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MICROBIOLOGICAL, THERMAL INACTIVATION, AND SENSORY  
CHARACTERISTICS OF BEEF EYE-OF-ROUND SUBPRIMALS AND STEAKS  
PROCESSED WITH HIGH-PRESSURE NEEDLELESS INJECTION

by

Laura Kahealani Jefferies

A dissertation submitted in partial fulfillment  
of the requirements for the degree

of

DOCTOR OF PHILOSOPHY

in

Nutrition, Dietetics, and Food Sciences

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Logan, Utah 2011

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## ABSTRACT

Microbiological, Thermal Inactivation, and Sensory Characteristics of  
Beef Eye-of-Round Subprimals and Steaks  
Processed with High-Pressure Needleless Injection

by

Laura Kahealani Jefferies, Doctor of Philosophy

Utah State University, 2011

Major Professor: Dr. Conly L. Hansen  
Department: Nutrition, Dietetics, and Food Sciences

High-pressure needleless injection (HPNI) is a process where small-diameter, high-velocity burst of liquid, penetrate foods at pressures  $\leq 10,000$  psi. The potential of HPNI as an enhancing technique for meat was studied. In study 1, HPNI translocated surface *E. coli* O157 into the interior of beef eye-of-round subprimals with an incidence of 40 ( $\pm 7$ ), 25 ( $\pm 8$ ), and 25 ( $\pm 8$ )% for meat that had been surface-inoculated with a four-strain cocktail at 0.5, 1, and 2  $\log_{10}$  CFU/cm<sup>2</sup>, respectively. Run-off water contained 2, 2, and 3  $\log_{10}$  CFU/ml and was used for HPNI of additional subprimals, which resulted in a cross-contamination incidence of 83 ( $\pm 4$ ), 60 ( $\pm 15$ ), and 37 ( $\pm 6$ ) %, respectively. Incidence of translocation and cross-contamination was similar at all sampled levels below the inoculated surface. Study 1 results indicate that surface microflora will be translocated from the surface into the interior of HPNI-treated beef by the injection fluid and by cross-contamination with recycled fluid.

In study 2, *E. coli* was undetected in cooked steaks (63°C internal) cut from subprimals inoculated with  $2 \log_{10}$  CFU/cm<sup>2</sup> and HPNI processed (study 1). Although cooking reduced *E. coli* counts, determination of complete kill was not possible because the detection limit for bacterial recovery was about  $1 \log_{10}$  CFU/g. Steaks cut from HPNI-processed subprimals took longer ( $p < 0.05$ ) to reach 63°C with grilling or broiling, compared to control steaks, possibly due to increased moisture in enhanced steaks.

In study 3, sensory acceptance of steaks was evaluated by a consumer panel. Appearance, flavor, and overall acceptance were similar among the untreated control, HPNI steaks, blade tenderized steaks (BT steaks), and steaks cut from subprimals that had been needle-injected with 0.35% (wt/vol) sodium tripolyphosphate using needle injection (NI-subprimal steaks) or HPNI (HPNI-subprimal steaks). Texture of BT steaks ( $6.5 \pm 1.9$ ) was more liked than control steaks ( $5.8 \pm 1.8$ ), while texture was similar for all other comparisons. Conversely, Warner-Bratzler shear force was NI-subprimal steaks < control < HPNI steaks = HPNI-subprimal steaks = BT steaks. Lack of correspondence between texture acceptance data and WBSF suggests that sensory scores were influenced by factors other than the force required for mechanical shear.

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Laura Kahealani Jefferies

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## CHAPTER 1

### INTRODUCTION AND OBJECTIVES

#### Introduction

Steak palatability and value are most often determined by juiciness, flavor and texture (tenderness vs. toughness), but of these characteristics, texture is consistently ranked most important by consumers (Brady and Hunecke 1985; Belew and others 2003; Caine and others 2003). Despite general guidelines of predicting beef cut tenderness, USDA grading standards can result in inconsistent tenderness categorization of beef (Wheeler and others 1999). Consequently, some consumers are frustrated with the unpredictability of getting the same quality or tenderness when re-purchasing that same cut (Maltin and others 2003). The lack of consistent predictability of beef tenderness has encouraged researchers and processors to develop ways of increasing beef quality and consistency of cut to meet consumer expectations. Current methods are simple and economical and include the use of tenderizing agents such as marinades, rubs, and glazes, as well as mechanical tenderization and enhancement processes such as tumbling, blade tenderization, and needle-injection of flavoring solutions, water binding ingredients and tenderizing agents.

Mechanical tenderization and enhancement processes, such as blade tenderization and needle injection, use sharp blades or needles, respectively, to penetrate the meat's surface to improve texture and overall palatability by severing muscle and connective tissue and/or introducing enhancing liquids into its interior. In the United States, nearly all beef steaks and roasts served in restaurants, hotels, and those for institutional use may

be mechanically tenderized (USDA-FSIS 2002). Some might also be sold to the public through retail stores (USDA-FSIS 2004) or door-to-door vendors (Laine and others 2005). Of the cuts available, one that can benefit greatly from mechanical tenderization is the eye of the round (Jeremiah and others 1999). This elongated, naturally boneless cut comes from the semitendinosus muscle at the rear of the animal and is characterized as being very tough due to high amounts of connective tissue.

In addition to blade tenderization and needle injection, there exists an emerging beef enhancement process of high-pressure needleless injection (HPNI) (Hendricks and Hansen 1991; Hansen and Watts 2004; Jefferies and Hansen 2010). HPNI is a process that uses small diameter, high velocity liquid jets to penetrate soft foods without the use of needles, blades, or other contacting devices. High-pressure liquid bursts that can be controlled to 10,000 psi penetrate the product surface to introduce enhancing fluids into its interior. HPNI has been used to add moisture, oil, flavors, spices, color, salt, enzymes, preservatives, acidulants, and minerals to cheese, meat, poultry, fish, vegetables and fruits (Lee and others 1978; Hendricks and Hansen 1991; Berry 2002; Pastorino and others 2003a,b,c; Hansen and Watts 2004).

With HPNI processing, a liquid injectant is placed in a balance tank and is pumped through the system using a high-pressure, positive displacement piston pump which runs on relatively low pressure compressed air. The solution is then directed to one or more injection heads via high-pressure hoses and tubing; its flow is regulated by a solenoid-controlled high-pressure air-actuated valve. Each injection head typically has several nozzles arranged side-by-side, 1 cm apart. Products to be injected are placed on a

conveyor belt which passes the product under the injection heads. Solution is discharged simultaneously from all nozzles within a single head while the conveyor belt pauses so that the product remains stationary during injection. After each burst, the conveyor belt advances, then pauses again, so that the food may receive another injection of liquid. A small number of studies confirm that *E. coli* (Sporing 1999; Luchansky and others 2008) and other natural microflora (Hajmeer and others 2000) can be translocated (moved from the surface to the interior) during blade tenderization of beef. The associated hazard is that such bacteria may not be exposed to the recommended minimum cooking temperatures that ordinarily kill those on the surface, and instead, remain viable, causing illness or even death (De Zuniga and others 1991; Tompkin and others 2001; USDA-FSIS 2002; Gill and McGinnis 2004; Stopforth and others 2006; Sofos and others 2008).

Thermal inactivation of translocated *E. coli* O157 in HPNI processed beef is unknown. The recommended endpoint temperature for highest eating quality of beef eye-of-round steaks is 63°C (NCBA 2007). USDA-FSIS recommends that intact steaks be cooked to a minimum internal temperature of 63°C/145°F (medium rare) (USDA-FSIS 2002), and recommends that non-intact beef products be cooked to a minimum internal temperature of 68°C/155°F (between medium rare and medium) regardless of cooking method (USDA-FSIS 2009).

Furthermore, subjective and objective data regarding the effect of HPNI in improving beef sensory acceptance is limited, although findings by Ricks and others (1998) indicated that beef tenderness, as measured by Warner-Bratzler Shear Force was

improved using HPNI. Considering the need to understand the characteristics of this emerging technology, the objectives of this research were as follows:

### Objectives

1. To determine the incidence and depth to which *Escherichia coli* O157 strains are translocated from the inoculated surface of beef eye-of-round subprimals and to determine the incidence and depth to which *Escherichia coli* O157 strains in recycled enhancing fluid are injected into beef eye-of-round subprimals by high-pressure needleless injection.
2. To determine the degree of bacterial kill realized by oven broiling and gas grilling beef eye-of-round steaks that have been previously inoculated *Escherichia coli* O157 strains, followed by high-pressure needleless injection processing.
3. To determine sensory acceptance high-pressure needleless injection processed beef eye-of-round steaks and subprimals processed and to compare them to steaks and subprimals processed using blade tenderization and needle injection and an untreated control.

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## CHAPTER 2

### LITERATURE REVIEW

#### **Beef Overview**

“Beef” is the term used to describe meat from mature cattle. When beef is harvested from cattle, the carcass is fabricated (sliced) into preliminary groups of muscle. These initial subdivisions are called primal or wholesale cuts because it is at this stage that they are usually boxed and sold to wholesale meat markets or butchers to be portioned for retail sale or to be furthered processed. The four main primal cuts are the round, loin, rib, and chuck. Smaller cuts of beef taken from a primal cut are called subprimals. Subprimals can be sold “as is” or can be divided for retail sale.

Americans eat an average of about 60 lbs of beef annually (USDA- FSIS 2007). Results from a 2005 survey concluded that beef is most often eaten as ground beef, followed by consumption as deli products and steaks (Melusky 2006). According to this same survey, nearly half of Americans choose steak as their most preferred form of beef. Steak palatability and value are most often determined by juiciness, flavor, and texture (tenderness vs. toughness), but of these characteristics, texture is consistently ranked most important (Brady and Hunecke 1985; Belew and others 2003; Caine and others 2003). In general, there are four variables that are central in determining meat texture: post-mortem proteolysis, amount of intramuscular fat, type and amount of connective tissue, and the contractile state of the muscle (Belew and others 2003). Beef cuts may consist of a single muscle or several muscles and cuts of beef from certain muscles are more tender than others. In general, the most tender, and therefore, more expensive cuts

come from the loin and the rib. The toughest cuts of meat come from the front and the rear of the animal (chuck and round, respectively) because those muscles are used for movement. One cut from the round that can benefit greatly from tenderization techniques is the eye of the round (Jeremiah and others 1999) which is the elongated, naturally boneless cut that comes from the semitendinosus muscle. It is considered to be very tough due to high amounts of connective tissue.

All beef in the United States is inspected by the U.S. Department of Agriculture (USDA) for wholesomeness, and while such inspection is mandatory, quality grading of beef is voluntary. Present USDA quality grading standards are based primarily on the amount and distribution of intramuscular fat or marbling in the rib eye muscle at the sliced surface between the 12<sup>th</sup> and 13<sup>th</sup> rib. Quality categories of beef sold at the retail level are Prime, Choice, and Select. USDA Prime beef comprises approximately 2% of graded beef and consists of the most tender and flavorful cuts because they have more fat marbling. USDA Choice and Select are the quality grades of beef most often sold in grocery stores. The majority of beef carcasses consist of lower-valued, less tender cuts (Molina and others 2005). Research shows that economic value from the lower rated cuts has not increased as much as those from the more tender and expensive loin and rib which consumers perceive as having greater value. Although providing general guidelines of predicting beef cut tenderness, USDA grading standards can result in inconsistent tenderness categorization of beef (Wheeler and others 1999). As a result, some consumers are frustrated with the unpredictability of getting the same quality or tenderness of beef when re-purchasing that same cut (Maltin and others 2003).

The lack of consistent predictability of beef tenderness has encouraged researchers and processors to develop ways of increasing beef quality to meet consumer expectations. Current methods are simple and economical and include the use of tenderizing agents such as marinades, rubs, and glazes and mechanical tenderization processes such as tumbling, blade tenderization, and needle-injection of flavoring solutions and tenderizing agents. In the United States, nearly all beef steaks and roasts served in restaurants, hotels, and those for institutional use may be mechanically tenderized (USDA-FSIS 2002). Some may also be sold to the public through retail stores (USDA-FSIS 2004) or door-to-door vendors (Laine and others 2005). Meats that are mechanically tenderized are defined as non-intact meats, while those whose interior has not been cut or penetrated are called intact meats.

### **Mechanical Tenderization and Enhancement**

Two common mechanical tenderization and enrichment processes are that of blade tenderization and needle injection. Blade tenderization is performed with one or more sets of dozens of double-edged stainless steel blades or knives that penetrate beef subprimals or steaks to cut muscle and connective tissue and thereby tenderize them. Meat is placed on a conveyor belt, and depending on the unit, blades enter the meat perpendicular to its surface or at an angle. Meat may be passed more than once under the blades.

Needle injection is a tenderizing and enhancing process where either a single or multiple hollow needles inject various whole muscle products such as ham, roasts, and turkey with curing brine, marinades, tenderizing solutions, or other ingredients. This

process also cuts muscle tissue to improve tenderness while increasing moisture content. Needles are pierced perpendicular to the product surface after which solutions are injected. In multiple needle systems, injection pressure and frequency can usually be adjusted which results in very uniform and consistent distribution of brine and marinade solutions (Brandt 1996). In addition to blade tenderization and needle injection, an emerging enhancing process called high-pressure needleless injection (HPNI) offers another option.

HPNI is an enhancement process that uses small diameter, high velocity liquid jets to penetrate soft foods without the use of needles, blades, or other contacting devices (Hendricks and Hansen 1991; Hansen and Watts 2004; Jefferies and Hansen 2010). High-pressure bursts of liquid that can range between 1,000 to 10,000 psi penetrate the product surface to introduce enhancing fluids into its interior. HPNI has been used to add moisture, oil, flavors, spices, color, salt, enzymes, preservatives, acidulants and minerals to cheese, meat, poultry, fish, vegetables and fruits (Lee and others 1978; Hendricks and Hansen 1991; Berry 2002; Pastorino and others 2003a,b,c; Hansen and Watts 2004). Non-food applications of high velocity liquid jets include the cutting and fragmenting of hard materials such as stone and ice, cleaning processing equipment, injecting fluids into soft materials and measuring physical properties (Robertson and Berry 1976).

Published consumer acceptance data for HPNI processed foods is lacking, although consumers report that injection holes in the surface of injected meat are very slight. Cheese injected with blueberry and sour apple flavors were accepted positively by an informal test of children ages 10-12 (Berry 2002).

HPNI systems have been manufactured for continuous or batch processes. Units are designed so that the liquid injectant is placed in a balance tank and is pumped through the system using a high-pressure, positive displacement piston pump which runs on relatively low pressure compressed air. Due to the small diameter of nozzles used to create the injection jets, injecting solutions must be particulate free. To ensure this, the solution flows through a gravity-fed filter followed by a high-pressure-high-output in-line filter. The solution is then directed to one or more injection heads via high-pressure hoses and tubing; its flow is regulated by a solenoid-controlled high-pressure air-actuated valve. Each injection head typically has several nozzles arranged side-by-side, 1 cm apart. Solution is discharged simultaneously from all nozzles within a single head which can be aimed at different angles, if desired. While nozzle diameter is fixed, jet diameters generally range from 0.005 – 0.5 mm, depending on the pressure of the liquid.

In the continuous system, products to be injected are placed on a conveyor belt which passes the product under the injection heads. During each injection burst, the conveyor belt pauses. A control panel regulates injection pressure and burst duration, delay between injections, and conveyor belt speed. In turn, these variables determine the density of the injection pattern and depth of liquid penetration. Injected from the meat side, HPNI has injected fish 6 mm thick to turkey breast > 150 mm thick; however, it has been most successful in food no greater than 100 mm thick (Hansen and Watts 2004). Cleaning is performed with a clean in place system and through the application of topical cleaners and antimicrobials.

## Microbiological Safety of Mechanically Tenderized Beef

The Centers for Disease Control and Prevention (CDC) estimate that 5 million cases of food borne illness annually in the United States are due to undercooked meats and meat products (CDC 2008). Of these, it is estimated that at least one third of the 5,000 deaths attributable to food-borne illnesses are due to contaminated meat and poultry. Primary pathogens of concern in meat products in the United States are *Salmonella*, *Campylobacter jejuni*, *Listeria monocytogenes*, and *Escherichia coli* O157:H7.

*Escherichia coli* O157:H7 is one of hundreds of *E. coli* bacterium strains and is the most common Shiga toxin-producing strain. While some strains are harmless, *E. coli* O157:H7 can cause human diseases such as diarrhea, severe stomach cramps, vomiting, and fever, and can result in serious, life threatening illnesses such as hemorrhagic colitis (bloody diarrhea) and hemolytic uremic syndrome (HUS), particularly in young children and the elderly. It is a facultative anaerobic, Gram-negative, single-celled rod that grows between 7 - 50°C and optimally at 37°C. It can survive at pH 4.4 and in foods with  $a_w = 0.95$ . Incubation after exposure ranges from 3 – 8 days.

*E. coli* O157:H7 is part of the natural microbial flora of ruminant animals including cattle, goats, sheep, deer, and elk. Infection in humans occurs from ingesting the microorganism through contaminated raw or undercooked food, untreated water and unpasteurized milk or juices. In beef, contamination can occur anywhere between the farm, to manufacture, processing and preparation. *E. coli* O157:H7 was first identified as a food borne pathogen in 1982 when it was associated with undercooked ground beef, a

source that continues to be linked to numerous outbreaks and recalls involving this bacterium (Rangel and others 2005). Since 2000, there have been five reported outbreaks, with one as recent as December 2009, associated with *E. coli* O157:H7 in beef that had been mechanically tenderized rather than ground (USDA-FSIS 2007, 2009).

A small number of studies confirm that *E. coli* (Luchansky and others 2008; Spring 1999) and other natural microflora (Hajmeer and others 2000) can be translocated (moved from the surface to the interior) during blade tenderization of beef. The associated hazard is that such bacteria may not be exposed to the recommended minimum cooking temperatures that ordinarily kill those on the surface, and instead, remain viable, causing illness or even death (De Zuniga and others 1991; Tompkin and others 2001; USDA-FSIS 2002; Gill and McGinnis 2004; Stopforth and others 2006; Sofos and others 2008). While quantification of a definitive infectious dose of *E. coli* O157:H7 is complex, some researchers suggest that it is low (Mead and Griffin 1998), with estimates of <50 organisms (Tilden and others 1996) and even < 10 organisms (Greig 2010) although specific foods or portions are not specified. While a “serving” was not defined in this estimate, USDA quantifies a serving of beef steak to be 99 – 113 grams (USDA 2011).

While there is no industry-wide baseline on the incidence of *E. coli* on beef cuts, it is estimated that the national incidence of *E. coli* O157:H7 in ground beef is 0.17% - 0.18% which is thought to translate to about the same degree of frequency on the surface of whole muscle beef cuts (Stopforth and others 2006). Kennedy and others (2006) concluded that the incidence of *E. coli* O157:H7 on subprimal beef cuts intended for



mechanical tenderization was <0.83%. Likewise, Heller and others (2007) reported that the incidence of *E. coli* on subprimal beef cuts is minimal (0.2% out of 1014 samples) with a mean concentration of the bacteria of <0.375 CFU/cm<sup>2</sup> on samples testing positive. USDA estimates that 98% of time, steaks contaminated with *E. coli* O157:H7 have a single *E. coli* organism per serving prior to cooking (USDA 2002).

### **Efforts to Reduce the Risk of *E. coli* O157:H7 Contamination in Beef**

All segments of the beef industry, from calf/cow producers, feedlot operators, fabricators and processors, to retail and foodservice companies, have worked separately and collectively to address and try to eliminate risks posed by *E. coli* O157:H7. In 1993, there were no regulations on this pathogen. By 1994, the U.S. Department of Agriculture, Food Safety and Inspection Service (USDA-FSIS) declared *E. coli* O157:H7 to be a food adulterant, and in 1999 the policy was expanded to include non-intact beef products (USDA-FSIS 1999). In 2002, USDA-FSIS required manufacturers of mechanically tenderized beef products to reassess their Hazard Analysis Critical Control Point (HACCP) plans to take into account *E. coli* O157:H7 contamination risk.

The following year, the National Cattleman's Beef Association (NCBA) organized the Beef Industry *E. coli* Summit Meeting where approximately 200 beef industry leaders, representing every segment from farm to market, met with the objective of working to reduce and eventually eliminate *E. coli* O157:H7 in the beef supply. Among the focus and research needs identified were to develop science-based performance standards for non-intact products, to verify safe cooking temperatures for blade/needle-injected products, to validate cooking equipment temperature/time, and to

determine intervention and decontamination strategies of raw products (USDA-FSIS 1999). Despite these efforts, the risk of *E. coli* contamination in non-intact beef remains significant; in 2007 the CDC recently reported that incidences of *E. coli* O157:H7 infections had increased since 2004 (CDC 2008). The increase in *E. coli* O157:H7 recalls prompted USDA-FSIS to again reassess the prevalence of the pathogen and instructed beef processors to reassess their HACCP programs and implemented a food safety checklist (USDA-FSIS 2007). Presently, the USDA-FSIS requires beef processors to perform *E. coli* enumeration counts to confirm the control of the slaughter process, yet no guidelines regarding the microbial load for fresh beef cuts are in effect (USDA-FSIS 1999).

Previous research regarding *E. coli* O157:H7 translocation and cross-contamination in beef by Stopforth and others (2006), determined that beef cut contamination type and amount may be influenced by the part of the carcass from which the cut originated and concluded that when contamination occurs, its levels range from 0.8 – 1.0 mean log CFU<sub>10</sub>/ml. The study also concluded that the incidence of *E. coli* O157:H7 on whole muscle cuts (0.3%) was similar to that estimated in ground beef. It also suggested that while there are regulatory efforts to control *E. coli* O157:H7 in ground beef, that the similar incidence of it in mechanically processed beef warrants greater attention.

A benchmark study performed at Kansas State University (Spring 1999) confirmed that *E. coli* O157:H7 was translocated by blade tenderization throughout beef muscle and concluded that 3 – 4% of surface organisms were pushed into the center of

the meat, as deep as 6 cm, regardless of initial starting concentrations. The conclusions of this study have strongly influenced subsequent USDA research, protocol and recommendations. Today, the incidence and prevention of *E. coli* O157:H7 is still of considerable interest to USDA-FSIS where studies continue to confirm the translocation of *E. coli* O157:H7 in blade tenderized steaks (Luchansky and others 2008; Ray and others 2010).

The effect of HPNI on microbial translocation and cross-contamination using equipment made for the purpose of mechanically tenderizing and enhancing meat is minimal. Ray and others (2010) used a manual, single-injection-at-a-time instrument, ordinarily used for livestock injections, on beef strip loins and concluded that *E. coli* could be translocated at 25 psi. Similarly, a study on the effect of high-pressure water jets on the penetration of bacteria during beef carcass washing concluded that bacteria were more likely to be driven deeper into tissue as pressure increased (De Zuniga and others 1991). A similar study by Anderson and others (1991) demonstrated that surface bacteria were translocated into muscle at fluid pressures > 100 psi. Some report that high-pressure jets cause less cross-contamination in foods than needle injection (Robertson and Berry 1976; Lee and others 1978; Ricks and others 1998). One such study compared the level of cross-contamination of natural microflora on chicken breasts enhanced with recycled injection fluid using HPNI to that of needle injection (Ricks and others 1998). Results showed that the degree of cross-contamination in chicken breasts by HPNI was significantly less than that by needle injection.

The degree and depth of bacterial translocation by any mechanical tenderization process depends on a variety of factors, such as the specific tenderizing method, variations within that method (Gill and McGinnis 2004), the injectant, and the specific matrix of the target food (Lee and others 1978; Anderson and others 1991). Likewise, the incidence of *E. coli* O157:H7 translocation by HPNI is likely to depend such characteristics of the jet velocity and resulting pressure (Smith and Kinslow 1976; Anderson and others 1991; De Zuniga and others 1991), injection density and pattern (Hansen and Watts 2004), volume delivered (Robertson and Berry 1976; Lee and others 1978), nozzle type (De Zuniga and others 1991) and diameter (Lee and others 1978), as well as residence time of the jet against the target medium. Injectant characteristics such as viscosity, temperature (Nezgada 1973; Lee and others 1978), and perhaps dissolved particles may also play a role, as well as physical and chemical characteristics of the injected medium including pump yield and initial microbial type, levels, and distribution (Ray and others 2010).

Some investigators have concluded that the risk of *E. coli* O157:H7 translocation in mechanically tenderized beef is nominal, due in part to the increased risk management measures directed at the bacteria from feedlot to market that have taken place in recent years which make its incidence and surface concentrations very low (Gill and others 2005; Heller and others 2007; Ray and others 2010). Nevertheless, the potential microbial translocation risks associated with mechanical tenderization continue to be a source of attention and concern.

### **Thermal Inactivation of Translocated *E. coli* O157**

Americans overwhelmingly prefer outdoor grilling over other methods when cooking steaks; oven broiling is the next preferred method (Melusky 2006). This same survey reported that 49% of respondents preferred their steaks medium rare to medium and that 45% preferred their steaks medium to well done. USDA-FSIS recommends that intact steaks be cooked to a minimum internal temperature of 63°C/145°F (medium rare) (USDA-FSIS 2002). It further recommends that non-intact beef products be cooked to a minimum internal temperature of 68°C/155°F (between medium rare and medium) regardless of cooking method (USDA-FSIS 2009). It also reports that there is sufficient anecdotal evidence that consumers frequently eat blade tenderized meat, particularly steaks, cooked to rare or medium rare endpoints, and believes that these levels of doneness are insufficient to destroy *E. coli* O157:H7 in the interior of the meat (USDA-FSIS 2002). Related challenges are that many consumers do not measure the internal temperature of their steaks to determine doneness and rely instead on visual clues, such as the color of the interior of the meat (Neely and others 1999) and that consumers are unaware that beef has been mechanically tenderized at all (Stopforth and others 2006).

Data reporting the thermal inactivation of translocated *E. coli* O157:H7 when heated to various endpoint temperatures by different cooking methods differs widely. Consequently, the effectiveness of heat in destroying *E. coli* O157:H7 translocated by mechanical tenderization or moisture enhancement is still uncertain (Mukherjee and others 2008). Spring (1999) studied the effectiveness of various cooking methods (commercial gas grill, electric skillet, and oven broiling) in reducing numbers of

translocated *E. coli* O157:H7 in steaks. It was concluded that oven broiling was most effective in reducing the pathogen's level while the electric skillet was least effective. The study also reported a 5 log<sub>10</sub> CFU/g reduction of *E. coli* O157:H7 in beef sirloin steaks broiled to 60°C while Ortega-Valenzuela and others (2001) observed a 2.70 log<sub>10</sub> CFU/g reduction when restructured beef steaks were broiled to 63°C. Both studies agreed that thermal death of the bacteria was less effective by grilling as Sporing observed that steaks needed to be cooked to 65.6 °C in order to achieve the same log reduction as that achieved through broiling. Ortega-Valenzuela and others (2001) reported a reduction of only 1.25 log<sub>10</sub> CFU/g *E. coli* O157:H7 by grilling. These differences were believed to be due to the higher cooking temperatures achieved during grilling which allowed the meat to reach the target temperature faster than by broiling. Therefore, the meat was not exposed to heat as long and, consequently, fewer bacteria were destroyed. Sporing concluded that blade tenderized steaks should be cooked to an internal temperature of 60°C by oven broiling to eliminate risk of this pathogen and that by doing so, beef so tenderized does not pose a greater risk to consumers than intact meat.

Other studies report that even steaks cooked to 71.1°C on an open hearth Faberware electric grill still had translocated *E. coli* O157:H7 present (Patel and others 2005). Conversely, Luchansky and others (2008) determined that a grilling temperature as low as 48.8°C was sufficient enough to reduce that initial *E. coli* load of approximately 4.0 log<sub>10</sub> CFU/g by 2.6 to 4.2 log<sub>10</sub> CFU/g. Gill and others (2005) determined that low levels of bacteria in needle injected pork brine was likely destroyed at 61°C and were

completely destroyed at 70°C. They, therefore, concluded, that cooking to the USDA-recommended temperature of 63°C would render the product safe.

USDA-FSIS believes that additional research to quantify *E. coli* O157:H7 survival in blade-tenderized meat is required and currently recommends that beef that has not been mechanically tenderized, be cooked to a minimum of 63°C and that mechanically tenderized beef be cooked to yet an even higher endpoint of 68°C (USDA-FSIS 2009), regardless of cooking method. Of the few studies published on thermal inactivation of *E. coli* O157:H7 in non-intact beef (Spring 1999; Patel and others 2005; Luchansky and others 2008), none have addressed this issue with regard to HPNI processed beef.

A number of factors influence heat resistance of translocated bacteria in beef, such as microbial species, product attributes such as muscle type, pH, the presence and distribution of fat, the presence of additives, and tenderizing method. One study compared the thermal resistance of ground vs. whole muscle cuts of beef purposely contaminated with *Salmonella* and concluded that whole muscle may offer more protection for embedded bacteria than ground muscle because more homogeneous fat distribution in ground meat may “dilute” its ability to shield the bacteria from heat (Orta-Ramirez and others 2005). Moreover, additives such as salts, lactates, and phosphates may increase thermal resistance of pathogens (Orta-Ramirez and others 2005; Mukherjee and others 2008). Additionally, levels of surviving *E. coli* O157:H7 depend on the initial level of contamination, the cooking method used, the cooking temperature, and the duration of cooking (USDA-FSIS 2002). Similarly, the rate of heat penetration into meat can be influenced by the energy supply rate, heat conduction within the meat, changes that occur

in the meat due to heating, size and shape of the product, meat composition, and muscle fiber orientation in relation to the heat source (Obuz and others 2001). Furthermore, studies conclude that thicker steaks require longer cooking times than thinner steaks, which therefore, leads to greater destruction of bacteria in thicker steaks because of their longer exposure to heat (Spring 1999; Ortega-Valenzuela and others 2001; Luchansky and others 2008).

### **Beef Tenderness**

Studies that assess the tenderness of beef typically compare the findings of human sensory evaluation to those of objective tests (Brooks and others 2000; Peachey and others 2002; Caine and others 2003). Questions posed through sensory tests typically assess acceptance of a food's attributes such as overall, appearance, aroma, flavor and texture. Questions about the ideality of certain levels of other attributes such as color or flavor intensity may also be asked. Panelists may also rank samples in order of preference. Consumer sensory data is nearly always essential in predicting product improvement, quality, or market potential.

The most commonly used objective tool for assessing beef tenderness is the Warner-Bratzler (WBSF) shear force method (Brady and Hunecke 1985). The WBSF method is performed by coring a sample of cooked meat and measuring the force required to shear it perpendicular to the meat grain. WBSF values  $<3.9$  and  $>4.6$  kg<sub>f</sub> are considered slightly tender and slightly tough, respectively (Shackelford and others 1991).

Beef tenderness studies often compare the data from human sensory evaluation and objective tests such as WBSF (Brooks and others 2000; Peachey and others 2002;



Caine and others 2003). The purpose such comparison is often done to determine whether WBSF is an accurate predictor of human perception and consumer acceptance of texture. Nevertheless, numerous attempts have frequently resulted in a wide range of inconsistent correlations ranging from 10 – 89% (Caine and others 2003; Lorenzen and others 2003). Standardized procedures (AMSA 1995; Wheeler and others 1999) for performing WBSF tests and conducting sensory analysis are attempts to increase consistency among researchers. Still, correlations and conclusions between them continue to vary. Correlations between these objective and subjective tests may be best when the samples of the same muscle fiber orientation were used for both tests (Poste and others 1993).

Further challenges to correlation may be inherent when attempting to compare subjective and objective data between and among muscle types (Belew and others 2003) including those specifically from the round. Kolle and others (2004) reported that steaks from the round were inconsistent in tenderness. A study of different tenderization treatments of chuck muscles determined that tenderness was not consistent between muscle types and concluded that inconsistencies may be due to physical and chemical variations within muscle types and cooking methods (Molina and others 2005). Brooks and others (2000) concluded that choice and select quality grades had no effect on WBSF values or sensory scores for eye-of-round samples.

Cooking method also influences beef tenderness data. Kolle and others (2004) reported that when eye-of-round subprimals were cooked using dry heat, such as clam shell grilling to 71°C, that there was no improvement in WBSF tenderness scores and that

steaks from eye-of-round subprimals produced lower (more tender) WBSF readings when they were cooked using moist heat methods.

Since tenderness is the primary factor in determining consumer satisfaction of beef, both producers and researchers are interested in safely and economically providing this. Traditional tenderization and enhancement techniques have been shown to translocate and cross-contaminate bacteria during processing (Spring 1999; Hajmeer and others 2000; Luchansky and others 2008), which poses a potential safety concern if beef products are undercooked (De Zuniga and others 1991; Tompkin and others 2001; USDA-FSIS 2002; Gill and McGinnis 2004; Stopforth and others 2006; Sofos and others 2008). The microbiological, thermal inactivation, and sensory characteristics of the emerging beef technology of high-pressure needleless injection have not been studied, and are therefore, the impetus for this research.

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CHAPTER 3

TRANSLOCATION AND CROSS-CONTAMINATION OF  
*ESCHERICHIA COLI* O157 IN BEEF EYE-OF-ROUND SUBPRIMALS  
PROCESSED WITH HIGH-PRESSURE NEEDLELESS INJECTION

Abstract

High-pressure needleless injection (HPNI) is an emerging enhancing process where small-diameter, high-velocity bursts of liquid penetrate soft foods at pressures up to 10,000 psi. The incidence and depth of translocated surface *E. coli* O157 in HPNI processed beef eye-of-round subprimals was determined. HPNI translocated *E. coli* O157 from the surface to the interior of eye-of-round subprimals with incidence of 40 ( $\pm 7$ ), 25 ( $\pm 8$ ), and 25 ( $\pm 8$ ) % for subprimals that had been surface-inoculated with a four strain cocktail at 0.5, 1, and 2  $\log_{10}$  CFU/cm<sup>2</sup>, respectively. The run-off water was collected and found to contain 2, 2, and 3  $\log_{10}$  CFU/ml *E. coli* O157, respectively. The runoff was used for HPNI of additional eye-of-round subprimals, and this resulted in a cross contamination incidence of 83 ( $\pm 4$ ), 60 ( $\pm 15$ ), and 37 ( $\pm 6$ ) %, respectively. Incidence of translocation and cross contamination was similar at 0 - 1, 1 - 2, 2 - 3, 3 - 4, 4 - 6, and 6 - 8 cm below the inoculated surface. Results indicate that surface microflora on beef will be carried to the interior of HPNI treated beef by initial translocation from the surface with the injected fluid and by cross contamination with recycled fluid.



## Introduction

*Escherichia coli* O157:H7 was first identified as a food borne pathogen in 1982 when it was associated with undercooked ground beef, a source that continues to be linked to numerous outbreaks and recalls involving these bacteria (Rangel and others 2005). Since 2000, there have been five reported *E. coli* O157:H7 outbreaks, with one as recent as December 2009, associated with beef that had been mechanically tenderized (USDA-FSIS 2007, 2009). Mechanical tenderization processes, such as blade tenderization and needle injection, use sharp blades or needles, respectively, to penetrate the meat's surface to improve texture and/or introduce enhancing liquids into its interior. In the United States, nearly all beef steaks and roasts served in restaurants, hotels, and for other institutions may be mechanically tenderized (USDA-FSIS 2002). Some are available through retail stores (Gill and McGinnis 2004; USDA-FSIS 2004) or door-to-door vendors (Laine and others 2005).

A small number of studies confirm that *E. coli* (Luchansky and others 2008; Spring 1999) and other natural microflora (Hajmeer and others 2000) can be translocated (moved from the surface to the interior) during blade tenderization of beef. The associated hazard is that such bacteria may not be exposed to the minimum recommended cooking temperatures needed to destroy them and instead, remain viable, causing illness or even death (De Zuniga and others 1991; Tompkin and others 2001; USDA-FSIS 2002; Gill and McGinnis 2004; Stopforth and others 2006; Sofos and others 2008). While quantification of a definitive infectious dose of *E. coli* O157:H7 is complex, some researchers suggest that it is low (Mead and Griffin 1998), with estimates

of <50 organisms (Tilden and others 1996) and even < 10 organisms (Greig 2010) although specific foods or portions were not stated. It is estimated that 98% of time, steaks contaminated with *E. coli* O157:H7 have a single *E. coli* organism per serving prior to cooking (USDA 2002). While a “serving” was not defined in this estimate, USDA quantifies a serving of beef steak to be 99 – 113 grams (USDA 2011).

High-pressure needleless injection (HPNI) is an emerging enhancement process that uses multiple small diameter, high-velocity, discontinuous liquid jets instead of traditional needles or blades (Jefferies and Hansen 2010; Hendricks and Hansen 1991; Hansen and Watts 2004). Liquid bursts can be controlled between 1000–10,000 psi and are dispensed from nozzles above the product to penetrate its surface to introduce enhancing fluids into its interior. HPNI has been used to add moisture, oil, flavors, spices, color, salt, enzymes, preservatives, acidulants and minerals to cheese, meat, poultry, fish, vegetables and fruits (Lee and others 1978; Hendricks and Hansen 1991; Berry 2002; Pastorino and others 2003a,b,c; Hansen and Watts 2004;).

The effect of HPNI on microbial translocation using equipment made specifically for the purpose of enhancing meat with high-pressure liquid jets is minimal. Ray and others (2010) used a manual, single-injection-at-a-time instrument, ordinarily used for livestock injections, on beef strip loins and concluded that *E. coli* could be translocated at 25 psi. A study where the effect of high-pressure water jets on the penetration of bacteria during beef carcass washing concluded that bacteria were more likely to be driven deeper into tissue as pressure increased (De Zuniga and others 1991). A similar washing study

by Anderson and others (1991) demonstrated that surface bacteria were translocated into muscle at fluid pressures > 100 psi.

With regard to cross contamination, some report that high-pressure jets cause less cross-contamination in foods than needle injection (Robertson and Berry 1976; Lee and others 1978; Ricks and others 1998). One such study compared the levels of cross-contamination of natural microflora on chicken breasts processed with HPNI to that of needle injection (Ricks and others 1998). Results showed that the degree of cross-contamination, caused by using recycled injection fluid, was significantly less by HPNI than that by needle injection.

The objective of this study was to determine the incidence and depth to which *E. coli* O157 strains are translocated from the inoculated surface of beef eye-of-round subprimals processed with HPNI. It was of further interest to determine the incidence and depth of cross-contamination that occurred through recirculated enhancing. It was hypothesized that, like customary mechanical tenderization and enhancement methods, translocation and cross-contamination in HPNI treated beef would occur.

## Materials and Methods

### **Inoculum preparation**

A cocktail of two *E. coli* O157:H7 strains (93.0055, 93.0138), one O157:H12 strain (6.2571) and one O157:NM strain (99.1224) was prepared. All strains were isolated from beef and were obtained from The Pennsylvania State University *E. coli* Reference Center (University Park, Pa., U.S.A.). Individual cultures were prepared from thawed freezer stocks by inoculating separate Erlenmeyer flasks, each containing 50 ml

of Tryptic Soy Broth (TSB), with 1  $\mu\text{L}$  of culture. Cells were incubated at  $37^{\circ}\text{C}$  for 22 – 26 hours, without shaking, to obtain stationary-phase growth. The individual cultures were then combined to form a cocktail inoculum culture. Serial dilutions of the combined inoculum were plated onto Petrifilm™ Coliform Count Plates (3M Corp., St. Paul, Minn., U.S.A.) and incubated for 24 hours to determine viable cell counts. Three inoculum levels (3, 2, 1  $\log_{10}$  CFU/ml) were prepared from the cocktail to deliver final target surface counts of 2, 1, 0.5  $\log_{10}$  CFU/cm<sup>2</sup>. Inoculum levels were selected based on the levels used by Luchansky and others (2008). The cocktail was then transferred to a sterile, high density polyethylene, calibrated spray bottle (Sprayco, Detroit, Mich., U.S.A.) for surface inoculation of subprimals. Work using these bacterial strains was performed at Brigham Young University (Provo, Utah, U.S.A.) with approval from the University Risk Management Office.

### **Translocation Study**

Fresh, unfrozen eye-of-round subprimals (IMPS, NAMP #171c), ~8 cm thick, were obtained from a local meat packing facility within 24 hours of harvest and were stored at  $4^{\circ}\text{C} \leq 7$  days after receipt. Sections of surface fat, if any, were trimmed. Three subprimals were randomly selected to determine surface counts of naturally occurring *E. coli* O157:H7, if any. Three subprimals were randomly assigned to each of the 3 inoculum level treatments. One inoculum level treatment and injection was performed per day. Duplicate trials were performed on separate days and the order of each inoculum treatment and duplicate were randomized.

Each subprimal was mist inoculated individually, under a biological hood, using the previously mentioned spray bottle which was calibrated to deliver 0.75 ml/pump, according to the method of Sporing (1999). Four pumps were administered per subprimal. All working surfaces were sterilized with ethanol between inoculation of individual subprimals. Each inoculated subprimal was then aseptically transferred, inoculated side facing up, to separate, sterile, covered aluminum foil containers. To allow for bacterial adhesion the subprimals were held at 4°C for 30 - 60 min before HPNI processing (Sporing 1999).

### **High-pressure needleless injection**

Following the bacterial adhesion step, subprimals were removed from their containers and placed longitudinally to their direction of travel, in the center of the conveyor belt of a continuous, in-line process, high-pressure needleless injector (Hansen and Watts 2004; Hendricks and Hansen 1991) with the inoculated surface facing up. Subprimals were spaced ~2 cm apart, one behind the other.

Seven and a half L of sterilized, filtered water (AquaOne, Orem, Utah, U.S.A.) were placed in the balance tank. Water without typical enhancing ingredients was used in order to focus only on the effect of HPNI jets on microbial translocation, similar to Lee and others (1978) who studied the properties of high-pressure water jets on mozzarella cheese. The injectant first flowed through an inline FulFlo pleated, stainless steel wire cloth filter (Parker Hannifin Corp., Indianapolis, Ind., U.S.A.) with a micrometer rating >2, and was pumped to the injection head via Teflon® tubing reinforced with braided stainless steel casing, by a high-pressure, positive pressure piston pump driven by

compressed air. A single injection head traversed the width of the conveyor belt and was comprised of 13 0.0015 cm inner diameter sapphire nozzles (A.M. Gatti, Inc., Trenton, N.J., U.S.A) arranged side-by-side, 1 cm apart. The mean distance between the top surface of the subprimals and the nozzle openings was ~4 cm.

Liquid jet injection pressure was 3000 psi. Preliminary work determined that a combined injection pressure of 3000 psi and injection burst duration of 1.5 seconds, while the conveyor belt remained stationary, would allow the injectant to penetrate each subprimal 7.5 to 8 cm, without passing through. After each injection burst, the conveyor belt advanced 0.5 cm. Jet diameter varied, but was generally between 0.5 – 2.0 mm. Subprimals were passed once under the injection head. The run-off injectant was not recycled, but was recovered in a sterile container for use in each subsequent and accompanying cross-contamination trial. After injection, subprimals were aseptically removed from the conveyor belt and immediately returned, inoculated side up, to their original, covered containers. Each injected subprimal was held at -18°C for 2 hours to facilitate core sampling and slicing for microbial analysis.

### **Cross-contamination study**

Immediately following each translocation trial, three uninoculated subprimals were injected using the run-off liquid collected from the preceding translocation study. Aliquots of this injectant were sampled in duplicate immediately after the earlier treatment to determine *E. coli* concentration. Subprimal pre- and post-injection handling and injection parameters were otherwise identical to those of the translocation study. Contaminated injectant was recycled during injection.

Between accompanying translocation and cross-contamination trials, the injector was completely disassembled, cleaned and sanitized with a commercial detergent and quaternary ammonia. Sterilization was confirmed by swabbing both critical and random locations and by collecting and testing the first water to flow through the nozzles at the beginning of each trial, for presence of *E. coli*.

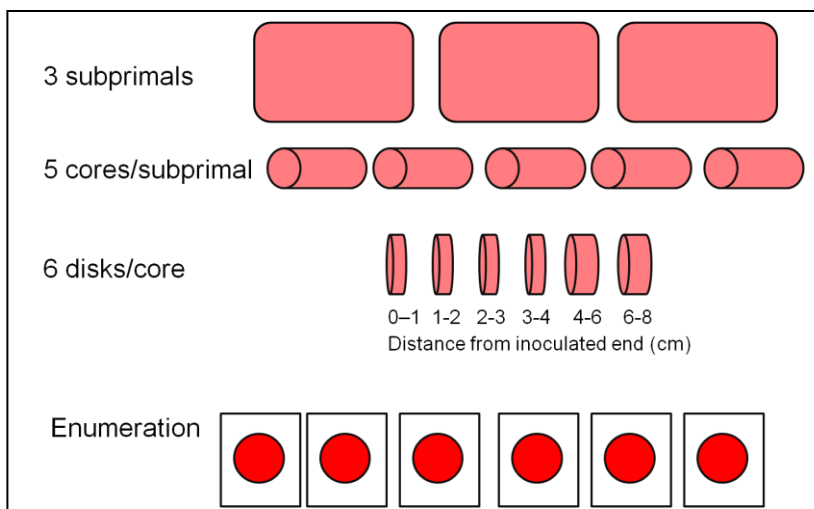
### **Microbial analysis**

For both studies, injected subprimals were then transferred from -18°C storage and held at 4°C ≤ 2 hours until they were sampled for microbial analysis. Core samples were aseptically removed from injected subprimals in order to recover translocated *E. coli*, if any. Sampling procedures were based on those used by other researchers (Sporing 1999; Luchansky and others 2008). To do this, each subprimal was aseptically transferred onto a sterile acrylic sheet surface under a biological hood with the inoculated surface face down. A stainless steel coring device (4.3 cm diam.) was pressed through the uninoculated surface, parallel to the direction of injection, to excise a core. The coring device was pressed through the uninoculated side of the subprimal to prevent surface inoculum from contaminating the cores. Five cores were sampled from the midline of each subprimal. The coring device was ethanol and flame sterilized between each sampling.

In order to determine if *E. coli* were translocated to various depths of the subprimals, the cores were aseptically sliced across muscle fibers using an ethanol and flame sterilized scalpel and a sterile cutting guide into disks 0 - 1, 1 - 2, 2 - 3, 3 - 4, 4 - 6, and 6 - 8 cm from the inoculated surface (Figure 3-1). After coring, ~2 mm of the non-

inoculated surface was removed from its respective core to remove any inoculum, if any, that may have touched it during any previous step. Disks were aseptically transferred to individual sterile filter bags (Nasco, Modesto, Calif., U.S.A.), and weighed. A 0.1% peptone (Biotrace International, Muncie, Ind., U.S.A.) solution was added to each disk at a 1:10 w/w dilution. Contents were stomached (Smasher, AES Laboratoire, Rennes, France) for 2 minutes. One ml of filtered slurry was transferred onto Petrifilm Coliform Count Plates (3M Corp., Minneapolis, Minn., U.S.A.) and incubated for 22-26 hours at 37°C before testing for presence of *E. coli*. The detection limit of the Petrifilm™ Coliform Count Plates used, with a single replication, is such that samples with fewer than 10 CFU/ml cannot be detected. Consequently, Petrifilm™ with no discernable growth was counted as negative for incidence of *E. coli*.

Figure 3-1: Sampling procedure to quantify number of disks testing positive for *E. coli* O157 at various subprimal depths.





Actual surface inoculum concentrations were quantified by mist inoculating, but not processing with HPNI, two subprimals per inoculation level. Five cores were removed from each subprimal, with *E. coli* counts determined for the top 1 cm disk and were reported as CFUlog<sub>10</sub>/g. The total time between coring and plating samples from a single subprimal was < 15 minutes. The procedures for determination of incidence and depth of translocated inoculum were likewise used to determine the same for cross-contamination by recycled run off liquid.

### **Statistical Analysis**

Data were analyzed for significance by Chi-square analysis to determine significant differences among the percent disks testing positive for *E. coli* O157 among sample core depth using Excel 2007 (New York, N.Y., U.S.A.). Significant differences were defined as  $P < 0.05$ . Percent positive samples were determined by dividing the number of total disks sampled per core depth into the number of disks testing positive for *E. coli*.

## **Results and Discussion**

### **Translocation**

Actual mean surface inoculum concentrations were 2 ( $\pm 0.30$ ), 1 ( $\pm 0.30$ ), and 0.5 ( $\pm 0.05$ ) log<sub>10</sub> CFU/cm<sup>2</sup>. Percentage of samples testing positive for translocated *E. coli* O157 is shown in Table 3-1. Samples from all subprimal depths tested positive for translocated *E. coli*. At the 2 CFU log<sub>10</sub>/cm<sup>2</sup> inoculum level, the amount of samples testing positive ranged from 27 – 47% throughout the depth of the subprimal with a mean

of 40 ( $\pm 7$ )%. At the 1 CFU  $\log_{10}/\text{cm}^2$  inoculum level, the amount of samples testing positive ranged from 13 – 33%, and at the 0.5  $\log_{10}/\text{cm}^2$  inoculum level, 13 – 43% of the samples tested positive throughout all subprimal depths. Mean translocation at these levels was 25 ( $\pm 8$ ) and 25 ( $\pm 12$ ) %, respectively. Trends in the quantity of positive disks at each depth of translocated *E. coli* in this study are not evident as the percentage of disks testing positive at each depth did not differ significantly within each surface inoculum concentration level ( $P < 0.05$ ).

Table 3-1. Translocation Study: Percent samples testing positive for *E. coli* O157 recovered from core samples at various depths in beef eye-of-round subprimals inoculated at different initial surface concentrations and processed with HPNI.

Mean initial surface concentrations $\log_{10}$ CFU/cm <sup>2</sup> (SD) of <i>E. coli</i> O157 on control beef eye-of-round subprimals <sup>A</sup>			
	2 ( $\pm 0.30$ )	1 ( $\pm 0.5$ )	0.5 ( $\pm 0.05$ )
Depth of core samples (cm)	Percentage of samples testing positive for <i>E. coli</i> (%) <sup>B</sup>		
Surface to 1	37 <sup>a</sup>	23 <sup>a</sup>	17 <sup>a</sup>
1 - 2	27 <sup>a</sup>	30 <sup>a</sup>	13 <sup>a</sup>
2 - 3	43 <sup>a</sup>	33 <sup>a</sup>	13 <sup>a</sup>
3 - 4	43 <sup>a</sup>	33 <sup>a</sup>	33 <sup>a</sup>
4 - 6	47 <sup>a</sup>	17 <sup>a</sup>	33 <sup>a</sup>
6 - 8	43 <sup>a</sup>	13 <sup>a</sup>	43 <sup>a</sup>
Mean % translocation	40 ( $\pm 7$ )	25 ( $\pm 8$ )	25 ( $\pm 12$ )

Values with like superscripts within each column are not significantly different from one another. ( $P < 0.05$ ),  $\chi^2$  (5, n=180) = 0.28, 0.01, and  $8.1 \times 10^{-7}$  for surface concentrations of 2, 1, and 0.5  $\log_{10}$  CFU/cm<sup>2</sup>, respectively.

<sup>A</sup> n=10 for each inoculum level

<sup>B</sup> n=30 for each core depth

The observation that surface *E. coli* are translocated throughout the entire depth of the subprimal seems likely attributable to the high-pressures used in this study, as the combined injection pressure of 3000 psi and injection burst duration settings were intentionally calibrated to allow the injectant to penetrate its entire depth. This agrees with a beef carcass washing study by De Zuniga and others (1991) where it was concluded that higher fluid pressures result in the translocation of more surface bacteria than lower fluid pressures and that bacteria are more likely to be driven deeper into muscle tissue as fluid pressure increases.

It also seems likely that the penetration holes created by the injecting jets create individual channels in the subprimal which in turn, allows for the flow of injectant throughout its depth. The movement of liquid within these channels could potentially carry *E. coli* and, therefore, the position of translocated bacteria at the time of sampling and may not reflect initial bacterial translocation depth. Ray and others (2010) also observed the development of such channels at pressures > 25 psi in their study using the one-dose-at-a-time needleless injector.

Blade tenderization studies (Spring 1999; Luchansky and others 2008), as well as the needleless single dose injector experiment (Ray and others 2010), generally concluded that *E. coli* counts are highest near the inoculated surface and decrease with increasing depth. Spring (1999) reported that 3 – 4% of surface *E. coli* was translocated to the geometric center of blade tenderized beef top butt subprimals where surface inoculum concentrations were 3 and 6 log<sub>10</sub> CFU/cm<sup>2</sup>. These studies do not report the

penetration depth of the blades or liquid jets and the pressures used, if reported, were far below the 3000 psi used in the present study.

### **Cross-Contamination**

Recovery of positive samples of *E. coli* O157 injected into the interior of uninoculated subprimals through contaminated injectant collected is shown in Table 3-2. Mean initial concentration of *E. coli* O157 in run-off injectant from the initial  $2 \log_{10}$  CFU/cm<sup>2</sup> surface contamination was  $3 \log_{10}$  CFU/ml. At this level of injectant contamination, the number of samples testing positive at each core depth ranged from 77 – 90% with a mean of 83 ( $\pm 4$ )%. Mean initial concentration of *E. coli* O157 in run-off injectant for both 1 and  $0.5 \log_{10}$ /cm<sup>2</sup> initial surface concentrations was  $2 \log_{10}$  CFU/ml. The number of samples testing positive ranged from 30 – 80% and 30 – 47% at the 1 and  $0.5 \log_{10}$ CFU/cm<sup>2</sup> original surface contamination levels, respectively. Mean cross contamination at these levels was 60 ( $\pm 15$ ) and 37 ( $\pm 6$ )% respectively. There was no significant difference in the percentage of positive samples at each depth at each original inoculation level ( $P < 0.05$ ).

Generally, the percentage of samples testing positive are higher than those from the translocation data, since the run-off water is a combination of all the surface inoculation run-off and suggests that contaminated run-off water injected directly into subprimals results in higher contamination than that which occurs solely by translocation. Cross-contamination results also show no specific trends in percentage of positive disks at each depth, and may again, be due to the free-movement of fluid in the channels created by the liquid jets, referred to earlier. Results indicate that recirculating solutions

Table 3-2. Cross-contamination Study: Percent samples testing positive for *E. coli* O157 recovered from core samples at various depths in beef eye-of-round subprimals processed with HPNI using recirculated, contaminated run-off liquid from the corresponding translocation study.

Mean initial surface concentrations $\log_{10}$ CFU/cm <sup>2</sup> (SD) of <i>E. coli</i> O157 on control beef eye-of-round subprimals <sup>A</sup>			
	2 ( $\pm$ 0.30)	1 ( $\pm$ 0.5)	0.5 ( $\pm$ 0.05)
Mean initial concentrations $\log_{10}$ CFU/ml (SD) of <i>E. coli</i> O157 in run-off liquid injected into non surface inoculated beef eye-of-round subprimals processed with HPNI <sup>A</sup>			
	3 ( $\pm$ 2.3)	2 ( $\pm$ 0.9)	2 ( $\pm$ 1.2)
Depth of core samples (cm)	Percent samples testing positive for <i>E. coli</i> (%) <sup>B</sup>		
Surface to 1	83 <sup>a</sup>	63 <sup>a</sup>	40 <sup>a</sup>
1 - 2	77 <sup>a</sup>	60 <sup>a</sup>	30 <sup>a</sup>
2 - 3	90 <sup>a</sup>	63 <sup>a</sup>	37 <sup>a</sup>
3 - 4	83 <sup>a</sup>	63 <sup>a</sup>	40 <sup>a</sup>
4 - 6	83 <sup>a</sup>	80 <sup>a</sup>	47 <sup>a</sup>
6 - 8	83 <sup>a</sup>	30 <sup>a</sup>	30 <sup>a</sup>
Mean % cross-contamination	83 ( $\pm$ 4)	60 ( $\pm$ 15)	37 ( $\pm$ 6)

Values with like superscripts within each column are not significantly different from one another. ( $P < 0.05$ ),  $\chi^2$  (5, n=180) = 0.96,  $5.0 \times 10^{-4}$ , and 0.32 for run-off liquid concentrations of 3, 2, and 2  $\log_{10}$  CFU/cm<sup>2</sup>, respectively.

<sup>A</sup> n=2 for each inoculum level

<sup>B</sup> n=30 for each core depth

that become contaminated with bacteria can be carried into a meat piece upon injection.

As for the bacteria not accounted for in either the translocation or cross-contamination study, other research offers a possible explanation. Luchansky and others (2008) found that 45 – 63% of inoculated bacteria were recovered on the blades of the tenderizer, and that *E. coli* not accounted for was assumed to be on various contact surfaces including the conveyor belt. Other investigators have shown that recirculation of microorganisms in injectant solutions during needle injection can cross-contaminate other products such as

pork loins (Greer and others 2003) yet could possibly be minimized through filtration of large particles which may harbor more bacteria (Gill and others 2005). In the present study, particles of beef and fat were observed in the run-off liquid. In the translocation study, injecting liquid was not allowed to recirculate; in the cross-contamination study, where run-off liquid was allowed to recirculate, particulates were filtered. Bacteria that may have been on particulate matter or equipment contact surfaces was not quantified.

### Conclusions

It is concluded that HPNI, with pressures as high as 3000 psi and a penetration density of 0.5 x 1 cm, can translocate *E. coli* O157 from the surface of beef subprimals at inoculation levels above those that are typically found and that bacteria can be distributed as deep as the jets penetrate. As demonstrated in other studies where injecting solutions were recirculated, it is not surprising that cross-contamination of foods that follow later in a process occurs when preceding products contaminate the injectant. There was no evidence of trends in the depth of contamination in samples testing positive, in either the translocation or cross-contamination studies, other than that positive samples were found at every level. This may suggest that development of channels by high-pressure liquid jets allows for the movement of enhancing fluid to move throughout the depth to which it has penetrated. Continued efforts to minimize bacterial contamination of beef during pre-fabrication steps, the use of good manufacturing practices during fabrication and processing, the application of antimicrobial agents on the surface of meat or in enhancement solutions are recommended steps toward minimizing surface contamination that could lead to translocation incidence during processing.

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CHAPTER 4

THERMAL INACTIVATION OF *ESCHERECHIA COLI* O157  
IN BEEF EYE-OF-ROUND STEAKS PROCESSED  
WITH HIGH-PRESSURE NEEDLELESS INJECTION

Abstract

High-pressure needleless injection (HPNI) is a novel process where soft foods are penetrated by small-diameter, high-velocity bursts of liquid up to 10,000 psi to enhance them with liquids. Thermal inactivation of an *E. coli* O157 cocktail in beef eye-of-round steaks processed using HPNI was determined by cooking by consumer oven broiling and gas grilling. It was hypothesized that at an initial *E. coli* O157 surface concentration of  $2 \log_{10}$  CFU/cm<sup>2</sup> that any microorganisms translocated into the interior of subprimals treated with HPNI would be reduced to about 1 log when the steaks from the subprimals were cooked to an internal temperature of 63°C the recommended endpoint temperature for highest eating quality of beef eye-of-round steaks and the USDA minimum recommended endpoint temperature for intact beef. A mixture of 4-strain *E. coli* strains was applied to the surface of the subprimals at a  $2 \log_{10}$  CFU/cm<sup>2</sup> concentration. Inoculated subprimals were injected with filtered, sterile water using HPNI at 3000 psi, then divided into 2.54 cm thick steaks. HPNI processed and control steaks were cooked to 63°C by both methods. No microorganisms were recovered from steak samples, indicating a log reduction of translocated *E. coli* of at least  $0.5 \log_{10}$  CFU/g. As the detection limit for the bacterial enumeration method used is  $1 \log_{10}$  CFU/g, it is not possible to state that because *E. coli* was not recovered, that it was completely destroyed.

For both cooking methods, HPNI processed steaks took significantly longer ( $13.98 \pm 2.4$  minutes) to reach  $63^\circ\text{C}$  compared to control steaks ( $12.73 \pm 3.76$  minutes) which was likely due to the increased moisture content of the injected meat. Grilled, control steaks reached the endpoint temperature significantly faster ( $9.74 \pm 2.02$  minutes) than HPNI processed grilled steaks ( $13.48 \pm 2.5$  minutes), HPNI processed broiled steaks ( $14.48 \pm 2.29$  minutes), and control broiled steaks ( $15.73 \pm 2.4$  minutes). Since grilling temperatures were higher than broiling temperatures, beef steaks reached the endpoint temperature faster when they were grilled. It was concluded that subprimals processed with HPNI that are subsequently sliced into steaks that are consumer oven-broiled or gas grilled to the suggested endpoint temperature for highest eating quality of beef eye-of-round steaks and the minimum USDA-FSIS recommended temperature for intact beef of  $63^\circ\text{C}$ , reduced surface *E. coli* of  $2 \text{ CFU log}_{10}/\text{cm}^2$  to an undetectable quantity of about  $1 \text{ log}_{10} \text{ CFU/g}$ . It was also concluded that gas grilling is a faster cooking method for 2.54 cm thick steaks that have been HPNI processed than oven broiling, due to the higher temperatures associated with gas grilling.

### Introduction

In 1982, *Escherichia coli* O157:H7 was first identified as a food borne pathogen when it was associated with undercooked ground beef, a source that continues to be linked to numerous outbreaks and recalls (Rangel and others 2005). There have been five reported outbreaks (USDA-FSIS 2007) since 2002 associated with *E. coli* O157:H7 in beef that has been mechanically tenderized, the most recent of which was in December 2009 (USDA-FSIS 2009). Blade tenderization and needle injection are mechanical

tenderization processes that use sharp blades or needles, respectively, to penetrate the meat's surface to improve texture and/or introduce enhancing liquids into its interior.

High-pressure needleless injection (HPNI) is an emerging enhancement process (Hendricks and Hansen 1991; Hansen and Watts 2004; Jefferies and Hansen 2010) where multiple small diameter, high-velocity, discontinuous liquid jets penetrate the meat instead of blades or needles. Liquid bursts can be controlled between 1,000–10,000 psi and are dispensed from nozzles above the product to penetrate its surface. HPNI has been used to add moisture, oil, flavors, spices, color, salt, enzymes, preservatives, acidulants and minerals to cheese meat, poultry, fish, vegetables and fruits (Lee and others 1978; Berry 2002; Pastorino and others 2003a,b,c; Hansen and Watts 2004).

A handful of studies confirm that *E. coli* (Spring 1999; Luchansky and others 2008) and other natural microflora (Hajmeer and others 2000) can be translocated (moved from the surface to the interior) during blade tenderization of beef. The associated risk is that such bacteria may not reach the recommended minimum cooking temperatures needed to destroy them (De Zuniga and others 1991; Tompkin and others 2001; USDA-FSIS 2002; Gill and McGinnis 2004; Stopforth and others 2006; Sofos and others 2008). Quantification of a definitive infectious dose of *E. coli* O157:H7 is complex, though some researchers suggest that it is low (Mead and Griffin 1998), with estimates of <50 (Tilden and others 1996) and even <10 organisms (Greig 2010) although specific foods or portions were not reported. It is estimated that 98% of time, steaks contaminated with *E. coli* O157:H7 have a single *E. coli* organism per serving prior to

cooking (USDA 2002). While a “serving” was not defined in this estimate, USDA quantifies a serving of beef steak to be 99 – 113 grams (USDA 2011).

According to surveys, Americans overwhelmingly prefer outdoor grilling over other methods when cooking steaks; oven broiling is the next preferred method (Melusky 2006). The United States Department of Agriculture – Food Safety Inspection Service (USDA-FSIS) reports that there is sufficient anecdotal evidence that consumers frequently eat blade tenderized meat, particularly steaks, cooked to rare (60°C) or medium rare (63°C) endpoints and believes that these endpoint temperatures are insufficient to destroy *E. coli* O157:H7 that may be in the interior of the meat (USDA-FSIS 2002). Yet, the National Cattlemen’s Beef Association (2007) recommends that beef eye-of-round steaks be cooked to a maximum endpoint temperature of 63°C for optimum eating quality.

Based on the research of Sporing (1999), it was determined that mechanically tenderized beef does not pose a greater risk to consumers when it is cooked to a minimum internal temperature of 60°C. Yet, current USDA recommendations are that mechanically tenderized beef be cooked to yet an even higher endpoint of 68°C (USDA-FSIS 2002), regardless of cooking method. The USDA minimum recommended temperature for intact beef is 63°C (medium rare). Of the few studies published on thermal inactivation of *E. coli* O157:H7 in non-intact beef (Sporing 1999; Patel and others 2005; Luchansky and others 2008), none have addressed this issue with regard to HPNI tenderized beef. Consequently, the effectiveness of heat in destroying *E. coli*

O157:H7 translocated by mechanical tenderization or moisture enhancement is unknown (Mukherjee and others 2008).

The objective of this study was to determine the degree of bacterial kill realized by oven broiling and gas grilling beef eye-of-round steaks that had been previously inoculated with *E. coli*, followed by HPNI processing. It was hypothesized that at an initial *E. coli* O157 surface concentration of  $2 \log_{10}$  CFU/cm<sup>2</sup> that any bacteria translocated into the interior of the beef would be reduced to about 1 log when steaks were cooked to 63°C for intact steaks by both oven broiling and gas grilling. It was of further interest to determine whether the cooking time to an internal temperature of 63°C would differ between the two cooking methods and between HPNI processed and untreated control steaks.

## Materials and Methods

### **Inoculum preparation**

A cocktail of two *E. coli* O157:H7 strains (93.0055, 93.0138), one O157:H12 strain (6.2571) and one O157:NM strain (99.1224) was prepared. All strains, which were isolated from beef, were obtained from The Pennsylvania State University *E. coli* Reference Center (University Park, Pa., U.S.A.). Individual cultures were prepared from thawed freezer stocks by inoculating separate Erlenmeyer flasks, each containing 50 ml of Tryptic Soy Broth (TSB), with 1 µL culture. Cells were incubated at 37°C for 20 – 22 hours, without shaking, to obtain stationary-phase growth. The individual cultures were then combined to form a cocktail inoculum culture. Serial dilutions of the combined inoculum were plated onto Petrifilm™ Coliform Count Plates (3M Corp., St. Paul, Minn.,

U.S.A.) and incubated for 24 hours to determine viable cell counts. One inoculum level of  $3 \log_{10}$  CFU/ml was prepared from the cocktail to deliver final target surface counts of  $2 \log_{10}$  CFU/cm<sup>2</sup>. The cocktail was then transferred to a sterile, high density polyethylene, calibrated spray bottle (Sprayco, Detroit, Mich., U.S.A.) for surface inoculation of subprimals. Work using these bacterial strains was performed at Brigham Young University (Provo, Utah, U.S.A.) with approval from the University Risk Management Office.

### **Subprimal inoculation**

Fresh, unfrozen eye-of-round subprimals (IMPS, NAMP #171c), ~8 cm thick, were obtained from a local meat packing facility within 24 hours of harvest and were stored at  $4^{\circ}\text{C} \leq 7$  days after receipt. Sections of surface fat, if any, were trimmed. Each subprimal was mist inoculated individually, under a biological hood, using the calibrated spray bottle, according to the method of Sporing (1999). Four pumps were administered per subprimal. All working surfaces were sterilized with ethanol between subprimals. Inoculated subprimals were then aseptically transferred, inoculated side facing up, to separate, sterile, covered aluminum foil containers. To allow for bacterial adhesion the subprimals were held at  $4^{\circ}\text{C}$  for 30 - 60 minutes before HPNI processing (Sporing 1999).

### **High-pressure needleless injection**

Following the bacterial adhesion step, subprimals were removed from their containers and placed longitudinally to the direction of travel, in the center of the conveyor belt of a continuous, in-line process, high-pressure needleless injector (Hansen

and Watts 2004; Hendricks and Hansen 1991) with the inoculated surface facing up.

Subprimals were spaced one behind the other, ~2 cm apart.

Seven and a half L of sterilized, filtered water (AquaOne, Orem, Utah, U.S.A.) were placed in the balance tank. Water without typical enhancing ingredients was used in order to focus only on the thermal destruction of *E. coli* O157 without the potential shielding effects of added ingredients (Orta-Ramirez and others 2005; Mukherjee and others 2008; Byelashov and others 2010). The injectant first flowed through an inline FulFlo pleated, stainless steel wire cloth filter (Parker Hannifin Corp., Indianapolis, Ind., U.S.A.) with a micrometer rating  $>2$ , and was pumped to the injection head via Teflon® tubing reinforced with braided stainless steel casing, by a high-pressure, positive pressure piston pump driven by compressed air. The injection head traversed the width of the conveyor belt and was comprised of 13 0.0015-cm inner diameter sapphire nozzles (A.M. Gatti, Inc., Trenton, N.J., U.S.A) arranged side-by-side, 1 cm apart. The mean distance between the top surface of the subprimals and the nozzle openings was ~4 cm.

Liquid jet injection pressure was 3000 psi. Preliminary work determined that a combined injection pressure of 3000 psi and injection burst duration of 1.5 seconds while the conveyor belt with the subprimals remained stationary, would allow the injectant to penetrate each subprimal 7.5 to 8 cm, without passing through. After each injection burst, the conveyor belt advanced 0.5 cm. Jet diameter varied, but was generally between 0.5 – 2.0 mm. Subprimals were passed once under the injection head and the injectant was not recirculated. After injection, subprimals were aseptically removed from the conveyor belt and immediately returned, inoculated side up, to their original covered



containers. Each injected subprimal was held at  $-18^{\circ}\text{C}$  for 2 hours to facilitate their being sliced into steaks.

After HPNI treatment and holding at frozen temperatures, subprimals were sliced perpendicular to the injection surface using a sharp knife and acrylic cutting guide into individual, 2.54 cm thick steaks. Steaks were randomly assigned to be cooked by electric oven broiling or gas grilling to a final temperature of  $63^{\circ}\text{C}$ . Untreated control steaks that were neither inoculated or HPNI processed were sliced into 2.54 cm-thick widths using the same procedure as those that had been treated.

### **Oven broiling**

General Electric model JSP34 electric ovens (General Electric Company, Louisville, Ky., U.S.A.) were set to “high” and pre-heated for  $\geq 15$  minutes. Steaks were placed on a broiler pan lined with aluminum foil in batches of four from the same treatment. Foil was molded to the pans and slits cut so that juices could drip to the lower pan. Broiler pans were placed on an oven rack 10.5 cm below the heat source. Oven temperature was  $\sim 132^{\circ}\text{C}$ .

### **Gas Grilling**

A propane gas grill (Kenmore Master Flame, Sears, Roebuck and Co., Hoffman Estates, Ill., U.S.A.) was used for grilling. Batches of 4 steaks from the same treatment were placed 11.5 cm above the heat source.

For both cooking methods, internal temperature of each steak was were monitored using 32 gage (0.02 cm), type T (copper and constantan) thermocouples probed through the side, into the geometric center of each steak and data were recorded using a CALPlex

data logger and CalSoft 32 heat penetration software (TechniCal New Orleans, La, U.S.A.). Oven and grill surface temperature was also monitored. Temperature measurements were taken every 15 seconds. For both cooking methods, steaks were turned after they were half way to the target endpoint temperature, after which they continued to cook until they reached the endpoint temperature. Control steaks were cooked using the same cooking methods, to compare heating data between them and for both HPNI processed control steaks. Each cooking method was replicated several times until 21 usable data sets were obtained as some data was deemed unusable for various reasons, such as thermocouple failure, the initial temperature of the steak was too high, or the steak did not reach the target endpoint temperature. Due to variations in initial steak temperature between trials, a standardized start time of when steaks were 21°C was employed to determine cooking time to 63°C. Grill temperature was ~189°C.

### **Microbial analysis**

At the endpoint temperature, steaks were aseptically removed from the oven or grill, immediately quartered using a sterilized knife and immersed in 100 g chilled 0.1% peptone (Biotrace International, Muncie, Ind. U.S.A.) solution in individual sterile sample filter bags (Nasco, Modesto, Calif., U.S.A.). Additional chilled peptone solution was added as needed to result in a 1:10 w/w sample:peptone dilution. Contents were stomached (Smasher, AES Laboratoire, Rennes, France) for 2 minutes. One ml of filtered slurry was transferred onto Petrifilm Coliform Count Plates (3M Corp., Minneapolis, Minn., U.S.A.) and incubated for 22-26 hours at 37°C before enumeration. The same

procedures for determination of *E. coli* remaining after heating by broiling were used to determine the *E. coli* remaining after grilling.

### **Statistical Analysis**

Data were analyzed with two -way analysis of variance (ANOVA) using the general linear model of XLSTAT 2008.7.03 (New York, N.Y., U.S.A.) at a significance level of  $P < 0.05$ . Statistically significant differences between the time to reach the endpoint temperature between cooking methods and mechanical processing treatment were further analyzed using the Tukey's HSD test.

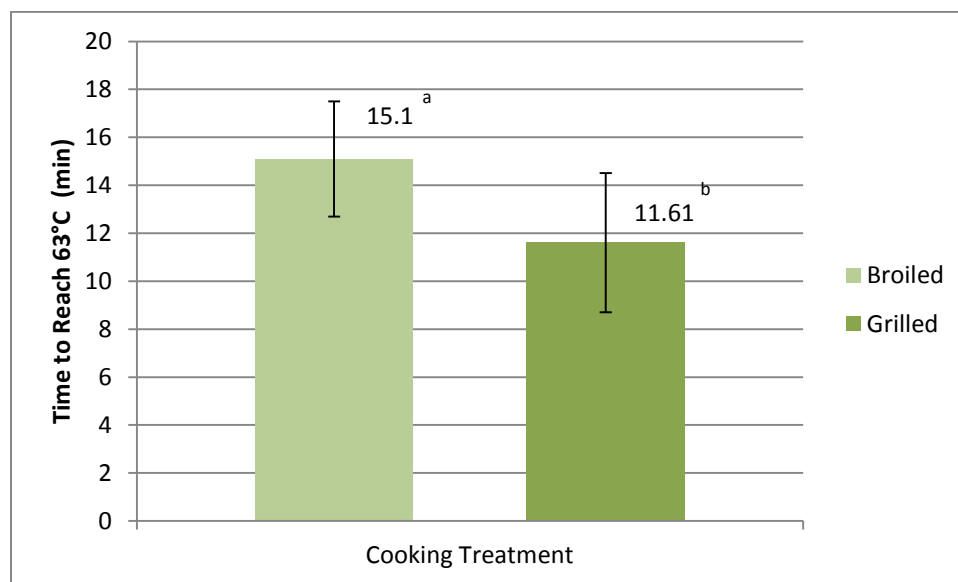
### **Results and Discussion**

Results from a previous study by the authors (Jefferies and others 2011) indicate that initial *E. coli* O157 surface contamination of  $2 \log_{10}$  CFU/cm<sup>2</sup> on beef eye-of-round subprimals resulted in a mean of  $1.53 (\pm 0.11) \log_{10}$  CFU/g bacterial translocation to all depths of the subprimal interior. It is assumed that similar levels of *E. coli* were translocated in the present study. Results from this study show that no *E. coli* was recovered from any of the steaks heated to 63°C, regardless of cooking method. Considering the very low levels of surface inoculum and translocated bacteria, the observation that none could be detected after cooking to 63°C is not surprising. However, it should be noted that the detection limit for the bacterial enumeration method used is 1 log; therefore, it is not possible to say that because *E. coli* was not recovered, that it was completely destroyed. Log reduction of *E. coli* O157, therefore, was at least

0.5. These findings are consistent Ortega-Valenzuela and others (2001) who observed a 2.70 log<sub>10</sub> CFU/g reduction when restructured beef steaks were broiled to 63°C.

Time to reach 63°C by cooking method was compared with results shown in Figure 4-1. For both HPNI and control steaks, the average time for those that were oven broiled to reach 63°C was 15.10 (±2.4) minutes. This was significantly longer (P<0.05) than the average time of 11.61 (±2.9) minutes for steaks to reach that temperature by gas grilling. This agrees with others who have reported that grilled steaks reach their endpoint temperature faster than by oven broiling due to the higher cooking temperatures typically achieved with grilling (Spring 1999; Ortega-Valenzuela and others 2001) although each study varied in the types of cooking equipment and temperatures used.

Figure 4-1. Time to reach 63°C (min) by cooking treatment (P<0.05).



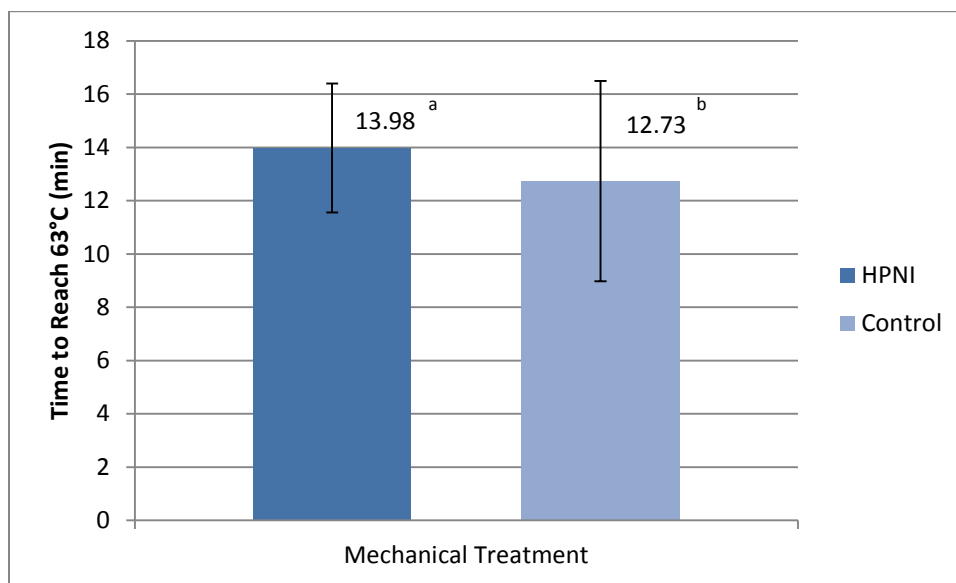
Means with like superscripts are not significantly different from one another.

n = 42 for each treatment

The time to reach 63°C by processing treatment is shown in Figure 4 – 2. For both cooking methods, HPNI processed steaks took an average of 13.98 ( $\pm 2.4$ ) minutes to reach the endpoint temperature which was significantly longer than the 12.73 ( $\pm 3.7$ ) minutes for untreated control steaks. This is thought to be because of the added moisture in the HPNI treated samples, as water has a much higher heat capacity (1 cal/g°C) than beef (0.68 cal/g °C) (The Engineering Toolbox 2011). Consequently, more energy would be required to heat a water-containing beef sample than one without water which would in turn, take more time to reach a certain endpoint temperature. However, Pietrasik and others (2010) reported that steaks from enhanced semitendinosis beef steaks cooked faster than unenhanced controls when cooked using an electric grill while Savell and others (1977) determined that blade tenderized steaks cooked faster than the control.

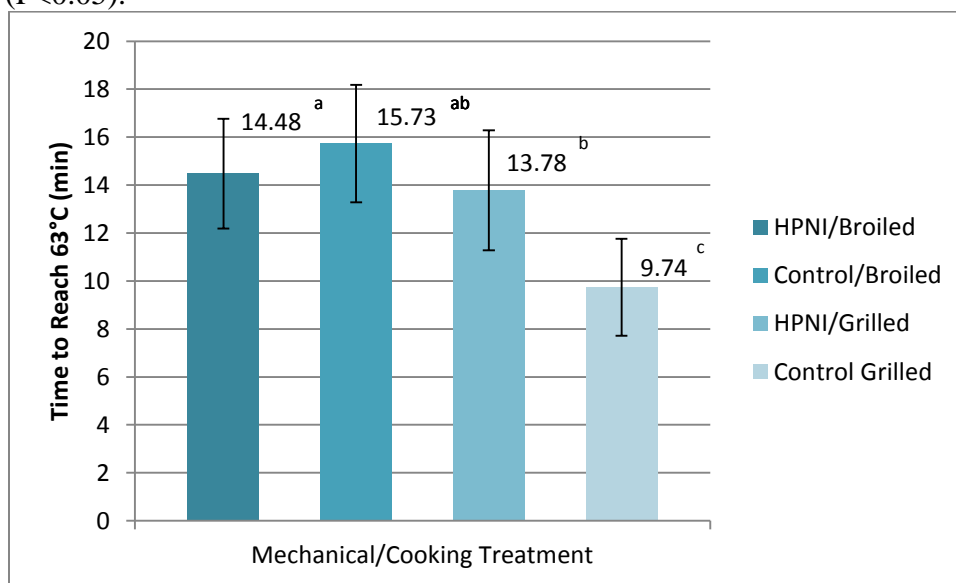
The interaction between processing and cooking methods was determined as shown in Figure 4 – 3. There was no significant difference in the average amount of time it took for broiled steaks to reach 63°C, regardless of whether they had been HPNI treated or not. However, untreated control steaks that were grilled, cooked significantly faster than the other three treatments. Again, this supports the observation that the higher temperatures achieved by grilling cooks steaks faster than by oven broiling, regardless of processing treatment. As thermal destruction of bacteria is a time/temperature relationship, the significance that one cooking method could take less time to heat beef is that most consumers prefer grilling to broiling (Melusky 2006) and do not always use thermometers to determine steak doneness (Jefferies and Hansen 2011).

Figure 4-2. Time to reach 63°C (min) by mechanical treatment ( $P < 0.05$ ).



Means with like superscripts are not significantly different from one another.  
 $n = 42$  for each treatment

Figure 4-3. Time to reach 63°C (min) by mechanical and cooking treatments ( $P < 0.05$ ).



Means with like superscripts are not significantly different from one another.  
 $n = 42$  for each treatment

## Conclusions

It is concluded that subprimals processed with high-pressure needleless injection that are subsequently sliced into steaks that are consumer oven broiled or gas grilled to the recommended endpoint temperature of 63°C for maximum eating quality and the minimum USDA-FSIS recommended temperature for intact tenderized beef, will reduce surface *E. coli* of 2 log<sub>10</sub>/cm by at least 1 log. Therefore, heating to the USDA recommended internal endpoint temperature for non-intact beef of 68°C should likewise, be sufficient to result in a similar or greater bacterial kill, although this temperature is inconsistent with recommendations for highest eating quality of eye-of-round steaks. Further work using higher inoculum concentrations is needed to determine greater log reduction of initial translocated populations. It is also concluded that moisture-enhanced steaks using HPNI take significantly longer to cook than their unenhanced counterparts, by both consumer oven broiling and gas grilling. The addition of enhancement liquid could also increase cooking time to the desired endpoint temperature. Enhanced steaks that were grilled reached their endpoint temperature significantly faster than oven broiled steaks.

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CHAPTER 5  
SENSORY AND INSTRUMENTAL EVALUATION OF  
BEEF EYE-OF-ROUND STEAKS  
PROCESSED WITH HIGH-PRESSURE NEEDLELESS INJECTION

Abstract

High-pressure needleless injection (HPNI) is a novel technique used to enhance meat with moisture. The effect of HPNI on the sensory acceptance of beef eye-of-round steaks was evaluated and compared to steaks processed using conventional tenderization and enhancement techniques. Treatments were untreated control steaks (untreated), steaks processed using HPNI (HPNI), and blade tenderization (BT), and subprimals that were needle (NI-cut from processed subprimals) or high-pressure needleless (HPNI-cut from processed subprimals) injected with 0.35% (wt/vol) sodium tripolyphosphate solution and then cut into steaks. Sensory characteristics were evaluated by 80 consumer panelists using a discrete 9-point hedonic scale. Mean overall, appearance, and flavor acceptance scores between all treatments were not significantly different. Texture acceptance was considered to be significantly more acceptable in BT steaks ( $6.5 \pm 1.9$ ) when compared to the untreated control ( $5.8 \pm 1.8$ ) while HPNI ( $6.2 \pm 1.8$ ) and HPNI – cut from processed subprimals ( $6.0 \pm 1.9$ ) when compared to the untreated control ( $5.8 \pm 1.8$ ) ( $P < 0.05$ ). Sensory ranking data showed that BT and HPNI steaks were ranked better than the other treatments. WBSF mean peak force ( $\text{kg}_f$ ) for HPNI – cut from processed subprimals, HPNI steaks and BT steaks ( $4.2 \pm 1.7$ ,  $4.4 \pm 1.2$ , and  $4.4 \pm 1.5$ , respectively) were significantly higher (required more shear force) than that of NI ( $2.4 \pm 1.1$ ) and the

control ( $3.4 \pm 1.4$ ). Correlation between sensory and WBSF was low ( $r=0.31$ ) suggesting that sensory tenderness liking scores are influenced by other factors than force required for mechanical shear. Sensory results support the hypothesis that consumers would rate HPNI treated beef higher than that of an untreated control.

## Introduction

Beef steak palatability and value are most often judged by its juiciness, flavor and texture (tenderness vs. toughness), but of these characteristics, texture is consistently ranked most important by consumers (Brady and Hunecke 1985; Belew and others 2003; Caine and others 2003). Due to limitations in beef quality grading, some consumers are frustrated with the unpredictability of getting the same quality or tenderness of cut when re-purchasing that same cut (Maltin and others 2003). This lack of consistent predictability has encouraged researchers and processors to develop ways of increasing beef quality and consistency to meet consumer expectations. Current methods are simple and economical and include the use of tenderizing agents such as marinades, rubs, and glazes, as well as mechanical tenderization and enhancement processes such as tumbling, blade tenderization, and needle injection.

Mechanical tenderization processes, such as blade tenderization and needle injection, use sharp blades or needles, respectively, to penetrate the meat's surface to improve texture by severing muscle and connective tissue and/or introduce enhancing and flavoring liquids into its interior. High-pressure needleless injection (HPNI) is an emerging mechanical enhancement process (Hendricks and Hansen 1991; Hansen and Watts 2004; Jefferies and Hansen 2010) where multiple small diameter, high-velocity,

discontinuous liquid jets penetrate the meat instead of blades or needles. Liquid bursts that can be controlled between 1000–10,000 psi, are dispensed from nozzles above the product to penetrate its surface. HPNI has been used to add moisture, oil, flavors, spices, color, salt, enzymes, preservatives, acidulants, and minerals to cheese, meat, poultry, fish, vegetables and fruits (Lee and others 1978; Berry 2002; Pastorino and others 2003a,b,c; Hansen and Watts 2004). However, subjective and objective data regarding its effect on improving beef sensory quality is limited. One study determined that beef tenderness, as measured by Warner-Bratzler shear force, was improved after it had been processed using HPNI (Ricks and others 1998).

According to a 2005 survey (Melusky 2006), nearly half of Americans choose steak as their most preferred form of beef. Of the cuts available, one that can benefit greatly from mechanical tenderization is the eye of the round (Jeremiah and others 1999). This elongated, naturally boneless cut, with high levels of connective tissue, comes from the semitendinosus muscle at the rear of the animal and is considered to be very tough.

The objective of this study was to determine sensory acceptance of HPNI processed beef eye-of-round steaks and subprimals and to compare them to steaks and subprimals processed by blade tenderization and needle injection and an untreated control. It was of further interest to gather information from beef steak consumers about their practices related to purchasing and preparation of beef steaks. It was hypothesized that beef subprimals and steaks treated with HPNI would be liked more than an untreated control.

## Materials and Methods

### **Mechanical treatment of subprimals**

Thirty (6 per treatment group) fresh, eye-of-round subprimals (IMPS, NAMP #171c) ~8 cm thick, were obtained from a local meat packing facility within 24 hours of harvest and were stored at  $4^{\circ}\text{C} \leq 7$  days after receipt. Steers were Angus crosses 18 - 22 months old with choice to high select quality grades. Sections of visible surface fat, if any, were trimmed. Eighteen subprimals were subdivided into 2.54 cm steaks using a sharp knife and acrylic cutting guide, which were randomly assigned to the following three treatment groups: untreated control, steaks to be processed using HPNI (HPNI), and steaks to be processed using blade tenderization (BT). The remaining twelve subprimals were randomly assigned to the following two treatment groups: subprimals to be processed using HPNI and subsequently subdivided into steaks (HPNI – cut from processed subprimals), and subprimals and treated using needle injection and subsequently subdivided into steaks (NI). All processes were performed in a single day, ~72 hours before sensory analysis.

Blade tenderization was performed using a Hollymatic AMT-625B (Hollymatic Corp., Park Forest, Ill., U.S.A.). Two injection bridges, each with forty-eight, 3 mm-wide double-edged blades spaced 1 cm apart, were at 65 and 75 degree angles, respectively, to the steak surface. Penetration depth of the blades was ~3 mm. BT steaks were passed through the blade tenderizer twice, once on each side, in accordance with industry practice.

Both HPNI and NI subprimals and steaks were injected with ambient temperature 0.35% (wt./vol) sodium tripolyphosphate (Nutrifos® 088, ICL Performance Products, St. Louis, Mo., U.S.A.) filtered water (AquaOne Orem, Utah, U.S.A.) solution. Needle injected subprimals were passed once through a Fomaco model FGM (Robert Reiser Co., Inc. Canton, Mass., U.S.A.) set at 40 psi. Needles were spaced 2 cm apart on a single needle bridge. NI subprimals were passed once through the needle injector. HPNI injection of both steaks and subprimals was performed using a high-pressure needleless injector (Hansen and Watts 2004; Hendricks and Hansen 1991). Liquid jet injection pressure was 3000 psi from each of 13 nozzles arranged side by side. Nozzles were 1 cm apart and after each injection burst, the conveyor belt advanced 0.5 cm. Jet diameter varied, but was generally between 0.5 – 2.0 mm. Both steaks and subprimals were passed once through the high-pressure needle injector.

After treatment, subprimals were sliced perpendicular to the injection surface using the sharp knife and acrylic cutting guide described earlier to produce individual, 2.54 cm steaks. All steaks were individually wrapped in Saran™ plastic wrap (SC Johnson, Racine, Wis., U.S.A.) and stored in a single layer at 4° C for <72 hours before cooking and sensory analysis.

### **Sensory analysis**

Sensory analysis was conducted at the Brigham Young University Sensory Laboratory (Provo, Utah, U.S.A.) Eighty consumer panelists, who had positive feelings about and ate steak regularly, evaluated the sensory acceptance of a sample from each treatment. Panelists were recruited from a database of campus and local communities

and were selected based on their willingness to evaluate beef steak. Both genders were equally represented with approximately equal representation among age categories from 18 – 29, 30 – 39, 40 – 49, 50 – 59, and  $\geq 60$  years. The study was approved by the University's Institutional Review Board and panelists provided informed consent. Panelists were compensated monetarily for their time.

Steaks were removed from refrigerated temperature storage and were cooked prior to sensory analysis by oven broiling according to American Meat Science Association (AMSA 1995) guidelines. Five General Electric model JSP34 electric ovens (General Electric Company, Louisville, Ky., U.S.A.) were set to “high” and pre-heated to 163°C for 15 – 20 minutes before cooking. In order to minimize differences in heat distribution and temperature fluctuations in individual ovens, each treatment batch was rotated to cook in a different oven for each of five preparations. Steaks were cooked in batches from the same treatment with four steaks at a time placed on a broiler pan lined with aluminum foil. Foil was molded to the pans and slits cut in the foil to allow juices to drip to the pan below. Each broiler pan was placed on an oven rack 10.5 cm below the heat source. Steaks were turned after reaching an internal temperature halfway between the initial and endpoint temperatures, after which they continued to cook until they reached an internal temperature of 71°C (medium doneness). Steak temperatures were monitored using 32 gauge (0.02 cm), type T (copper and constantan) thermocouple wire inserted into the geometric center of each steak. Oven temperatures were monitored in like manner, with a thermocouple wire placed in the oven. Thermocouples were connected to either a multiple channel data logger (TechniCAL, New Orleans, La.,



U.S.A.) or hand-held digital thermometer (Fluke 51 II, Fluke Corp, Everett, Wash, U.S.A.). Batches of steaks were cooked continuously throughout the sensory panel. After broiling, steaks were sliced parallel to the cooked surface, into 2.54 cm cubes. If not served immediately, sample cubes were held in a covered stainless steel pan on a 77 - 82 °C steam table for no longer than 20 minutes.

The panel was conducted in a single afternoon session within an approximate three hour period. Panelists received all five samples side-by-side using a Williams design to balance the order of presentation (Macfie and others 1989). Each sample was served on individual 15.24 cm diameter Styrofoam plates labeled with three-digit blinding codes. Panelists were instructed to use a bite of unsalted cracker and a sip of bottled water to refresh their sense of taste between samples. Samples were received through bread box-style pass-through compartments in isolated booths under normal 17 Watt fluorescent lighting.

Questions were presented one-at-a-time on a computer screen and data was collected using Compusense<sup>®</sup>5 (version 4.6) software (Compusense Inc., Guelph, Ontario, Canada). Before receiving samples, panelists were asked questions regarding their habits related to purchase and preparation of steak. Panelists were asked how often they consumed steak at home, what preparation methods they used when doing so, their preferred level of doneness, what method they used to determine steak doneness, the cut of beef they purchase most often, and whether or not they use a tenderizing method before cooking steaks at home. Panelists evaluated first impression of overall liking, appearance, flavor, and texture using a discrete 9-point hedonic scale where 9 = like

extremely, 5 = neither like nor dislike, 1 = dislike extremely and tenderness/toughness and moistness/dryness ideality using a 5-point “just about right” scale (5 = definitely too tender/moist, 3 = just about right, 1 = definitely too tough/dry). After assessing all attributes, panelists were then asked to rank the samples in order of preference. After sample evaluation, panelists were asked questions regarding the likelihood of purchasing the steaks sampled.

### **Warner-Bratzler Shear Force**

Steaks for Warner-Bratzler Shear Force (WBSF) were also cooked and evaluated using AMSA (1995) protocol, cooled to room temperature, and then wrapped individually in Saran™ plastic wrap. They were placed in a single layer with no overlapping and cooled to 4°C overnight before testing. WBSF was measured using a TA-XT 2 Analyzer (Texture Technologies Corp., Ramona, Calif., U.S.A.) to measure the force ( $\text{kg}_f$ ) required to shear a sample core, where  $\text{kg}_f$  is the customary unit with which to report WBSF data.

Six 1.27 cm diameter cores were sampled from each of four randomly selected cooked steaks from each treatment group using a handheld coring device, parallel to the orientation of the muscle fibers. For the untreated sample, data from three cores from a single steak were removed for analysis because their shear force values were outliers. All cores were free of significant amounts of connective tissue and were uniform in diameter. Each core was sheared once through its center using a TA-7 USDA Warner-Bratzler shear blade, perpendicular to the longitudinal orientation of the muscle fibers which is standard for this method. Crosshead speed was set at 200 mm/min. The peak force ( $\text{kg}_f$ )

required to shear each core was recorded and reported as the mean value of all cores for that treatment.

### **Statistical analysis**

Sensory hedonic and ideality scores were evaluated by one-way analysis of variance using Compusense®5 version 4.6 (Compusense, Inc., Guelph, Ontario, Canada). Tukey's HSD procedure determined significant differences among sample means for each attribute. Statistical significance was defined as  $P < 0.05$ . Correlation between sensory hedonic tenderness scores and Warner-Bratzler shear force data was performed using XLSTAT 2008.7.03 (New York, N.Y., U.S.A.).

## **Results and Discussion**

### **Sensory analysis**

Mean hedonic and ideality scores for each treatment are shown in Table 5-1. Hedonic scores ranged among the treatments from 6.3 to 6.7 for first impression of overall liking, 6.5 to 6.8 for appearance liking, and 6.2 to 6.6 for flavor liking, with no significant differences in the scoring of these attributes. No significant differences in appearance scores may suggest that panelists were either unable to detect visual effects imparted by the processing treatments after the beef was cooked or that they did not find them objectionable. Significant differences did exist, however, in texture liking with BT steaks rating significantly higher (6.5) than control steaks (5.8). NI subprimal and HPNI steak and subprimal texture were not rated significantly different than either the BT or control steaks. One possible explanation for the significantly higher acceptance of the

texture of BT steaks over the control were that the BT steaks were processed on both sides. It is of interest to note that there was no significant difference between the texture liking scores between beef that had been injected parallel (steaks) versus perpendicular (subprimals) to the muscle grain.

Ideality results show that all of the samples were judged to be slightly tough and slightly dry as mean ideality scores were 0.7 to 1.0 points below the ideal or “just about right” score of 3.00 for both attributes. This is consistent with Jeremiah and others (1999) who studied 33 muscles or muscle groups using a trained sensory panel, and determined that eye-of-round roasts were deficient in juiciness, flavor, and texture. In this study, all beef was cooked to 71°C using the dry cooking method of oven broiling, in keeping with the recommended temperature and cooking method published by AMSA (1995) which likely influenced mean sensory scores. As far as eating-quality, it is suggested that the eye-of-round be cooked using moist cooking methods (Neely and others 1999; NCBA 2007).

After assessing all characteristics, panelists ranked BT (207) and HPNI (211) significantly better than the untreated control (265) and HPNI-cut from processed subprimals (281) (lower scores equal higher ranking). Needle injected samples were not ranked significantly different from either BT, HPNI or the control ( $P>0.05$ ).

Treatments ranked least favorably were the untreated control and the HPNI – cut from processed subprimals. Ranking results suggest that after all of the sample attributes had been considered individually, panelists were able to establish preferences among samples. Since panelists were only asked to rank the samples in order of preference, it is not

known which attributes were most influential in their ranking; however, because tenderness plays the primary role in beef sensory satisfaction among consumers (Brady and Hunecke 1985; Belew and others 2003; Caine and others 2003) it is assumed that texture acceptance was of considerable influence.

The majority of panelists (79%) indicated that they ate steak in their homes at least once every three months and that the cooking methods used most frequently were grilling (59%), pan frying (19%), and broiling (10%). Kerth and others (2003) reported that broiling is the most common method for cooking beef steaks, but the panelists in this study used broiling the least. The majority of panelists preferred their steaks cooked medium well (40%) or medium (40%). Panelists reported that their primary method of determining when steaks were “done” were visual cues such as muscle or juice color (72%), temperature as determined by a thermometer (9%), textural cues (6%) and cooking time (6%). Most panelists (31%) indicated that when purchasing steaks, they were generally inexpensive cuts, such as those from the chuck or round and that they used no tenderizing method during preparation. Such results suggest that these consumers typically purchased and prepared steaks from tougher cuts, but tended to use dry heat cooking methods to prepare them, although this is generally not recommended for highest eating quality (Neely and others 1999; Kollé and others 2004; NCBA 2007;). Furthermore, consumers tend to rely on subjective methods to determine when steak is done. Panelists were most willing to purchase BT and HPNI steaks.

Table 5-1. Mean hedonic scores (SD), Just About Right difference, and ranking of mechanically tenderized beef eye-of-round steaks.

	Overall Acceptance	Appearance Acceptance	Flavor Acceptance	Texture Acceptance	Tenderness/ Toughness Difference from Just About Right (3.0)	Moistness/ Dryness Difference from Just About Right (3.0)	Rank sum
Untreated Control	6.3 <sup>a</sup> (1.5)	6.6 <sup>a</sup> (1.5)	6.3 <sup>a</sup> (1.6)	5.8 <sup>b</sup> (1.8)	-1.0	-1.0	265 <sup>bc</sup>
Blade tenderized	6.7 <sup>a</sup> (1.5)	6.6 <sup>a</sup> (1.4)	6.6 <sup>a</sup> (1.5)	6.5 <sup>a</sup> (1.9)	-0.7	-0.7	207 <sup>a</sup>
Needle injected - cut from processed subprimals	6.4 <sup>a</sup> (1.5)	6.5 <sup>a</sup> (1.4)	6.5 <sup>a</sup> (1.5)	6.0 <sup>ab</sup> (1.9)	-0.8	-0.9	236 <sup>ab</sup>
HPNI	6.3 <sup>a</sup> (1.5)	6.8 <sup>a</sup> (1.4)	6.3 <sup>a</sup> (1.6)	6.2 <sup>ab</sup> (1.8)	-0.8	-0.8	211 <sup>a</sup>
HPNI - cut from processed subprimals	6.3 <sup>a</sup> (1.5)	6.6 <sup>a</sup> (1.4)	6.2 <sup>a</sup> (1.6)	6.0 <sup>ab</sup> (1.7)	-0.7	-1.0	281 <sup>c</sup>

Means with like superscripts within each column are not significantly different from one another. n=80, (P<0.05)

### Warner-Bratzler Shear Force

WBSF mean peak force data is shown in Table 5-2. Mean scores ranged from 4.4 kg<sub>f</sub> for both BT and HPNI steaks and 2.4 kg<sub>f</sub> for NI steaks. The control had a mean of 3.4 kg<sub>f</sub>. WBSF values < 3.9 and > 4.6 are considered to be slightly tender and slightly tough, respectively (Shackelford and others 1991). Eye-of-round steaks that have not been mechanically processed have been found to have WBSF readings between 4.08 and 4.55 kg<sub>f</sub> (Otremba and others 1999; Brooks and others 2000). Seideman and others (1977) reported that WBSF values for beef eye-of-round improved (were lowered) by 0.7 kg<sub>f</sub> compared to an untreated control when treated with single and multiple passes through a blade tenderizer.

Table 5- 2. Mean Warner-Bratzler Shear Force peak force (SD) of mechanically tenderized beef eye-of-round steaks.

Treatment	WBSF peak force (kg <sub>f</sub> )
Control	3.4 (1.4) <sup>b</sup>
Blade tenderized	4.4 (1.5) <sup>c</sup>
Needle injected (cut from processed subprimals)	2.4 (1.1) <sup>a</sup>
HPNI	4.4 (1.2) <sup>c</sup>
HPNI (cut from processed subprimals)	4.2 (1.7) <sup>c</sup>

Means with like superscripts within each column are not significantly different from one another.

n=24 for all treatments except Untreated, where n=21. (P<0.05)

WBSF results of this study are inconsistent with as the aforementioned findings, as readings were 1.0 kg<sub>f</sub> higher for BT steaks when compared to the control. It is

uncertain why WBSF values for the control steaks in this study were so low in comparison to the treated samples, but similar results were observed in other measurements taken in this study. It is likely that the cooking method and endpoint temperature influenced the objective beef tenderness data. Kolle and others (2004) reported that when eye-of-round subprimals were cooked using dry heat, such as the dry heat method of clam shell grilling to 71°C, that there was no improvement in WBSF tenderness scores and that steaks from eye-of-round subprimals produced lower WBSF readings when they were cooked using moist heat methods. The cooking methods used in this study were dry heat methods, which could explain why WBSF scores were generally not improved. The exception to this is the improved scores for NI subprimals. Perhaps there was more injectant solution present in the NI samples, although, because pump yield was not measured, it is not possible to know this conclusively. Also, since only one level of sodium tripolyphosphate was used and only one set of processing conditions per mechanical tenderizing treatment, it is not known how results may have varied with different variables.

The National Cattlemen's Beef Association's (2007) recommends that beef eye-of-round steaks be cooked to an internal endpoint temperature of 63°C for maximum eating quality. The endpoint temperature of 71°C used in this study was well-above this and likely influenced both subjective as well as objective findings. As the USDA-FSIS recommended endpoint temperature for non-intact beef (68°C) is also well-above the NCBA's recommendation, there is a discrepancy between heating requirements for both



quality and safety. This is of particular concern in instances where consumers are unaware that beef steaks have been mechanically treated.

Correlation between the sensory acceptance data for tenderness and WBSF values is low ( $r = 0.31$ ) which suggests that sensory tenderness liking scores are influenced by other factors than force required for mechanical shear. This low correlation is consistent with many similar studies, thereby illustrating limitations when comparing Warner-Bratzler shear force to sensory scores, both consumer and descriptive (Shackelford and others 1995; Caine and others 2003; Lorenzen and others 2003). In this study, WBSF data was not an accurate predictor of sensory response for tenderness acceptance.

Standardized procedures (AMSA 1995; Wheeler and others 1999) for performing WBSF tests and conducting sensory analysis are attempts to increase consistency among researchers. Still, correlations and conclusions between them continue to vary. Correlations between the objective and subjective tests may be best when the samples of the same muscle fiber orientation were used for both tests (Poste and others 1993). In the present study, muscle fibers were severed perpendicular to the WBSF blade. Panelists were not instructed on how to orient or chew the samples.

### Conclusions

It is concluded that steaks processed using HPNI can be ranked at parity to those processed using blade tenderization, as judged by consumers. Variations to specific mechanical tenderization and cooking techniques will likely result in differences in tenderness and overall sensory results, as well as WBSF values. Correlation between sensory and WBSF is not always a helpful predictor of consumer liking of mechanically

tenderized or enhanced beef. Therefore, further research should attempt to improve methods to correlate sensory and instrument methods. Consumers likely need more education on the ideal way to prepare various cuts of beef, particularly those that are inherently tough, and to be encouraged to use a thermometer to determine end point temperature. Recommended internal endpoint temperatures for beef safety and eating quality should support one another, and therefore, further efforts to reduce the risk of illness due to microbial contamination are necessary.

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## CHAPTER 6

### GENERAL CONCLUSIONS

#### Overall Summary

This research was designed to increase understanding of the microbiological, heating, and sensory characteristics of the novel enhancing process of high-pressure needleless injection.

The following conclusions summarize the major findings of this research.

1. High-pressure needleless injection can translocate *E. coli* O157 from the surface of beef subprimals at inoculation levels above those that are found naturally to the depth to which the liquid jets can penetrate.
2. There was no significant difference between the percentage of samples testing positive at each subprimal depth regardless of initial surface inoculum concentration ( $P < 0.05$ ).
3. Recirculating solutions that become contaminated with pathogens during the injection process are a potential source of cross-contamination in high-pressure needleless injection.
4. Grilling and broiling were cooking methods that were effective in reducing the translocated microbial load of subprimals that were surface inoculated with  $2 \log_{10}$  CFU/cm<sup>2</sup> to an undetectable quantity of about  $1 \log_{10}$  CFU/g when steaks were cooked to an internal endpoint temperature of 63°C.

5. Beef steaks cooked by broiling are a slower heating method than gas grilling due to the higher cooking temperatures generally associated with grilling. Steaks cooked to an internal temperature of 63 °C by broiling, took significantly longer (15.10 ( $\pm$ 2.4) minutes) than gas grilling (11.61 ( $\pm$ 2.9) minutes) ( $P < 0.05$ )
6. Moisture-enhanced steaks by HPNI take significantly longer to cook (13.98 ( $\pm$ 2.4) minutes) than their untreated counterparts (12.73 ( $\pm$ 3.8) minutes), by both oven broiling and gas grilling which is likely due to the increased moisture content of injected steaks.
7. Discrepancies between suggested endpoint temperatures for beef eye-of-round quality and safety require further study to make them more consistent with each other.
8. Steaks processed using HPNI can be ranked at parity to those prepared using blade tenderization, as judged by beef consumers.
9. The majority of sensory panelists preferred their steaks cooked medium well (40%) or medium (40%); however vast majority of them also reported using subjective methods to determine when steaks were “done”, such as visual (72%) and textural (6%) cues. Only 6% reported using a meat thermometer.
10. Most panelists (31%) indicated that when purchasing steaks, they were generally inexpensive cuts that they used no tenderizing method during preparation and that they tended to use dry heat cooking methods to prepare them, although this is generally not recommended.

11. To summarize, when the results of this research are collectively considered, high-pressure needleless injection offers a potential alternative to common beef tenderizing methods.

### **Recommendations for Future Research**

1. Further research is needed to study the incidence and depth of translocated *E. coli* O157:H7 cocktail and cross-contamination through recycled run-off injectant in beef subprimals using higher inoculum concentrations so that percent translocation can be more accurately determined.
2. Future studies could consider the addition of antimicrobial agents in injecting solutions as a means of controlling translocated and cross-contaminated bacteria.
3. Studies could be performed to determine the effect of high-pressure liquid jets on the survivability of surface *E. coli*.
4. Considering the versatility of HPNI units with respect to variables such as pressures used, jet residence time, and jet diameter further work could be done to maximize tenderization and enhancement fluid retention in beef.
5. Additional research is needed to confirm whether greater tenderization may occur when beef is severed parallel to the muscle grain instead of perpendicular to it.
6. Experiments could also be performed to determine the water-binding properties of high-pressure needleless injected beef as a function of muscle disintegration when various additives, such as salt and phosphates are added to the injectant.

7. Thermal destruction of translocated *E. coli* in high-pressure needleless injected steaks at various moisture enhancement levels using commercial and consumer cooking equipment can also be studied.
8. Further research could be done to *study the* log reduction of translocated *E. coli* O157:H7 during various cooking methods by using much higher concentrations of surface inoculum.



APPENDIX

Table A-1. Translocation *Study*: Summary of statistical data for percent samples testing positive for *E. coli* O157 recovered from core samples at various depths in beef eye-of-round subprimals inoculated at different initial surface concentrations and processed with HPNI.

<b>Surface concentration of <i>E. coli</i></b>	<b>2 log<sub>10</sub> CFU/cm<sup>2</sup></b>	<b>1 log<sub>10</sub> CFU/cm<sup>2</sup></b>	<b>0.52 log<sub>10</sub> CFU/cm<sup>2</sup></b>
DF	5	5	5
χ <sup>2</sup> Test Statistic	0.83	0.60	0.37
Observed value	0.9606	0.0005	0.3223
Critical value	1.15	1.15	1.15
P	0.05	0.05	0.05

Table A-2. Cross-contamination Study: Summary of statistical data for percent samples testing positive for *E. coli* O157 recovered from core samples at various depths in beef eye-of-round subprimals processed with HPNI using recirculated, contaminated run-off liquid from the corresponding translocation study.

<b>Concentration of <i>E. coli</i> in run-off liquid</b>	<b>3 log<sub>10</sub> CFU/ml</b>	<b>2 log<sub>10</sub> CFU/ml</b>	<b>2 log<sub>10</sub> CFU/ml</b>
DF	5	5	5
$\chi^2$ Test Statistic	0.40	0.25	0.23
Observed value	0.2849	0.0122	$8.092 \times 10^{-7}$
Critical value	1.15	1.15	1.15
P	0.05	0.05	0.05

Table A-3 Heat Penetration Statistical Summary

**Comparison of Cooking Methods**

B = Broiling

G = Grilling

Sample	Mean	Standard deviation (n-1)	Standard error of the mean
Time to 63.00   B	15.102	2.427	0.375
Time to 63.00   G	11.607	2.938	0.453

**Comparison of Processing Methods**

T = HPNI Processed

U = Untreated

Sample	Mean	Standard deviation (n-1)	Standard error of the mean
Time to 63.00   T	13.976	2.424	0.374
Time to 63.00   U	12.733	3.757	0.580

**Cooking x Processing Interaction**

Sample	Mean	Standard deviation (n-1)	Standard error of the mean
Time to 63.00   BT	14.476	2.290	0.500
Time to 63.00   BU	15.729	2.452	0.535
Time to 63.00   GT	13.476	2.505	0.547
Time to 63.00   GU	9.738	2.021	0.441

Source	DF	Sum of squares	Mean squares	F	Pr > F
Model	3	419.740	139.913	25.893	< 0.0001
Error	80	432.279	5.403		
Corrected Total	83	852.018			

Table A-4  
Demographics – Beef Steak Consumer test

What is your age category?	
Under 20	2
20 to 29 years	22
30 to 39 years	16
40 to 49 years	15
50 to 60 years	13
Over 60	<u>12</u>
Total	80
What is your gender?	
Female	40
Male	<u>40</u>
Total	80
What is your attitude about beef steak?	
I like it	77
I neither like nor dislike it	3
I dislike it	<u>0</u>
Total	80
How often do you eat beef steak at a restaurant, cafe, etc...?	
More than once a week	3
Once a week to every two weeks	9
Once every two weeks to once a month	27
Once a month to once every three months	25
Less than every three months	15
I don't eat steak at restaurants, cafes, etc...	<u>1</u>
Total	80
How often do you eat beef steak at home?	
More than once a week	3
Once a week to every two weeks	14
Once every two weeks to once a month	14
Once a month to once every three months	32
Less than every three months	10
I don't eat beef steak at home	<u>7</u>
Total	80
What cooking method do you use most often when preparing beef steak at home?	
Pan frying	15
Grilling	47
Broiling	8
Clamshell type grill (i.e. George Foreman Grill)	3
Baking	0
Other	1
I don't prepare steak at home	<u>6</u>

Total	80
Which of the following choices best describes your preferred level of beef steak “doneness”?	
Well done	3
Medium well	54
Medium	54
Medium rare	7
Rare	<u>2</u>
Total	80
When preparing beef steak at home, what is your primary method of determining when it is “done”?	
Visual cues, such a muscle or juice color	58
Temperature, as determined by a thermometer	7
Textural cues	5
Cooking time	5
No method	0
I do not prepare steak at home	<u>5</u>
Total	80
Which of the following best describes how you purchase steak from the grocery store?	
I purchase inexpensive steaks (round or chuck) and tenderize them at home	19
I purchase inexpensive steaks (round or chuck) And use no tenderizing method	25
I purchase more expensive steak cuts (loin or rib)	23
I do not purchase steak	<u>13</u>
Total	80
How likely on unlikely would you be to purchase pre-tenderized or pre-marinated steaks from the grocery store, assuming the cost was affordable?	
Definitely likely	27
Somewhat likely	36
Neither likely nor unlikely	7
Somewhat unlikely	7
Definitely unlikely	<u>3</u>
Total	80

**Table A-5****Frequency Tables – Beef Steak Consumer Test**

Sample 1 – 105: Untreated Control steaks

Sample 2 – 234: Blade Tenderized steaks

Sample 3 – 420: Needle Injected subprimals

Sample 4 – 673: HPNI steaks

Sample 5 – 849: HPNI subprimals

**Table 3A – Overall first impression**

Sample	1 [9]	2 [8]	3 [7]	4 [6]	5 [5]	6 [4]	7 [3]	8 [2]	9 [1]	Total
1 - 105		15	27	21	7	5	4		1	80
2 - 234	3	25	23	15	7	2	4	1		80
3 - 420	2	11	33	18	6	7	1		2	80
4 - 673	2	10	34	17	4	8	4	1		80
5 - 849	1	13	28	18	8	9	2		1	80
<b>TOTALS</b>	8	74	145	89	32	31	15	2	4	400

**Table 3B - Appearance acceptance**

Sample	1 [9]	2 [8]	3 [7]	4 [6]	5 [5]	6 [4]	7 [3]	8 [2]	9 [1]	Total
1 - 105	4	23	18	20	5	7	2	1		80
2 - 234	5	16	27	19	5	5	2	1		80
3 - 420	2	19	21	23	8	4	2		1	80
4 - 673	7	19	25	15	7	5	2			80
5 - 849	2	20	27	15	7	7	1	1		80
<b>TOTALS</b>	20	97	118	92	32	28	9	3	1	400

**Table 3C – Flavor acceptance**

Sample	1 [9]	2 [8]	3 [7]	4 [6]	5 [5]	6 [4]	7 [3]	8 [2]	9 [1]	Total
1 - 105		14	32	17	5	5	5	1	1	80
2 - 234	3	24	22	14	8	5	3	1		80
3 - 420	2	18	27	18	7	5	1		2	80
4 - 673	3	16	26	18	5	4	6	1	1	80
5 - 849		16	24	17	10	9	1	2	1	80
<b>TOTALS</b>	8	88	131	84	35	28	16	5	5	400

**Table A-5 continued**  
**Frequency Tables – Beef Steak Consumer Test**

Sample 1 – 105: Untreated Control steaks  
 Sample 2 – 234: Blade Tenderized steaks  
 Sample 3 – 420: Needle Injected subprimals  
 Sample 4 – 673: HPNI steaks  
 Sample 5 – 849: HPNI subprimals

**Table 3D – Texture acceptance**

Sample	1 [9]	2 [8]	3 [7]	4 [6]	5 [5]	6 [4]	7 [3]	8 [2]	9 [1]	Total
1 - 105	1	10	25	17	6	11	5	3	2	80
2 - 234	6	22	16	21	5	3	2	3	2	80
3 - 420	3	13	21	19	7	8	4	2	3	80
4 - 673	1	21	22	13	5	10	5	1	2	80
5 - 849	3	11	25	16	7	13	2	2	1	80
<b>TOTALS</b>	14	77	109	86	30	45	18	11	10	400

**Table 3E – Tenderness/Toughness level ideality**

Sample	1 [5]	2 [4]	3 [3]	4 [2]	5 [1]	Total
1 - 105			26	29	25	80
2 - 234		1	37	28	14	80
3 - 420		1	29	31	19	80
4 - 673			35	26	19	80
5 - 849		2	21	37	20	80
<b>TOTALS</b>		4	148	151	97	400

**Table 3F – Moistness/Dryness level ideality**

Sample	1 [5]	2 [4]	3 [3]	4 [2]	5 [1]	Total
1 - 105		2	18	39	21	80
2 - 234		3	37	24	16	80
3 - 420		2	24	32	22	80
4 - 673			35	28	17	80
5 - 849		1	20	34	25	80
<b>TOTALS</b>		8	134	157	101	400



**Table A-5 continued**  
**Frequency Tables – Beef Steak Consumer Test**

Sample 1 – 105: Untreated Control steaks  
 Sample 2 – 234: Blade Tenderized steaks  
 Sample 3 – 420: Needle Injected subprimals  
 Sample 4 – 673: HPNI steaks  
 Sample 5 – 849: HPNI subprimals

**Table 3G - Preference ranking**

Sample	1	2	3	4	5	Total
1 - 105	9	20	11	17	23	80
2 - 234	28	14	15	9	14	80
3 - 420	18	15	14	19	14	80
4 - 673	21	16	22	13	8	80
5 - 849	4	15	18	22	21	80
<b>TOTALS</b>	80	80	80	80	80	400

**Table 3H – Likelihood of purchase**

Sample	1 [5]	2 [4]	3 [3]	4 [2]	5 [1]	Total
1 - 105	9	23	9	22	17	80
2 - 234	19	30	8	10	13	80
3 - 420	11	21	14	20	14	80
4 - 673	8	32	14	13	13	80
5 - 849	8	17	16	14	25	80
<b>TOTALS</b>	55	123	61	79	82	400

**Table 3I – Likelihood of purchasing pre-marinated or pre-tenderized steaks**

Sample	1 [5]	2 [4]	3 [3]	4 [2]	5 [1]	Total
n/a	27	36	7	7	3	80
<b>TOTALS</b>	27	36	7	7	3	80

## CURRICULUM VITAE

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**Education**

Utah State University	Ph.D.	2011	Food Science
Brigham Young University	M.S	1998	Food Science
Brigham Young University	B.S.	1994	Food Science, Chemistry minor

**Professional Experience**

- 1987 – Present Research Associate, Dept. of NDFS, Brigham Young Univ
- 2003 – Present Graduate Student, Dept. of NDFS, Utah State Univ
- 1995 – 1998 Graduate Student, Dept. of NDFS, Brigham Young Univ

**Honors and Awards**

- Distinguished Service Award, College of Life Sciences, 2010
- Nominated for Brigham Award, 2010
- Nominated for Outstanding Thesis Award, Society of Sigma Xi, 1999
- Team Captain and National Champion, IFT Food Science College Bowl, 1997
- Ara O. Call Scholarship, 1997
- L. Reed Freeman Scholarship, 1996, 1995
- College of Biology and Agriculture Scholarship, 1993
- Leadership Scholarship, Brigham Young University – Hawaii, 1983
- Computer Scholarship, Brigham Young University – Hawaii, 1983

**Professional Studies/Continuing Education**

- Compusense FCM® Descriptive Analysis Short Course, 2009
- Response Surface Methodology Design Strategy Course, ECHIP, 2004
- Flavor 101®, Flavors of North America, Inc., 2001
- Sensory Consumer Testing, Sensory Spectrum, 2001
- Thermal Process Development, NFPA, 2000
- Thermal Processing Deviation, NFPA, 2000
- Better Process Control School, University of Tennessee, 1999
- ServSafe®, Sysco, 1999

### Membership in Professional Societies

- Professional Member, Institute of Food Technologists, 2003 – Present
- Member, Institute of Food Technologists, 1993 – 2003
- Member, Institute of Food Technologists, Bonneville Section, 1993 – Present
- Member at Large, Institute of Food Technologists Bonneville Section, 2010 - Present
- Elected Secretary, Institute of Food Technologists Bonneville Section, 1998 – 2001

### Publications

Johnston, NP, **Jefferies, LK**, Rodriguez, B. 2011. The Acceptance of Brown-shelled Eggs in a White-shelled Egg Market. *Poultry Sci.* 20:1074-1079.

**Jefferies, Laura K.** and Hansen, Conly. 2010. In: Heldman, Dr, editor. High-pressure Injection Technologies. Encyclopedia of agricultural, food, and biological engineering. 2<sup>nd</sup> ed. CRC Press.

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Invited Speaker, IFT Rocky Mountain Section Annual Meeting, 2003

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Workshop Instructor, Expanding Your Horizons, Utah Valley State College, 1998-2003

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**Jefferies, L.K.** and L.V. Ogden. 1996. Casein Modification of Skim Milk for Improved Color and Body: A Comparison of Two Processes. Oral Presentation at Annual Meeting, American Dairy Science Association, Corvallis, OR. Vol. 79 (Suppl. 1), p.90.

### Teaching Experience

- 2005 – Present            NDFS 355L    “Food Engineering” Laboratory
- 2005 – Present            NDFS 350     “Food Sensory Analysis” Laboratory
- 2002 – Present            NDFS 250     “Essentials of Food Science”
- 2007 – 2008                IP&T 652     “Assessing Learning Outcomes”,  
Guest Lecturer
- 1994 - 1995                NDFS 251L    “Essentials of Food Science” Laboratory

### International Work

- 2008                            Instructor, Solar Drying Workshop, Suva, Fiji
- 2006                            Sensory Analysis of calcium-fortified quinoa cookies,  
La Paz, Bolivia

### **University, College and Department Committee and Administrative Assignments**

- 2010 – Present      Department Facilities & Resources Committee
- 2009 – Present      Department Scholarship Committee
- 2009                    Department Computer Representative
- 2005 - Present      Department Web Site Representative
- 2003 - 2004          Department Scholarship Committee
- 2000 - 2009          Chair, Department Social Committee
- 1999 - Present        Resource Committee to LDS Welfare Services

### **Grants Received**

- 2007                    Mentoring Environment Grant, BYU, \$10, 800

### **Military Service**

- 1991                    Recipient, Army Commendation Medal
- 1990 – 1991          Veteran, Operation Desert Storm
- 1989 – 1995          Medical Specialist, 144<sup>th</sup> Evacuation Hospital,  
Utah Army National Guard
- 1990                    Advanced Individual Training, Fort Sam Houston, Texas
- 1989                    Basic Training, Fort Jackson, South Carolina