05$) reductions than the lower temperatures, but also were not different ($p>0.05$) from each other, at 4.3, 5.3, and 5.2 log reductions, respectively. These results are similar to those reported by Jackson et al. (1995), who observed between 4 and 6 log reductions of *E. coli* O157:H7 in 114 g ground beef patties cooked to 68.3°C (155°F) on a snap action grill. In the present



^{a,b} Differences in tenderization within each thickness/temperature group are indicated by different letters.
^{x,y,z} Reduction differences between temperatures are indicated by different letters.
¹ Reduction means were grouped by thickness to eliminate thickness*temp interaction.

study, a 5 log reduction or greater was achieved in 1.3 cm steaks only at temperatures of 71.1°C and 76.7°C. In this thickness group, blade tenderization was not a significant factor.

Similar trends in reductions were observed in 1.9 cm steaks (Fig. 12b), and again, tenderization was not significant within each temperature group. At 60.0°C, reductions of 4.2 logs (T) and 5.4 logs (NT) were greater ($p \leq 0.05$) than those at 54.4°C, but were not statistically different from 65.6° or 71.1°C. Five log reductions or greater were observed at target temperatures of 65.6°C and above. However, standard deviations within these temperatures ranged from 0.4 logs at 65.6° to 1.8 logs at 71.1°, suggesting that the margin of safety provided by these relatively high cooking temperatures may be somewhat less than indicated by mean log reductions.

3.2 cm steaks produced the greatest reductions (Fig. 12c), likely due to the long cook times required to reach the desired internal temperatures, which is discussed in the following section. In this group, tenderization did have an effect ($p \leq 0.05$) at endpoints of 54.4° and 60.0°C, suggesting that while the external surface temperatures reached during these cooking processes were sufficient to control 5-6 logs cfu/g, the low internal temperatures were not adequate to control organisms carried into the center of the muscle via the blade tenderization process. A temperature of 54.4°C produced reductions of 3.8 and 5.1 logs for T and NT steaks, respectively. At 60.0°C, reductions for T and NT steaks were 4.8 and 6.0 logs, respectively. Reductions at 54.4°C were lower ($p \leq 0.05$) than those observed at 60.0°, 65.6°, 71.1°, and 76.7°C, all of which achieved reductions of nearly 5 logs or greater. Standard deviations of up to 2 logs in bacterial reduction were observed throughout the grill study. This high degree of variability may reduce the margin of safety indicated by the

results of this study and suggests that this particular method of cookery lacks consistency.

Evaluation of Heat Injury

A similar statistical analysis was conducted using *E. coli* O157:H7 counts enumerated on MSA. In general, the same trends were observed (Appendix 4a-c). However, reductions did tend to be somewhat higher, suggesting that recovery of injured cells was not as great as with PRSA. To examine this occurrence more thoroughly, the percent of the total recovered *E. coli* O157:H7 population that was injured was determined (Table 1). Although the percent recovery was quite low and generally decreased with each increase in target temperature, the degree of injury observed was substantial. In thin (1.3 cm) steaks, injured cells were recovered at every endpoint temperature. Injury was also quite substantial in 1.9 cm steaks, observed at endpoint temperatures from 54.4 - 71.1°C, but no organisms were recovered at 76.7°C, suggesting total destruction of the *E. coli* O157:H7 population. In 3.2 cm steaks, injured cells were recovered at 54.4°, 60.0° and 65.6°C, but no organisms were recovered at 71.1° and 76.7°C. Although these findings suggested that PRSA was a more conservative and accurate recovery medium than MSA, both agars were evaluated in the following studies to further investigate the differences in recoverability. These data support the results obtained by Ahmed and Conner (1995), wherein PRSA with 0.005% 4-methylumbelliferyl- β -d-glucuronide (MUG) and PRSA with 1% pyruvic acid (PA) produced consistently higher recovery of heat-treated *E. coli* O157:H7 populations than did MSA. Ahmed and Conner also determined that the addition of MUG, PA, or MgSO₄ to MSA further compromised this medium's recoverability. Abdoul-Raouf et al. (1993) reported that MSA-MUG was unable to resuscitate some heat-stressed *E. coli* O157:H7 cells from ground,

roasted beef cooked to

TABLE 1. Percent of *E. coli* O157:H7 population, including the percent injured by cooking on a gas grill, recovered on PRSA

media.

		54.4°C (130°F)		60.0°C (140°F)		65.6°C (150°F)		71.1°C (160°F)		76.7°C (170°F)	
		% RECOV ¹	% INJURED ²	% RECOV	% INJURED	% RECOV	% INJURED	% RECOV	% INJURED	% RECOV	% INJURED
T	1.3	0.00762	37.91	0.00073	67.11	0.00006	79.83	0.00001	68.23	0.00485	99.99
	cm										
NT	1.3	0.02081	74.48	0.07803	73.31	0.00137	55.44	0.00014	65.96	0.00005	0.00
	cm										
T	1.9	0.04289	52.18	0.00019	93.64	0.00009	90.34	0.00015	99.27	0.00000	0.00
	cm										
NT	1.9	0.00769	62.44	0.00005	83.18	0.00001	72.51	0.00156	85.55	0.00000	0.00
	cm										
T	3.2	0.00011	72.84	0.00003	96.89	0.00000	80.05	0.00000	0.00	0.00000	0.00
	cm										
NT	3.2	0.00010	49.85	0.00000	60.19	0.00000	60.19	0.00000	0.00	0.00000	0.00

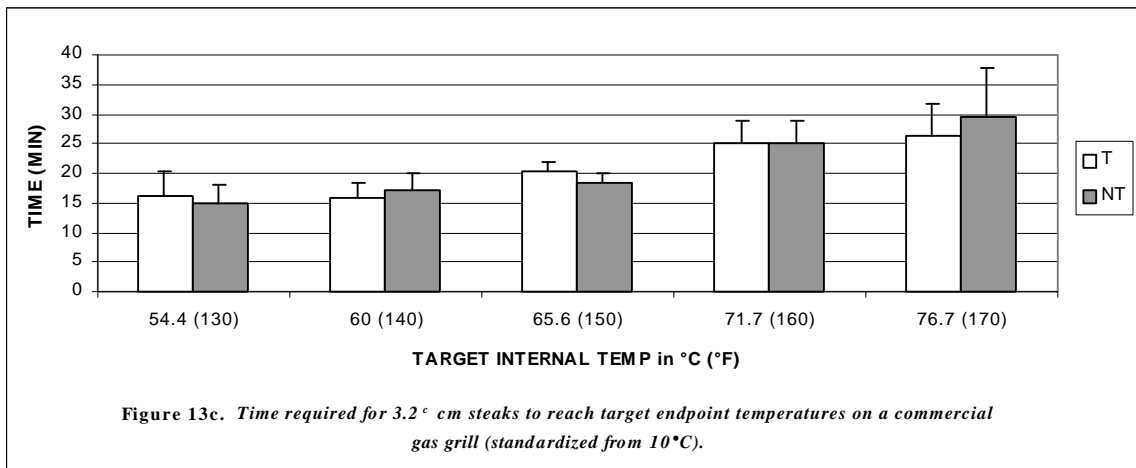
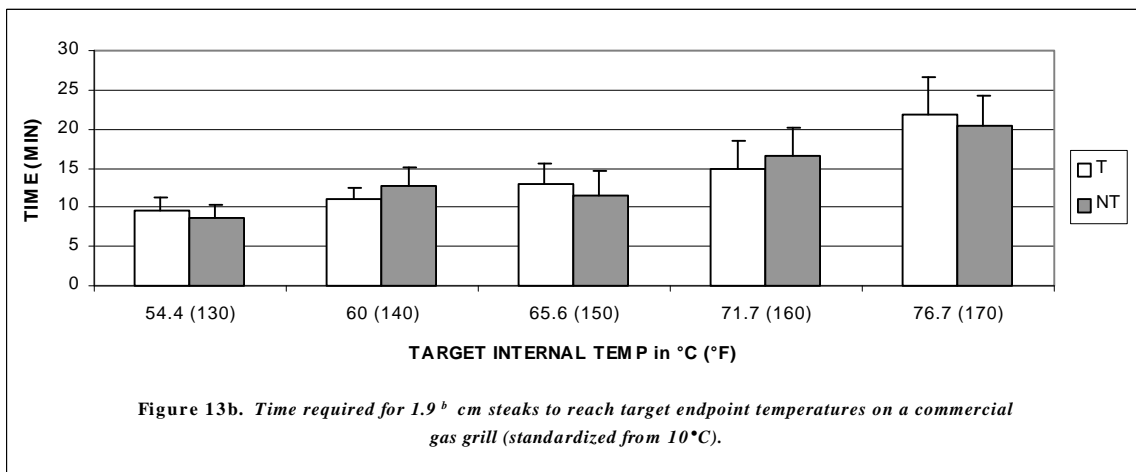
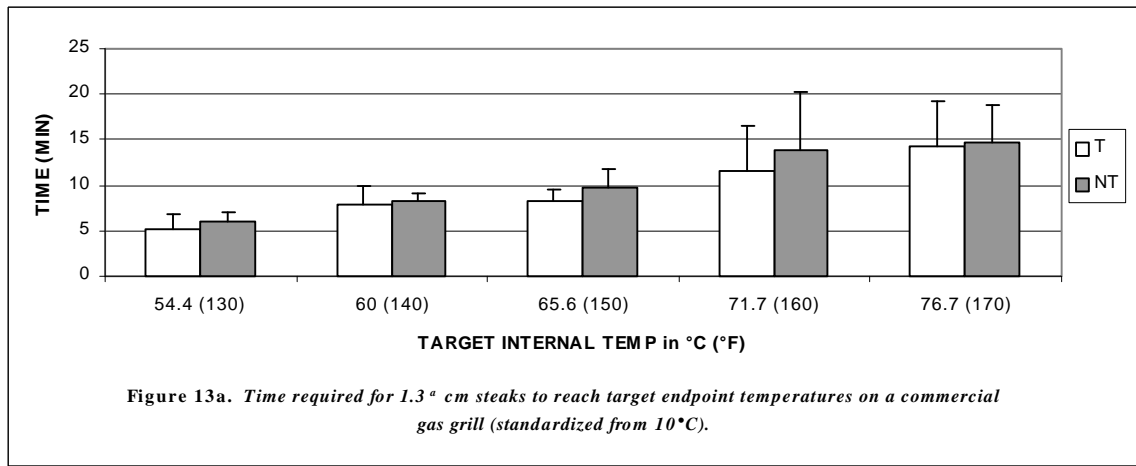
¹ % recovery was calculated as the number of bacteria recovered on PRSA from cooked samples / number of bacteria recovered on PRSA from raw control sample X 100.

² % injury was calculated as the difference in bacterial counts recovered on PRSA vs MSA / number of bacteria recovered on PRSA from raw control sample X 100.

an internal temperature of 77°C (compared to a Tryptic Soy Agar recovery system). This study also evaluated 20% cooked beef slurries, with pH adjusted to 4.70, 5.00, and 5.40 (± 0.03) using acetic, citric and lactic acids. Again, MSA-MUG was unable to resuscitate injured cells, illustrating its limited application for acid-stressed *E. coli* O157:H7, as well.

Cooking Profile

Differences ($p \leq 0.05$) in cooking time (standardized from an initial temperature of 10°C), were detected within temperature and thickness groups, but were not effected by tenderization. When grouped by endpoint temperature, 1.3, 1.9, and 3.2 cm steaks were always statistically different from each other (Figs. 13a-c), even though standard deviations over the six replications were fairly high. As expected, 1.3 cm steaks required the shortest time to reach each of the desired internal temperatures, while 3.2 cm steaks required the longest time on the grill, due to the difference in thickness. In general, cook time appeared to increase by ca. five minutes with each increase in steak thickness (Figs. 13a-c). Based on the concept of integrated lethality, which describes the time-temperature relationship that dictates bacterial destruction, this increase in cook time likely explains the greater log reductions observed in thicker steaks than in thin steaks. For example, at 54.4°C, *E. coli* O157:H7 populations were reduced by 2.4 log cfu/g in tenderized 1.3 cm steaks, which were on the grill for 5 minutes. Reductions in 3.2 cm steaks were 3.8 logs over a 16 minute cook time. These differences in total lethality were more prevalent at lower target temperatures of 54.4-65.6°C; at 71.1° and 76.7°C, bacterial destruction is virtually instantaneous. It is also apparent from Figs. 13a-c that variability in cook time within each thickness group tended to increase as target temperature increased. This study did not support findings which suggest that blade tenderization increases cooking rates (Glover et al., 1977).

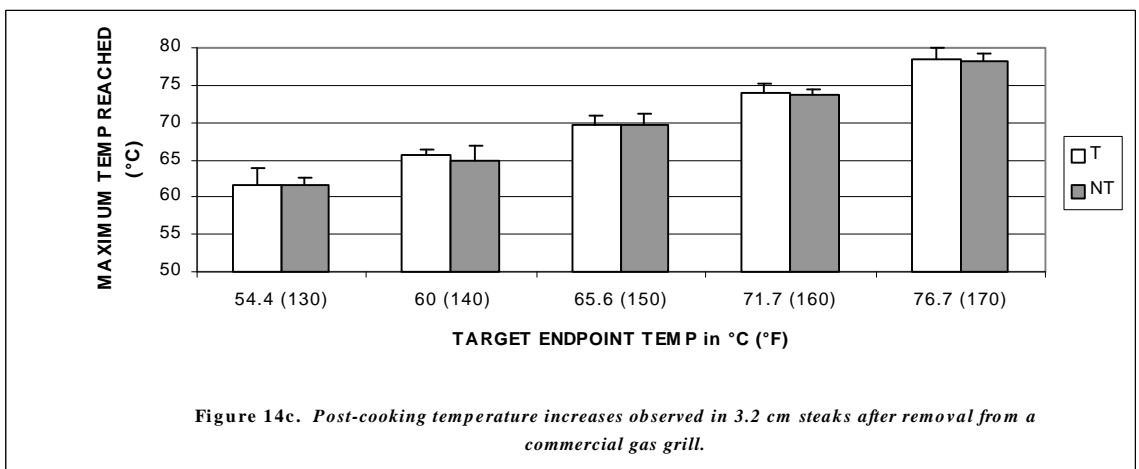
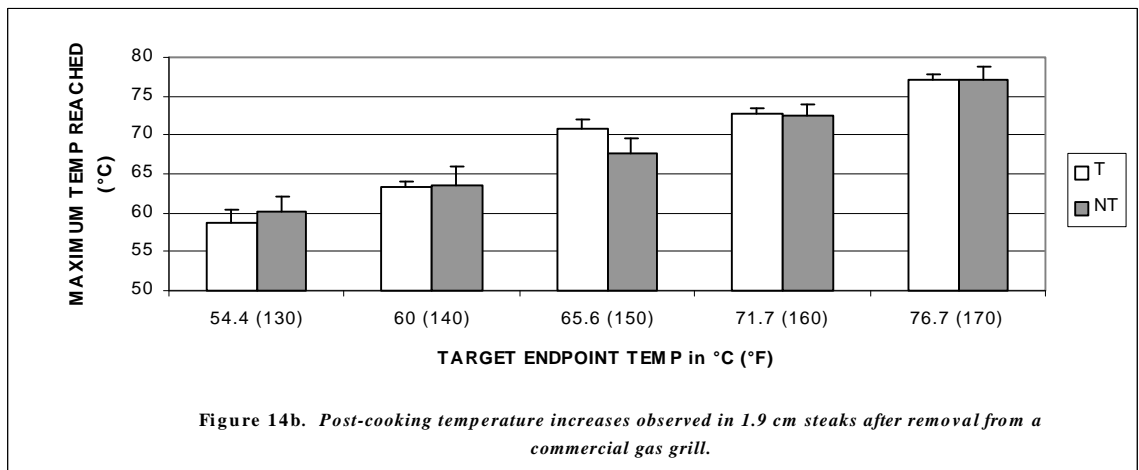
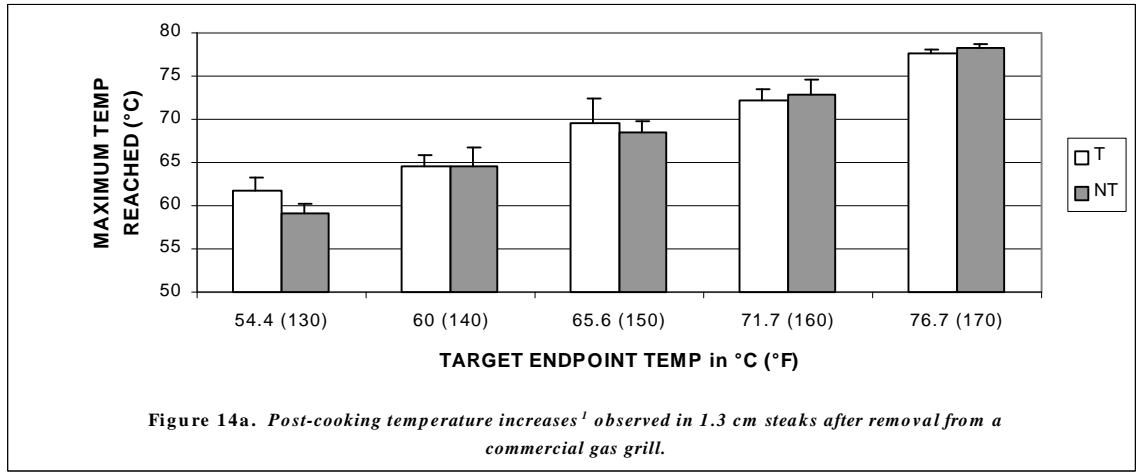


^{a,b,c} Cook time differences between thicknesses are indicated by different letters. Tenderization was not found to be significant.

After removal from the grill surface, steaks were held at room temperature for a five-minute holding time, during which internal temperature was monitored. Within this period, temperature continued to rise above the target temperature (Figs. 14a-c). This phenomenon provided an added margin of safety, the extent of which varied with thickness and target endpoint.

To statistically analyze the maximum endpoint temperatures reached after removal from the grill, treatment data were grouped by target temperatures, all of which were statistically different ($p < 0.05$) from each other. Tenderization was not shown to have an effect on the maximum endpoint. Within all temperature groups, the thicker the steak, the greater post-cooking temperature rise. In general, however, this rise did get smaller as target temperature increased. For instance, 3.2 cm steaks (Fig. 14c) cooked to 54.4°C increased 7°C, while 170°C 3.2 cm steaks only increased 1.5°C during the five-minute holding time. Similar trends were seen for 1.3 and 1.9 cm steaks (Figs. 14a and 14b).

The five-minute holding time was incorporated into the design of the experiment to simulate restaurant/food service holding times, and it should be noted that five minutes was generally sufficient time for steaks to reach their maximum temperature. However, due to a somewhat time-consuming sampling process, during which samples were cut and weighed, steaks remained at relatively high temperatures for an extended period, prior to being submersed in cold peptone water to halt thermal lethality. The additional lethality associated with this sampling process likely resulted in slightly higher bacterial reductions than that of a controlled D-value study. To design a more controlled study, wherein bacterial reductions



would be more accurately, and perhaps more conservatively, represented by experimental design, the five-minute cool-down was eliminated from the following studies. Instead, each steak was placed in a plastic bag immediately upon removal from the cooking surface, and submersed in an ice bath. The steak's internal temperature was monitored until it fell below 100°C, a temperature contributing no significant lethality.

Study 3: Efficiency of Cooking by the Electric Skillet Method for *Escherichia coli* O157:H7 Elimination in Blade Tenderized Steaks

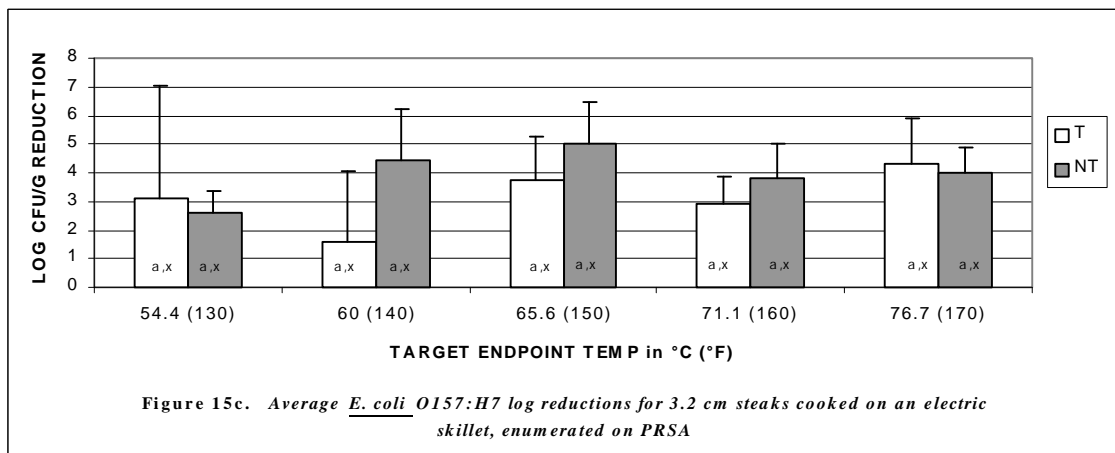
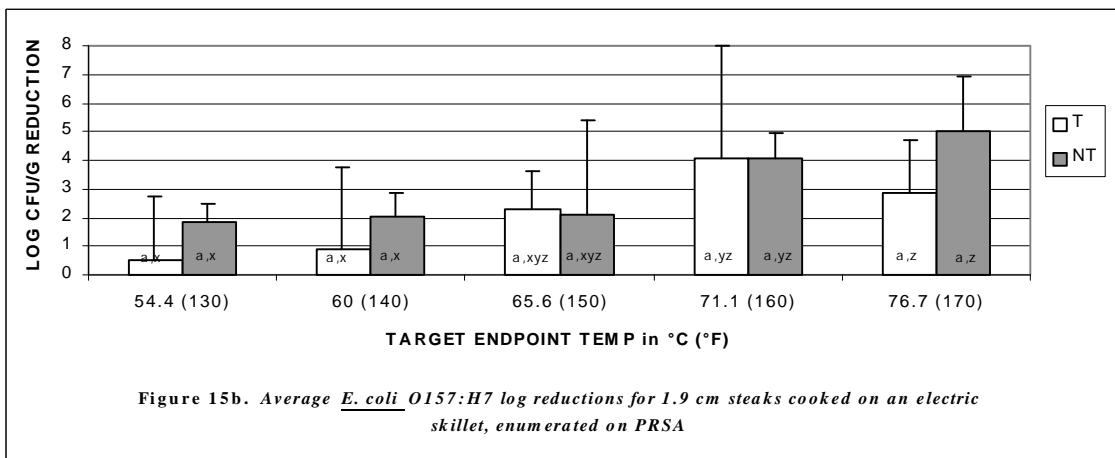
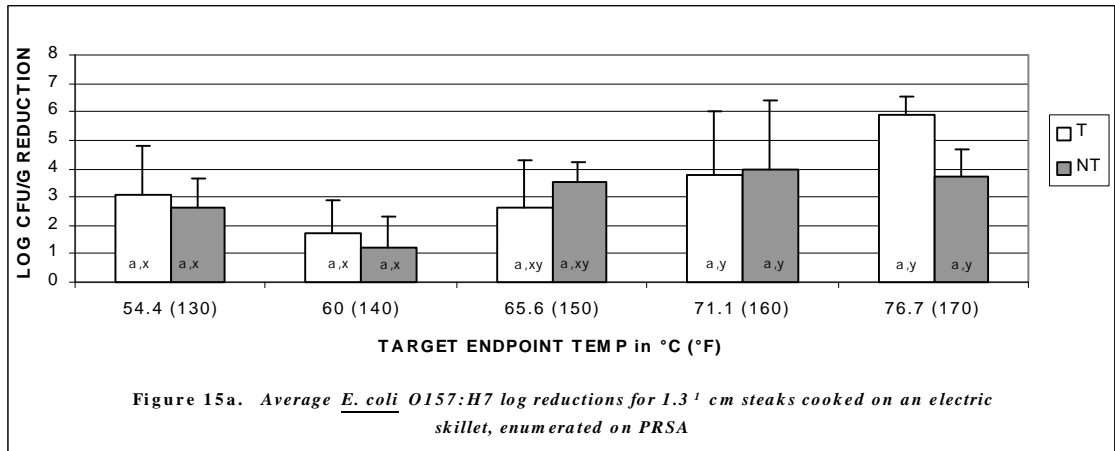
Bacterial Reduction Analysis

Pooled reduction data, based on PRSA enumeration of the surviving *E. coli* O157:H7 population, indicated that temperature was the only significant ($p \leq 0.05$) variable in regard to bacterial reduction, and no interactions were detected. Thickness and tenderization were not found to be significant factors in this study. However, the inability to detect differences may be due to the large degree of variability associated with this cooking method. (Note that the graphic interpretation of this data is not pooled across thickness or tenderization, to facilitate comparison of data to other cooking methods.)

Analysis of endpoint temperatures (Figs. 15a-c) indicated that reductions at 54.4°C and 60.0°C (2.3 and 2.0 logs, respectively), were not different ($p > 0.05$). These reductions are similar to those obtained in a study by Juneja et al. (1997), who reported that internal temperatures of

¹ No differences in post-cooking temperature increase were observed between T and NT steaks.

133-134°C in ground beef patties cooked on an electric skillet resulted in a 2 log cycle destruction of *E. coli* O157:H7. The authors concluded that for every 5°C increase in internal temperature above 57.2°C, an additional one log reduction was achieved. In the current study, an internal endpoint of 65.6°C resulted in a reduction of only 3.2 logs.



^{a,b} Differences in tenderization within each thickness/temperature group are indicated by different letters.
^{x,y,z} Reduction differences between temperatures are indicated by different letters.

Evaluation of Heat Injury

The electric skillet method of cookery did not consistently achieve a 5 log reduction (Figs. 15a-c), even at target temperatures up to 76.7°C, as was demonstrated in the commercial grill study. *E. coli* O157:H7 were recovered at every endpoint temperature, and degree of injury was extensive, ranging from 25 - 100% of the recovered populations (Table 2). Temperatures from 65.6 - 76.7°C resulted in the highest percentage of injured cells. Standard deviations in log reductions at 76.7°C were nearly 2 logs in both T and NT steaks. These results indicated that this method is not adequate for controlling high levels of *E. coli* O157:H7.

Cooking Profile

Statistical analysis of skillet cooking times reaffirmed the inconsistency of this cooking method (Figs. 16a-c). Variability in skillet cookery also was reported by Liu and Berry (1996), who evaluated the time on a skillet required for frozen ground beef patties to reach specified endpoint temperatures. They found that considerable variability in cooking properties exists, even when control is exerted over a selected parameter, such as cook time or internal temperature, in order to standardize the cooking procedure. Their study also reported that endpoint temperatures varied greatly when patties were cooked for a predetermined length of time. As in the commercial grill study, cooking times on the electric skillet were different ($p < 0.05$) between each steak thickness and standard deviation generally increased as target temperature increased. Again, blade tenderization did not have a significant effect on cook time.

Interestingly, trends related to post-cooking temperature rise (Figs. 17a-c) were quite

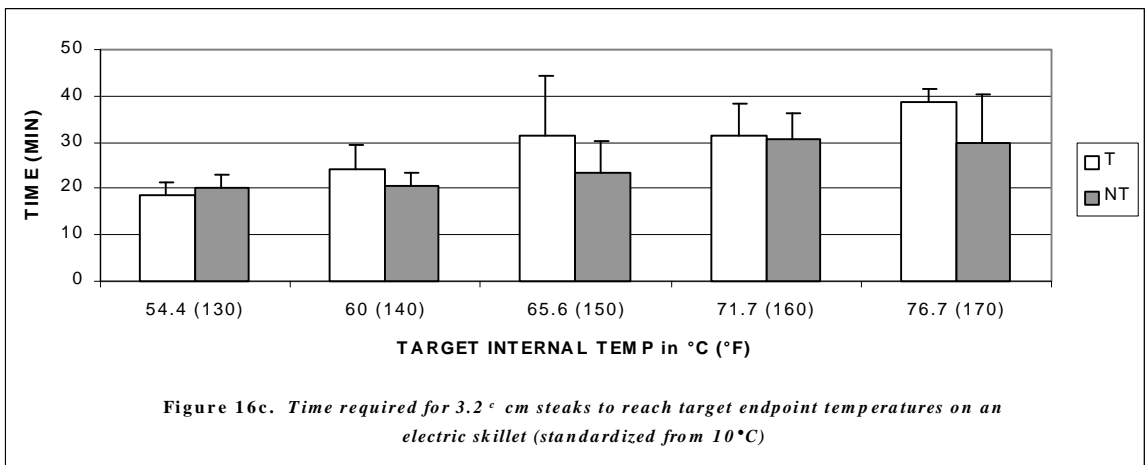
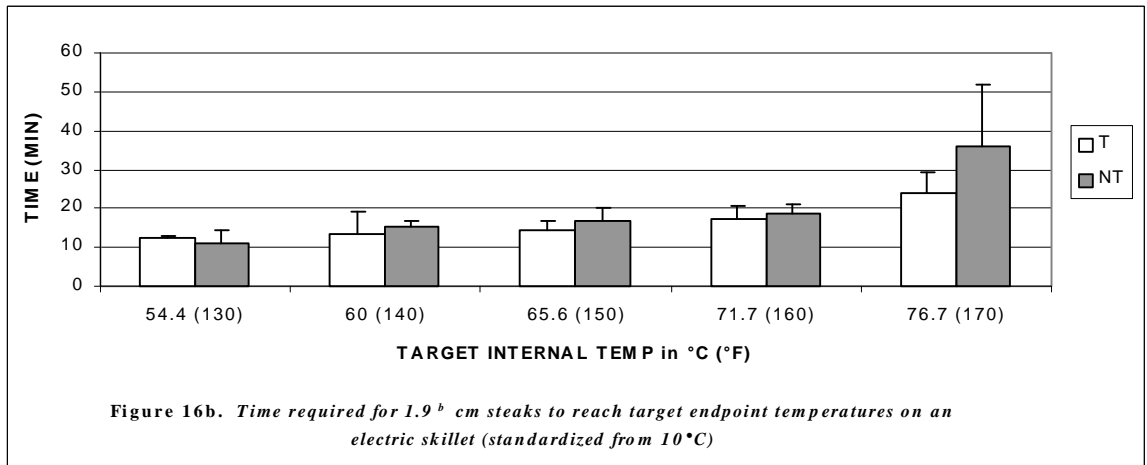
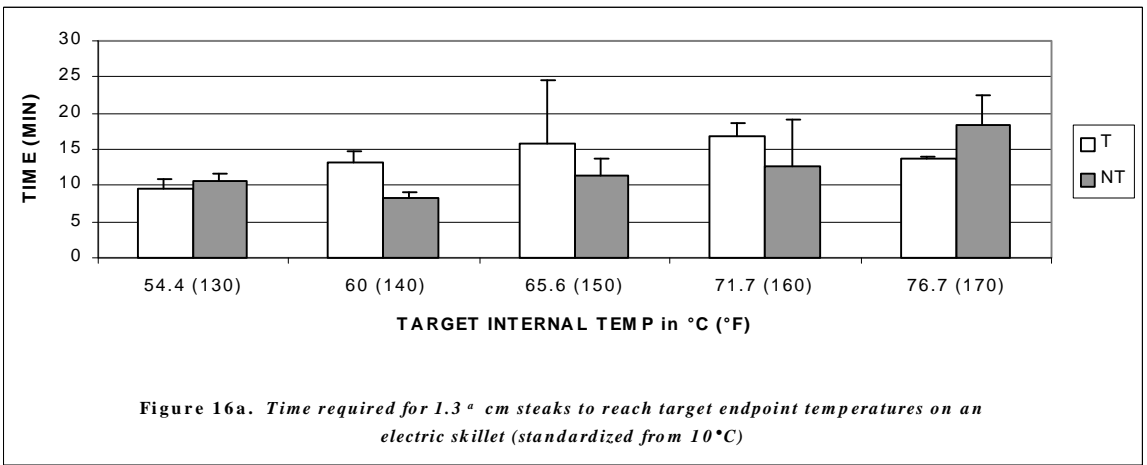
TABLE 2. Percent of *E. coli* O157:H7 population, including the percent injured by cooking on an electric skillet, recovered on PRSA media.

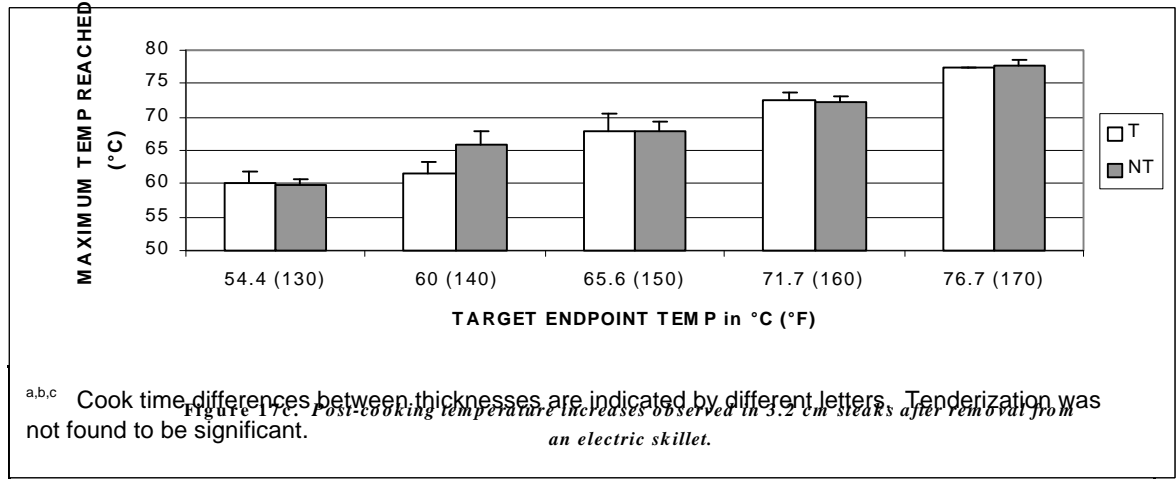
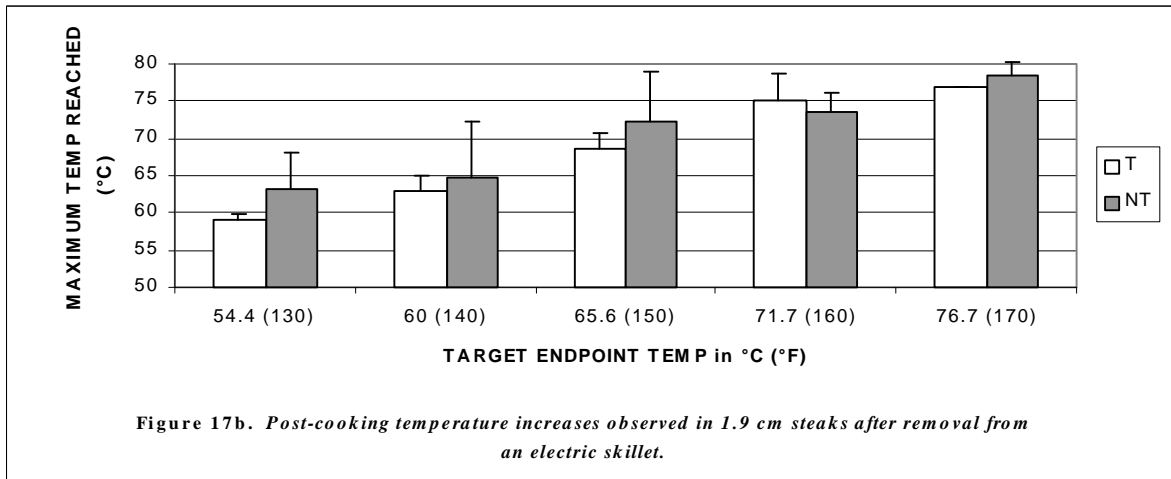
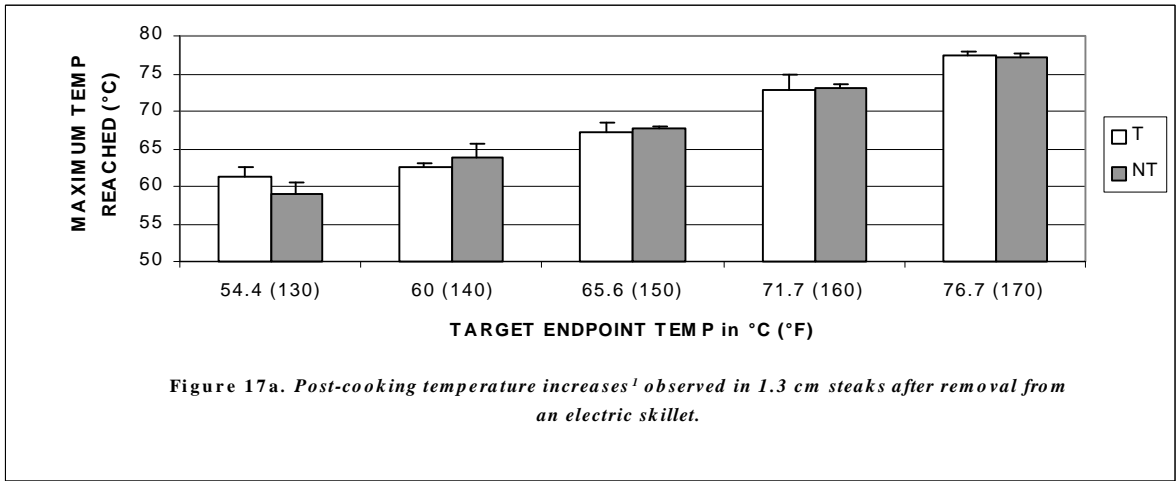
		54.4°C (130°F)		60.0°C (140°F)		65.6°C (150°F)		71.1°C (160°F)		76.7°C (170°F)	
		% RECOV ¹	% INJURED ²	% RECOV	% INJURED	% RECOV	% INJURED	% RECOV	% INJURED	% RECOV	% INJURED
T	1.3	2.8035	0.00	12.5321	82.50	1.5231	84.03	0.4162	55.57	0.0002	100.00
	cm										
NT	1.3	0.5325	81.17	13.4057	92.57	0.0729	76.57	0.2629	61.03	0.0760	77.72
	cm										
T	1.9	7.1467	84.77	1.5825	55.80	0.3312	75.88	0.0168	56.35	0.0450	92.22
	cm										
NT	1.9	1.1530	87.53	0.3119	80.37	2.3437	87.25	0.0104	83.06	0.0005	80.05
	cm										
T	3.2	0.2114	66.53	0.3760	33.28	0.0032	64.86	0.6918	89.61	0.0018	58.94
	cm										
NT	3.2	0.4291	25.98	0.1814	0.00	0.0245	85.50	0.1652	79.65	0.0244	42.70
	cm										

¹ % recovery was calculated as the number of bacteria recovered on PRSA from cooked samples / number of bacteria recovered on PRSA

from raw control sample X 100.

² % injury was calculated as the difference in bacterial counts recovered on PRSA vs MSA / number of bacteria recovered on PRSA from raw control sample X 100.





¹ No differences in post-cooking temperature increase were observed between T and NT steaks.

similar to those seen with the commercial grill, although methodology for monitoring this variable was changed considerably. Even upon immediate immersion of the steaks into an ice bath, internal temperatures rose rather dramatically, especially at lower target endpoint temperatures, where the increase was as much as 13°C.

By monitoring internal temperatures every 10 seconds, an unexpected series of downward spikes was observed in the temperature profiles of several steaks cooked on the electric skillet. These spikes (Appendix 7) were, on occasion, as large as 17°C and tended to occur in thick steaks cooked to high internal temperatures. A possible explanation for the spikes may be related to the solid cooking surface, which allowed no space for juices and fat to drip. Although temperature steadily increased as the fluids heated and expanded, the build-up and eventual release of pressure in these fluids may have created a “micro-explosion”, which resulted in severe temperature decrease, seen as a downward spike on the temperature profiles. This spiking effect increased the likelihood of human error during the cooking process, especially in regard to removal of steaks precisely when the desired internal temperature was reached. Therefore, a third method of cookery, oven broiling, was evaluated to eliminate this unexpected variability.

Study 4: Efficiency of Oven Broiling for *Escherichia coli* O157:H7 Elimination in Blade Tenderized Steaks

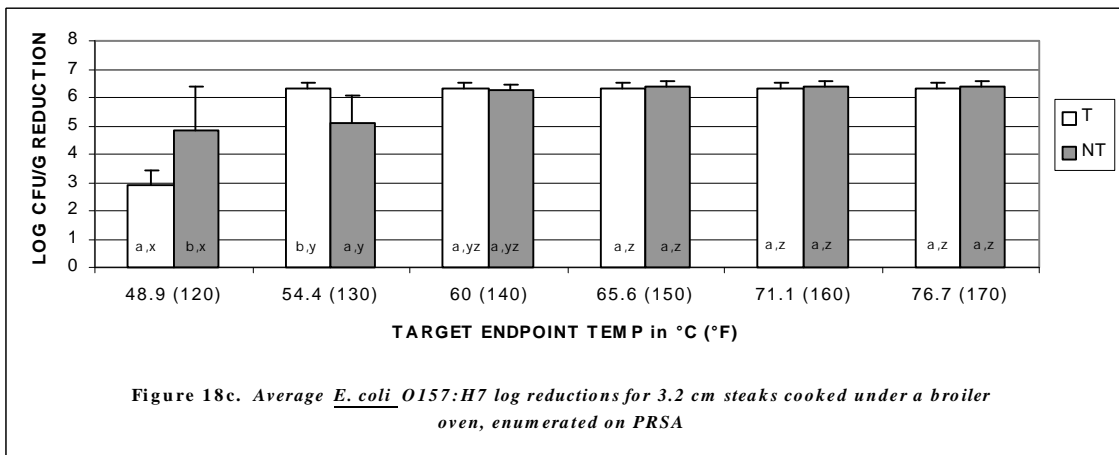
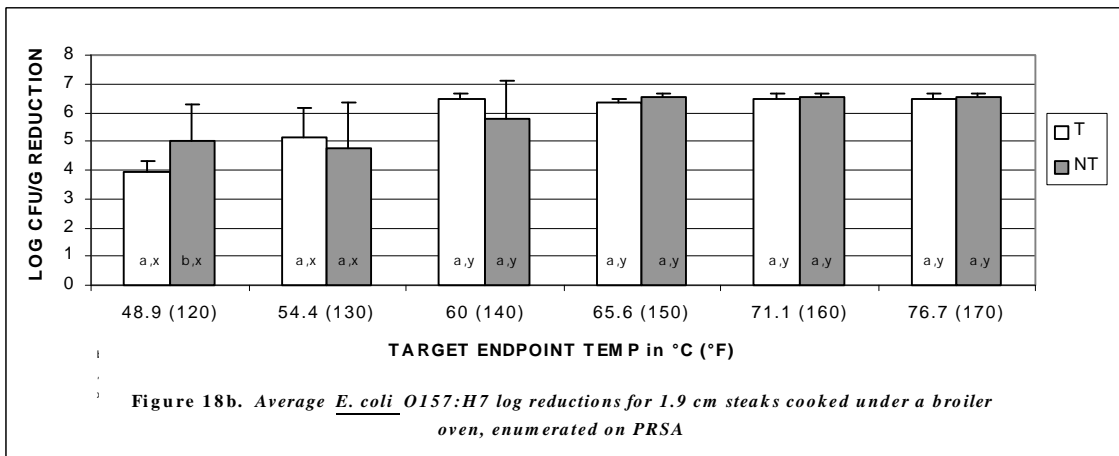
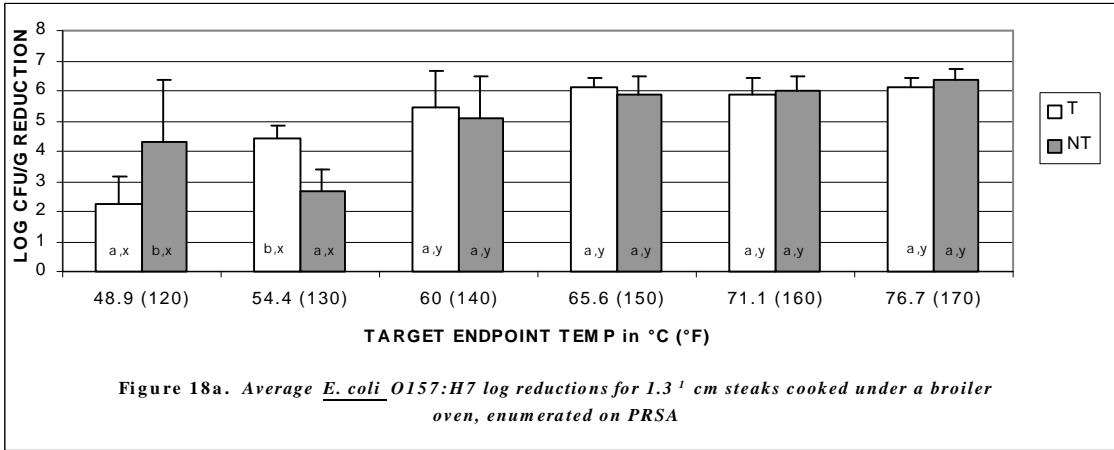
Bacterial Reduction Analysis

Preliminary studies with the broiler oven indicated that this cooking method was more effective for reducing *E. coli* O157:H7 populations than the other cooking methods evaluated. In fact, reductions of up to 6 log cfu/g were observed at the low target temperature of 54.4°C. Due

to the significant increases in steak temperatures after removal from the heat source observed in the previous studies, wherein 54.4°C steaks actually reached final temperatures of 60-62°C, an internal temperature of 48.9°C was added to the experimental design to simulate undercooking. It was only at this low temperature that differences ($p \leq 0.05$) due to blade tenderization were detected (Figs. 18a-c).

Statistical analysis of main effects (blade tenderization, thickness, and temperature) and all interactions, with bacterial reduction as the dependent variable, revealed an interaction ($p \leq 0.05$) between tenderization and temperature. When PRSA reduction data were pooled across thickness and analyzed within each individual temperature, it was indeed observed that at 48.9°C, NT steaks produced a significantly greater reduction (4.7 log reduction) than T steaks (3.0 log reduction). Figs. 18a-c illustrate this finding, but do not include pooled data, so as to show differences across thicknesses at other endpoint temperatures. This finding shows that while an internal temperature of 48.9°C yields a surface temperature sufficient to kill nearly 5 logs of *E. coli* O157:H7, all of which are on or near the surface of NT steaks, it is not high enough to eliminate bacteria carried into and protected by the interior of the muscle.

At 54.4°C, this observation was reversed in 1.3 and 3.2 cm steaks, with T steaks achieving greater ($p \leq 0.05$) reductions than NT steaks (Figs. 18a and 18c). The same trend was observed in 1.9 cm steaks, although the difference was not significant (Fig. 18b). Overall, 1.3 cm reductions were lower ($p \leq 0.05$) than 1.9 and 3.2 cm reductions. At this low temperature, standard deviations were up to 1.0 log in T steaks and as high as 1.6 logs in NT steaks. Although reductions greater than 4 logs were observed for both treatments at each



^{a,b} Differences in tenderization within each thickness/temperature group are indicated by different letters.
^{x,y,z} Reduction differences between temperatures are indicated by different letters.
¹ Steak thickness did not have a significant effect on *E. coli* O157:H7 reductions.

thickness, the high degree of variation would reduce the margin of safety associated with a 54.4°C cooking protocol.

From 60.0° to 76.7°C, no significant differences ($p>0.05$) due to blade tenderization were detected, and each temperature group achieved *E. coli* O157:H7 reductions of greater than 5.5 log cfu/g. Standard deviations also decreased within this higher temperature range, indicating a more consistent level of safety.

Based on an initial inoculum level of ca. 6 logs cfu/g detected on the raw control steaks, it was observed that at 65.6°C, virtually complete destruction of *E. coli* O157:H7 was achieved in both T and NT 3.2 cm steaks. At 71.1°C, this was observed in the 1.9 cm steaks as well, and by 76.7°C, complete elimination of the pathogen was achieved in all thickness groups (Figs. 18a-c).

Evaluation of Heat Injury

As seen in the previous studies, MSA produced similar trends (Appendix 6a-c). However, with this method of cookery, differences in recovery between the two media were only prevalent at low temperatures of 48.9°, 54.4° and 60.0°C, since higher temperatures produced total destruction of *E. coli* O157:H7. Percent injury data (Table 3.) corresponds closely to the reduction graphs (Figs. 18a-c), in that 3.2 cm steaks showed complete destruction (i.e. no injured cells) at 65.6°C, 1.9 cm at 71.1°C, and 1.3 cm at 76.7°. When injury was apparent, NT steaks tended to result in a higher percent of injured cells than T steaks, although this was not statistically confirmed.

Results of the oven broiler study appear to contradict observations made by Carpenter and

Harrison (1989) in an evaluation of oven-roasted chicken breasts inoculated with 10^5 - 10^6

TABLE 3. Percent of *E. coli* O157:H7 population, including the percent injured by cooking under a broiler oven, recovered on PRSA media.

		48.9°C (120°F)		54.4°C (130°F)		60.0°C (140°F)		65.6°C (150°F)		71.1°C (160°F)		76.7°C (170°F)	
		% RECOV ¹	% INJURED ²	% RECOV	% INJURED	% RECOV	% INJURED	% RECOV	% INJURED	% RECOV	% INJURED	% RECOV	% INJURED
T	1.3 cm	3.3821	41.10	0.0031	66.12	0.0016	61.10	0.0000	0.00	0.0001	100.00	0.0000	0.00
NT	1.3 cm	0.0977	29.48	0.2658	88.07	0.0020	70.74	0.0001	100.00	0.0001	100.00	0.0000	0.00
T	1.9 cm	0.0188	57.34	0.0042	70.13	0.0000	0.00	0.0000	100.00	0.0000	0.00	0.0000	0.00
NT	1.9 cm	0.0048	74.62	0.0095	68.25	0.0024	100.00	0.0000	0.00	0.0000	0.00	0.0000	0.00
T	3.2 cm	0.2733	0.00	0.0000	0.00	0.0000	0.00	0.0000	0.00	0.0000	0.00	0.0000	0.00
NT	3.2 cm	0.0062	49.38	0.0029	68.08	0.0000	100.00	0.0000	0.00	0.0000	0.00	0.0000	0.00

¹ % recovery was calculated as the number of bacteria recovered on PRSA from cooked samples / number of bacteria recovered on PRSA from raw control sample X 100.

² % injury was calculated as the difference in bacterial counts recovered on PRSA vs MSA / number of bacteria recovered on PRSA from raw control sample X 100.

cfu/g *Listeria monocytogenes*. These authors reported that internal product temperatures of 65.6-82.2°C, obtained by a dry heat cookery method, were insufficient to totally eliminate the pathogen. The study also indicated that dry heat was less effective for killing microorganisms than moist heat. It was suggested that evaporation occurring at the surface of the chicken breast due to progressive drying of the surface during heating may reduce the surface water activity (A_w). Juices from the interior of the muscle could then flow to the surface and provide a buffer from increasing temperatures. A similar evaporative phenomenon was reported by Blankenship (1978), regarding *Salmonella typhimurium* survival on the surface of beef roasts.

In the current study, the oven broiler method, which is a dry heat method, proved very effective at reducing *E. coli* O157:H7 populations. Results obtained from the previous studies may reflect greater thermal resistance of *Listeria monocytogenes* and *Salmonella*, which has been documented in studies by Line et al. (1991), Goodfellow and Brown (1978) and an unpublished, USDA-contracted study entitled, “Lethality of Heat to *Listeria monocytogenes* Scott A and *Escherichia coli* O157:H7, Part I: D Value Determination in Ground Beef and Turkey”, which was conducted by ABC Research in 1988 (USDA-FSIS, 1993).

Discrepancies between the previous and current research may also be explained by differences in the cooking methods, both of which were dry-heat. Oven broiling applies constant and direct heat from slightly above the surface of the product, while oven roasting relies on the temperature of ambient air within the oven, which is regulated and maintained by a thermostat. Prolonged cooking times allow for greater evaporative cooling and drying on the surface of the product. In contrast, broiling is a very rapid cooking method, which reduces the degree of drying

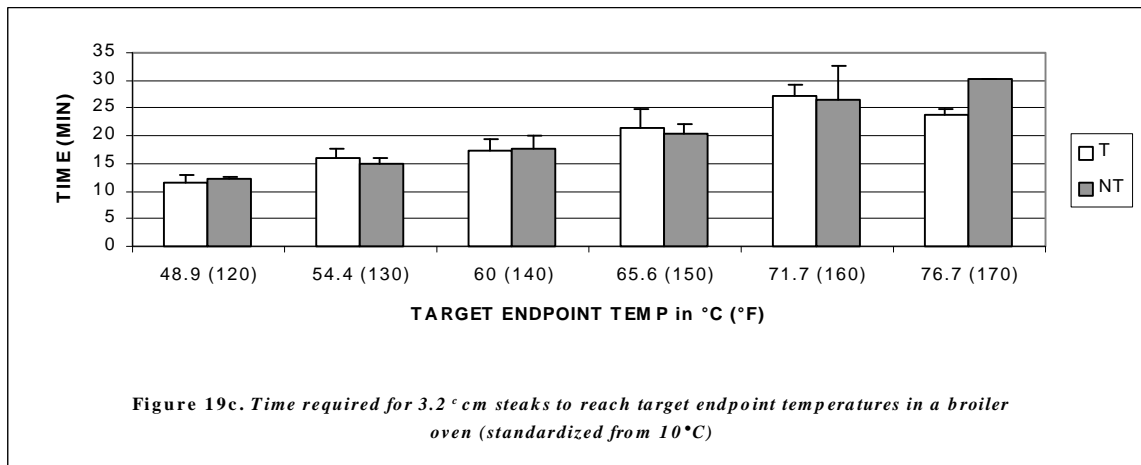
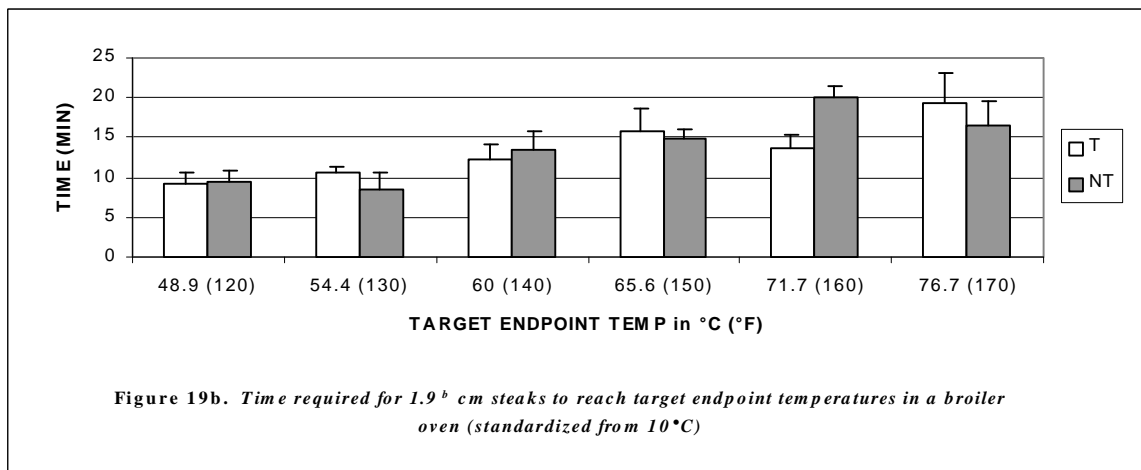
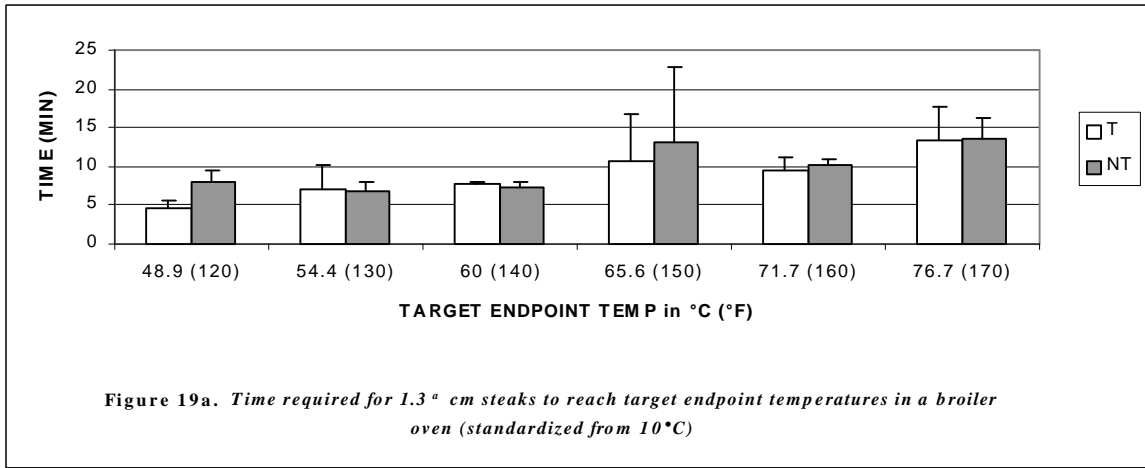
or crusting, unless the product is cooked to very high internal temperatures. As juice flows to the surface, it is immediately exposed to direct heat from the broiler element, which likely provides sufficient lethality to kill bacteria carried with the juice.

Cooking Profile

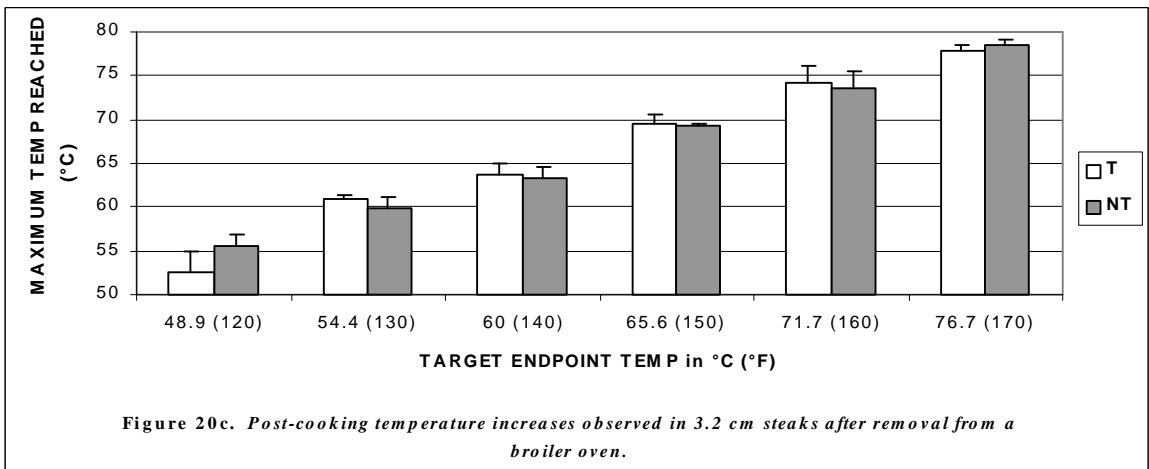
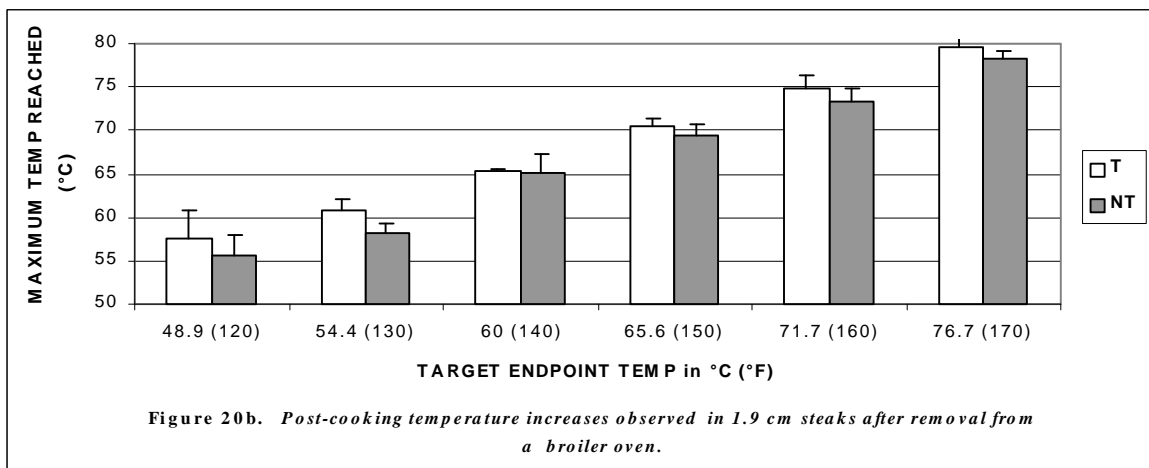
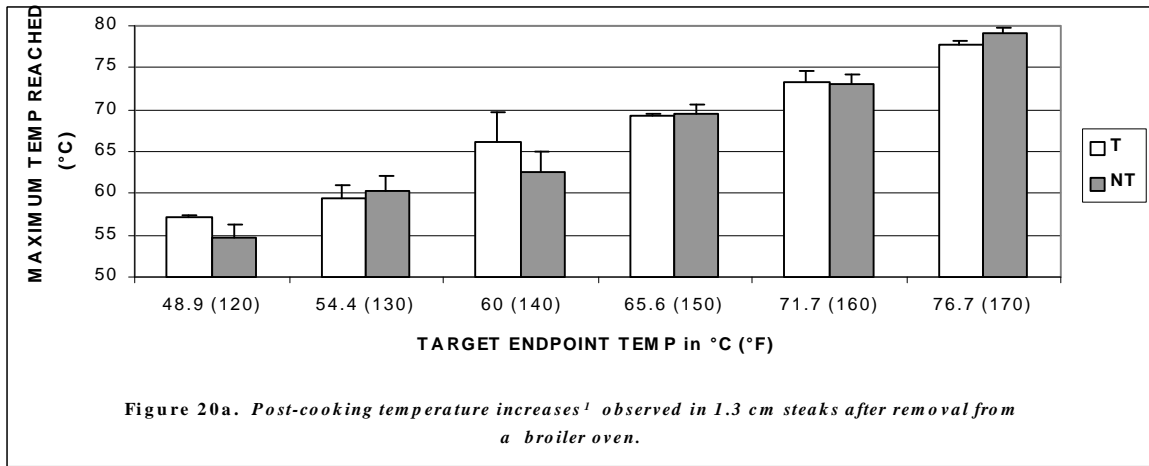
Cook times (Figs. 19a-c) recorded for the oven broiler method were shorter and more consistent than those observed in the gas grill and electric skillet studies. Maximum temperatures (Figs. 20a-c) obtained after removal from the broiler, however, were very similar to the other studies. Even when cooked to a 48.9°C target temperature and submersed in ice, steak temperature increased ca. 5-8°C. Again, maximum endpoint temperatures decreased as target internal temperature increased. Again, blade tenderization did not have a significant effect ($p>0.05$) on cook time or maximum endpoint temperature.

F-Value Comparisons Between Cooking Methods

Due to slight variations in experimental designs, analysis of variance was not conducted to determine statistical differences between the three cooking methods evaluated. However, based on cooking and enumeration data collected, F-values, which represent total thermal lethality associated with each process, were computed and compared between the methods, using regression and correlation analysis. F-value calculations were based on a reference temperature of 65.6°C and a z-value of 8.37, which was determined for lean ground beef by Line et al. (1991). Regression analysis was conducted to determine the correlation between the F-values and reductions observed in the current study. Separate analyses were conducted based on PRSA and MSA reductions, respectively. The initial statistical model



^{a,b,c} Cook time differences between thicknesses are indicated by different letters. Tenderization was not found to be significant.

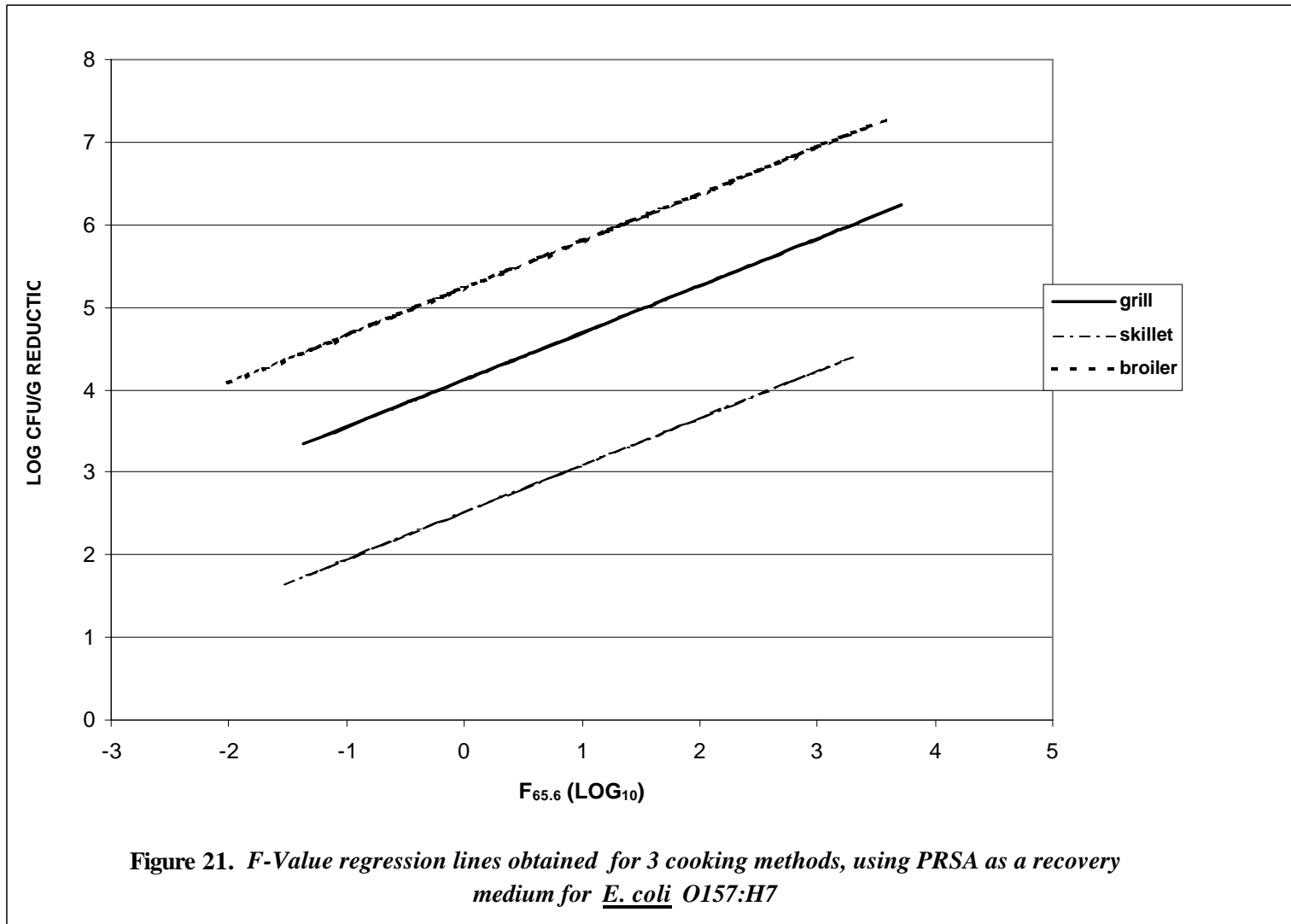


¹ No differences in post-cooking temperature increase were observed between T and NT steaks.

tested significance levels of cook method, tenderization, and $\log_{10} F_{65.6}$.

Results of the PRSA analysis determined that cooking method and F-value were the only significant effects in the model; blade tenderization was not significant and interactions between tenderization, thickness and endpoint temperature were not detected, indicating that the methods shared a common slope (Fig. 21). Because cooking method did effect ($p \leq 0.05$) the F-value/reduction correlation, further analysis was performed for each method separately. Across tenderization, correlation coefficients (R^2) for the grill, skillet, and broiler studies were 0.29, 0.20, and 0.40, respectively (Figs. 22-24). These values are fairly low, indicating that the lethality equations developed from the data are not strong predictors of bacterial reduction. However, it is important to note that correlations based on log reductions are limited by the initial inoculum level. As discussed in previous sections, temperatures ranging from 65.6-76.7°C were noted to achieve complete elimination of *E. coli* O157:H7 populations. Higher inoculum levels likely would have demonstrated that the thermal lethality associated with these high temperatures is sufficient for further destruction of the pathogen. It is also reasonable to assume that the large degree of random error associated with the cooking processes contributed to the poor correlations. The broiler method did produce the highest correlation, likely due to relatively low variability, again suggesting that it is the most consistent and effective cooking method in regard to food safety.

F-value/reduction correlation analysis based on MSA data showed a considerably different pattern than that of PRSA data. With this recovery system, there were significant interactions between F-value and cooking method, suggesting that the methods did not share a common slope, as illustrated in Fig. 25. Blade tenderization did not affect ($p > 0.05$) the F-



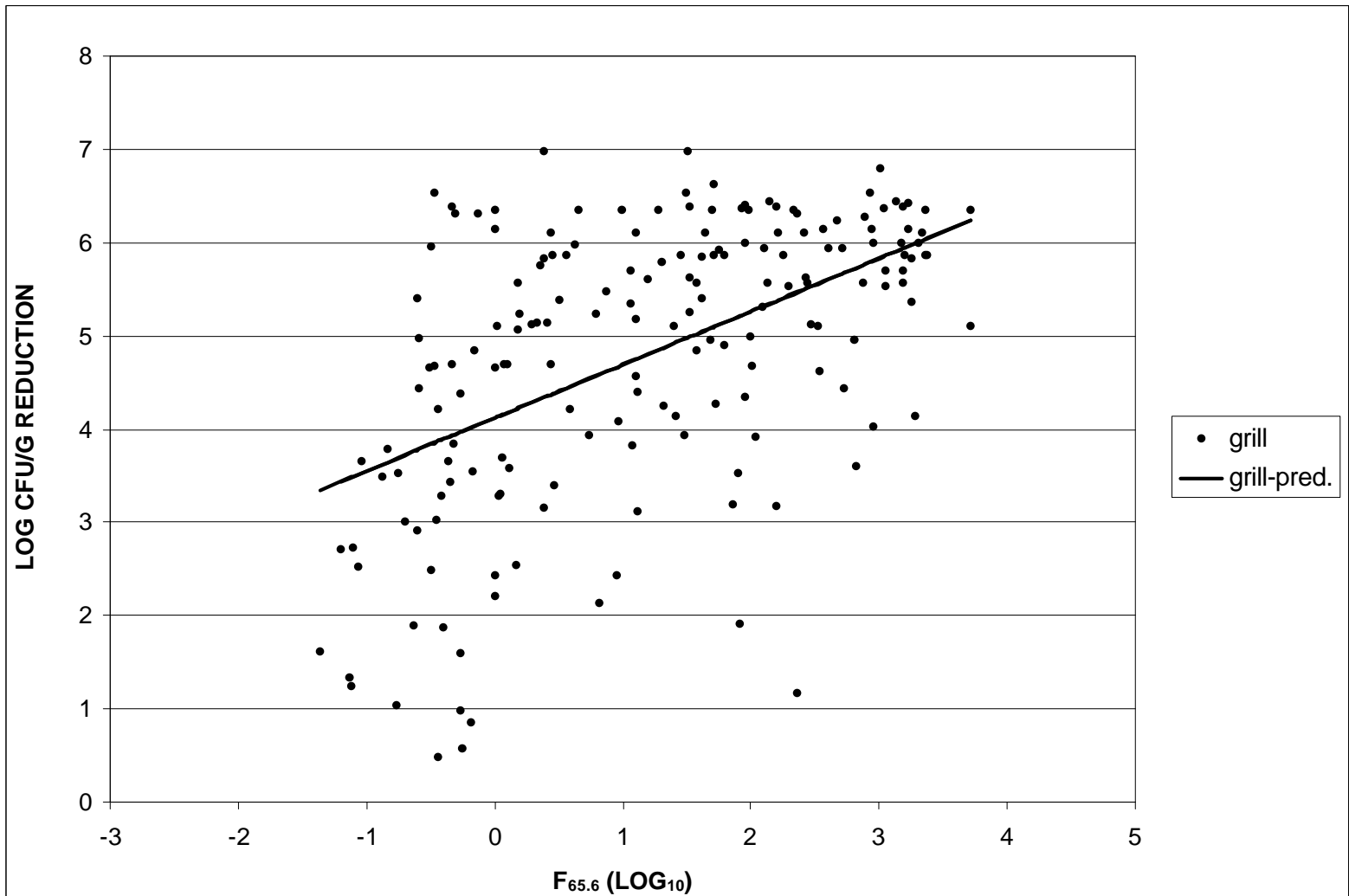
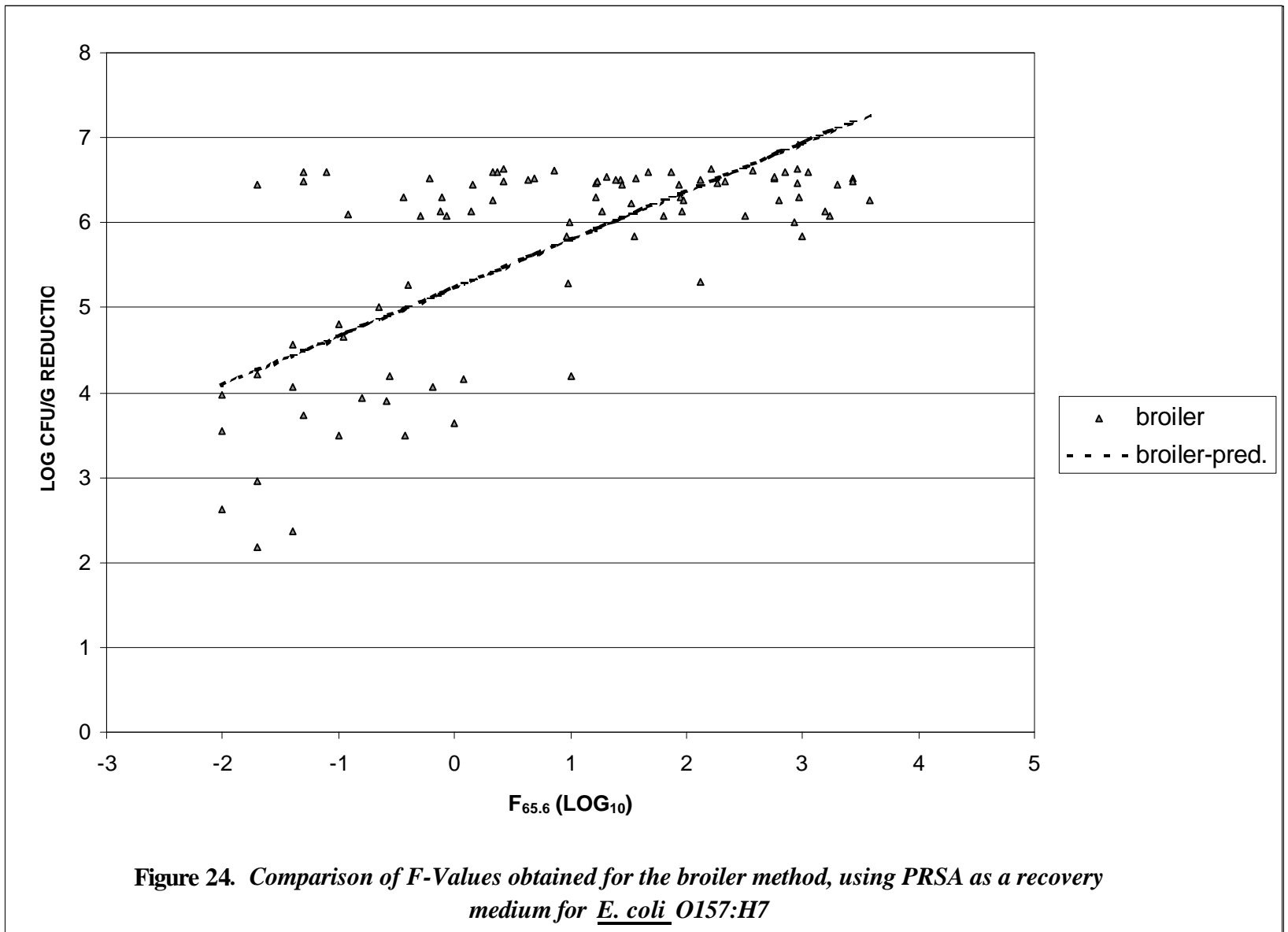
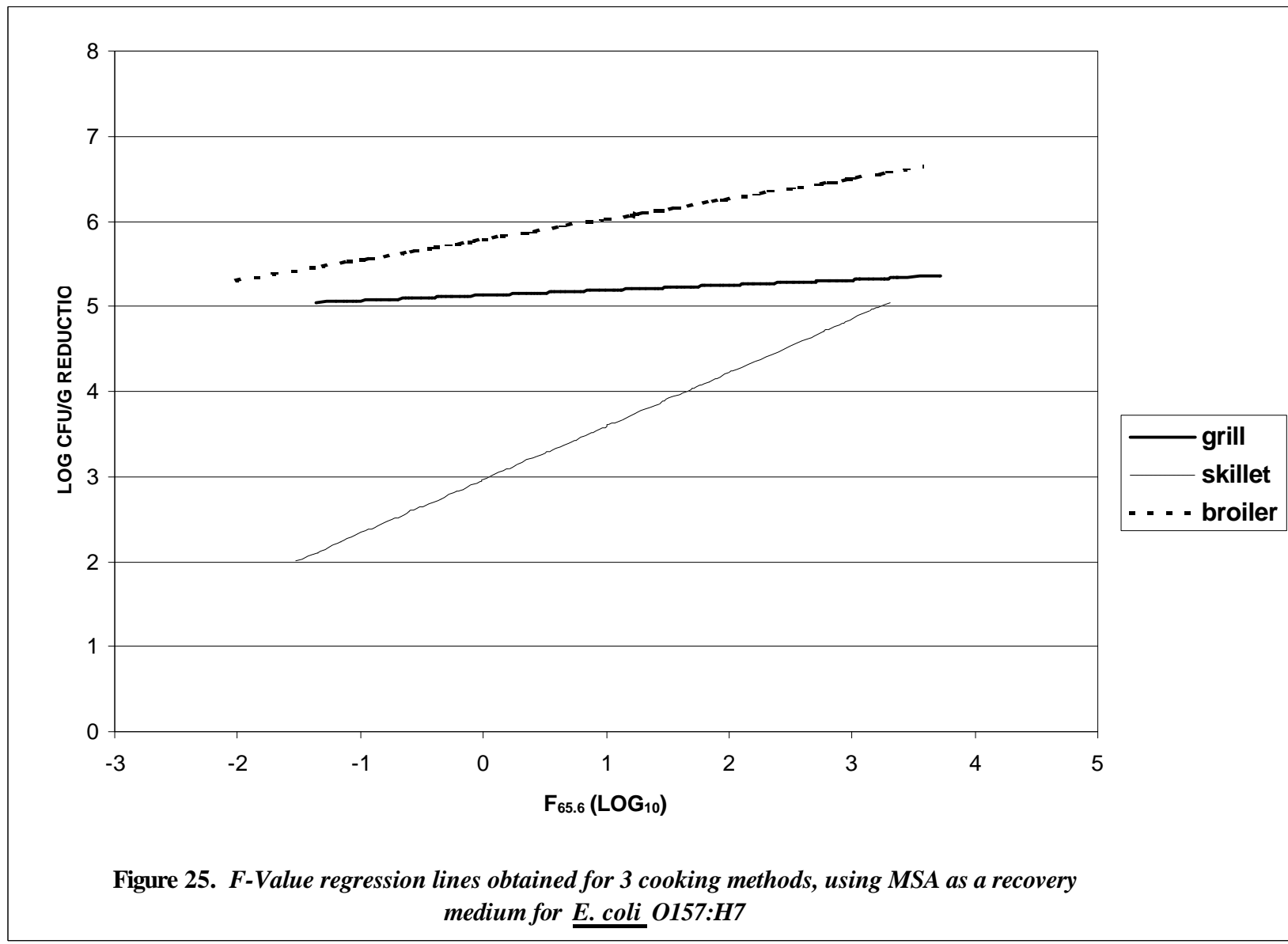


Figure 22. Comparison of F-Values obtained for the grill method, using PRSA as recovery medium for *E. coli* O157:H7



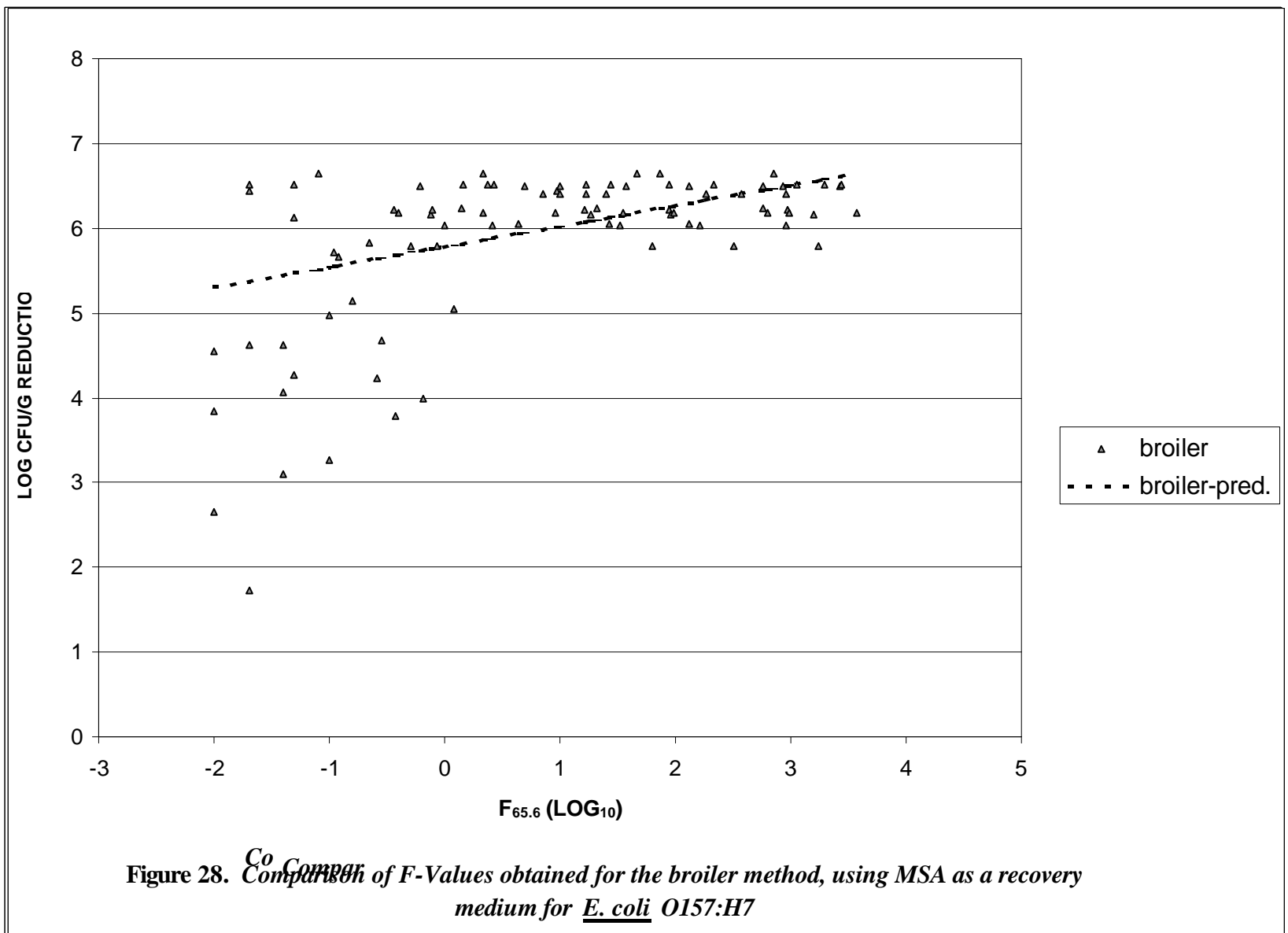
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oon. In this case, limitations created by inoculum level were even more significant, since MSA was not effective for recovering heat injured cells. The agar's low recoverability, paired with extremely high variability in log reductions and cooking parameters which determine F-value, resulted in low, if any correlation between the two variables. This is especially apparent in the grill scatter plot (Fig. 26), where the correlation coefficient was only 0.02. Although the broiler regression line was limited by initial inoculum level, the relatively low variability about the line again helped to improve the correlation slightly ($R^2 = 0.24$). Fig. 28 provides another clear depiction of the broiler method's effectiveness for reducing pathogen populations. This figure shows that the $\log F_{65.6}$ of zero, equivalent to 1 minute at 65.6°C ($\log 1 = 0$), is sufficient to reduce the population by 5 logs or greater.

F-value/reduction correlations observed in the skillet method were similar for both PRSA and MSA data (Figs. 23 and 27). As indicated in previous discussion, variability was quite high, regardless of the recovery method, and a 5 log cycle was not consistently achieved, even in the high range of thermal lethality. Correlation based on the skillet method was not limited by inoculum level, since complete destruction of the organisms was never achieved. Therefore, low correlation coefficients appear to be a result of the random error associated with the process. Overall, the correlation analysis indicates that thermal lethality depends greatly on the efficiency of the cookery method used.

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Blade tenderization was not found to significantly affect the safety of beef steaks when cooked to adequate internal temperatures of 60.0°C and above. Although a 5 log reduction was not necessarily achieved by each cooking method at these temperatures, reductions observed in T and NT steaks were not found to be different ($p>0.05$).

Overall, broiling was the most effective means for achieving 5 log reductions or greater in *E. coli* O157:H7 populations, even at endpoints as low as 60.0°C, and was the most consistent method in regard to cook time. In contrast, the electric skillet produced the least consistent and least effective results. The commercial grill was found to achieve 4-5 log reductions when steaks were cooked to endpoints of 65.6°C and above. However, standard deviations for reductions were rather high, indicating that the margin of safety associated with these temperatures is be less than what bacterial counts suggest. Maximum temperature rise after removal of steaks from the various heat sources were surprisingly similar across the three methods, in each case rising at least 5°C at lower target endpoints, but consistently decreasing as endpoint temperatures increased.

In each cook study, MSA consistently suggested higher reductions than PRSA, offering further support that MSA is not effective

at recovering heat injured cells. This recovery method may provide a false sense of security if used to validate a thermal process. Although PRSA is a less selective medium, allowing for the growth of other organisms present in the sample, it does provide a more accurate and conservative representation of viable *E. coli* O157:H7 cells.

The findings of this study indicate that regulatory requirements considered for the preparation of blade tenderized steaks must be clearly defined in their purpose. If the objective is to achieve a consistent 5 log reduction for *E. coli* O157:H7, as is the requirement for ground beef, internal steak temperatures greater than 60.0°C may be required, depending on the cooking method recommended. If, however, the objective of cooking guidelines is to ensure that the safety of blade tenderized products is equal to that of intact products, 60.0°C appears to be sufficient, regardless of the cooking method employed.

There is an obvious need to identify methods of reducing variability associated with cooking practices commonly used in food service preparation. The commercial gas grill and electric skillet studies clearly illustrate the variation, not only in cooking parameters, but also in bacterial reduction, that occurs from steak to steak, regardless of size or treatment. Unfortunately, the perception by food handlers remains that BT products are “whole muscle” and therefore, internal temperature is not considered a critical factor in regard to product safety. Often, endpoint temperature of these products is estimated by cook time, which may pose a problem when preparing products specified as “rare”. To reduce risks associated with this practice, it is in the best interest of the meat and food service industries to encourage the use of thermometers to determine degree of doneness in all meat products. This philosophy is rapidly being adopted for ground beef preparation,

and would likely prove beneficial for other non-intact products, as well.

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