INACTIVATION OF *ESCHERICHIA COLI* IN NON-INTACT BEEF STEAKS USING
RADIO FREQUENCY HEATING AND ITS QUALITY EVALUATION

by

ANGELA MARIA RINCON

(Under the Direction of Rakesh Singh)

ABSTRACT

Adequate cooking is one of the most effective means to eliminate foodborne pathogens from foods including Shiga toxin-producing *Escherichia coli* (STEC). The efficacy of radio frequency (RF) heating on inactivation of pathogens and quality studies on meat products has been previously reported. The effect of thickness (1.2 cm, 1.9 cm and 2.5 cm) and endpoint temperature (55°C, 65°C and 75°C) on quality of non-intact beefsteaks cooked in a RF oven was evaluated. Physicochemical characteristics were mainly affected by degree of doneness; however thickness had an effect on cooking time, heating rate, cooking and drip losses. The use of antibiotic resistant microorganisms as selective markers for meat products is a common approach in studies of new interventions. Validation of nalidixic (Nal) acid for STEC and nonpathogenic *Escherichia coli* using RF heating in a model solution was conducted. Buffers were heated to three different endpoint temperatures (55°C, 60°C and 65°C). STEC strains O157:H7, O26:H11 and O111 as well as nonpathogenic *E. coli* showed no significant differences compared to parent strains either before treatment or after heating therefore validating the use of Nal acid resistance as selective marker in RF studies. Results also
showed RF heating being more effective at temperatures $\geq 65^\circ C$. Thermal inactivation of STEC and nonpathogenic *E. coli* in packaged non-intact steaks heated up to 60$^\circ C$ or 65$^\circ C$ was also carried out. Results indicated a log reduction of 0.99, 3.08, 2.85 and 5.02 for O157:H7, O26:H11, O111 and non-pathogenic *E. coli* respectively at 60$^\circ C$ and a 5.0 log reduction at 65$^\circ C$. A study for the USDA-FSIS cooking guidelines for mechanically tenderized product at 63$^\circ C$ holding at room temperature for five minutes was also performed for STEC strains. Validation of the protocol was effective for *E. coli* O157:H7 and *E. coli* O111 but not for *E. coli* O26:H11. Therefore, either adjustments to the present protocol or more heat treatment needs to be conducted for *E. coli* O26:H11 strains. Heating steaks to 65$^\circ C$ and holding at room temperature for five minutes resulted in a 5 log reduction for all strains; therefore, showing a potential validation temperature for non-intact steaks cooked with RF. The cooking protocol developed for the present study has a practical relevance for the industry since the experiments were carried on a pilot-scale RF oven and also the pathogens were tested under realistic processing conditions.

INDEX WORDS: *Escherichia coli* O157:H7; non-O157 STEC, blade tenderization, quality, radio frequency, thermal inactivation
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By

ANGELA M RINCON

B.S., America University, Bogota, Colombia, 1999

M.S., The University of Georgia, 2004

A Dissertation Submitted to the Graduate Faculty of The University of Georgia in Partial Fulfillment of the Requirements for the Degree

DOCTOR OF PHILOSOPHY

ATHENS, GEORGIA

2014
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By

ANGELA M RINCON

Major Professor: Rakesh K. Singh
Committee: Mark A. Harrison
Alex M. Stelzleni
Yen-Con Hung

Electronic Version Approved:

Julie Coffield
Interim Dean of the Graduate School
The University of Georgia
December 2014
DEDICATION

I would like to dedicate this dissertation to the memory of my beloved dad. I miss him every day of my life but I feel his presence on every single thing that I do. I would also like to dedicate this hard work to my husband Ivan. His unconditional support and love during these years have helped me during hard times. He is an inspiration for my professional life, such a great dad to our daughter and a loving spouse. I am so blessed to be by his side. I have a very especial dedication to my sweet daughter Daniela. She is the center of my whole life teaching me every day with her innocence and pure love. To my mom and my sister for their invaluable help and advice during the completion of this project. Without them this dream would not have been possible. I also thank them for loving my Daniela with such huge heart and also for being such great role models for her. I could not be more proud of that. Thanks to my family in Athens, my uncle Pedro Villegas and Angela Villegas for opening the door for me to this wonderful opportunity that changed my life. Thanks for the support. Last but not least, to God for giving me the strength to finish this journey and to help me believe it was possible to achieve this challenge.
AKNOWLEDGEMENTS

I would like to express my deepest gratitude to my major professor Dr. Rakesh Singh for his guidance and encouragement during the development of the present project. Thanks to Dr. Mark Harrison for being part of my committee, for his advice and for allowing me to work on his lab during an important part of my research. I want to express my gratitude to Dr. Alex Stelzleni for accepting to be part of my graduate advisory committee and also for his priceless advice and guidance during my doctorate studies. I want to thank Dr. Yen-Con Hung for being part of my committee and for allowing me to be part of this interesting project.

My sincere thanks to my whole family for giving me the strength, encouragement and inspiration for the completion of this degree. I would also like to thank people that at one point or the other were of huge help assisting me with the project: Austin Bernard for his efficiency and ability to multitask, Kerrianne Fisher for her help as lab assistant, Carl Ruiz for his help with Radio Frequency troubleshooting, Gwen Hirsch for facilitating lab logistics during my time at Dr. Harrison’s lab. Special thanks to lab mates that helped me with valuable advice with the development and execution of this project: Chi-Ching Lee and Bilal Kirmaci. I would also like to thank my friends Maria Sohail, Ganashree Nagaraj, Jaideep, Lee Carella, Jaideep Sidhu, Sofia Santillana, Leslie Kleiner, Stephanie Barnes and Lisa Trimble for their help with my experiments and constant moral encouragement. Lastly, I would like to thank the USDA-AFRI Integrated Food Safety Project for funding this research.
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CHAPTER 1
INTRODUCTION

Cattle are the primary source of Shiga toxin-producing *Escherichia coli* (STEC) infection in humans. Meat products, particularly non-intact beef may carry a significant risk of contamination from STEC due to potential translocation of the pathogen from the skin and rumen of the carcass to the interior of the tissues during slaughter and dressing operations (6). Because of risks associated with consumption of contaminated beef, *Escherichia coli* O157:H7 as well as *Escherichia coli* serotypes O26, O45, O103, O111, O121 and O145 have been declared as adulterants in non-intact beef products by the United States Department of Agriculture, Food Service Inspection Service (USDA-FSIS).

Adequate cooking is one of the most effective means to eliminate STEC and other pathogens from food (1); however, traditional cooking in non-intact beef products may not be sufficient to inactivate pathogens internally without overheating the surface (5). In contrast, radio frequency (RF) uses electromagnetic energy to generate heat within the food (volumetric heating) due to molecular friction of polar molecules generating rapid temperature distribution and deep penetration; thus, avoiding overheating. The energy and heat are absorbed by the food; therefore the cooking not only saves time but also yields a product with improved quality and increased shelf life (5).

Meats precooked in heat resistant pouches that only need to be warmed or heated to desire temperatures are currently offered at the grocery stores and restaurants. Thus, RF cooking has potential application for value added products fabricated from non-intact
muscle allowing faster heating rates, reducing pre and post processing contamination and offering better quality. To date, several studies pertaining to the application of RF cooking of beef products has been documented in terms of quality of product (2, 3, 7, 8) and inactivation of non-pathogenic E. coli and Bacillus cereus and Clostridium perfringens (1, 4, 5). However, not much is known about the effect of RF heating on quality and Shiga toxin producing E. coli inactivation on whole muscle non-intact beef products. Therefore, the scope of this dissertation includes the development of cooking procedures, the quality analysis and also the inactivation of STEC and non-pathogenic E. coli on non-intact beefsteaks cooked in a RF oven to different endpoint temperatures. In order to meet the overall goals of this investigation, the study was divided on three portions:

1. **Develop a RF cooking protocol for packaged steaks fabricated from mechanically tenderized beef muscle in order to achieve different target endpoint temperatures and evaluate their physicochemical and quality parameters.**

2. **Validate the use of nalidixic acid resistant strains of E. coli (STEC and non-pathogenic) in studies of RF heating and determine the effectiveness of RF at different endpoint temperatures using model solutions.**

3. **Evaluate the thermal inactivation of various STEC and non-pathogenic E. coli in packaged whole muscle non-intact steaks using RF and validate the minimum USDA-FSIS cooking recommendation requirements for inactivation of STEC using RF heating.**

Arcing and thermal runaway are the main problems of RF heating despite its many advantages (e.g. deep penetration, faster heating rates). Objective number one addresses
those issues in Chapter 3. A RF cooking protocol for whole muscle non-intact beefsteaks was developed. Heat penetration, cooking properties and quality evaluation were performed on steaks cooked with RF to different endpoint temperatures. Antibiotic resistance of bacteria is a commonly used practice for selective markers in food matrix environments. Suitability of antibiotic resistant microorganisms should be determined for all new interventions which is addressed in objective number two and developed in Chapter 4. The use of nalidixic acid resistant \(E.\ coli\) strains in studies of RF heating was validated at different endpoint temperatures using model solutions. Finally, objective number three covered in Chapter 5 focuses on evaluation of inactivation of pathogens using RF on blade-tenderized steaks. Mechanically tenderized products have been involved in STEC outbreaks as a result of internalization of STEC during blade tenderization of the product. Conventional heating methods are effective on the elimination of STEC and other pathogens from meat products. However, traditional cooking requires the food to be heated externally leading to longer cooking times and lower quality of the product. RF heating uses electromagnetic energy with rapid heat distribution and lower energy consumption. Thermal inactivation of STEC and non-pathogenic \(E.\ coli\) was evaluated at different endpoint temperatures using a RF oven. Also, a validation of the USDA-FSIS recommendation for the minimum internal cooking temperature requirements for RF was performed.
References


CHAPTER 2
LITERATURE REVIEW

_Escherichia coli_

_Escherichia coli_ are a predominant intestinal microflora found in the gut of humans and other mammals (5). Part of the _Enterobacteriaceae_ family _E. coli_ is a facultative anaerobic, rod-shaped, gram-negative bacterium (67). Most _E. coli_ bacteria are harmless; however some strains can cause illnesses such as diarrheal disease, urinary tract infections, sepsis and meningitis (32, 77).

_E. coli_ isolates are serologically differentiated based on three major surface antigens, which enable serotyping: the O (somatic), H (flagella) and K (capsule) antigens. A total of 173 O antigens, 56 H antigens, and 103 K antigens have been identified to date (29). Diarrheagenic _E. coli_ isolates are categorized into specific groups based on virulence properties, mechanisms of pathogenicity, clinical syndromes, and different O:H serotypes. These categories include enteropathogenic _E. coli_ (EPEC), enterotoxigenic _E. coli_ (ETEC), enteroinvasive _E. coli_ (EIEC), diffuse-adhering _E. coli_ (DAEC), enteroaggregative _E. coli_ (EAEC) and enterohemorrhagic _E. coli_ (EHEC)/Shiga toxin-producing _E. coli_ (STEC) (29). Among diarrheagenic _E. coli_ strains, STEC strains are distinguished by the ability to cause severe life-threatening complications such as hemolytic-uremic syndrome (HUS) and thrombotic thrombocytopenic purpura (TTP) (104).
Shiga Toxin-Producing *Escherichia coli* (STEC)

STEC have been recognized as a cause of serious disease and mortality in outbreaks of foodborne illnesses (100). STEC are transmitted to humans mainly through consumption of contaminated food and water (49). Domestic and wild ruminant animals, in particular cattle, are considered the main reservoir of STEC and the main source of contamination from the food supply (104). The pathogenic capacity of STEC resides in a number of virulence factors, including Shiga toxins (Stx1 and Stx2), intimin (an outer membrane protein) (49), enterohaemolysin, and the STEC autoagglutinating adhesion (Saa) (81). Although Stx1 and Stx2 are different proteins, encoded by different genes, their biological activities are quite similar. Their activities involve depurinating specific residues of the host ribosomes, blocking the binding of aminoacyl tRNA to the ribosomes, and inhibiting protein synthesis (44). The toxins bind to Gb3 receptors, expressed at high levels in renal vascular endothelial cells, and inhibit peptide synthesis, ultimately causing cell death (65, 73). The toxins are produced by the pathogen in the colon and cause local cell damage as well as travel to the kidney where it is thought to play a role in causing HUS (100). However, the ability to cause disease is not solely dependent on the expression of Shiga toxins. STEC have been isolated from asymptomatic individuals; therefore, multiple factors must play roles in causing disease, such as additional virulence factors, ingested dose and immune status of the individual (55). *E. coli* serotype O157:H7 and several non-O157 serogroups are considered major foodborne pathogens worldwide (88) due to their designation as adulterants in non-intact beef and the consequences of such policies for the beef processing industry (55).
*Escherichia coli O157:H7*

*Escherichia coli* O157:H7 is known as an important cause of bloody diarrhea (hemorrhagic colitis) and renal failure (HUS) in humans (30). This bacterium is capable of producing large quantities of Shiga toxin that can cause severe damage to the intestinal lining (55). *E. coli* O157 grows best within a temperature range of 30-42°C with the optimal temperature being 37°C (31). The organism does not, however, grow well above 44°C (31); which is noteworthy because most standard detection procedures for fecal coliforms are conducted in this higher range, and would thus fail to detect the pathogen (89). *E. coli* O157 enters the food chain by inhabitation in the gastrointestinal tract of dairy cattle and other animals, particularly ruminants. Numerous steps in the slaughter process may contribute to carcass contamination (89).

*E. coli* O157 was first identified as a human pathogen in 1982 when it was associated with two outbreaks of hemorrhagic colitis in the states of Oregon and Michigan (77). Recent data from the Centers for Disease Control and Prevention (CDC) revealed that there is an estimate of 63,153 annual cases of *E. coli* O157 infection in the United States (51, 82). *E. coli* O157 outbreaks have been associated with minced beef and other beef products, raw milk and milk products (e.g. cheese, yogurt), apple juice and raw vegetables and salads (25). Person-to-person contact, environmental exposure (e.g. farm visits) and drinking or swimming in polluted water are also thought to be important sources of contamination (90). Some of the outbreaks of *E. coli* O157 in the United States associated with foods other than meat products include the outbreak in the fall of 1991 associated with the consumption of fresh-pressed apple cider in southern Massachusetts (7, 77), the outbreak in the summer 1995 associated with leafy lettuce
consumption in Washington (1, 77) and the outbreak in the summer of 1997 associated with eating alfalfa sprouts in Michigan and Virginia (9, 77). Other multistate outbreaks include: the outbreak associated with frozen pizza in the fall 2007 (12), the outbreak linked to eating raw refrigerated prepackaged cookie dough in the spring 2009 (13), the outbreak associated with cheese in the fall 2010 (14), the outbreak associated with in-shell hazelnuts in the spring 2011 (15), the outbreak linked to romaine lettuce in the fall 2011 (17), the outbreak of infections linked to organic spinach and spring mix blend in the fall 2012 (19) and the outbreak linked to ready to eat salads in the fall 2013 (22).

*Escherichia coli* non-O157

In the United States, it is estimated that non-O157 *E. coli* cause more illnesses than *E. coli* O157. The majority of non-O157 *E. coli* infections are due to serogroups O26, O45, O103, O111, O121 and O145, referred to as the top six non-O157 STEC. The diseases of non-O157 *E. coli* are generally milder than those induced by *E. coli* O157; nonetheless, non-O157 *E. coli* strains have also been associated with serious illnesses as well as death (88). Foods associated with non-O157 *E. coli* illness include sausage, ice cream, milk and lettuce among others (67).

The true incidence of non-O157 adulterants is not known but it is estimated that they account for between 20 and 50% of STEC infections in the United States, outnumbering the number of illnesses caused by *E. coli* O157 in certain geographic regions (80). The CDC estimated that non-O157 *E. coli* are responsible for about 112,752 cases of illness annually (51, 82). Unfortunately, only a limited number of laboratory tests for non-O157 *E. coli* is available; therefore, the public health risk associated with their occurrence is likely underestimated (49). Outbreaks associated with products other
than meat include: a multistate outbreak of STEC O145 linked to shredded romaine lettuce in the spring 2010 (18), the outbreak of STEC O104 (STEC O104:H4) infections associated with travel to Germany in the spring 2011 (16), the outbreak of STEC O145 in the spring 2012 (20), the outbreak of STEC O121 infections linked to Farm Rich Brand Frozen Food Products in the spring 2013 (21) and most recently a multistate outbreak of STEC O26 infections linked to raw clover sprouts at Jimmy John’s restaurants in the spring 2014 (23).

**Blade Tenderization Process and Internalization of Pathogens**

Human perception of meat palatability is derived from a complex interaction of sensory and physical processes during chewing. Of the various subjective characteristics determining meat palatability, tenderness is the most important (11). Thus, the meat industry uses various tenderization techniques such as aging, use of proteolytic enzymes, marination, electrical stimulation, flaking and forming, injection enhancement and mechanical tenderization to improve tenderness (105). Meat from cattle is primarily destined for consumption as ground beef or beef cuts. Beef cuts are described as whole muscle cuts commonly consumed as steaks or roasts. These cuts are derived from subprimal cuts, which can be subjected to a tenderization process before fabrication into steaks or roasts which are referred as whole muscle non-intact beef cuts (87). The USDA-FSIS defines non-intact beef products as ground beef, beef injected with solution, beef that has been mechanically tenderized by needling, cubing, or pounding devices and beef that has been reconstructed into formed entrees (98).

Mechanical tenderization of beef by piercing the meat with blades is a common practice used in North America to overcome the inadequacy on tenderness (48). Blade
tenderization involves a series of very sharp, double-edged blades, which are capable of penetrating meat by cutting through muscle tissues and fibers, rather than tearing the tissue or “punching holes” (79). The product to be tenderized is placed fat-side-down on a conveyor system, which moves in tandem with the blade heads, advancing when the heads are retracted, and stopping as the head drop to allow penetration. The blades are usually arranged in a perpendicular configuration to prevent a sliced or sheared appearance (89). Intrinsic factors such as myofibrillar proteins, muscle cytoskeletal proteins, intramuscular connective tissue, marbling and intrafiber water content also affect meat tenderness (28); however, research has shown that needle or blade mechanical tenderization can improve the tenderness of less tender and typically less expensive meat cuts. This process makes the less tender cuts more marketable to consumers. The mechanically tenderized products are widely available to consumers in the marketplace (99). In the United States at least 18% of beef products at the retail level have been mechanically tenderized or injected with solutions for enhancement of tenderness and/or flavor (71, 105).

The dehiding process during slaughter may transport fecal matter and debris from the hides and hooves of the animal to the carcass. Evisceration may also contribute to the contamination when rumen or intestinal contents are dispelled (89). During blade tenderization, mechanical blades are inserted into sub-primal cuts, disrupting the structure of beef muscle. During this disrupting process, bacteria present on the surfaces of beef cuts are carried and translocated into the interior, leading to internal contamination (43). Research has revealed that blade tenderizers allow surface bacterial to penetrate the center of subprimals in top butts, striploins, and inside rounds. Only an estimated 0.2%
(47, 103) to <0.083% (47, 52) of subprimals destined for mechanical tenderization are positive for *E. coli* O157 (46). It has been publicized that approximately 3-4% of *E. coli* O157 inoculated onto meat surfaces can be translocated internally (89). Other studies, (61, 63) have also reported internal translocation of *E. coli* O157:H7 after blade tenderization. In these studies, blade tenderization transferred *E. coli* O157 cells primary to the topmost 1 cm of surface-inoculated beef subprimals, but also into deeper tissues. It was reported specifically that 33% of the inoculum was recovered from the topmost 1 cm, and 3.04%, 3.09%, 0.44%, 0.10% and 0.82% was recovered from depths of 1 to 2, 2 to 3, 3 to 4, 4 to 6 and 6 to 8 cm respectively (2). Another previous work (39), showed that 2 log CFU/ 25g of aerobic microorganisms were recovered from the deep tissues of mechanically tenderized beef striploins that had surface contamination levels of 2.8 log CFU/ 25cm². The main concern with the previous results is that internalized *E. coli* O157 cells may survive and result in illness, if such products are intentionally (consumer preference) or unintentionally (accidental) undercooked (98, 105).

**STEC Outbreaks in Non-Intact Beef and Regulations**

*E. coli* O157 was first implicated in infectious disease in the early 1980s (32). Since then it has been repeatedly implicated in outbreaks caused by consumption of inadequately cooked contaminated beef (31, 50). In 1993, a multistate outbreak of *E. coli* O157 associated with hamburgers at a national fast food chain increased the awareness about this pathogen (6). In response, the USDA-FSIS declared *E. coli* O157:H7 as an adulterant in ground beef in October of 1994 under the Federal Meat Inspection Act (78). Additionally, all beef processors and plants that produced raw, ground meat and poultry were required to target and reduce all significant hazards by adopting the Hazard
Analysis and Critical Control Point (HACCP) system, a program that allows the identification and control of the hazards associated with their respective process (33). Under this policy, non-intact beef products, if contaminated with E. coli O157:H7, must be processed into ready-to-eat products, or they would be considered adulterated (43).

Since 2000, the Centers for Disease Control and Prevention has received reports of six outbreaks attributable to needle or blade tenderized beef products prepared in restaurants and homes. Among these outbreaks there were a total 176 E. coli O157 cases that resulted in 32 hospitalizations and 4 cases of HUS. In the first outbreak in August 2000, the Michigan Department of Community Health (MDCH) found fecal E. coli O157:H7 isolates from two individuals with matching Pulse-Field Gel Electrophoresis (PFGE) patterns. Both individuals had consumed needle tenderized sirloin steak cooked to order with a red or pink center at different locations of a local restaurant chain (77). In 2003, Minnesota Department of Health (MDH) identified seven infection cases plus four single cases reported in a single state linked to a boneless beef filet bacon-wrapped steak product injected with marinade sold by door-to-door vendors. The establishment involved voluntarily recalled approximately 335,204 kg of the product from the market (57). In 2004, the Colorado Department of Public Health and Environment (CDHPE) confirmed a new outbreak associated with blade tenderized steaks marinated in vacuum tumbler. Four cases of E. coli O157:H7 had similar PFGE patterns by the individuals affected and the establishment, which produced the mechanically tenderized beef, recalled 184,158 kg of product (77, 99). As a result of those outbreaks, in May 2005, USDA-FSIS mandated that establishments producing mechanically tenderized whole
muscle, non-intact product (with or without marinade) to reassess their HACCP plans (77).

Needle tenderized seasoned tri-tip beef was involved in a non-intact beef outbreak in 2007 affecting 124 people in Fresno, California. Illnesses were associated with product served through the restaurant food-to-go operation that had some sanitary violations. Notes indicate that a seasoned marinade was used in the needling process. No recall was made (99). Eight patients were infected with *E. coli* O157:H7 between may and august 2007 after consumption of needle injected and marinated steaks. The Pennsylvania establishment that produced the steaks voluntarily recalled approximately 117,184 kg of beef products (99). In a new outbreak, a national Steak and Poultry establishment in Oklahoma recalled approximately 112,490 kg of beef products suspected to be contaminated with *E. coli* O157:H7. A cluster of illnesses was investigated and the USDA-FSIS determined that the outbreak was associated with non-intact steaks (blade tenderized) (99). In many cases, patients associated with outbreaks reported preparing or ordering steaks as “rare” or “medium rare” (26, 42). Changes in non-intact beef product regulations during the outbreaks included the HACCP systems validation document draft guidance. This document addresses the importance of validating the entire HACCP system, including prerequisite programs. It also stipulates what was necessary for validation and defined validation as “the process of demonstrating that the HACCP system as designed can adequately control identified hazards to produce a safe, unadulterated product” (76, 94).

Historically *E. coli* O157 has been linked to several recalls and outbreaks of foodborne illness involving meat products, while non-O157 *E. coli* has only rarely been
associated with illness when meat was the vehicle (62). However, non-O157 outbreaks attributed to food containing beef products have been reported (56). Consequently, and due to potential risks to consumers, USDA-FSIS declared six additional pathogenic *E. coli* serogroups comprising of O26, O45, O103, O111, O121 and O145 adulterants in raw beef trim, the major component of ground beef in 2012 (80).

For products that are chemically enhanced or that contain added substances, the product must declare all added ingredients and include an appropriate qualifying statement such as “ Injected with up to 10% of a flavoring solution”. In contrast, regardless of how products are mechanically tenderized, such products were not required by the USDA-FSIS to be labeled as “Blade Tenderized”. Given the demonstrated potential for transfer of pathogens from the surface to the deeper tissues of the meat via mechanical or chemical tenderization, in addition to safe handling instructions consideration should be given to labeling such products as tenderized and/or enhanced and to educating consumers, restaurants and/or food service personnel about proper cooking and handling of non-intact meats (62). As a consequence, the USDA-FSIS published a proposed a rule to require the use of the descriptive designation “mechanically tenderized” on the labels of raw or partially cooked needle or blade tenderized beef unless such products are destined to be fully cooked in an official establishment on June of 2013 (99). Also, USDA-FSIS issued a Compliance Guideline For Validating Cooking Instructions for Mechanically Tenderized Beef Products designed to help establishments that manufacture non-intact beef products to identify the minimum components of validated cooking instructions (95).
**Thermal Inactivation of STEC in non-intact beef products**

The measured thermal resistance of a species can be influenced by many factors, including growth conditions, growth phase of the cells, composition, pH and water activity of the growth medium, growth temperature, holding period before and after heat treatment, heat shock and the heating method \((90)\). Several researchers have shown that *E. coli* O157 does not possess an unusual heat resistance and D-values at 50-64°C ranging from 92.67 to 0.16 min have been reported \((24)\). Estimates of the infectious dose of STEC are as low as 1-100 cells \((73)\) thus it is vital to eliminate this pathogen from food, rather than merely to prevent its growth. Heat treatment is the method of bacterial destruction most frequently used in food processing. Accurate information on thermal death rates is important for food processors in order to achieve the desired safety margins while avoiding over processing \((90)\). Cooking conditions believed to be adequate for assuring the microbiological safety of injected or blade tenderized meats have been specified by US regulators. The specifications are that all parts of the food be heated to 63, 65 or 68°C for times of 180, 60 or 15 s, respectively or to \(\geq 70°C\) without the need of maintenance of that temperature \((38)\). Those specifications based on decimal reduction times \(\text{(D-values)}\) for *Salmonella* are calculated to reduce the numbers of those organisms by 6.5 log CFU \((97)\).

A comparative risk assessment of non-intact and intact beefsteaks indicated that oven broiling to an internal temperature of more than 60°C would result in safe blade tenderized beefsteaks. The same study indicated that broiling was more effective than grilling or frying when meat samples were cooked to 60 or 65°C \((89)\); consequently, it is important to conduct studies of validation for each cooking method. Shen and others, \((85)\)
studied the thermal inactivation of *E. coli* O157:H7 in non-intact beef steaks of different thicknesses by different cooking methods and appliances. The study concluded that the extent of thermal inactivation in undercooked (65°C) non-intact steaks depend on steak thickness and in the way by which the cooking method transferred heat into the steak, affecting the rate of heat penetration and the total time needed to reach the target endpoint temperature. Roasting in a kitchen oven was the most effective cooking method in reducing *E. coli* O157:H7, whereas the thicker the steaks, the greater inactivation of the pathogen present internally into the product. In a different study, a lean beef model system was used to evaluate the effect of different meat binding or restructuring formulations, both with or without added lactic acid, on thermal inactivation of *E. coli* O157:H7 embedded in the meat, when samples were cooked to internal temperatures of 60°C (rare) or 65°C (medium-rare) simulating product undercooking (69). Thermal destruction of the pathogen was greater at 65°C than 60°C as expected. At both cooking temperatures, thermal inactivation of the pathogen was not significantly different on any of the meat binding formulations compared to the control. Therefore, it was suggested that the restructuring formulations might not protect or enhance thermal inactivation of the internalized pathogen. Research evaluating the effect of sodium citrate plus sodium diacetate (SC+D) or buffered vinegar on *E. coli* O157:H7 when incorporated on brine solutions for injected beef was conducted (78). After injection and storage half of the muscles were samples raw and the other half cooked to an internal temperature of 60°C with a 12-minute hold time. For raw samples, a significant reduction of 0.6 and 1.0 log CFU/g of the pathogen was reported for CD+D and for vinegar respectively, whereas for cooked samples there was no presence of the pathogen under the detection level.
Therefore, it was concluded that the thermal processing of injected beef treated with enhanced solutions was effective at eliminating internalized *E. coli* O157:H7 and also controlling the growth of psychotropic microorganisms during aerobic storage. Another study evaluated the inactivation of *E. coli* O157:H7 in moisture-enhanced non-intact beef by pan-broiling or roasting with various cooking appliances set at different temperatures (86). At the lowest temperature setting evaluated (149°C), pathogen destruction ranged from 1.5 to 2.4 log CFU/g. At the maximum possible temperature settings of each appliance (204 to 260°C), thermal inactivation of *E. coli* O157 increased as the temperature setting increased with the highest reduction of 5.5 log CFU/g. A previous study evaluated the thermal inactivation of *E. coli* O157:H7 inoculated at different depths of non-intact blade-tenderized beef steaks (2). Findings indicated that the extent of thermal inactivation of *E. coli* O157 was affected by cooking method, depth of inoculation, and in some instances, non-intact steak thickness, but not by whether the steak was cooked from a frozen state or after thawing. The fate of STEC O157 and non-O157 cells inoculated on blade tenderized steaks after cooking on a commercial open-flame gas grill was evaluated (62). Regardless of temperature or thickness, both STEC O157 and non-O157 behaved similarly in response to heat, in that cooking eliminated significant numbers of both pathogen types; however, some survivors were recovered due presumably to uneven heating of the blade tenderized steaks.

The microbial safety of raw beef often relies on a proper heat treatment at the end user stage. In the case of intact beef, proper heat treatment of the meat surface by cooking, frying or grilling is then sufficient to kill pathogenic bacteria and the beef is microbiologically safe to eat even if is not cooked thoroughly. Many consumers may not
be aware though that injection or blade tenderization process may transfer bacteria including pathogens from the surface to the interior and prepare non-intact meat as intact meat. Therefore, future research should focus on optimization and streamlining of combined enhanced meat processes to obtain improved food safety and high quality non-intact beef products. This work should also include the implementation of new technologies, marinades and novel packaging technologies (58).

**Radio frequency heating technology overview**

Radio Frequency (RF) heating or capacitative dielectric heating is an innovative technique among several that are based on electro-technologies, including ohmic heating, microwave dielectric heating, inductive-ohmic combinations and radiative-magnetic heating (75). Microwave (MW) and RF energy are transmitted as electromagnetic waves and the depth to which they penetrate foods is determined by both their frequency and the characteristics of the food. MW has a range of frequencies from 300 MHz to 3000 GHz whereas RF energy has lower frequencies from 10 to 300 MHz. However, because these frequencies are also used for communications and navigation, an international agreement has allocated the following bands for industrial, scientific and medical use (RF 13.56 MHz, 27.12 MHz and 40.68 MHz) (34). RF heating involves the transfer of electromagnetic waves directly into the product, initiating volumetric heating due to frictional interaction between molecules. Heating with microwave and RF involves primarily two mechanisms: Dielectric and ionic. Due to its dipolar nature, water in the food is often the primary component responsible for dielectric heating. The second major mechanism of heating with microwaves and RF is through the oscillatory migration of
ions in the food that generates heat under the influence of the oscillating electric field (27).

The RF oven comprises of a RF generator that produces oscillating fields of electromagnetic energy. This RF energy is applied via two parallel electrodes to heat the food placed between the electrodes. One of these electrodes is grounded to set up a capacitor configuration for the storage of electric energy. RF results from constant polarity reversal of the field. Dipolar molecules like water present in the material, also continuously realign with the changing field. This phenomenon of dipole rotation further contributes to heat generation within the product (66, 70). The dielectric properties of foods determine the interaction between the food stuff and electromagnetic energy and are key factors in determining efficiency of RF and MW heating (64). The dielectric constant ($\varepsilon'$) is the characteristic of the material that describes the ability to absorb, transmit and reflect energy from the electric portion of the RF waves. The dielectric loss factor ($\varepsilon''$) describes how well the material absorbs energy from electric fields passing through it and converts that energy to heat (3). A third important concept in the dielectric heating is the power penetration depth ($d_p$), which is a measure of the electric field penetration of a material (3). When electromagnetic waves propagate to a material, part of the waves is reflected. The remaining part will penetrate into the substrate and the strength of penetration is reduced with distance (102). The dielectric properties are usually function of temperature, frequency, density, moisture content, and other compositions of the food. Thus, for a given material, the dielectric properties may vary during heating, and the heating behavior may also change accordingly. Therefore, knowing the dielectric properties as a function of temperature, moisture content and other
properties before running experiments may help to predict possible thermal run away and temperature distribution in the bulk food (45).

**Thermal effects on quality of meat**

Today, most meat and meat-based products are cooked before being eaten. The cooking process not only destroys pathogenic or spoilage microorganisms but also develops sensorial properties which are specific of the cooked product (54). Myofibrillar and connective tissue proteins undergo several temperature and time dependent structural changes during cooking which impact directly product yield, texture and overall eating quality. Cooking causes denaturation of the myofibrillar components, which results in toughening. Conversely, it may also promote structural alterations and solubilization of collagen, which results in more tender meat. Thus, cooking can cause either tenderization or toughening of beef with the effect being dependent on the inherent composition characteristics of the muscles, the method of heating and the time/temperature combination employed (101). Mass transfer take place as a result of shrinkage in dimensions to fluid extrusion (sarcoplasmic fluid loss) from the meat and evaporation from the meat surface producing both mass and volume loss (72). The heat treatment has a significant impact on the composition and physicochemical characteristics of final products; indeed, it is well known that meat product composition and cooking techniques are among the factors affecting the quality attributes of the product (84). Color measurement in cooked meat can also provide reliable information about eating quality attributes. The myoglobin protein is the primary heme pigment responsible for meat color (35). During cooking three forms of myoglobin are degraded through oxygenation and oxidation and reduction reactions ultimately influencing the
appearance of meat color (60). The denaturation temperature of protein is dependent of
the protein pH and concentration, redox state, and any ingredients that modify thermal
stability of myoglobin. Therefore; cooked color may not always be an indicator of safety,
especially in ground beef where the pigment forms can be oxygenated or oxidized (91).

Cooking is energy intensive, so manufacturers are interested in processes that
reduce the energy required (8). The main factors that differ among cooking techniques
are the temperature in the meat surface and the method of heat transfer that are important
for odor, flavor and color of meat; and the temperature profile through the meat which
influences the rate and the extent of changes in meat protein structures (74). The heat
transfer in conventional cooking methods takes place from the outside of the product to
its interior; making products undergo long cooking procedures where the surface may
look overcooked in order to ensure the center is well cooked, which in turn can
potentially reduce product quality and nutritional value (83). By contrast, RF heating
generates heat rapidly within the product, due to the frictional interactions of polar
dielectric molecules rotating in the space charge displacement in response to an
externally applied AC electric field (41). This method therefore has the potential to be a
good alternative from conventional heating technologies performing a more uniform
heating and also resulting in a desired microbial lethality without altering or degrading
the overall food quality (107).

The use of RF heating in meat processing

Meat products are complex food systems, mainly due to the presence of fat and
proteins, a variable number of additives and moisture and salt being the key factors that
account for their dielectric properties (4). Several works have been done to understand
the role of different parameters such as geometry and composition, dielectric properties and thermal properties of food products during RF. Heating of beef rolls of *biceps femoris* muscle was compared in a steam oven vs. a RF oven (27.12 MHz) under recirculating water at 80°C. The RF protocol reduced cooking times compared to steam cooking times on meat that was not injected and also in rolls prepared with curing brines possessing similar dielectric properties. Cooking yields were significantly higher (P<0.05) on RF cooked rolls compared with steam-cooked rolls. Also, participants in a 50 untrained sensory panel were unable to detect texture differences, which had been indicated by previously with instrumental analysis (93). Another study evaluated the RF and steam cooking effect on quality parameters such as Water Holding Capacity (WHC), Texture Profile Analysis (TPA), penetration test ($d_p$), Warner Bratzler shear (WB), color, sensory evaluation and cooking time of two types of pork products (106). RF cooking resulted in a shorter cooking time, higher cooking yield (P<0.05) but a lower WHC (P<0.05) compared to steam cooking. TPA indicated that RF cooked samples were harder (P<0.05) for leg hams. This also agreed with the sensory evaluation where panelists also could differentiate between RF and steam cooked samples (P<0.05). A significant reduction in cooking times for leg and shoulder hams using RF was reported (68). However, on this particular study a number of quality-attributes such as hardness, penetration test and Warner-Bratzler maximum loads were higher on RF samples. Also, RF cooked hams reported lower WHC and higher yields than steam-cooked hams. In addition, RF cooking resulted in less well-done color, which was, attributed to the level of protein denaturation which was confirmed with Differential Scanning Calorimetry (DSC) analysis. RF and steam heating was also applied on turkey breast rolls (92). The
time to end point temperature (minimum 73°C) was 40 min compared to 150 min for steam-cooked product. Proximate analysis of macro components, assays of the B-vitamins, thiamine and riboflavin and TPA revealed no significant difference (P<0.05) between the two cooking methods. However, RF turkey rolls had lower Hunter a value (redness) than their steam cooked counterparts. In addition, the rate of lipid oxidation in RF cooked rolls during refrigerated storage at 5°C was significantly slower than in the steam-cooked products. Laycock and others (59), compared the heating rate, time temperature profiles and quality of three meat products (ground, comminuted and whole muscle) cooked in water bath or by a RF heater. RF cooking was found to reduce cooking times up to a quarter of conventional cooking times in a water bath. The results of this work also indicated that the surface of the RF cooked products heated at a faster rate than the center, with differences in temperature of 10-20°C at the end of the process, which the authors attributed to an uneven salt distribution for comminuted product.

Instrumental quality measurements indicated that RF cooked samples had lower juice losses than water bath cooked samples and were also acceptable in terms of color and water holding capacity. Instrumentally measured textural attributes on RF cooked whole muscle were not significantly different to water bath cooked samples. However some textural differences were noted between RF and water bath ground and comminuted meat samples.

**RF mechanism of inactivation for microorganisms**

The effects of electromagnetic energy on food components are similar to those found using other methods of heating; however, research has shown the benefits of bacterial destruction with reduced damage to sensory and nutritional properties due to
shorter processing times (34). The energy absorption from MW and RF is capable of raising the temperature of the food high enough to inactivate microorganisms for effective pasteurization or sterilization. MW and RF propose two mechanisms for inactivation of microorganisms. The first, states that microwaves inactivate microorganisms entirely by heat through mechanisms comparable to other biophysical processes induced by heat, such as denaturation of enzymes, proteins, nucleic acids, or other vital components as well as disruption of membranes (27). A second more controversial mechanism for inactivation by microwaves involves non-thermal effect. Four predominant theories have been used to explain non-thermal inactivation or “cold pasteurization”: selective heating, electroporation, cell membrane rupture and magnetic field coupling (10). The selective heating theory states that solid microorganisms are heated more effectively by RF waves than the surrounding medium and are thus killed more readily. Electroporation is caused when pores form in the membrane of microorganisms due to the potential across the membrane, resulting in leakage. Cell membrane rupture is related in that the voltage drop across the membrane causes it to rupture. In the fourth theory, cell lysis occurs due to coupling electromagnetic energy with critical molecules within the cells, disrupting internal components of the cell (27). Few publications have attempted to address the non-thermal mechanism on microbial inactivation through RF on apple cider, apple juice and orange juice (36, 37, 96).

Pathogen inactivation studies using RF

Different studies have evaluated inactivation of pathogens using RF heating in different food matrices without changing color and sensory characteristics. One study evaluated the efficacy of RF heating to inactivate Salmonella typhimurium and
Escherichia coli O157:H7 on black and red pepper spice (53). RF heating resulted in a reduction of 2.8 to 4.29 log CFU/g of S. typhymurium and E. coli O157:H7 for black pepper respectively and a reduction of 3.38 log CFU/g to more than 5 log CFU/g (below the detection limit) of S. typhymurium and E. coli O157:H7 for red pepper without affecting the color. A recent investigation (41) evaluated inactivation of S. typhymurium and E. coli O157:H7 in peanut butter cracker sandwiches using RF heating. After 90 s of RF treatment, the log reductions of S. typhymurium and E. coli O157:H7 were significant with 4.29 and 4.39 log CFU/g respectively, in creamy peanut butter and with 4.55 log CFU/g and 5.32 log CFU/g respectively in chunky peanut butter.

The efficacy of RF cooking on inactivation of Escherichia coli in ground beef and the effect on the shelf life stability of ground beef were investigated with a comparison to hot water bath cooking (40). E. coli K-12 was used as target bacterium instead of E. coli O157:H7 since the use of the first requires less stringent controlled working conditions as compared to the second strain, which requires a biosafety lab category 2 apt to handle human pathogens. In this particular study, E. coli K-12 was introduced with the ampicillin resistance gene (amp') to facilitate detection and enumeration of the target bacterium. Ground beef samples were inoculated with E. coli K-12 (amp') and heated until target temperature of 72°C. The samples were stored at 4°C up to 30 days. Both methods (RF and water bath cooking) reduced E. coli K-12 (amp'); however, RF had a significantly shorter cooking time and more uniform heating (P<0.01). For the shelf life studies enumeration of E. coli K-12, background E. coli and coliform counts were carried out for shelf life studies. The study concluded that RF cooking showed significant effects on reducing E. coli, thus maintaining shelf life and saving cooking time. The authors also
noted that RF would be a potential substitute for hot water bath cooking since RF was significantly faster and the difference between center and side temperature was significantly lower than hot water bath. In another study, *Bacillus cereus* and *Clostridium perfringens* vegetative and spore cocktails were inoculated into pork luncheon meat to challenge a previously developed RF cooking protocol (10). After RF cooking and cooling microbial enumeration results showed a reduction in *B. cereus* and *C. perfringens* vegetative cells and spores. However, the authors stated that the post cooking temperature was lower than anticipated as a consequence of addition of ingredients that altered the dielectrical properties of the meat contributing to reduced and less uniform temperatures. A major limitation of the RF technologies is lack of uniform heating distribution patterns due to the low number of efficient electromagnetic modes achievable in a tight band of frequencies, which may cause inconsistent profiles. Therefore, inactivation of foodborne pathogens in ground beef by cooking with highly controlled radio frequency energy was studied (83). Authors artificially inoculated meatballs with *Escherichia coli* O157:H7, *Salmonella typhymurium* and *Listeria monocytogenes* as well as spores of *Bacillus cereus* and *Bacillus thuringiensis*. Three cooking methods were compared: Convection cooking (external cooking) at 220°C for 40 min until core temperature of meatballs reached 73°C (±1°C); RF cooking (7.5 min) and combined convection-RF treatment (5.5 min). Cooking of meatballs using conventional heating reduced *E. coli* population by 5.5 log CFU/g while RF treatment reduced population to undetectable levels. Combined treatment reduced the level of *S. typhimurium*, and *L. monocytogenes* to undetectable levels. Authors concluded that combination RF and
convection cooking resulted in similar or even better effects on selected foodborne pathogens compared to convection only, while cooking times were reduced by 86%.
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CHAPTER 3

EFFECTS OF ENDPOINT TEMPERATURE AND THICKNESS ON QUALITY OF WHOLE MUSCLE NON-INTACT STEAKS COOKED IN A RADIO FREQUENCY OVEN

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1 Rincon, Angela, Singh, Rakesh K.
To be submitted to the *Journal of Food Science.*
Abstract

Non-intact beef steaks (NIBS) of different thicknesses were cooked to different endpoint temperatures using a radio frequency (RF) oven. Beef boneless short loin muscle was blade tenderized, cut into roasts and frozen until further use. Steaks were cut from thawed muscle into 1.2 cm, 1.9 cm and 2.5 cm thickness and then randomly assigned and cooked in a 6 kW 27.12 MHz RF oven until the center reached rare (55°C), medium rare (65°C) or well done (75°C) temperatures. High variability during cooking was detected for the 1.2 cm steaks; therefore, only cooking properties (cooking time, heating rate, cook and drip losses) of those steaks were evaluated. Physicochemical characteristics were mainly affected by degree of doneness for most of the parameters evaluated; however, thickness had an effect on cooking time, heating rate, cooking and dripping losses of NIBS. Post thermal results indicated that steaks cooked to 75°C showed greater cooking and drip losses, as well as greater moisture loss and shear value compared to steaks cooked to lower endpoint temperatures. Expressible moisture (EM) was highest at 55°C and further decreased as the endpoint temperature increased due to structural changes in the muscle and less moisture lost during cooking. Visual appearance of the product confirmed the volumetric heating as the color distribution of the steaks appeared bright pink at 55°C, slight pink at 65°C and completely brown at 75°C throughout the tissue. Experiments were conducted in pilot scale oven; therefore, our work offers the opportunity to develop a baseline for value added non-intact beef products cooked in thermal pouches to be delivered to customers for finishing cooking or reheating.
Introduction

Non-intact beef steaks (NIBS) are value added products made from subprimal beef cuts that are usually “tough” in structure and less desirable for consumption as regular steaks (14). Blade tenderization increases the value of tougher muscles by increasing the perceived tenderness of beef (2) making subprimal beef cuts more palatable by disruption of the structure of the beef muscles (14). In the United States at least 18% of beef products available at the retail level have been mechanically tenderized or injected with solutions for enhancement of tenderness and/or flavor (45). Products from non-intact muscle are sold at lower prices than intact beef products; therefore, consumed in large quantities in many restaurants (14). However, NIBS tenderized with blades have the potential of transferring external meat surface contamination, if present, to the interior of the normally sterile deep tissue (2). Thus, it is important to find alternatives to conventional methods of heat processing that reach greater penetration depth and potentially lead to more uniform heating without higher energy consumption.

Conventional methods applied to transfer heat to food include hot air or water, steam grilling by direct conduction, frying with oils and radiant heating (9). Meat products are relatively poor thermal conductors and, as most conventional methods rely on heat penetration via conduction, convection or radiation from the outside to the inside of the product, the cooking times can be relatively long in industrial scale processing operations (39). Radio Frequency (RF) heating is a promising alternative to conventional methods of thermal processing in which heat is generated within the product, which reduces cooking times and could potentially lead to a more uniform heating (22). The mechanism of RF heating relates to the fact that the molecules within a product placed in
a RF environment reorient themselves continuously in response to the applied field (i.e. dipole heating). This response initiates volumetric heating within the entire product due to frictional interaction between the molecules. Thus, RF heating is accomplished through a combination of dipole heating and electric resistance resulting from the movement of dissolved ions present in the food (3). RF heating has been used in the food processing industry for more than 60 years. Heating uniformity is one of the most important considerations in the development of heating and drying processes when using RF energy (42). The most successful application is in post-bake drying of biscuits and cookies (40), blanching of vegetables (28, 39) and thawing of frozen products (7). Other applications have also been explored in disinfestation, enzyme inactivation, pasteurization and sterilization (16). For this technology to become commercial, food technologists and engineers will require detailed knowledge of the effect factors such as the meat type, temperature, recipe, state of meat before cooking dielectric properties (21), geometry and shape of product (42). RF heating of meat has been studied with uncased meat products (12, 19) and also meat products cooked in plastic cases or inside thermal resistant bags (5, 12, 17, 20, 39, 47).

One of the problems with RF heating in the food industry is the risk of arcing and thermal runaway during processing (48). Those problems can be overcome by packing the product in proper plastic bags prior to cooking (17) and immersion of product in water (39). To our knowledge, there are only a limited number of studies that have evaluated RF cooking in whole muscle meat products (17, 19). RF offers the advantages of providing more uniform heating due to deep penetration and simple uniform field patterns (11), which makes it potentially ideal to cook value added products such as
NIBS. RF technology may also offer inactivation of pathogens that may have migrated to the center of the tissue during tenderization. Thus, the objectives of this work were first to: (i) develop a Radio Frequency cooking protocol for steaks fabricated from non-intact beef muscle in order to achieve different target endpoint temperatures and (ii) evaluate physicochemical and quality parameters of NIBS cooked with RF.

**Materials and Methods**

**Sample preparation**

Short loin (NAMP 180) subprimals were purchased at a local store (Restaurant Depot, Atlanta, GA) and transported (2 ± 2°C) to the University of Georgia, Department of Food Science and Technology (Athens). Whole muscle cuts were prepared by trimming of fat, connective tissue and silver skin. Subprimals were then transported to the University of Georgia Meat Science Technology Center (Athens) to be tenderized. Each subprimal was passed once on each side through a blade tenderizer (Ross TC700M, Midland, VA). The tenderizer produced approximately 10 incisions per cm² with a blade width of 3 mm. Handling and transportation of the meat was done at 4 ± 2°C. Subprimals were then cut into roasts of about 1.3 kg, packed in multilayer PD-900 plastic bags (Cryovac, Duncan, SC), vacuum sealed (Henkelman, Hertogenbosch, Netherlands) and stored in a blast freezer at -20°C until testing. Before cooking, roasts were tempered at (2 ± 2°C) during 18-24 h and then sliced perpendicular to the muscle fibers into three different thicknesses including 1.2, 1.9 and 2.5 cm (n = 9) using a commercial meat slicer (model 808, Berkel Incorporated, Laporte, Indiana, US). Steaks were also selected for a weight range before cooking of 100 ± 5 g for the 1.2 cm steaks, 185 ± 5 g for the 1.9 cm steaks and 225 ± 5 g for the 2.5 cm steaks; using a scale (Fairbanks Scale, Kansas City, MO).
**RF Equipment**

All treatments were conducted at a constant frequency of 27.12 MHz and a power of 6 kW in a RF oven (Model S061B Stray-field Ltd, Reading, UK). The equipment consists of a RF generator, two electrodes, and a conveyor belt. Steaks were placed in between the upper and lower electrodes.

**Temperature measurement**

Fiber optic temperature probes (Fiso Tech. Inc., Quebec, Canada) were used to monitor the steak and water temperature during RF cooking. In order to place thermocouple inside of package and steak, a hole was made on the bags with a #5 brass cork borer. Then, a stuffing box (C-5.2D, Eucklund-Harrison Tech., Fort Myers, FL) was placed on the pouches so that the fiber optic cable could be inserted through the package and then at the visual center of the steak. Packages were then sealed in a chamber vacuum sealing machine (Henkelman, Hertogenbosch, Netherlands). Temperature was recorded using a multichannel data logger (Fiso Tech. Inc., Quebec Canada) continuously during RF heating.

**RF cooking protocol**

Preliminary studies were performed for selection of thermal resistant pouches and trays invisible to the electromagnetic waves. Steaks (4 ± 2°C) were individually packed inside of a nylon/linear low density polyethylene pouch (Winpak, Minneapolis, MN) and placed into polysulfone trays (H-Pan™, Cambro, Huntington Beach, CA), then covered with tap water. A special grid elaborated with Polyetherimide was designed to maintain the steak immersed inside of the water preventing packages from floating caused by expansion of vapor inside the tissue during cooking. This step reduced the risk of arcing.
during processing but did not completely eliminate it (Figure 3-1). The RF oven was turned off once the center of the steaks reached the desired endpoint temperature. The USDA-FSIS recommends minimum internal temperature of 62.8°C for cooking beef steaks (23). The chosen internal temperatures were below and above the recommended temperature and corresponded to very-rare (55°C), medium-rare (65°C) and well-done (75°C) cooking of the product (1).

**Temperature distribution following RF**

Once the RF cycle was over the meat packages destined for quality measurements were removed from the hot water and center temperature was monitored for an additional 5 minutes to further characterize post-cooking temperature rise differences due to steak thickness and tenderization as previously assessed (36). Temperature history results were analyzed for each batch in order to determine heating rate at different points (upper end and bottom end) within the package to evaluate heat distribution as described on a previous study (17). A K-type thermocouple (type K, Fisher Scientific, Pittsburgh, PA) was used to measure temperature distribution at points different than the center within the pouch at the end of RF cycle. The temperature was recorded on the upper side of the steak (2 cm from the upper edge) and lower side (2 cm from the bottom edge of steak). All thermocouples were calibrated once per processing day using a mixture of ice water (0°C ± 2).

**Meat Quality Measurements**

As soon as the endpoint temperature was reached, cooked steaks were allowed to cool at room temperature (about 21°C). Analysis of cooking measurements, proximate composition, pH and expressible moisture were conducted at day zero. After those tests
were conducted, cooked steaks were placed into polyethylene bags, cooled and refrigerated overnight at 4 ± 2°C. The following day Warner-Brazler shear test and instrumental color evaluations were performed.

**Cooking Measurements**

Total cooking loss and drip loss were determined by calculating the weight differences for the steaks before cooking (raw weight) and post thermal cooled samples (cooked weight) as follows:

\[
\text{Total Cooking loss} (%) = \frac{\text{raw weight} - \text{cooked weight}}{\text{raw weight}} \times 100
\]

\[
\text{Total Drip Loss} (%) = \frac{\text{drip weight}}{\text{raw weight}} \times 100
\]

**Proximate analysis**

Proximate analysis was performed in raw and cooked samples. **Moisture content** was determined in duplicate per sample \((n = 9)\) using the Association Official Analytical Chemists (AOAC) 950.46 method (AOAC, 1995). About 3-3.5 g samples were placed in appropriate pre-dried Whatman paper (No 1 diameter 185mm) and dried in a vacuum oven (Cole-Parmer Instrument Co., Vernon Hills, IL) at 10.7 kPa pressure and 99°C for 24 h. **Fat** was determined in duplicate per sample \((n = 9)\) using the solvent extraction method 920.153 (AOAC, 1995). **Protein content** was determined with an Automatic Kjeldahl nitrogen analyzer (Kjtech 2300 Analyzer Unit, Foss Tecator AB). **Ash content** was determined by heating the sample in a furnace at 400°C for 4 h and weighing the residue (47).

**pH value**

The pH values of fresh and cooked samples were measured in triplicate per sample \((n = 9)\) according to a direct probe method. A pH meter was used and inserted
with a meat surface probe (I.Q. Scientific Instruments, Loveland, CO). The probe and the pH meter were calibrated using pH 4.00 and 7.00 buffer solutions (Fisher Scientific, Pittsburg, PA).

**Expressible moisture**

The expressible moisture was measured in duplicate per sample \((n = 9)\) according to the filter press method \((43)\). Samples having a fixed diameter and 300 ± 10 mg were obtained from raw and cooked meat using a brass cork borer. Samples were placed on pre-weighed Whatman paper (no. 1, diameter 90 mm) between two plexiglass plates and compressed by a 1 kg load cell for 1 min. After that time, the filter paper was weighed to determine amount of water released from the sample. Expressible moisture was calculated with the following formula:

\[
Expressible Moisture (EM) = 100 \times (W_{\text{final}} - W_{\text{initial}})
\]

\(W_{\text{final}} = \text{weight of paper after compression}\)

\(W_{\text{initial}} = \text{initial weight of filter paper}\)

**Warner-Bratzler shear test**

Cooked steaks were cooled for 24 h at 4\(^\circ\)C. Six round cores (1.27 cm) per sample \((n = 9)\) were removed parallel to the long axis of the muscle fibers using a hand-held coring device. Each core was sheared once through the center using a Warner-Bartzler shear attachment (V-notch blade) connected to a texture analyzer (TMS-PRO, Food Technology Corporation, Sterling, VA). A 490 N compression load cell was used and the crosshead speed was 200 mm/min.
Instrumental color measurement

Color measurements for cooked samples were made using the Hunterlab MiniScan EZ spectrophotometer (D/8-S, 45/0 LAV, 14.3 mm diameter aperture 10° standard observer, Illuminant A; Hunter Associates Laboratory, Inc., Reston, VA, USA). Lightness ($L^*$), $a^*$ (redness/greenness) and $b^*$ (yellowness/blueness) were determined. The measurements were reported as a mean of six different random areas on each steak per endpoint temperature and thickness. The color hunter was calibrated using a fixed input standard.

Statistical analysis

Sample size was estimated using Statistical Solutions, LLC software (37) to avoid Type II error (Power test = 0.80). Sample size corresponded to 1.2, 1.9 and 2.5 cm ($n = 9$) thick steaks cooked to 55, 65 and 75° C endpoint temperatures ($n = 9$); therefore, the experimental design was a $3 \times 3$ completely random design conducted over three replicates. Two-way ANOVA using the General Linear model procedure of SAS statistical package (SAS Version 9.3, SAS Institute, Cary, NC) was chosen to verify the effect of thickness and endpoint temperature on the data. The Tukey Studentized Range (HSD) test was set to alpha = 0.05 in order to determine differences between treatment means.

Results and Discussion

RF Cooking Protocol

Heating rate of product using RF depends on several parameters such as anode and grid current, dielectric properties and heating medium. Therefore, preliminary experiments were conducted to evaluate different factors such as effect of power (anode
current) on the heating profile of steaks, packaging performance and integrity and selection of trays invisible to electromagnetic waves. Decreasing the distance between electrodes in the RF can increase anode and grid current. Thus, as the distance decreases the risk of arcing and melting of package also increases (17). It was determined that the optimal power for heating steaks was at anodic current of 0.6 A. Selection of packaging materials suitable for RF processing was also evaluated. Low density polyethylene pouches and polysulfone resin trays (24), were found to be very stable during RF processing and they also showed a significant reduction in arcing. Previous studies have reported failure to cook in the air medium when using RF (5, 17, 20); therefore, water immersion cooking was implemented. However, it has been reported that it was difficult to control the anode current when the water surrounding the product increased above 95°C (17). Hence, the water temperature was cooled down with ice to 6 ± 2°C before processing so water temperature would not exceed 90°C after the RF cycle was finished.

Most protocols for safe and efficient RF cooking have been previously developed for uniformly shaped encased meats and emulsions (20, 39, 40) as well as whole muscle products (17, 19). The amount of heat absorbed by a food, the heating rate and the location of cold spots depend on the food composition, the shape and the size of the food as well as frequency used during cooking (8). For a heterogeneous food matrix system such as steaks, the composition of the food (moisture content, intramuscular fat) not only changes across the muscle but also changes as cooking progresses. Thus, in order to determine best settings to develop a RF cooking protocol, it was necessary to monitor additional factors besides power such as initial steak and water temperature, amount of water covering the steaks, thickness, weight, and location of the steak within the tray.
during processing. Combining of all factors minimized arcing of equipment but did not completely eliminate the problem. The effect of thickness on the heating profile showed that when cooking 1.2 cm steaks, the equipment was unstable. In order to maintain power settings constant (0.6 A), the distance between electrodes had to be decreased, which in turn increased the risk of arcing in the equipment. Compositional properties, pH, EM, color and shear force would have increased variation of the results with the current sample size \((n = 9)\) for 1.2 cm steaks; therefore, analysis of these parameters was not performed at such thickness. Time-temperature diagrams, heating rate, temperature distribution and cooking properties were however, analyzed for 1.2 cm steaks.

**Time-temperature profiles**

Time-temperature profile during RF cooking of non-intact beefsteaks was measured using fiber optic sensors, until the center reached the designated temperature (55°C, 65°C or 75°C). A descriptive statistics time-temperature diagram of steaks heated to 75°C is displayed on Figure 3-2. It was observed that for the first few minutes, there was a characteristic initial linear profile of a RF heated product and it also indicated a faster heating rate for the steaks compared to surrounding water. This behavior agrees with time-temperature profiles from previous studies \((5, 17, 19, 29)\). As cooking progressed, the heating pattern gradually shifted to resemble more of a curvilinear profile, typical of a product where heat transfer takes place mostly by conduction from surrounding water. At RF frequencies, the conductivity of water and hence the amount of energy absorbed increases at high temperatures \((8)\). Thus, it is expected that as the temperature of the water surrounding the product increases, its heating rate also increases which was also observed in the present study.
Heating rate and endpoint temperature distribution

Table 3-1 shows the effect of thickness and endpoint temperature on the heating rate (°C/min) of whole muscle non-intact steaks. A significant interaction between thickness and endpoint temperature was observed; however, there was no temperature trend across all thicknesses. The heating rate was not significantly different ($p \leq 0.05$) across temperatures for 1.2 cm steaks while for 1.9 cm and 2.5 cm steaks, the heating rate at 55°C was significantly higher than heating rate at 65°C or 75°C. RF generates heat rapidly within the product, due to frictional interactions of polar dielectric molecules (water) rotating in the space charge displacement in response of an externally applied AC electric field (11). At 55°C water was still being entrapped within muscle and the electromagnetic energy was being directed to those molecules. As the temperature increased the water started to migrate out of tissue and the heating rate decreased. When RF energy heats water in foods, it increases the vapor pressure and causes movement of the moisture from the interior to the surface (8). The heating pattern of any food subjected to electromagnetic radiation depends first on the way energy is absorbed by the product as determined by dielectric properties, and second on the manner in which the absorbed energy is transferred to the material as influenced by the thermophysical properties (7, 29). The dielectric properties can be described by the dielectric constant ($\varepsilon'$) that means the ability of the product to store electromagnetic energy, and the dielectric loss factor ($\varepsilon''$) or its ability to dissipate the electromagnetic energy (29), which results in heat generation (35). The amount of thermal energy converted in the food is proportional to the value of the loss factor (38). The temperature dependence of the dielectric constant is quite complex but generally loss factor increases, with increasing
temperature at low frequencies due to ionic conductance (35). The current study shows that as temperature increased, probably the $\varepsilon''$ also increased which resulted in lower heating rates.

The endpoint temperature distribution of steaks is shown in Table 3-2. There was a significant effect ($p \leq 0.05$) of final temperature measured in the upper and lower edges of the steak for thickness but this effect was not significant for the final temperature at the center. Temperatures at the center, upper area (2 cm from edge position), and lower area (2 cm from edge position) were recorded in order to study the average temperature differential ($\Delta T$) within the package after cooking. RF cooking is in theory a volumetric method of heating with all parts of the product receiving equal amounts of heat and therefore temperature differentials should not develop (47). However, this will only be the case if the product to be heated is completely dielectrically homogeneous which differs from a product such as whole muscle steak. Temperature differentials can occur for a number of reasons. A lack of dielectric uniformity caused by areas of products with high capacities for RF absorption can lead to runaway heating and even product arcing in these areas (30, 46). Temperature of the cooked steaks near to the edges was expected to be greater than the temperature at the center due to shape or corner effect where edges are more exposed to more energy than the middle part resulting in runaway heating (17). However, in this particular case, temperatures of steaks were few degrees lower at the edges compared to the center. The reason explaining this change may be that while the center temperature was evaluated permanently during and after cooking, the temperature at the edges was measured once the cooking process was over and the steaks were taken out of the water. Some of the heat could have escaped from the
edges faster than at the center. Also, even though the thermocouples were calibrated at the beginning of each processing day, copper-constantan thermocouples were used on the edges while fiber optic thermocouples were used at the center, which may have contributed to a few degrees difference.

**Cooking properties**

No interaction was found between thickness and endpoint temperature with respect of cooking time (Table 3-3). There was a significant effect of cooking time as the endpoint temperature increased across all thicknesses \( p < 0.0001 \). It would be expected that as the mass of the product increases, the steak would require more time to cook; however, there was a 12% increase in cooking time \( p \leq 0.05 \) for 1.2 cm steaks with respect to 1.9 and 2.5 cm steaks. These results indicate that the coupling of the RF energy with the 1.2 cm steaks was not optimal and some of the energy was being lost creating high variability during cooking. A previous study also reported problems cooking 1-2 cm carrot sticks in thin layers at the frequency of 27.12 MHz \( (28) \). A reduction in thickness close to 25% was also observed for the thin steaks as the vacuum sealing of the samples took place. The RF manufacturer advises a rod electrode for samples 0.6 cm thick; thus, implementation of such electrode would have perhaps decreased variability during processing for such samples. The present study reports that the average cooking time for 75°C steaks was 18.67 ± 0.57 minutes. Previous work \( (19) \), reported whole muscle product that was cooked to 72°C to have an average cooking time of beef whole muscle as 13.25 minutes, which resulted in a power efficiency of 43% compared to water bath cooking. Another study indicated a 79% reduction in cooking time achieved with RF compared with steam cooking for a pork leg and shoulder ham and a comminuted meat
product \((46, 47)\). It is important to notice though, that the maximum output power of the former studies was 1.5 kW (695W) and 500 W respectively compared to 6 kW output power used in the present study.

Loss of juices during meat cooking is known to be due to heat-induced protein denaturation which causes less water to be entrapped within the protein structures held by capillary forces \((29)\). It can be observed (Table 3-3) that there was no significant interaction between thickness and endpoint temperature observed for cooking loss and drip loss. In regards of thickness, the present study shows a higher drip loss \((p \leq 0.05)\) for 2.5 cm steaks compared to 1.2 cm and 1.9 cm steaks. It is expected that cooking losses increase as thickness of steaks increase due to the fact that thicker steaks need more time to reach endpoint temperatures \((32)\). In this particular case, even though cooking times were higher for 1.2 cm steaks compared to 2.5 cm steaks, the high cooking loss and drip losses were probably due to previous blade tenderization and posterior freezing thawing of the muscle; which made steaks more susceptible to release water during cooking. In fact, blade tenderization disrupts and opens muscle structure, which allows moisture to escape from the interior of the meat to the exterior more easily \((25)\).

A strong \((p < 0.0001)\) endpoint temperature effect existed for cooking loss and drip loss (Table 3-3), showing a significant increase as the endpoint temperature increased. Several researchers \((6, 27, 29)\) have also reported increased cooking loss and drip loss with increased endpoint temperatures using different cooking technologies. The present results display a relative increase on cooking loss becoming more pronounced at endpoint temperatures >60°C, which agrees with other authors \((4, 26)\). A drip loss of 20.36 ± 1.45 % was obtained for steaks cooked to 75°C with RF, while Laycock and
others (19) obtained higher juice losses for whole muscle product cooked to 72°C with RF reporting 28.7%. These losses depend on the mass transfer process during thermal treatment, which in turn is influenced by the characteristics of the cooking procedure (i.e. heating rate, endpoint temperature) and of the meat system (i.e. moisture, fat, protein, size, shape, etc) (31).

**Proximate composition analysis**

Total percent of moisture, fat, protein and ash analyses of the raw meat and the cooked steaks are presented in Table 3-4. Endpoint temperature influenced ($p \leq 0.05$) the percentage of moisture as opposed to the thickness, which did not have an effect on moisture content. As the endpoint temperature increased, the percent of moisture generally decreased. This was because the cooking process caused a loss of moisture as previously seen with the drip loss. Similar trends for degree of doneness have been observed for beef products (34). There was no significant effect ($p \leq 0.05$) for protein as the thickness or endpoint temperature changed (Table 3-4). However, when comparing the mean levels of the two main compositional components of the steaks (i.e., moisture and protein contents) the steaks had lower moisture and protein contents as the endpoint temperature increased which is consistent with lower moisture loss and a more “dilute” product as seen in previous studies (40). Fat percentage was relatively constant among degrees of doneness. This trend was not evident across thickness but the effect was not significant ($p \leq 0.05$). The ash content was not significantly different for either the thicknesses or the endpoint temperatures.
**pH and Expressible Moisture**

In our study, the endpoint temperature had a significant effect \((p \leq 0.05)\) on pH (Table 3-5). Cooking induced an increase on the pH of the steaks as previously supported \((31, 33)\). The net charge of muscle proteins may affect the retention of water through interactive forces between charges. Changes in pH value of meat during heating was likely a result of the dynamic balance of acid-base groups at the surface of sarcoplasmic proteins that were being degraded as temperature increased \((13)\). The endpoint temperature had a significant effect on EM; while the thickness effect was not significant (Table 3-5). As the steaks reached 55°C and 65°C, there was a significant increase \((p \leq 0.05)\) in EM compared to raw steak and 75°C steaks. The review of Tornberg \((41)\) summarizes the structural changes that explain results obtained in the present research. The amount of water around fiber bundles increases up to 50°C, in comparison to the raw meat, which seems to be in accordance with the transverse shrinkage of fibers and fiber bundles in the muscle. Above 50°C, this widened gap diminishes again up to 70°C, probably due to the shrinkage of the connective tissue of the meat causing the gradual reduction in EM.

**Visual appearance and Instrumental color**

Figure 3-6 shows internal color (cut across the grain) of the steaks after each endpoint temperature compared to raw steak. It can be seen that the internal color distribution corresponds to a product heated with electromagnetic energy. There was no browning on the outside and a pinker color at the center and a more uniform color across the tissue; which confirms the volumetric heating nature of the process. Visually, the steaks looked drier as the endpoint temperature increased particularly at 75°C. Although
sensory analysis was not performed on these steaks, it could be anticipated from their
drip losses, moisture content and expressible moisture results that 75°C heat treated
samples would not be as juicy as 65°C or 55°C samples. Several changes in appearance
and physical properties of meat occur during heating processes, such as discoloration of
meat due to the oxidation of the pigment heme group (29). Myoglobin changes were
visually observed starting with raw product being bright red, transitioning to 55°C with
bright pink, followed by slight pink at 65°C until it looked completely brown at 75°C.
Color change is initially due to myoglobin denaturation, shifting from deep red to pink
and then to a greyish color before finishing in a light brown. It is recognized that those
changes occur near 60°C, between 60 and 70°C and between 70 and 80°C respectively
(18).

There was a thickness effect for \( p \leq 0.05 \) \( L^* \) and \( b^* \) values; at the same time
there was an endpoint temperature effect \( p \leq 0.05 \) for \( a^* \) and \( b^* \) but no interaction
effects (Table 3-6). Lightness for 1.9 cm steaks was significantly higher than 2.5 cm
steaks leading to a paler colored beef which also indicated a higher degree of myoglobin
denaturation (29). Regarding degree of doneness, \( L^* \) values slightly decreased as the
temperature increased which has been previously observed (29, 44). A higher \( L^* \) value
indicates a lighter color, which is desirable in order to ensure that the meat products will
have high consumer acceptance (10). During cooking, denaturation of the bright
oxymyoglobin at the surface and of the purple red myoglobin in the interior takes place,
resulting in color changes in the cooked product that are dependent on both, the type of
meat and also on the severity of the cooking process (39). It was observed that as the
endpoint temperature increased, the redness \( a^* \) significantly decreased \( p \leq 0.05 \) which
is in agreement with previous studies (29, 33). The decrease in $a^*$ values is also related to the myoglobin denaturation that is produced in the range of 55-80°C (15). Laycock and others (19) cooked whole muscle to 72°C using RF reporting values for whole muscle of $L^*$ value of 49.83 ± 0.86 and $a^*$ value of 7.35 ± 0.34. Values from the present study showed $L^*$ value of 47.65 and $a^*$ value of 6.92 which indicates that myoglobin changes took place around the 55-75°C range. Degree of doneness also had a significant effect reflecting lower $b^*$ (less yellow) values as the endpoint temperature increased also likely due to more myoglobin oxidation at higher temperatures (27).

**Shear Force**

The effect of thickness and endpoint temperature on Warner-Bratzler shear force (WBSF) values is shown in Table 3-6. There were no interactions ($p \leq 0.05$) among thickness and endpoint temperature for the texture of the steaks. Thickness had no effect ($P<0.05$) on WBSF while endpoint temperature significantly ($p \leq 0.05$) affected WBSF. Steaks cooked to rare (55°C) and medium rare (65°C) degree of doneness reported significantly lower shear values ($p \leq 0.05$) compared to those cooked to medium well (75°C) that had the highest ($P<0.05$). Earlier studies have also reported that shear force increases as the endpoint temperature increases. Obuz et al. (26), found that the shear force increased as the endpoint temperature increased above 55°C in *longissimus* beef steaks cooked in a water bath and on a belt grill. Yancey et al. (44) also reported increased shear force values as *longissimus* steaks were cooked to 65.5°C and 76.6°C. During heating, the different meat proteins denature and they cause structural changes such as destruction of cell membranes, transversal and longitudinal shrinkage of meat fibers, aggregation and gel formation of sarcoplasmic proteins and the shrinkage and
solubilization of the connective tissue (41). In addition, heating method is an important factor affecting the tenderness of the meat. For example, the tenderness of meat heated in water bath is better than that of microwave heated meat, which may be due to the higher loss of water from muscle during microwave heating resulting in greater hardening of myofibrillar proteins (13).

Conclusions

The RF cooking of non-intact steaks was successfully carried out by packing the steaks in low-density polyethylene pouches inside polysulfone trays with the integration of a customized polyetheremide grid and water immersion. Control of different parameters such as initial temperature of steak and water, steak thickness, location of the steaks inside trays, steak thickness and volume of water added minimized arcing but did not completely eliminate the problem. RF cooking was heterogeneous resulting in a temperature differential within the steak; however, there were no detectable differences in meat color and tenderness throughout the product. In summary, thickness had an effect on cooking properties (cooking time, cooking loss, yield loss and heating rate). However, physicochemical characteristics of NIBS were affected mainly by endpoint temperature. The specific degree of doneness (55°C, 65°C or 75°C) had a significant effect on cooking time, heating rate, cooking and drip losses, moisture content, pH, EM, $a^*$ value and shear force. Higher drip and cooking losses as well as lower moisture in 75°C steaks were due to sample pretreatments (blade tenderization, freezing and thawing), as well as meat structural changes caused by protein denaturation as the temperature rose. All changes were confirmed by visual appearance of the samples, which looked, drier on samples cooked to well done compared to medium well and rare
temperature. Expressible moisture (EM) was highest at 55°C and further decreased as the endpoint temperature increased and also due to structural changes in the muscle. Our experiments may be the starting point for the industry to develop RF cooking for value added meat products fabricated from non-intact muscle allowing faster heating rates (energy savings), reducing pre-processing contamination (from potential translocation of surface bacteria to the interior of the muscle during tenderization), post-processing contamination and in general offering better quality. In addition it is recommended that supplemental studies be conducted for marinated steaks since marinade ingredients will change the dielectric properties of the system. Due to the potential risk transferring surface contamination (Shiga toxin-producing *Escherichia coli*) from the surface to the interior of the tissue of NIBS, further studies will involve challenge studies at different temperatures to evaluate the present cooking protocol in terms of food safety (highest log reduction of pathogens) on the steaks.
References


TABLE 3-1 Effects of thickness and endpoint temperature on cooking time and heating rate of steaks cooked with RF\textsuperscript{a}

<table>
<thead>
<tr>
<th>Main Effects</th>
<th>Cooking Time (min)</th>
<th>Heating Rate (°C/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Thickness (cm)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.2</td>
<td>16.18\textsuperscript{A}</td>
<td>4.10\textsuperscript{C}</td>
</tr>
<tr>
<td>1.9</td>
<td>14.42\textsuperscript{B}</td>
<td>4.67\textsuperscript{B}</td>
</tr>
<tr>
<td>2.5</td>
<td>14.12\textsuperscript{B}</td>
<td>5.25\textsuperscript{A}</td>
</tr>
<tr>
<td><strong>p-value</strong></td>
<td>0.0072</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td><strong>Endpoint Temperature\textsuperscript{b} (°C)</strong></td>
<td>\textbf{0.57}</td>
<td>\textbf{0.18}</td>
</tr>
<tr>
<td>55</td>
<td>10.44\textsuperscript{C}</td>
<td>5.81\textsuperscript{A}</td>
</tr>
<tr>
<td>65</td>
<td>15.61\textsuperscript{B}</td>
<td>4.17\textsuperscript{B}</td>
</tr>
<tr>
<td>75</td>
<td>18.67\textsuperscript{A}</td>
<td>4.04\textsuperscript{B}</td>
</tr>
<tr>
<td><strong>p-value</strong></td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td><strong>Thickness*Endpoint T</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SEM\textsuperscript{c}</td>
<td>0.57</td>
<td>0.18</td>
</tr>
</tbody>
</table>

\textsuperscript{a} Means within the same effect with different letters in a column are significantly different (p \leq 0.05)

\textsuperscript{b} Degree of doneness according to the Beef Steak Color Guide (National Live Stock and Meat Board) Chicago, IL and are described as Very rare (Approx. 55°C), Medium rare (Approx. 65°C) and Well done (Approx. 75°C)

\textsuperscript{c} SEM = Standard Error of the Mean (n = 9)
### TABLE 3-2 Effects endpoint temperature on temperature distribution of steaks cooked with RF

<table>
<thead>
<tr>
<th>Main Effects</th>
<th>Upper Temperature</th>
<th>Center Temperature</th>
<th>Lower Temperature</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2 cm from edge (°C)</td>
<td>Visual center (°C)</td>
<td>2 cm from edge (°C)</td>
</tr>
<tr>
<td><strong>Thickness (cm)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.2</td>
<td>61.44&lt;sup&gt;B&lt;/sup&gt;</td>
<td>65.23&lt;sup&gt;A&lt;/sup&gt;</td>
<td>63.00&lt;sup&gt;A&lt;/sup&gt;</td>
</tr>
<tr>
<td>1.9</td>
<td>60.62&lt;sup&gt;B&lt;/sup&gt;</td>
<td>64.69&lt;sup&gt;A&lt;/sup&gt;</td>
<td>59.26&lt;sup&gt;B&lt;/sup&gt;</td>
</tr>
<tr>
<td>2.5</td>
<td>65.72&lt;sup&gt;A&lt;/sup&gt;</td>
<td>65.27&lt;sup&gt;A&lt;/sup&gt;</td>
<td>65.12&lt;sup&gt;A&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>p-value</strong></td>
<td>0.0005</td>
<td>0.2504</td>
<td>0.0005</td>
</tr>
<tr>
<td><strong>Endpoint Temperature</strong>&lt;sup&gt;b&lt;/sup&gt; (°C)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>55</td>
<td>52.14&lt;sup&gt;C&lt;/sup&gt;</td>
<td>54.90&lt;sup&gt;C&lt;/sup&gt;</td>
<td>52.30&lt;sup&gt;C&lt;/sup&gt;</td>
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<td>63.99&lt;sup&gt;B&lt;/sup&gt;</td>
<td>65.03&lt;sup&gt;B&lt;/sup&gt;</td>
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<td>71.07&lt;sup&gt;A&lt;/sup&gt;</td>
</tr>
<tr>
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<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td><strong>SEM</strong></td>
<td>1.17</td>
<td>0.35</td>
<td>1.20</td>
</tr>
</tbody>
</table>

<sup>a</sup> Means within the same effect with different letters in a column are significantly different (<i>p</i> ≤ 0.05)

<sup>b</sup> Degree of doneness according to the Beef Steak Color Guide (National Live Stock and Meat Board) Chicago, IL and are described as Very rare (Approx. 55°C), Medium rare (Approx. 65°C) and Well done (Approx. 75°C)

<sup>c</sup> SEM = Standard Error of the Mean (<i>n</i> = 9)
TABLE 3-3 Effects of thickness and endpoint temperature on cooking loss and drip loss of steaks cooked with RF

<table>
<thead>
<tr>
<th>Main Effects</th>
<th>Cooking Loss (%)</th>
<th>Drip Loss (%)</th>
<th>p-value</th>
<th>SEMc</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Thickness</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.2 cm</td>
<td>16.56B</td>
<td>11.21B</td>
<td>0.016</td>
<td>1.55</td>
</tr>
<tr>
<td>1.9 cm</td>
<td>17.11B</td>
<td>12.80AB</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.5 cm</td>
<td>21.48A</td>
<td>15.82A</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Endpoint Temperature</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>55°C</td>
<td>10.94C</td>
<td>6.95C</td>
<td>&lt;0.0001</td>
<td></td>
</tr>
<tr>
<td>65°C</td>
<td>17.20B</td>
<td>12.52B</td>
<td></td>
<td></td>
</tr>
<tr>
<td>75°C</td>
<td>27.00A</td>
<td>20.36A</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Means within the same effect with different letters in a column are significantly different (p ≤ 0.05)

Degree of doneness according to the Beef Steak Color Guide (National Live Stock and Meat Board) Chicago, IL and are described as Very rare (Approx. 55°C), Medium rare (Approx. 65°C) and Well done (Approx. 75°C).

SEM = Standard Error of the Mean (n = 9)
TABLE 3-4 Effects of thickness and endpoint temperature on proximate composition of steaks cooked with RF

<table>
<thead>
<tr>
<th>Main Effects</th>
<th>Moisture (%)</th>
<th>Protein (%)</th>
<th>Fat (%)</th>
<th>Ash (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thickness</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.9 cm</td>
<td>67.85&lt;sup&gt;A&lt;/sup&gt;</td>
<td>13.48&lt;sup&gt;A&lt;/sup&gt;</td>
<td>13.97&lt;sup&gt;A&lt;/sup&gt;</td>
<td>4.03&lt;sup&gt;A&lt;/sup&gt;</td>
</tr>
<tr>
<td>2.5 cm</td>
<td>67.71&lt;sup&gt;A&lt;/sup&gt;</td>
<td>16.24&lt;sup&gt;A&lt;/sup&gt;</td>
<td>9.37&lt;sup&gt;B&lt;/sup&gt;</td>
<td>4.43&lt;sup&gt;A&lt;/sup&gt;</td>
</tr>
<tr>
<td>p-value</td>
<td>0.864</td>
<td>0.4730</td>
<td>0.0024</td>
<td>0.098</td>
</tr>
<tr>
<td>Endpoint temperature</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Raw</td>
<td>71.71&lt;sup&gt;A&lt;/sup&gt;</td>
<td>13.89&lt;sup&gt;A&lt;/sup&gt;</td>
<td>9.02&lt;sup&gt;A&lt;/sup&gt;</td>
<td>4.21&lt;sup&gt;A&lt;/sup&gt;</td>
</tr>
<tr>
<td>55&lt;sup&gt;°&lt;/sup&gt;C</td>
<td>69.04&lt;sup&gt;AB&lt;/sup&gt;</td>
<td>11.88&lt;sup&gt;A&lt;/sup&gt;</td>
<td>12.14&lt;sup&gt;A&lt;/sup&gt;</td>
<td>4.18&lt;sup&gt;A&lt;/sup&gt;</td>
</tr>
<tr>
<td>65&lt;sup&gt;°&lt;/sup&gt;C</td>
<td>66.39&lt;sup&gt;BC&lt;/sup&gt;</td>
<td>14.38&lt;sup&gt;A&lt;/sup&gt;</td>
<td>12.16&lt;sup&gt;A&lt;/sup&gt;</td>
<td>4.15&lt;sup&gt;A&lt;/sup&gt;</td>
</tr>
<tr>
<td>75&lt;sup&gt;°&lt;/sup&gt;C</td>
<td>63.99&lt;sup&gt;C&lt;/sup&gt;</td>
<td>16.28&lt;sup&gt;A&lt;/sup&gt;</td>
<td>13.36&lt;sup&gt;A&lt;/sup&gt;</td>
<td>4.39&lt;sup&gt;A&lt;/sup&gt;</td>
</tr>
<tr>
<td>p-value</td>
<td>&lt;0.0001</td>
<td>0.2396</td>
<td>0.1485</td>
<td>0.8705</td>
</tr>
<tr>
<td>SEM&lt;sup&gt;c&lt;/sup&gt;</td>
<td><strong>0.82</strong></td>
<td><strong>1.89</strong></td>
<td><strong>2.02</strong></td>
<td><strong>0.25</strong></td>
</tr>
</tbody>
</table>

<sup>a</sup> Means within the same effect with different letters in a column are significantly different (p ≤ 0.05)

<sup>b</sup> Degree of doneness according to the Beef Steak Color Guide (National Live Stock and Meat Board) Chicago, IL and are described as Very rare (Approx. 55°C), Medium rare (Approx. 65°C) and Well done.

<sup>c</sup> SEM = Standard Error of the Mean (n = 9)
TABLE 3-5 Effects of thickness and endpoint temperature on expressible moisture and pH of steaks cooked with

<table>
<thead>
<tr>
<th>Main Effects</th>
<th>Expressible Moisture (%)</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Thickness</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.9 cm</td>
<td>14.21&lt;sup&gt;A&lt;/sup&gt;</td>
<td>5.71&lt;sup&gt;A&lt;/sup&gt;</td>
</tr>
<tr>
<td>2.5 cm</td>
<td>16.19&lt;sup&gt;A&lt;/sup&gt;</td>
<td>5.70&lt;sup&gt;A&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>p-value</strong></td>
<td>0.1884</td>
<td>0.7819</td>
</tr>
<tr>
<td><strong>Endpoint Temperature</strong>&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Raw</td>
<td>8.35&lt;sup&gt;B&lt;/sup&gt;</td>
<td>5.54&lt;sup&gt;B&lt;/sup&gt;</td>
</tr>
<tr>
<td>55°C</td>
<td>22.67&lt;sup&gt;A&lt;/sup&gt;</td>
<td>5.68&lt;sup&gt;AB&lt;/sup&gt;</td>
</tr>
<tr>
<td>65°C</td>
<td>18.74&lt;sup&gt;A&lt;/sup&gt;</td>
<td>5.76&lt;sup&gt;A&lt;/sup&gt;</td>
</tr>
<tr>
<td>75°C</td>
<td>11.03&lt;sup&gt;B&lt;/sup&gt;</td>
<td>5.84&lt;sup&gt;A&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>p-value</strong></td>
<td>&lt;0.0001</td>
<td>0.0029</td>
</tr>
<tr>
<td><strong>SEM</strong>&lt;sup&gt;c&lt;/sup&gt;</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1.66</td>
<td>0.03</td>
</tr>
</tbody>
</table>

<sup>a</sup> Means within the same effect with different letters in a column are significantly different (*p* ≤ 0.05)

<sup>b</sup> Degree of doneness according to the Beef Steak Color Guide (National Live Stock and Meat Board) Chicago, IL and are described as Very rare (Approx. 55°C), Medium rare (Approx. 65°C) and Well done (Approx. 75°C)

<sup>c</sup> SEM = Standard Error of the Mean (*n* = 9)
TABLE 3-6 Effects of thickness and endpoint temperature on color ($L^*$, $a^*$ and $b^*$) and shear of steaks cooked with RF$^a$

<table>
<thead>
<tr>
<th>Main Effects</th>
<th>$L^*$ value</th>
<th>$a^*$</th>
<th>$b^*$</th>
<th>Shear (N)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Thickness</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.9 cm</td>
<td>54.07$^A$</td>
<td>9.03$^A$</td>
<td>17.01$^A$</td>
<td>21.61$^A$</td>
</tr>
<tr>
<td>2.5 cm</td>
<td>47.65$^B$</td>
<td>8.96$^A$</td>
<td>14.97$^B$</td>
<td>22.74$^A$</td>
</tr>
<tr>
<td>$p$-value</td>
<td>0.0085</td>
<td>0.9084</td>
<td>0.0007</td>
<td>0.3518</td>
</tr>
<tr>
<td><strong>Endpoint Temperature</strong>$^b$</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>55°C</td>
<td>51.11$^A$</td>
<td>11.31$^A$</td>
<td>16.57$^A$</td>
<td>19.01$^B$</td>
</tr>
<tr>
<td>65°C</td>
<td>50.95$^A$</td>
<td>8.75$^B$</td>
<td>16.56$^A$</td>
<td>21.51$^B$</td>
</tr>
<tr>
<td>75°C</td>
<td>50.50$^A$</td>
<td>6.92$^B$</td>
<td>14.83$^B$</td>
<td>26.01$^A$</td>
</tr>
<tr>
<td>$p$-value</td>
<td>0.9698</td>
<td>0.0001</td>
<td>0.0126</td>
<td>0.0009</td>
</tr>
<tr>
<td>SEM$^c$</td>
<td>0.86</td>
<td>0.34</td>
<td>0.31</td>
<td>0.98</td>
</tr>
</tbody>
</table>

$^a$Means within the same effect with different letters in a column are significantly different ($p \leq 0.05$)

$^b$Degree of doneness according to the Beef Steak Color Guide (National Live Stock and Meat Board) Chicago, IL and are described as Very rare (Approx. 55°C), Medium rare (Approx. 65°C) and Well done (Approx. 75°C)

$^c$SEM = Standard Error of the Mean ($n = 9$)
FIGURE 3-1 Picture of steaks packaged inside thermal resistant bags threaded with a fiber optic thermocouples inserted at the center of non-intact beefsteaks prior to cooking inside of a RF oven
FIGURE 3-2 Average and standard deviation for time-temperature profile at the center of 1.2 cm, ○ 1.9 cm and △ 2.5 cm of non-intact beefsteaks cooked to 75°C by RF heating (n = 3)
FIGURE 3-3 Visual appearance comparison of raw versus cooked non-intact beefsteaks (55°C, 65°C and 75°C) using RF heating
CHAPTER 4

EFFECT OF RADIO FREQUENCY HEATING ON NALIDIXIC ACID ADAPTED SHIGA TOXIN-PRODUCING AND NON-PATHOGENIC Escherichia coli STRAINS IN BUFFER²

² Rincon, Angela, Singh, Rakesh K.
To be submitted to the Journal of Food Protection
Abstract

This study aimed to validate the use of nalidixic acid-adapted strains of three major Shiga toxin-producing *Escherichia coli* (STEC) and non-pathogenic *Escherichia coli* for the use of radio frequency (RF) heating using phosphate buffer saline (PBS). The effectiveness of RF was evaluated on cocktails of various STEC serotypes (O157:H7, O26:H11, O111) and non-pathogenic *E. coli* at different endpoint temperatures (55°C, 60°C and 65°C). All strains were successfully adapted to nalidixic acid (Nal). In general, the results indicated that Nal resistant strains were not significantly different from Nal sensitive strains evaluated either before treatment or at the endpoint temperatures. Nal adapted strains were therefore validated to be used as marker organisms in studies involving the use of RF. Results also showed that the thermal inactivation of strains was more effective as the treatment temperature increased, particularly at 65°C, which showed a 6.0 log reduction log CFU/ml. Results of the present study will serve as baseline to study RF as a potential intervention technology for non-intact beef products.


Introduction

Shiga toxin-producing *Escherichia coli* (STEC) are important foodborne pathogens worldwide (15). Cattle have been implicated as the primary reservoir of STEC and contamination of any beef product with feces is often attributed to STEC infection (4). Because of risks associated with consumption of contaminated beef, the U. S. Department of Agriculture Food Safety and Inspection Service has declared *E. coli* O157:H7 (30) and six other serogroups (O26, O45, O143, O111, O121, O145) (31) as adulterants in some beef products. STEC infections can lead to serious health issues such as hemolytic uremic syndrome (HUS) and kidney failure (13). Numerous steps in the slaughter process may contribute to carcass contamination. The de-hiding process may transport fecal matter and debris from the hides and hooves of the animal to the carcass. Evisceration may also contribute to the contamination when rumen or intestinal contents are dispelled (25). Therefore, it is important for beef producers to review their current pathogen mitigation strategies and new intervention steps on their efficacy to inactivate both O157 STEC and non-O157 STEC (14).

Radio frequency (RF) heating forms part of a group of innovative techniques based on electromagnetic heating (e.g. infrared and microwave), and other non-thermal methods such as high pressure, pulsed electric and ultrasonic waves (1). Unlike conventional heating methods where heat transfer takes place through conduction, convection or radiation, RF is achieved through a combination of ionic displacement and ionic rotation (7). The heat is generated within the product due to molecular friction resulting from oscillating molecules and ions caused by the applied alternating electric field. The energy and heat are absorbed directly by the food; therefore cooking not only
saves time but also energy (24). In addition, RF offers the advantages of providing more uniform heating due to deep penetration and simple uniform field patterns (11). RF cooking can be used to reduce high levels of microbial contamination and improve quality in meat (10).

Studies of interventions to remove or inactivate bacteria are complicated by the relatively high level of background microflora that the food may contain (21). Meat is a rich nutrient matrix that provides a suitable environment for proliferation of meat spoilage microorganisms and common foodborne pathogens (2). Therefore; antibiotic resistance of inoculated bacteria is commonly used as a selective marker on those food matrix environments (22). Blackburn and others (3), developed antibiotic resistant strains and antibiotic amended media for the study of foodborne pathogens using the quinolone antibiotic nalidixic acid (Nal). Previous studies have demonstrated that Nal-resistant (Nalr) strains had similar growth rates, stress tolerances and so forth as the Nal-sensitive (Nals) strains on chemical interventions and growth parameter measurements (27). However another study testing ionic radiation revealed that those Nal-r strains showed an increased sensitivity compared to Nal-s strains. The mode of action of Nal is disruption of DNA replication of bacteria (22); while ionizing radiation can cause damage to DNA and interfere with DNA repair; thus, it was hypothesized that the Nal-resistant bacteria may have altered the nucleic acid synthesis making them more susceptible to ionizing radiation (21). It is clear then that suitability of Nal-resistant strains as marker organisms should be determined for all new interventions (13) such as RF. The objectives of this study were first, to validate the use of Nal-resistant strains of *E. coli* (STEC and non-pathogenic) in studies of the efficacy of RF heating to be used in further experiments on
beef and second, to determine the effectiveness of RF heating at different endpoint
temperatures using model solutions.

**Materials and Methods**

**Bacterial Strains and Inoculum preparation**

A total of 19 strains of STEC *E. coli* O157:H7, O26 and O111 as well as non-
pathogenic *E. coli* were used in this study. *Escherichia coli* pathogenic strains were
provided by the Center for Food Safety, University of Georgia (CFS-UGA), Griffin, GA.
*E. coli* O157:H7 isolates tested were as follows: ATCC 43895 (hamburger isolate),
ATCC 35150 (human isolate), E009 (beef isolate), E0018 (cattle isolate) and 932 (human
isolate). *E. coli* O26:H11 isolates tested were: DEC10E (cattle isolate), DEC9E (cattle
isolate), DEC10B (cattle isolate) 3079-97 (human isolate) and 0261 (human isolate). *E.
coli* O111 isolates tested were: NM:3208-95 (human isolate), NM:0944-95 (cattle
isolate), NM:3287-97 (human isolate), NM:4543-95 (cattle isolate), NM:0073-92 (cattle
isolate). Also, four nonpathogenic *E. coli* beef cattle isolates, previously characterized
and selected based on their heat resistance similar to *E. coli* O157:H7 (18, 20), were used
on this study: ATCC BAA-1427 (P1), ATCC BAA-1428 (P3), ATCC BAA-1430 (P14)
and ATCC BAA-1431 (P68).

**Nalidixic Acid (Nal) resistant strains**

The method described by Taormina and Beuchat (27) was used to adapt all the parent
strains to Nal (Sigma Chemicals Co., St. Louis, MO) up to 50µg/mLs. Briefly, each
parent strain was cultured separately in 10 ml tryptic soy broth (TSB, Difco, Becton
Dickinson, MD) for 24h at 37°C. Subsequently these overnight cultures were transferred
to TSB supplemented with Nal for 24 h. The amount of Nal was gradually increased (1,
5, 10, 20, 30, 40, and 50µg/mL) using sterile filtered (0.22µm) Nal stock solutions. After incubation 200µl of bacterial culture were transferred from the lower concentration to the next higher concentration in solution. Cultures were grown at 37°C in a shaking incubator at 150 rpm for 24h.

**Culture preparation and buffer inoculation**

All bacterial strains (resistant and sensitive) were stored at -80°C in Cryobeads (Cryobank™, Copan diagnostics, Murrieta, CA). One frozen bead of *E. coli* strain was aseptically transferred to 10 ml TSB or tryptic soy broth supplemented with Nal (TBSN) and incubated at 37°C for 24h. A loop full of broth was transferred to 10 ml TSB (or TBSN) and incubated at 37°C for another 24h to obtain a stationary phase culture containing approximately $10^9$- $10^8$ CFU/ml of *E. coli*. Three successive transfers were grown overnight before cocktail was prepared. The overnight cultures were washed and centrifuged twice on PBS (pH 7.2 Thermo Fisher Scientific Inc., MA) at relative centrifuge force of 2,300 x g (Eppendorf Centrifuge Model 5804R) for 15 min. The cell concentration of the individual cultures was evaluated by measuring the optical density (OD) of the cultures using a spectrophotometer. STEC cocktails were prepared by mixing 2 ml of each *Escherichia coli* serovar to produce a 10-ml cocktail, while non-pathogenic *E. coli* cocktail was prepared by mixing 2.5 ml of each serovar in order to produce a 10-ml cocktail. Sterile deionized (DI) water was used to prepare phosphate buffer saline (PBS). Once the buffer (250ml) was inoculated by taking 2.5 ml of the cocktail adding it to 247.5 ml of PSB to make for a 1:100 dilution. The bags (Sealed Air Corp., Duncan, SC) were sealed with an impulse sealer (Omcan Impulse bag sealer,
Hotel Supply Warehouse, Inc., Deerfield Beach, FL) and refrigerated at 4 ± 2°C overnight until processing.

**RF Equipment and heating protocol**

All treatments were conducted at a constant frequency of 27.12 MHz and a power of 6kW in a RF oven (Model S061B Stray-field Ltd, Reading, UK). The equipment consists of a RF generator, two electrodes, and a conveyor belt. A series of preliminary studies were conducted to characterize the tray, packaging material, heating medium, and the most effective power setting for optimum efficiencies for the RF process. Since it was not possible to monitor the temperature inside of the bags during RF heating due to possible contamination to the environment, two important steps were performed. First, the average time of four samples per endpoint temperature was calculated with fiber optic temperature probes (Fiso Tech. Inc., Quebec, Canada) on non-inoculated PBS and second, an external thermocouple was placed outside of bag to monitor temperature changes on the water surrounding the bag (Figure 4-1). A fiber optic thermocouple was inserted inside the heat resistant bags (Cryovac, Duncan, SC) by making a hole with a #5 bras cork borer. Then, a stuffing box (C-5.2D, Eucklund-Harrison Tech., Fort Myers, FL) was placed inside bag, filled with 250 ml of PBS, sealed and placed inside a polysulfone resin tray (CAMBRO, Huntington Beach, CA, US). A special grid elaborated with Ultem (Polyethirimide) was designed to maintain the bag immersed in water to prevent packages from floating due to ballooning effect caused by expansion of vapor inside the bag as the temperature increased. Once the bag was secured inside of the tray, tap water (10 ± 2°C) was placed on top of the bag before processing and then the RF oven was turned on. The electrodes were manually adjusted in such way that the anode current
was 0.6 A at all times during the RF cycle. Once the predetermined average time to reach the desired end point temperature was reached (55°C, 60°C or 65°C), the RF oven was turned off and the buffer bags were immediately taken out of the water and immersed inside sterile aluminum of trays containing ice-water in order to lower the temperature and be able to form time zero for the samples.

**Microbiological analysis**

A positive control (inoculated non-treated) per each serotype as well as the treated samples were serially diluted in 9-ml volumes of PBS and spiral plated in duplicates \((n = 6)\) (Autoplate Spiral Plating System®, Norwood, MA) on tryptic toy agar (TSA, Difco, beckton Dickinson, MD) for the parent strains or on TSAN (TSA supplemented with Nal) for the Nal adapted strained. Plates were incubated overnight at 37°C for 24h and enumerated using an automatic colony counter (QCount, Model 530, Advanced Instruments Inc., Norwood, MA).

**Statistical Analysis**

Sample size was estimated using Statistical Solutions, LLC software \((26)\) to avoid for type II error (power test = 0.80). Experimental design consisted on a 8 X 3 factorial design. Treatments consisted of four cocktails of STEC \((E. coli\ O157:H7, E. coli\ O26:H11\ and\ E. coli\ O111)\) and non-pathogenic \(E. coli\) of Nal resistant and four cocktails of corresponding parent strains (Nal sensitive) inoculated on PSB and heated to three different target end-point temperatures: 55°C, 60°C and 65°C \((n = 6)\). Experiments were carried out three times. Statistical analysis was performed using SAS (SAS Version 9.3, SAS Institute, Cary, NC). Nal resistant strains and Nal sensitive strains were statistically compared at each stage (before cooking and each endpoint temperature) using a two
sample t-test on counts transformed to log cfu/ml with the minimum level of statistical significance was set at alpha = 0.05. Net log reductions (treatment temperature – positive control) samples were used to study the efficacy of RF to inactivate strains. Mean comparisons were performed with the pairwise t-test at alpha = 0.05.

**Results and Discussion**

**Nalidixic acid adaptation**

All cultures were successfully adapted to 50 mg/L nalidixic acid and consecutively subcultured three times in TSBN to confirm stability of nalidixic acid adaptation. After that, an overnight subculture was used to determine the relationship between the OD$_{600}$ values and the log number of colony forming units of each particular strain on each serotype in order to adjust cell concentrations of the individual cultures in order to prepare cocktails with equal proportions of strains of respective serotype.

**RF heating protocol and average time-temperature**

The heating rate of any product using RF depends on several parameters such as anode and grid current, dielectric properties and a secondary medium surrounding the product. Therefore, preliminary experiments were conducted to evaluate different factors such as effect of power (anode current) on the heating profile of the buffers, packaging performance and integrity and selection of trays transparent to electromagnetic waves. The power of RF applied to the product changes with the electrode gap. As the gap between electrodes decreases, the power increases; however the risk of arcing and melting of the package also increases (16). In order to be in compliance with the food safety protocols implemented, it was important to maintain integrity of the package during processing and transportation. Previous studies (16, 19) have shown that the use of
water as a medium surrounding the product instead of air during RF heating significantly decreases arcing which usually compromises the integrity of the package. The heating time to reach three different endpoint temperatures was calculated and averaged as: 9.55 ± 0.96 min for 55°C, 10.41 ± 1.48 min for 60°C and 11.58 ± 1.09 min for 65°C.

The heating rate of the water surrounding the pouches was calculated as 4.24 ± 4.6°C/min, 4.19 ± 5.20°C/min and 4.05 ± 3.69°C/min while the heating rate of the buffers was 4.03 ± 3.86°C/min, 4.00 ± 4.34°C/min and 3.96 ± 3.57°C/min corresponding to 55°C, 60°C and 65°C respectively. From these results, it can be inferred that the heating rate for water surrounding the bags fell closely within the range for the buffer; thus, both samples appear to heat equally. Interaction between RF and the material to be heated is mostly a function of the dielectric properties. The dielectric constant (ε’) determines the amount of energy reflected from the product and transmitted into the product while the dielectric loss factor (ε”) is related to how well a material absorbs energy from electric fields passing through it and how it well converts that energy to heat (33). Phosphate buffer saline is a water-based salt solution (sodium chloride and sodium phosphate) used as standard diluent on studies of survival and recovery of microorganisms (32). Lyng et al. (17) studied dielectric properties in different types of meat and ingredients commonly used in meat products. It was determined that the presence of ionic compounds such as salts increase the dielectric loss factor altering the penetration pattern of electromagnetic waves. The heating rate of the buffers was slightly lower than the water perhaps due to the dielectric nature of the PSB present which likely reduced the ε’ and increase the ε” (23).
Survival of Nalr and Nals on PBS after RF heating

In general, the results indicated that resistance of Nalr strains was not significantly different from Nals strains for any of the serotypes either before treatment or at the temperatures tested. *Escherichia coli* O157:H7, *Escherichia coli* O26:H11 and *Escherichia coli* O111 results (Table 4-1 to 4-3) showed no significant differences between Nalr and Nals either before processing or when heated to 55°C, 60°C or 65°C. Non-pathogenic *Escherichia coli* exhibited similar results (Table 4-4) with the exception of samples heated to 55°C where there was a significant difference between Nalr and Nals strains. The present results therefore validated the use of Nalr STEC strains and non-pathogenic *E. coli* strains as marker organisms to substitute the parent strains for RF experiments.

One of the mechanisms proposed for inactivation of microorganisms by electromagnetic energy (e. g. microwave and radio frequency) involve non-thermal effects where destruction of microorganisms has been attributed to selective heating, electroporation, cell membrane rupture, and magnetic field coupling (5, 8, 28). However, there is some controversy as to the additional inactivation of microorganisms over the thermal effects of electromagnetic energy, since this additional non-thermal inactivation has been shown to be small and inconsistent (6). In this particular study, preliminary experiments were performed on buffers heated to 45°C but no reduction was detected compared to initial inoculation levels (results not shown). Therefore, selection of endpoint temperatures were set based on *E. coli* regulations and thermal reductions for Salmonellae spp. (9, 29) and inactivation mechanisms were attributed to thermal effects triggered by RF energy.
Thermal inactivation by radio frequency occurs mostly through mechanisms comparable to other technologies induced by heat such as denaturation of enzymes, proteins, nucleic acids or other vital components as well as disruption of membranes (12). Nalidixic acid on the other hand, inhibits bacterial growth by affecting topoisomerase IV and DNA gyrase hence, inhibiting DNA replication (13, 21). Initial levels of inoculation (before processing) for Nalr were slightly lower compared to Nals strains; however, results were not significantly different ($p \leq 0.05$) on any of the serotypes tested. Similar results were also found as the buffers were heated up to 55°C and 60°C, which confirms that the inactivation of *E. coli* (STEC and non-pathogenic) was due mainly to thermal effects of RF rather than nalidixic acid adaptation.

**Efficacy of RF heating to inactivate Nals and Nalr strains**

Tables 4-5 and 4-6 show the pairwise $t$-test results for RF temperature effects on Nalr and Nals strains at 55°C and 60°C respectively. Initially there was a gradual log reduction of all *Escherichia coli* strains as the temperature increased to 55°C (0.5 to 1.0 log CFU/ml) and also as the temperature increased to 60°C (1.0 to 1.5 log CFU/ml). However, when the buffers were heated up to 65°C there was a substantial reduction of at least 6.0 log CFU/ml. An effective combination of electromagnetic energy generated and also convection from surrounding water as the whole system heated up allowed for a more even distribution of the heat at higher temperatures making the RF very effective at such temperatures. Previous experiments have also shown and effective combination of RF and convection heating resulting in a significant pathogen reduction on meatballs (24).
Conclusion

Shiga toxin-producing (O157:H7, O26:H11 and O111) and non-pathogenic E. coli strains were successfully adapted to nalidixic acid. Standard sanitation procedures for treatment and transportation of inoculated samples to be treated in RF oven were also created and strictly followed (Appendix B). There was no significant difference between Nalr and Nals strains either below or at treatment temperatures; thus, the results of this research validated the use of nalidixic acid resistant strains as marker organisms for RF trials. This study has important implications for further work on thermal inactivation of STEC using Radio Frequency energy. The log reduction at 55°C ranged from 0.5 to 1.0 log CFU/ml while the log reduction at 60°C ranged from 1.0 to 1.5 log CFU/ml. Experiments were successful in inactivation of all STEC and non-pathogenic E. coli at temperatures ≥ 65°C with a 6.0 log reduction for all strains.

Experiments were carried out in a pilot scale oven, which could be the starting point for creation of protocols for effective thermal inactivation of foodborne pathogens using RF. Inoculation studies involving pathogen testing are necessary for Hazardous Analysis of Critical Control Points (HACCP) verification. Nevertheless, introduction of pathogens into a processing facility is not suitable because of public health significance. Instead, indicator (non-pathogenic) microorganisms are used for verification purposes (18). An additional finding of the present research is that non-pathogenic E. coli response in buffer was not different from STEC strains. This opens the possibility for further in-plant challenge studies where surrogates can be used instead of pathogenic strains when using RF. In particular for non-intact beef products, which have the potential of transferring external surface contamination with STEC to the interior of the
normally sterile tissue (1). Therefore, the heat resistance of the potential surrogates
should be tested also in the food matrix (i.e. non-intact beef products) since the survival
of pathogens and other bacteria in food ingredients and products is significantly
influenced by intrinsic elements of the food ecosystem (6)
References


**TABLE 4-1** Shiga toxin-producing *Escherichia coli* O157:H7 recovered on tryptic soy agar for nalidixic acid sensitive (Nals) and tryptic soy agar supplemented with nalidixic acid for nalidixic acid resistant (Nalr) strains before and after RF heating samples to 55°C, 60°C and 65°C in a model solution.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Escherichia coli O157:H7 Nals (log CFU/ml)</th>
<th>Escherichia coli O157:H7 Nalr (log CFU/ml)</th>
<th>Significance level (p-value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before Processing</td>
<td>6.87 ± 0.10</td>
<td>6.68 ± 0.06</td>
<td>0.2077</td>
</tr>
<tr>
<td>55°C</td>
<td>6.39 ± 0.16</td>
<td>6.23 ± 0.16</td>
<td>0.4491</td>
</tr>
<tr>
<td>60°C</td>
<td>5.11 ± 0.38</td>
<td>4.71 ± 0.74</td>
<td>0.6573</td>
</tr>
<tr>
<td>65°C</td>
<td>&lt; 2.0b</td>
<td>&lt; 2.0</td>
<td>N/A</td>
</tr>
</tbody>
</table>

*Mean ± Standard Error Mean (n = 6)*

*No colonies detected at the detection level (2.0 log CFU/ml)*
TABLE 4-2  Shiga toxin-producing *Escherichia coli* O26:H11 recovered on tryptic soy agar for nalidixic acid sensitive (Nals) and tryptic soy agar supplemented with nalidixic acid for nalidixic acid resistant (Nalr) strains before and after RF heating samples to 55°C, 60°C and 65°C in a model solution

<table>
<thead>
<tr>
<th>Treatment</th>
<th><em>Escherichia coli</em> O26:H11 Nals (log CFU/ml)&lt;sup&gt;a&lt;/sup&gt;</th>
<th><em>Escherichia coli</em> O26:H11 Nalr (log CFU/ml)</th>
<th>Significance level (p-value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before Processing</td>
<td>6.76 ± 0.03</td>
<td>6.72 ± 0.04</td>
<td>0.5171</td>
</tr>
<tr>
<td>55°C</td>
<td>6.25 ± 0.07</td>
<td>6.61 ± 0.36</td>
<td>0.1681</td>
</tr>
<tr>
<td>60°C</td>
<td>5.28 ± 0.32</td>
<td>5.12 ± 0.18</td>
<td>0.6865</td>
</tr>
<tr>
<td>65°C</td>
<td>&lt; 2.0&lt;sup&gt;b&lt;/sup&gt;</td>
<td>&lt; 2.0</td>
<td>N/A</td>
</tr>
</tbody>
</table>

<sup>a</sup>Mean ± Standard Error Mean (n = 6)

<sup>b</sup>No colonies detected at the detection level (2.0 log CFU/ml)
### TABLE 4-3

Shiga toxin-producing *Escherichia coli* O111 recovered on tryptic soy agar for nalidixic acid sensitive (Nals) and tryptic soy agar supplemented with nalidixic acid for nalidixic acid resistant (Nalr) strains before and after RF heating samples to 55°C, 60°C and 65°C in a model solution.

<table>
<thead>
<tr>
<th>Treatment</th>
<th><em>Escherichia coli</em> O111 Nal-sensitive (log CFU/ml)<em>a</em></th>
<th><em>Escherichia coli</em> O111 Nal-resistant (log CFU/ml)</th>
<th>Significance level (p-value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before Processing</td>
<td>6.82 ± 0.05</td>
<td>6.85 ± 0.02</td>
<td>0.6973</td>
</tr>
<tr>
<td>55°C</td>
<td>6.21 ± 0.22</td>
<td>6.25 ± 0.15</td>
<td>0.8986</td>
</tr>
<tr>
<td>60°C</td>
<td>5.25 ± 0.33</td>
<td>5.08 ± 0.30</td>
<td>0.7309</td>
</tr>
<tr>
<td>65°C</td>
<td>&lt; 2.0b</td>
<td>&lt; 2.0</td>
<td>N/A</td>
</tr>
</tbody>
</table>

*a*Mean ± Standard Error Mean (n = 6)

bNC = No colonies detected at the detection level (2.0 log CFU/ml)
TABLE 4-4 Shiga toxin-producing non-pathogenic *Escherichia coli* recovered on tryptic soy agar for nalidixic acid sensitive (Nals) and tryptic soy agar supplemented with nalidixic acid for nalidixic acid resistant (Nalr) strains before and after RF heating samples to 55°C, 60°C and 65°C in a model solution

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Non-pathogenic <em>Escherichia coli</em> Nal-sensitive (log CFU/ml)<em>a</em></th>
<th>Non-pathogenic <em>Escherichia coli</em> Nal-resistant (log CFU/ml)</th>
<th>Significance level (p-value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before Processing</td>
<td>6.68 ± 0.21</td>
<td>6.72 ± 0.04</td>
<td>0.8874</td>
</tr>
<tr>
<td>55°C</td>
<td>6.04 ± 0.07</td>
<td>6.42 ± 0.06</td>
<td>0.0196</td>
</tr>
<tr>
<td>60°C</td>
<td>5.50 ± 0.15</td>
<td>5.56 ± 0.15</td>
<td>0.8259</td>
</tr>
<tr>
<td>65°C</td>
<td>&lt; 2.0b</td>
<td>&lt; 2.0</td>
<td>N/A</td>
</tr>
</tbody>
</table>

*a*Mean ± Standard Error Mean (*n* = 6)

*b*No colonies detected at the detection level (2.0 log CFU/ml)
TABLE 4-5 Pairwise *t*-test for evaluation effects of RF on log reductions at 55°C (treated samples-positive control) of nalidixic acid resistant (Nalr) and nalidixic acid sensitive (Nals) STEC and non-pathogenic *E. coli* cell counts.

<table>
<thead>
<tr>
<th>Bacterial Strain</th>
<th>Log reduction (log CFU/ml)*</th>
<th>Significance value p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>E. coli</em> O157:H7 Nalr</td>
<td>0.479 ± 0.11</td>
<td>0.0554</td>
</tr>
<tr>
<td><em>E. coli</em> O157:H7 Nals</td>
<td>0.454 ± 0.06</td>
<td>0.0177</td>
</tr>
<tr>
<td><em>E. coli</em> O26:H11 Nalr</td>
<td>0.515 ± 0.37</td>
<td>0.0338</td>
</tr>
<tr>
<td><em>E. coli</em> O26:H11 Nals</td>
<td>1.103 ± 0.34</td>
<td>0.0840</td>
</tr>
<tr>
<td><em>E. coli</em> O111 Nalr</td>
<td>0.612 ± 0.21</td>
<td>0.1006</td>
</tr>
<tr>
<td><em>E. coli</em> O111 Nals</td>
<td>0.598 ± 0.13</td>
<td>0.0445</td>
</tr>
<tr>
<td>Non-pathogenic <em>E. coli</em> Nalr</td>
<td>0.533 ± 0.09</td>
<td>0.1563</td>
</tr>
<tr>
<td>Non-pathogenic <em>E. coli</em> Nals</td>
<td>0.302 ± 0.03</td>
<td>0.0147</td>
</tr>
</tbody>
</table>

*Mean ± Standard Error Mean (n = 6)*
TABLE 4-6 Pairwise *t*-test for evaluation effects of RF on log reductions at 60°C (treated samples-positive control) of nalidixic acid resistant (Nalr) and nalidixic acid sensitive (Nals) STEC and non-pathogenic *E. coli* cell counts.

<table>
<thead>
<tr>
<th>Bacterial Strain</th>
<th>Log reduction (log CFU/ml)</th>
<th>Significance value p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>E. coli</em> O157:H7 Nalr</td>
<td>1.274 ± 0.26</td>
<td>0.0412</td>
</tr>
<tr>
<td><em>E. coli</em> O157:H7 Nals</td>
<td>1.517 ± 0.71</td>
<td>0.1684</td>
</tr>
<tr>
<td><em>E. coli</em> O26:H11 Nalr</td>
<td>0.961 ± 0.34</td>
<td>0.1217</td>
</tr>
<tr>
<td><em>E. coli</em> O26:H11 Nals</td>
<td>0.493 ± 0.51</td>
<td>0.4387</td>
</tr>
<tr>
<td><em>E. coli</em> O111 Nalr</td>
<td>1.128 ± 0.54</td>
<td>0.1767</td>
</tr>
<tr>
<td><em>E. coli</em> O111 Nals</td>
<td>0.998 ± 0.45</td>
<td>0.1576</td>
</tr>
<tr>
<td>Non-pathogenic <em>E. coli</em> Nalr</td>
<td>0.302 ± 0.03</td>
<td>0.0308</td>
</tr>
<tr>
<td>Non-pathogenic <em>E. coli</em> Nals</td>
<td>0.857 ± 0.11</td>
<td>0.0175</td>
</tr>
</tbody>
</table>

*a*Mean ± Standard Error Mean (*n* = 6)
FIGURE 4-1 Picture with inoculated heat resistant pouch and thermocouple monitoring water surrounding the bag
CHAPTER 5

INACTIVATION OF SHIGA TOXIN-PRODUCING AND NONPATHOGENIC

*ESCHERICHIA COLI* IN NON-INTACT STEAKS COOKED IN A RADIO

FREQUENCY OVEN

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3 Rincon, Angela; Singh, Rakesh K. To be submitted to *Journal of Food Protection*
Abstract

This study evaluated the thermal inactivation of Shiga toxin-producing *Escherichia coli* (O157:H7, O26:H11 and O111) (STEC) and non-pathogenic *Escherichia coli* in non-intact beefsteaks cooked by radio frequency (RF). Previously blade tenderized steaks were inoculated with nalidixic (Nal) acid resistant *E. coli* strains, vacuum packaged in thermal pouches and cooked using pre-determined cooking times to 60°C (rare) or 65°C (medium-rare) inside a RF oven. Log reduction ranged from 0.99, 3.08, 2.85 and 5.0 for O157:H7, O26:H11, O111 and non-pathogenic *E. coli* respectively at 60°C and a 5.0 log reduction at 65°C for all strains. Non-pathogenic *E. coli* strains selected for the present study did not behave similar to the pathogenic strains showing more sensitivity; therefore, they were not deemed to be appropriate surrogates for RF treatment for in-plant validation studies. Validation for the USDA-FSIS recommendation for cooking to 63°C was effective for *E. coli* O157:H7 and *E. coli* O111 but not for *E. coli* O26:H11. Results for cooking to 65°C with a holding time at room temperature of 5 minutes before refrigeration would be a better temperature to validate the proposed cooking protocol instructions for non-intact whole muscle steaks using RF. The cooking protocol developed on the present study, has a practical relevance for the industry since the experiments were carried on a pilot-scale RF oven and also pathogens were tested under realistic processing conditions.
Introduction

Shiga toxin-producing *Escherichia coli* (STEC) in general and seven major STEC groups: O26, O45, O103, O111, O121, O145 and O157 have been recognized as major foodborne pathogens \( (9, 11) \). In the United States it has been estimated that O157 STEC causes approximately 62,000 cases of symptomatic infection, resulting in about 1,800 hospitalizations and 52 deaths \( (29) \) while non-O157 STEC cause approximately 112,752 cases annually \( (12) \). Cattle are hosts for these pathogens and a significant proportion of beef may become contaminated during slaughter and carcass breaking operations \( (14) \). Because of risks associated with consumption of contaminated beef, the U. S. Department of Agriculture Food Safety Inspection Service (USDA-FSIS) declared O157 STEC as an adulterant in ground beef in 1994 \( (26) \) and other six major non-O157 STEC as adulterants in non-intact beef products in 2012 \( (10) \). As defined by the USDA-FSIS, non-intact beef products include ground beef, mechanically or chemically tenderized meat cuts, restructured entrees and those meat products that have been injected with marination or brining solutions to enhance flavor or tenderness \( (31, 38) \). Mechanically tenderized beef is a value added product made from subprimal beef cuts. During blade tenderization, mechanical blades are inserted into meat disrupting the structure of beef muscle. As this process takes place, bacteria present of the surfaces of beef cuts are carried and translocated into the interior leading to internal contamination \( (8) \). In fact, needle or blade tenderized products have been involved in several STEC infection outbreaks in the United States since 2000 \( (15, 26, 39) \).

Adequate cooking is the most effective means to eliminate *E. coli* O157:H7 and other pathogens and to assure the microbiological safety of food \( (6) \). Blade tenderized
products may not be recognized as being non-intact by consumers and food service establishments; if intentionally or unintentionally undercooked, survival of internalized pathogens could result in human illness (2, 17). Many conventional methods of cooking including hot air, water, steam, frying and radiant heat are applied to meat by transferring the heat through convection, conduction or radiation (6). Conventional food heating methods require that food is heated externally leading to longer cooking time and non-uniform heating (16). Information pertaining to the effectiveness of different cooking methods on inactivation of *E. coli* O157:H7 internalized in non-intact beef products other than ground meat has been published. (1, 29, 31). However, the safety of proper cooking of meat and meat products is yet of increasing concern. Radio Frequency (RF) heating, or capacitive dielectric heating, is an emerging technology which uses electromagnetic energy to heat foods with rapid heat distribution, large penetration depth and lower energy consumption (35). In contrast to other heating technologies, RF cooks products volumetrically with the product forming a dielectric between two electrodes acting as capacitor plates. These electrodes are alternatively charged from positive to negative several million times a second (e.g. 27 MHz) and as a result the polar molecules in the food are constantly realigned causing constant friction to occur and leading to the production of heat (3). When compared to microwaves, RF waves provide higher penetration depth, which in turn allow heating of thicker products like whole muscle steaks. Overcooking on the surface is avoided while energy is transferred by longer wavelengths (7, 16, 28).

Meats pre-cooked inside thermal pouches that only need to be warmed or heated to desired endpoint temperature are currently offered at the grocery stores and
restaurants, and are usually seasoned, including sauce or gravy, so that they can be identified as dinner entrée (32). RF cooking has a potential application for these types of value-added meat products allowing faster heating rates (energy savings), reducing cross-contamination (food safety), and also offering better quality (lower juice losses and more retention of nutrients and flavors). In spite of the risk of arching and thermal runaway as the main problems that have limited the use of this technology in the industry; effective protocols for safe and efficient RF cooking have been developed for uniformly shaped encased meats and chicken breast meat (13, 35). Those protocols involve cooking the meat inside casings, thermal resistant packages and the use of water to avoid arching of the equipment. Studies of thermal inactivation of foodborne pathogens on meat products using RF are limited. However, previous studies on inactivation of E. coli, Salmonella and Listeria (28), generic E. coli (6) and Bacillus cereus and Clostridium perfringens (3) have been reported. The goals of this study were to evaluate the thermal inactivation of various STEC and non-pathogenic E. coli populations in non-intact steaks using an RF oven and to validate the USDA-FSIS recommendation (20) for the minimum internal cooking temperature requirements for RF.

Materials and Methods

Bacterial strains and preparation of cocktail

A total of 19 strains of STEC E. coli O157:H7, O26 and O111 as well as non-pathogenic E. coli were used in this study. Escherichia coli pathogenic strains were provided by the Center for Food Safety, University of Georgia (CFS-UGA), Griffin, GA. E. coli O157:H7 isolates tested were as follows: ATCC 43895 (hamburger isolate), ATCC 35150 (human isolate), E009 (beef isolate), E0018 (cattle isolate) and 932 (human
isolate). *E. coli* O26:H11 isolates tested were: DEC10E (cattle isolate), DEC9E (cattle isolate), DEC10B (cattle isolate) 3079-97 (human isolate) and 0261 (human isolate). *E. coli* O111 isolates tested were: NM:3208-95 (human isolate), NM:0944-95 (cattle isolate), NM:3287-97 (human isolate), NM:4543-95 (cattle isolate), NM:0073-92 (cattle isolate). Also, four nonpathogenic *E. coli* beef cattle isolates, previously characterized and selected based on their heat resistance similar to *E. coli* O157:H7 (19, 22) were used on this study: ATCC BAA-1427 (P1), ATCC BAA-1428 (P3), ATCC BAA-1430 (P14) and ATCC BAA-1431 (P68). All bacterial strains were adapted to nalidixic acid (Nal) (Sigma Chemicals Co., St. Louis, MO) up to 50µg/ml using the method described by Taormina and Beuchat (36) to allow for selective selection and enumeration of the inoculum from natural contaminating microflora. The thermotolerance of Nal resistant strains corresponding to parent strains was previously evaluated for RF treatment at 55°C, 60°C and 65°C in phosphate buffer saline (results not shown) and was found not to be different (*p* ≤ 0.05).

All bacterial strains were stored at -80°C in Cryobeads (Cryobank™, Copan diagnostics, Murrieta, CA). One frozen bead of *E. coli* strain was aseptically transferred to 10 ml of tryptic soy broth supplemented with Nal (TBSN) and incubated at 37°C for 24h. A loop full of broth was transferred to 10 ml TBSN and incubated at 37°C for another 24h to obtain a stationary phase culture containing approximately 10⁹-10⁸ CFU/ml of *E. coli*. Three successive transfers were grown overnight before cocktail were prepared. The overnight cultures were washed and centrifuged twice on phosphate saline buffer (pH 7.2 Thermo Fisher Scientific Inc., MA) at relative centrifuge force of 2,300 x g (Eppendorf Centrifuge Model 5804R) for 15 min. The cell concentration of the
individual cultures was evaluated by measuring the optical density (OD) of the cultures using a spectrophotometer. STEC cocktails were prepared by mixing 2 ml of each *Escherichia coli* serovar to produce a 10-ml cocktail, while non-pathogenic *E. coli* cocktail was prepared by mixing 2.5 ml of each serovar in order to produce a 10-ml cocktail. Preparations were serially diluted and spiral plated (Autoplate® 4000 Spiral Biotech, MA) onto tryptic soy agar (Difco, Beckton Dickinson, MD) supplemented with 50µg/ml Nal (TSAN). Plates were then incubated at 37°C for 24 h and enumerated using an automatic colony counter (QCount, Model 530, Advanced Instruments Inc., Norwood, MA).

**Meat procurement and inoculation**

Beef boneless short loin (NAMP 180) subprimals were purchased at a local store (Restaurant Depot, Atlanta, GA) and transported (2 ± 2°C) to the University of Georgia Department of Food Science and Technology (Athens). Whole muscle cuts were prepared by trimming of fat, connective tissue and silver skin. Subprimals were then transported to the University of Georgia Meat Science Technology Center (Athens) to be tenderized. Each subprimal was passed once on each side through a blade tenderizer (Ross TC700M, Midland, VA). The tenderizer produced approximately 10 incisions/cm² with a blade width of 3mm. Handling and transportation of the meat was done at 4 ± 2°C. Subprimals were then cut into roasts of about 1.3 kg, packed in multilayer PD-900 plastic bags (Cryovac, Duncan, SC), vacuum sealed (Henkelman, Hertogenbosch, Netherlands) and stored in a blast freezer at -20°C until testing. The day before processing, roasts were tempered at (2 ± 2°C) during 18-24 h and then sliced perpendicular to the muscle fibers.
into 1.9 cm (185 ± 5g) steaks using a commercial meat slicer (model 808, Berkel Incorporated, Laporte, Indiana, US).

Steaks (Figure 5-2) were individually placed on sterile stainless steel trays inside a biological safety cabinet (Bio Safe, NuAire, Class II Type A2, Plymouth, MN). The initial inoculum cocktail (200µL) of any single strain was spot inoculated on the surface of the steak using sterile inoculating loops (10 µL, Fisherbrand™, Thermo Fisher Scientific Inc., MA). The volume of inoculum was targeted for approximately 5.0 log CFU/g for the meat and was inoculated by piercing through the meat tissue with the needle part of the loop and then placing the loop with cocktail inside of the incision in order to randomly distribute the inoculum not only on the surface but also inside of the muscle. Sterile aluminum templates (5 X 5 cm² surface) were used as inoculation guide and the corners of the designated area were marked using meat dye (GL #80 purple Brite Great Lakes Meat Branding Ink, Kock Supplies Inc., Kansas City, MO) for ease of identification of the inoculated area during sampling. The steaks were rested for 1h to allow for attachment of the strain. Steaks (4 ± 2°C) were then individually packed inside of a nylon/linear low-density polyethylene pouch (Winpak, Minneapolis, MN) vacuum sealed (Promarks, Inc., Ontario, Canada) and kept overnight at 4 ± 2°C until processing.

**RF equipment and cooking of non-intact beef**

All treatments were conducted at a constant frequency of 27.12 MHz and a power of 6kW in a RF oven (Model S061B Stray-field Ltd, Reading, UK). The equipment consists of a RF generator, two electrodes, and a conveyor belt. A series of preliminary studies were conducted to characterize the tray, packaging material, heating medium, and the most effective power setting for optimum efficiencies for the RF process. It was not
possible to monitor the temperature inside of the steaks during heating due to potential exposure of foodborne pathogens onto the environment inside the pilot plant area. Also, the present protocol was intended to simulate industry processing conditions where monitoring of temperature of each product is not feasible. Therefore, the average heating time from six steaks that were not inoculated was collected at each endpoint temperature and used as cooking time. The protocol to collect cooking times was as follows: Steaks (4 ± 2°C) were individually packed inside of a nylon/linear low density polyethylene pouch (Winpak, Minneapolis, MN) and placed into polysulfone trays (H-Pan™, Cambro, Huntington Beach, CA). A special grid elaborated with Polyetherimide was designed to maintain the steak immersed inside of water preventing packages from floating caused by expansion of vapor inside the tissue during cooking. The heating medium selected was tap water (6 ± 2°C) since it has shown an important reduction in arcing of the equipment (13, 21). Fiber optic temperature probes (Fiso Tech. Inc., Quebec, Canada) were used to monitor the steak and water temperature during RF cooking. The RF oven was turned off once the center of the steaks reached the desired endpoint temperature. For the inoculated steaks the cooking protocol was performed as follows (Figure 5-3): once the predetermined average time to reach the desired end point temperature was reached, the RF oven was turned off, the steaks were immediately taken out of the water and allowed to rest at room temperature for 5 minutes for temperature equalization. Afterwards, a mixture of ice-water was poured on top to lower the temperature and it was labeled as time zero for the samples. Standard sanitation procedures for transportation and processing were strictly followed (Appendix B).
Steak sampling

Each steak was assigned a sterile plastic board, pair of tongs, knife, and cutting guide in order to avoid cross contamination. After use, all the tools that were in contact with the steaks were soaked in bleach water before being autoclaved and washed. A steak sample was aseptically obtained by cutting the 5 X 5 cm$^2$ impression made previously with the meat dye during inoculation (Figure 5-4) and carefully placing it into sterile stomacher bags (WhirlPak$^\text{®}$ filter bag, Nasco, Modesto, CA) along with the exudates expelled during the cooking process. Uncooked samples consisting on a negative control to check for background microflora of the thawed roast used on each processing day and also a positive control (inoculated with respective strain not cooked) were sampled using the same procedure. The cooked muscles were sampled as early as physically possible after thermal processing. All samples weighed approximately 50 ± 10 g.

*Escherichia coli* analysis

Samples were stomached (Stomacher 400 Circulator, Seward Laboratory Systems Inc., NY) for 2 min at 230 rpm after adding 225 ml of 0.1% peptone (Difco™, Beckton Dickinson, MD) to the stomacher bags, (Figure 5-4). Serial dilutions were made using 9 ml of 0.1% peptone and spread plated in duplicate onto TSA to enumerate total bacterial populations; TSAN to enumerate Nal acid adapted populations; Sorbitol McConkey supplemented with Nal acid (SMACN) for enumeration of *E. coli* O157:H7 populations and McConkey agar supplemented Nal acid and 0.1% sodium pyruvate (MACNP) for enumeration of *Escherichia coli* O26:H11 and *Escherichia coli* O111 populations. Cooked samples were also enriched by transferring 1 ml of macerated sample into 9 ml of EC medium (Difco, Becton Dickinson, MD) supplemented with 50µg/ml of Nal acid.
Each sample was incubated at 37°C for 24 h and then streaked onto TSAN, SMACN or MACPN plates and incubated at 37°C for 24 h for determining presence or absence of STEC or non-pathogenic *E. coli*. Presumptive STEC O157 and non-O157 colonies were confirmed using latex agglutination rapid detection kits (Dryspot *E. coli* O157 and non-O157 Latex kit, Oxoid Limited, Hampshire, UK).

**Statistical analysis**

Sample size was estimated using Statistical Solutions, LLC software (34) to avoid for type II error (power test = 0.80). For the first experiment the experimental design consisted on a 4 X 2 factorial design. Treatments consisted of four cocktails of STEC (*E. coli* O157:H7, *E. coli* O26:H11 and *E. coli* O111) and non-pathogenic *E. coli* inoculated on non-intact steaks and heated to two different target end-point temperatures: 60°C and 65°C (*n* = 6). For the validation study three cocktails of STEC were inoculated on non-intact steaks and heated to a single temperature of 60°C (*n* = 3). Colony counts were transformed to values expressed as CFU/g. Strains were compared before cooking for total bacterial population and Nal acid adapted strains using the GML procedure. The mixed procedure was used to test for normality of the residual distribution of the means and least square means was used to compare averages across strains for TSA. Tukey’s studentized test was used to analyze mean differences among strains before cooking for TSAN. A paired *t*-test was used to compare differences before and after cooking for specific STEC strains (O157:H7, O26:H11 and O111).
Results and Discussion

RF cooking protocol and time-temperature diagram

As mentioned before, average cooking times to reach three different endpoint temperatures were calculated in order to simulate plant processing where not all steaks can be monitored during cooking procedures and also to avoid exposure of pathogens in a food processing area (Table 5-1). In order to reduce variability during cooking it was necessary to control different factors such as initial temperature of the steak and water surrounding product, thickness and weight of steaks, location of steak inside of the tray and amount of water covering the steaks. The combination of the above factors minimized arching during heating and reduced variability in cooking time. Another challenge was that it was not possible to maintain the desired endpoint temperatures to generate dwell times with the current RF equipment. In order to further characterize post-cooking temperature rise differences due to steak thickness and tenderization as previously seen (33), the steaks were taken out of the water and left at room temperature for five more minutes (simulating restaurant hold times) and subsequently immersed in an ice-water bath to stop any further cooking (Figure 5-1).

Thermal inactivation of bacterial populations

The natural microbial counts of the non-inoculated steaks recovered on TSA were 3.01 ± 0.39 log CFU/g. Nal acid resistant bacteria were not detected (detection limit 0.3 log CFU/g) on TSAN. Initial counts of total bacterial populations and Nal acid resistant populations of *E. coli* O157:H7 were significantly higher (*p* ≤ 0.05) than the rest of the strains (Table 5-2). Therefore, the mixed procedure test was conducted to determine whether the residuals were normally distributed. There was not a significant departure
from normality on the data, which means that the variances among the four strains before cooking were not significantly different. It can also be observed that the count of different *E. coli* strains recovered on TSAN were similar to total bacterial populations recovered on TSA, indicating that the inoculum was the dominant source of steak contamination as seen in similar studies (1). A model that allowed for difference variances within each strain was used when testing in changes from uncooked to cooked (60°C) steaks for total bacterial populations (Table 5-3) and Nalidixic acid adapted populations (Table 5-4). In general, there were significant differences (*p* ≤ 0.05) among the strains when heated to 60°C (on TSA and TSAN). The microbial reduction at 60°C was 0.99, 3.08, 2.85 and 5.02 log CFU/g for *E. coli* O157:H7, O26:H11, O111 and non-pathogenic *E. coli* respectively recovered on TSAN. A pairwise test for the evaluation effects on log reductions of *E. coli* O157:H7, *E. coli* O26:H11 and *E. coli* O111 recovered with selective media was also performed (Table 5-5). The greater variation of the SEM of *E. coli* strains O26:H11 and O111 can be explained when the raw data is analyzed. At 60°C (almost 3°C below the USDA recommended temperature for inactivation of *E. coli*) the log reduction of those strains varies from 5.0 to 2.0 log CFU/g reduction. This could be due to the fact that at 60°C is the temperature where inactivation starts taking place but it still not effective enough to significantly kill most of bacterial populations; therefore, some bacteria may or may not have been injured and survive the treatment. Fluctuation of temperature of few degrees from the 60°C mark caused by using average cooking temperatures could have also caused variation in the log reduction on the steaks. Positive enrichment results of pathogenic samples at 60°C confirmed that injured bacteria can recover at this temperature (Table 5-6). Thus,
cooking steaks to 60°C with a holding time of 5 minutes using the RF oven can not ensure inactivation of STEC in mechanically tenderized steaks.

Cooking beef to 65°C resulted in greater reductions in bacterial populations than those obtained at 60°C with a 5.0 log reduction for all strains which was confirmed through enrichment (Table 5-6). Our results are in agreement with a previous research where inactivation of foodborne pathogens in ground beef using convection, RF or a combination of RF and convection were evaluated (28). Cooking the meatballs to 73°C with convection reduced the *E. coli* K-12 population by 5.5 log CFU/g, while treatment with only RF reduced the *E. coli* K-12 population to undetectable levels. Guo and co-workers (6) also reported that RF cooking of ground beef inoculated with *E. coli* K-12 cooked to 72°C resulted in undetectable levels of *E. coli* and longer shelf life of the product. The study also compared the RF cooking with water bath cooking and even though both methods resulted in similar inactivation, the cooking time of the RF was about 35 folds lower than the water bath.

Reports of evaluating the thermal inactivation efficacy of *E. coli* O157:H7 internalized in non-intact beef products have been published (2, 20, 30, 31, 40) along with some other research evaluating non-O157 strains in non-intact beef products (18, 27). A previous study that evaluated the extent of thermal inactivation of *E. coli* O157:H7 in blade tenderized steaks using different cooking methods (1), concluded that the cooking method is an important factor that needs to be included in lethality guidelines that are designed to ensure the safe preparation and consumption of non-intact beef products. Similar research studies have shown inactivation of *E. coli* O157:H7 by evaluation of different cooking methods. One study reported that the inactivation
decreased in the order of roasting > pan broiling ≥ double pan broiling (29), another study reported that the inactivation decreased in order of broiling > frying ≥ grilling (20) and a third study reported inactivation decreasing in the order of broiling > commercial grill > electric skillet (33). When comparing different cooking methods even under relatively controlled experimental conditions, there is a great variety in log reduction (27). Variation among cooking methods may be due in part to the inability of reaching the target endpoint temperature leading to the apparition of cold spots, reduction of heat penetration due to insulating effects of fat, and connective tissue causing variability of heating and heat transfer mechanism used (18). Radio frequencies heat food more uniformly and thus reduce heating time. The heat is generated within the product caused by polar molecules friction from the applied alternating electric field. The RF energy allows deeper penetration into the food without surface overheating (4). The killing of bacteria using RF has been mostly understood as heat related destruction due to heat generated on the substrate (28). One factor that needs to be considered though is the fact that the heating rate of samples during RF treatment is known to be affected by the chemical composition, moisture content and temperature among other factors (7, 23, 25).

The present protocol is intended for value added non-intact beef products cooked inside thermal bags, which most certainly would include marination ingredients such as phosphates and salts in their preparation. It has been shown that marinated products could have a higher absorption of power near the surface and a lower penetration depth of the electromagnetic waves compared to non-marinated products. The dielectric loss factor $\varepsilon''$ (which determines the ability of the material to dissipate the electromagnetic energy) would be altered and would result likely lower heating rates of the product (24).
Therefore, before commercial application of this method, further research needs to be conducted to fully understand the influence of ionic compounds present in marination ingredients on the heating rate and therefore on thermal inactivation of foodborne pathogens at temperatures \( \geq 65^\circ C \) in order to maximize the effectiveness of the protocol.

**Non-pathogenic *Escherichia coli* as potential surrogates for RF studies**

Previous experiments in phosphate buffer (results not shown) had indicated that the same non-pathogenic *E. coli* strains used on this study had the potential to be used as surrogates for STEC using RF technology. However, non-pathogenic *E. coli* strains had a significantly higher log reduction \((p \leq 0.05)\) when heated to 60°C compared to STEC strains on TSA and TSAN (Table 5-3 and 5-4). These results suggest that the selected non-pathogenic *E. coli* strains might not be a good choice to substitute the STEC strains for further in-plant validation studies using RF heating. The present results agree with a previous research (21) performed to determine the microbial inactivation of non-pathogenic *E. coli* strains (same strains from the present study) on beef homogenate samples cooked with RF along with antimicrobials. The study reported that no colonies were observed at temperatures \( \geq 60^\circ C \) below the detection limit. One reason that could explain the difference between studies in buffer solution compared to studies in the food matrix could be that liquid media studies may not be representative of the conditions in a food, particularly in heterogeneous foods such as whole muscle meat products, which are difficult to accurately reproduce in a model system as previously seen on different studies (3). Due to the high log reduction obtained at 60°C for non-pathogenic *E. coli* it was decided that non-pathogenic *E. coli* strains would not be included on the validation study at 63°C.
Validation Temperature Study

The USDA-FSIS compliance guideline for validating cooking instructions for mechanically tenderized beef products (37) recommends that the time for 5.0 log CFU reduction at 62.8°C (145°F) internal temperature in non-intact meat, chops and steaks needs to be maintained for minimum 3 minutes. This time-temperature recommendation is based on Thermal death curves for Salmonella in beef emulsions in tubes (5). The first step for validation based on this USDA-FSIS guideline is to provide scientific data and technical support that demonstrates that the cooking instructions can achieve the desired minimum temperature and rest time for the sample (37). Table 5-1 shows the expected average cooking time for the steaks to reach 63°C. Based on the average data there is a 95% confidence that 63°C will be reached between 14.2 to 16.5 minutes at the conditions tested. The second element for validation of cooking instructions based on the USDA-FSIS guideline is to implement critical operational parameters in-plant demonstration (37). The natural microbial counts of the non-inoculated steaks recovered on TSA were 2.98 ± 0.56 log CFU/g. Nal acid resistant bacteria were not recovered (detection limit 0.30 log CFU/g) on TSAN. Initial bacterial populations were not significantly different (p ≤ 0.05) among STEC strains for TSA or TSAN (Table 5-7). Initial populations on selective agar show an attachment close to 5.0 log CFU/g for each strain (Table 5-8). In general, validation at 63°C was effective for E. coli O157:H7 and E. coli O111 showing a 5.0 log reduction, but not for E. coli O26:H11, which showed a log reduction of 4.0 on one of the replicates. The reason behind this may be that the sample really did not reach 63°C due to slight fluctuation of temperature when using average cooking causing that some injured cells survived the treatment, or that E. coli O26:H11 was more resistant to
heat at this temperature and the protocol needs more adjustments for this particular strain. As a consequence, the USDA-FSIS recommendation at 63°C for this particular product may not be valid when using RF. Based on the results presented on this study, validation of RF heating instructions for blade tenderized steaks at 65°C and allowing 5 minutes of holding time before refrigeration of the product would be more effective for thermal inactivation of STEC. An example for a summary sheet from the validation trial at such temperature is shown on Figure 5-5.

Conclusions

Cooking of non-intact beefsteaks inside of a RF oven to 60°C resulted on significant log reduction with a variation from 5.0 to approximately 2.0 log CFU/g throughout the strains. Therefore, cooking mechanically tenderized steaks to 60°C with a holding time of 5 minutes may not be the best temperature for thermal inactivation of STEC and other pathogens. Cooking beefsteaks to 65°C resulted in a 5.0 log reduction for STEC and non-pathogenic strains. Verification and validation are important principles in a HACCP program that ensure that a specific process control is effective (19). However, one of the practical limitations of process validation is that the actual pathogens cannot be taken into a food-processing establishment to verify a specific process (22). The RF cooking protocol developed in the present study has practical relevance in the industry since whole steaks rather than beef homogenates were used; pilot-scale equipment instead of bench top equipment were tested and also real pathogens and surrogates were used under real processing conditions. At 60°C, non-pathogenic E. coli showed more sensitivity compared to STEC strains even though they had shown no differences when tested in a model solution on a previous study. This implies that the
selected strains may not be the correct choice to be used as surrogates for in-plant validation studies on RF and also that liquid media studies were not representative of the conditions tested in a heterogeneous system such as whole muscle. Validation for the USDA-FSIS recommendation for cooking to 63°C was effective for *E. coli* O157:H7 and *E. coli* O111 but not for *E. coli* O26:H11. Validation for *E. coli* O26 needs either more changes in the protocol or more thermal treatment to be done in order to be effective. Results for cooking to 65°C with a holding time at room temperature of 5 minutes before refrigeration would be a better temperature to validate the proposed cooking protocol instructions for non-intact whole muscle steaks using RF. However, it is recommended that validation of the process is to be conducted using marination ingredients since ions from phosphates and salts are capable of changing the heating profile and consequently the thermal inactivation of pathogens inoculated on the steaks.
References


TABLE 5-1 Expected average cooking times at each endpoint temperature of non-intact steaks cooked with a RF oven

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>Expected Cooking Time (min)(^a)</th>
<th>Confidence Interval (95%) (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>60</td>
<td>14.1 ± 1.35</td>
<td>13.2-15.4</td>
</tr>
<tr>
<td>63</td>
<td>15.4 ± 1.38</td>
<td>14.2-16.5</td>
</tr>
<tr>
<td>65</td>
<td>16.5 ± 1.15</td>
<td>14.3-17.4</td>
</tr>
</tbody>
</table>

\(^a\)Mean of cooking times ± Standard Error of the Mean (SEM) obtained from monitoring temperature inside of short loin non-intact beefsteaks of 1.9 cm thick and 185 ± 5 g (n = 6).
TABLE 5-2 Total bacterial populations and nalidixic acid-resistant *Escherichia coli* populations (log CFU/g), recovered with TSA and TSAN respectively for non-intact beefsteaks uncooked after overnight refrigeration (4°C ± 2) inside of a RF oven.

<table>
<thead>
<tr>
<th>Strain</th>
<th>Mean* (log CFU/g)</th>
<th>Mean (log CFU/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>TSA(^a)</td>
<td>TSAN</td>
</tr>
<tr>
<td>Escherichia coli O157:H7</td>
<td>5.66(^A)</td>
<td>5.59(^A)</td>
</tr>
<tr>
<td>Escherichia coli O26:H11</td>
<td>5.21(^B)</td>
<td>5.21(^A)</td>
</tr>
<tr>
<td>Escherichia coli O111</td>
<td>5.09(^B)</td>
<td>4.72(^B)</td>
</tr>
<tr>
<td>Non-Pathogenic <em>Escherichia coli</em></td>
<td>5.26(^AB)</td>
<td>5.32(^A)</td>
</tr>
<tr>
<td>SEM(^b)</td>
<td>0.028</td>
<td>0.022</td>
</tr>
</tbody>
</table>

\(^a\) Means within the same column with different superscript letters are different (\(p \leq 0.05\))

\(^b\) SEM = Standard Error of the Mean (\(n = 6\))
TABLE 5-3 Least Squares Means test measuring log reduction of total bacterial populations (log CFU/g) recovered from steaks heated to 60°C using a RF oven using the mixed procedure

<table>
<thead>
<tr>
<th>Strain</th>
<th>Mean(^a) (log CFU/g)</th>
<th>Significance ((p \leq 0.05))</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Escherichia coli O157:H7</em></td>
<td>0.81 ± 0.11</td>
<td>0.0001</td>
</tr>
<tr>
<td><em>Escherichia coli O26:H11</em></td>
<td>2.92 ± 1.19</td>
<td>0.0394</td>
</tr>
<tr>
<td><em>Escherichia coli O111</em></td>
<td>3.14 ± 1.12</td>
<td>0.0231</td>
</tr>
<tr>
<td>Non-Pathogenic <em>Escherichia coli</em></td>
<td>4.96 ± 0.08</td>
<td>&lt; 0.0001</td>
</tr>
</tbody>
</table>

\(^a\)Mean of log reduction (treatment – positive control) ± Standard Error of the Mean (SEM) \((n = 6)\).
**TABLE 5-4** Nalidixic acid-resistant *Escherichia coli* log reductions (treated samples-positive control) heated to 60°C using a RF oven and recovered with TSAN from non-intact beefsteaks (log CFU/g)

<table>
<thead>
<tr>
<th>Strain</th>
<th>Mean$^a$ (log CFU/g) TSAN</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Escherichia coli O157:H7</em></td>
<td>0.99$^B$</td>
</tr>
<tr>
<td><em>Escherichia coli O26:H11</em></td>
<td>3.08$^{A,B}$</td>
</tr>
<tr>
<td><em>Escherichia coli O111</em></td>
<td>2.85$^{A,B}$</td>
</tr>
<tr>
<td>Non-Pathogenic <em>Escherichia coli</em></td>
<td>5.02$^A$</td>
</tr>
<tr>
<td>SEM</td>
<td>1.45</td>
</tr>
</tbody>
</table>

$^a$Mean of log reduction (treatment – positive control) ± Standard Error of the Mean (SEM) ($n = 6$).
TABLE 5-5 Pairwise $t$-test for evaluation effects of RF on log reductions (treated samples-positive control) of *Escherichia coli* O157:H7 recovered with Sorbitol McConkey supplemented with nalidixic acid (SMACN) and *Escherichia coli* O26:H11 and O111 populations recovered with MacConkey agar supplemented with sodium pyruvate and nalidixc acid (MACNP) of non-intact beefsteaks cooked to 60°C.

<table>
<thead>
<tr>
<th>Strain</th>
<th>Mean log Reduction (log CFU/g)$^a$</th>
<th>Significance value $p$-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Escherichia coli</em> O157:H7</td>
<td>2.00 ± 1.05</td>
<td>0.1977</td>
</tr>
<tr>
<td><em>Escherichia coli</em> O26:H11</td>
<td>2.11 ± 1.23</td>
<td>0.2281</td>
</tr>
<tr>
<td><em>Escherichia coli</em> O111</td>
<td>2.49 ± 0.97</td>
<td>0.1241</td>
</tr>
</tbody>
</table>

$^a$ Mean log reduction ± SEM ($n = 6$)
TABLE 5-6 Levels of *E.coli* O157:H7, *E. coli* O26:H11 and *E. coli* O111 recovered from cores aseptically excised from non-intact beefsteaks after enrichment cooked to 60°C, 63°C or 65°C using a RF oven

<table>
<thead>
<tr>
<th>Strain</th>
<th>60°C</th>
<th>63°C</th>
<th>65°C</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Escherichia coli</em> O157:H7</td>
<td>6/6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0/3</td>
<td>0/6</td>
</tr>
<tr>
<td><em>Escherichia coli</em> O26:H11</td>
<td>6/6</td>
<td>1/3</td>
<td>0/6</td>
</tr>
<tr>
<td><em>Escherichia coli</em> O111</td>
<td>6/6</td>
<td>0/3</td>
<td>0/6</td>
</tr>
<tr>
<td>Non-pathogenic <em>Escherichia coli</em></td>
<td>4/6</td>
<td>-</td>
<td>0/6</td>
</tr>
</tbody>
</table>

<sup>a</sup> Number of positive samples after enrichment / total number of enriched samples)

Sample size for 60°C and 65°C *n* = 6

Sample size for 63°C *n* = 3

Detection limit = 0.30 log CFU/g
TABLE 5-7 Total bacterial populations and nalidixic acid-resistant *Escherichia coli* populations (log CFU/g), recovered with TSA and TSAN respectively for non-intact beefsteaks uncooked after overnight refrigeration (4°C ± 2) validation study at 63°C

<table>
<thead>
<tr>
<th>Strain</th>
<th>Mean (log CFU/g)</th>
<th>TSA</th>
<th>TSAN</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Escherichia coli</em> O157:H7</td>
<td>5.30&lt;sup&gt;A&lt;/sup&gt;</td>
<td>5.29&lt;sup&gt;A&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td><em>Escherichia coli</em> O26:H11</td>
<td>5.49&lt;sup&gt;A&lt;/sup&gt;</td>
<td>5.40&lt;sup&gt;A&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td><em>Escherichia coli</em> O111</td>
<td>5.49&lt;sup&gt;A&lt;/sup&gt;</td>
<td>5.51&lt;sup&gt;A&lt;/sup&gt;</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup>Means within the same column with different superscript letters are different ($p \leq 0.05$)

<sup>b</sup>SEM = Standard Error of the Mean ($n = 3$)
TABLE 5-8 *Escherichia coli* O157:H7 populations recovered with Sorbitol McConkey supplemented with nalidixic acid (SMACN) and *Escherichia coli* O26:H11 and O111 populations recovered with MacConkey agar supplemented with sodium pyruvate and nalidixc acid (MACNP) from uncooked non-intact beefsteaks validation study

<table>
<thead>
<tr>
<th>Strain</th>
<th>Mean(^a) (log CFU/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Escherichia coli</em> O157:H7</td>
<td>5.03 ± 0.05</td>
</tr>
<tr>
<td><em>Escherichia coli</em> O26:H11</td>
<td>4.83 ± 0.21</td>
</tr>
<tr>
<td><em>Escherichia coli</em> O111</td>
<td>5.04 ± 0.13</td>
</tr>
</tbody>
</table>

\(^b\) SEM = Standard Error of the Mean (n = 3)
FIGURE 5-1 Average and standard deviation for time-temperature profile at the center of 1.9 cm non-intact beefsteaks cooked to 65°C with a holding time of five minutes after cooking by RF heating (n = 6)
FIGURE 5-2 Flow diagram for steak inoculation and packaging

Uncooked thawed muscle → Steaks were cut and weighed → Sterile aluminum template to create 5x5 cm² impression → Vacuum steal and refrigerate at 4°C until processing → Spot inoculation of steaks and rest for 1 h
FIGURE 5-3 Flow diagram for packaged non-intact beefsteak cooking protocol using RF heating.
FIGURE 5-4 Flow diagram of non-intact beefsteak sampling protocol
**Validation Trial Product Summary Sheet**

**Product Name:** Radio Frequency Steaks

**Product Variables**

<table>
<thead>
<tr>
<th>Method of Tenderization</th>
<th>Blade Tenderized</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thickness of the Product</td>
<td>1.9 cm (0.75 in)</td>
</tr>
<tr>
<td>Type of Cut</td>
<td>Short Loin</td>
</tr>
</tbody>
</table>

**Testing Variables**

<table>
<thead>
<tr>
<th>Method of Cooking</th>
<th>Radio Frequency Oven Model #: SO61B</th>
</tr>
</thead>
<tbody>
<tr>
<td>State of the Product at the start of Cooking</td>
<td>Refrigerated (4°C)</td>
</tr>
<tr>
<td>Multiple Units</td>
<td>One one steak was tested at a time since a batch protocol was developed</td>
</tr>
<tr>
<td>Type of Cooking Container</td>
<td>The steaks were cooked inside thermal resistant bags contained inside of trays and covered with chilled water at 6°C</td>
</tr>
<tr>
<td>Number and location of temperature measurement sites during testing</td>
<td>One fiber optic thermocouple was inserted at the center of the steak and the other outside of the thermal pouch to measure water temperature surrounding the package</td>
</tr>
<tr>
<td>Number of replicates</td>
<td>The cooking times were calculated from an average of six samples. The testing methodology for inoculated product was tested three times per strain</td>
</tr>
<tr>
<td>Endpoint Temperature</td>
<td>65°C (149°F)</td>
</tr>
<tr>
<td>Resting time after cooking</td>
<td>5 minutes outside of the water</td>
</tr>
<tr>
<td>Rotation of product</td>
<td>None</td>
</tr>
</tbody>
</table>

**Figure 5-5** Example of validation of Radio Frequency instructions for 1.9 cm on-intact beefsteaks cooked to 65°C using a RF oven
CHAPTER 6

CONCLUSIONS

The results of the current study research revealed the following:

1) Cooking of non-intact beefsteaks using RF was successfully carried out by packing the steaks inside low-density polyethylene heat resistant pouches, placing them inside of a polysulfone tray containing a customized polyethirimide grid and using water immersion as heating media.

2) In terms of quality, thickness had an effect on cooking properties (cooking time, cooking loss, yield loss and heating rate); however, physicochemical characteristics of non-intact beefsteaks were affected mainly by endpoint temperature. The specific degree of doneness had a significant effect on cooking time, heating rate, cooking and drip losses, moisture content, pH, EM, $a^*$ value and shear force. All changes were confirmed by visual appearance of the samples, out of which, well done (75°C) looked drier as compared to medium well (65°C) and rare (55°C) steaks.

3) Shiga toxin-producing (O157:H7, O26:H11 and O111) and non-pathogenic E. coli strains were successfully adapted to nalidixic acid. There was no significant difference between Nal resistant and Nal sensitive strains at treatment temperatures; thus, the results of this research validated the use of nalidixic acid resistant strains as marker organisms for RF trials.
4) Thermal destruction of the pathogenic and non-pathogenic strains was greater at 65°C than 60°C, regardless of the initial level of inoculation on the steaks showing a 6.0 log reduction in all strains.

5) Thermal inactivation of non-intact beefsteaks at 60°C resulted in a log reduction of 0.99, 3.08, 2.85 and 5.02 log CFU/g for *E. coli* O157:H7, O26:H11, O111 and non-pathogenic *E. coli* respectively, while thermal inactivation at 65°C resulted in a 5.0 log reduction for all strains.

6) Non-pathogenic *E. coli* response in buffer solution was not significantly different from STEC strains at the temperatures tested; however, these strains were significantly more sensitive to temperature increase during RF heating than STEC strains on the steaks. These results are in line with previous research that shows that liquid media studies may be not representative of the conditions of heterogeneous food matrix, which are difficult to reproduce in model systems. As a result, the selected non-pathogenic strains may not be the correct choice to be used as surrogates for further in-plant validation studies with RF.

7) Validation for the USDA-FSIS recommendation for cooking to 63°C showed to be effective (5.0 log CFU/g reduction) for *E. coli* O157:H7 and *E. coli* O111 but not for *E. coli* O26:H11. However, cooking to 65°C with a holding time at room temperature of 5 minutes before refrigeration showed to be a better temperature to validate the proposed cooking protocol instructions for non-intact whole muscle steaks using RF.

The RF cooking protocol developed in the present study has practical relevance in the industry since whole steaks rather than beef homogenates were used; pilot-scale
equipment instead of bench top equipment were tested and also real pathogens and surrogates were used under real processing conditions. This study shows that RF cooking of packaged steaks was a promising process that can be used in the food industry specially at temperatures $\geq 65^\circ$C. Medium well steaks provided a better outcome in terms of quality and food safety compared to other temperatures. However, validation of the process has to be conducted using marination ingredients if the protocol is to be implemented since ions from phosphates and salts are capable of changing the heating profile and consequently the thermal inactivation of pathogens inoculated on the steaks.
CHAPTER 7
APPENDIX A
Figure A-1: Relationship between individual cell cultures of *Escherichia coli* O157:H7 counts and OD$_{600}$ before cocktail preparation
Figure A-2: Relationship between individual cell cultures of *Escherichia coli* O26:H11 counts and OD$_{600}$ before cocktail preparation
Figure A-3: Relationship between individual cell cultures of *Escherichia coli* O111 counts and OD$_{600}$ before cocktail preparation
Figure A-4: Relationship between individual cell cultures of non-pathogenic *Escherichia coli* counts and OD<sub>600</sub> before cocktail preparation.
Figure A-5: Efficacy of RF heating to inactivate NalR and NalS strains of various STEC and non-pathogenic *Escherichia coli* in PBS. Log reductions presented in this figure are net log reductions (Treatment temperature-positive control). Bars represent standard deviations.
CHAPTER 8
APPENDIX B
Sanitation Protocols

The RF equipment used for the present study, located inside the pilot plant at the Department of Food Science and Technology at the University of Georgia, is separated from the biosafety laboratory level 2 apt to handle human pathogens. Therefore, standard sanitation operation procedures (SSOP) had to be created and strictly followed for transportation and processing of samples. Prior to experimentation, the RF adjacent area was subjected to a full cleaning and sanitation process. The floors were washed with hot water, sanitized using quaternary ammonium sanitizer (300 ppm) and allowed to dry at room temperature. A full cleaning was performed before each replicate of the experiment. Samples were transported to the pilot plant area always using secondary sterile containers on a tray shelf plastic cart previously covered with biohazard plastic bags and disinfected with ethanol 70% before and after each use. Gloves were changed during the process as samples were placed inside of the trays, taken out of biosafety level II lab and transported to the pilot plant to be processed.

At the pilot plant the RF equipment was adapted for potential spills by placing removable plastic on the opposite ends of the conveyor belt and the front door. The plastic was sanitized after each use with 70% ethanol and allowed to dry at ambient temperature. Exposed drains that were close to the equipment were also sealed with plastic and labels indicating presence of biohazardous materials were placed around equipment area. The RF equipment and adjacent area were cleaned and disinfected with 70% ethanol and allowed to be air-dried before and after use. For validation of processing and transportation operational procedures environmental swabs were collected before and after cleaning for each processing day. Six random areas (inside and outside
of equipment) were swabbed and sampled for aerobic plate counts (3M St. Paul, MN) incubated at 37°C for 24h and general coliforms petrifilm (3M St. Paul, MN) incubated at 45°C for 24h. The experimental area in the biosafety 2 laboratory was also thoroughly cleaned and sanitized for steak slicing and packing operations. The meat slicer was dismantled, brushed with soap, rinsed with hot water and allowed to air dry. Then parts were assembled again and sprayed with 70% ethanol and allowed to dry before next processing day.