

FINAL REPORT

EFFECT OF TRADITIONAL AND MODIFIED ENHANCEMENT SOLUTION INGREDIENTS ON SURVIVAL OF *ESCHERICHIA COLI* O157:H7 DURING STORAGE AND COOKING OF MOISTURE-ENHANCED BEEF

APRIL, 2009

SUBMITTED TO:
AMERICAN MEAT INSTITUTE FOUNDATION

SUBMITTED BY:
CENTER FOR MEAT SAFETY & QUALITY
FOOD SAFETY CLUSTER
DEPARTMENT OF ANIMAL SCIENCES
COLORADO STATE UNIVERSITY, FORT COLLINS, CO

IFIGENIA GEORNARAS
NIKOS CHORIANOPOULOS
KYUNG YUK KO
JEREMY M. ADLER
OLEKSANDR A. BYELASHOV
SHIVANI GUPTA
CANGLIANG SHEN
KEITH E. BELK
GARY C. SMITH
JOHN N. SOFOS

TABLE OF CONTENTS

	Page
I. EXECUTIVE SUMMARY	4
II. TECHNICAL ABSTRACT.....	5
III. INTRODUCTION.....	7
IV. OVERALL PROJECT GOAL.....	9
V. SUPPORTING OBJECTIVES.....	9
VI. MATERIALS AND METHODS.....	9
A. Preliminary Studies.....	9
<i>Study 1: Heat inactivation kinetics of E. coli O157:H7 strains in a broth culture medium.....</i>	9
<i>Study 2: Selection of rifampicin-resistant E. coli O157:H7 variants and their thermotolerance as compared to the parental strains.....</i>	10
B. Studies Addressing Objective 1.....	11
<i>Study 3: Effect of brining ingredients on thermal inactivation of E. coli O157:H7 in a meat model system.....</i>	11
<i>Study 4: Effect of selected brining ingredients on E. coli O157:H7 in a meat model system following a 96 h storage period at 4°C and subsequent cooking to 65°C.....</i>	13
<i>Study 5: Antimicrobial activity of essential oils, alone or in combination with other antimicrobials, against E. coli O157:H7 in a beef extract containing sodium chloride and sodium tripolyphosphate.....</i>	14
C. Studies Addressing Objective 2.....	15
<i>Study 6: Survival/growth of E. coli O157:H7 during frozen, refrigerated or retail-abusive storage conditions, and subsequent thermal inactivation during cooking of moisture-enhanced beef steaks.....</i>	15
<i>Study 7: Effect of different brining formulations on survival during frozen storage, and thermal inactivation during cooking, of E. coli O157:H7 in moisture-enhanced beef roasts.....</i>	18
<i>Study 8: Inactivation of E. coli O157:H7 in moisture-enhanced beef steaks of different thickness by pan-broiling, double pan-broiling or roasting using different cooking equipment.....</i>	20
D. Studies Addressing Objective 3.....	21
<i>Study 9: Survival of E. coli O157:H7 in brining solutions, prepared in a meat homogenate, during storage at 4 or 15°C.....</i>	21
<i>Study 10: Survival of E. coli O157:H7 in fresh, non-recirculated brining solutions stored at 4 or 15°C.....</i>	22
E. Additional Studies.....	23
<i>Study 11: Translocation of E. coli O157:H7 during needle injection for moisture enhancement of beef.....</i>	23
<i>Study 12: Thermal inactivation of E. coli O157:H7 at different depths of nonintact steaks cooked to a sublethal temperature.....</i>	24
VII. RESULTS.....	26
A. Preliminary Studies.....	26

<i>Study 1</i> : Heat inactivation kinetics of <i>E. coli</i> O157:H7 strains in a broth culture medium.....	26
<i>Study 2</i> : Selection of rifampicin-resistant <i>E. coli</i> O157:H7 variants and their thermotolerance as compared to the parental strains.....	26
B. Studies Addressing Objective 1.....	27
<i>Study 3</i> : Effect of brining ingredients on thermal inactivation of <i>E. coli</i> O157:H7 in a meat model system.....	27
<i>Study 4</i> : Effect of selected brining ingredients on <i>E. coli</i> O157:H7 in a meat model system following a 96 h storage period at 4°C and subsequent cooking to 65°C.....	28
<i>Study 5</i> : Antimicrobial activity of essential oils, alone or in combination with other antimicrobials, against <i>E. coli</i> O157:H7 in a beef extract containing sodium chloride and sodium tripolyphosphate.....	28
C. Studies Addressing Objective 2.....	29
<i>Study 6</i> : Survival/growth of <i>E. coli</i> O157:H7 during frozen, refrigerated or retail-abusive storage conditions, and subsequent thermal inactivation during cooking of moisture-enhanced beef steaks.....	29
<i>Study 7</i> : Effect of different brining formulations on survival during frozen storage, and thermal inactivation during cooking, of <i>E. coli</i> O157:H7 in moisture-enhanced beef roasts.....	34
<i>Study 8</i> : Inactivation of <i>E. coli</i> O157:H7 in moisture-enhanced beef steaks of different thickness by pan-broiling, double pan-broiling or roasting using different cooking equipment.....	36
D. Studies Addressing Objective 3.....	38
<i>Study 9</i> : Survival of <i>E. coli</i> O157:H7 in brining solutions, prepared in a meat homogenate, during storage at 4 or 15°C.....	38
<i>Study 10</i> : Survival of <i>E. coli</i> O157:H7 in fresh, non-recirculated brining solutions stored at 4 or 15°C.....	38
E. Additional Studies.....	39
<i>Study 11</i> : Translocation of <i>E. coli</i> O157:H7 during needle injection for moisture enhancement of beef.....	39
<i>Study 12</i> : Thermal inactivation of <i>E. coli</i> O157:H7 at different depths of nonintact steaks cooked to a sublethal temperature.....	40
VIII. CONCLUSIONS.....	40
A. Preliminary Studies.....	40
B. Studies Addressing Objective 1.....	40
C. Studies Addressing Objective 2.....	41
D. Studies Addressing Objective 3.....	42
E. Additional Studies.....	43
IX. Recommendations for Future Research.....	43
X. Presentations and Publications.....	43
XI. REFERENCES.....	44
Figures.....	47
Appendix.....	63

I. EXECUTIVE SUMMARY

Project Title: Effect of Traditional and Modified Enhancement Solution Ingredients on Survival of *Escherichia coli* O157:H7 during Storage and Cooking of Moisture-Enhanced Beef

Principal Investigator: John N. Sofos

Co-Investigators: Ifigenia Geornaras, Nikos Chorianopoulos, Kyung Yuk Ko, Jeremy M. Adler, Oleksandr A. Byelashov, Shivani Gupta, Cangliang Shen, Keith E. Belk, and Gary C. Smith

Research Institution: Center for Meat Safety & Quality, Food Safety Cluster, Department of Animal Sciences, Colorado State University, 1171 Campus Delivery, Fort Collins, CO 80523-1171
Phone: 970-491-7703, Fax: 970-491-0278
email: john.sofos@colostate.edu

Submittal Date of Final Report to AMI: April, 2009

Moisture enhancement of beef products is one alternative used by the meat industry to improve tenderness and flavor of lower valued cuts, and involves multi-needle injection of a brine solution, comprised traditionally of salt, one or more of the sodium phosphates, and water, into the meat. A microbiological safety concern associated with this process is that it may lead to contamination of the interior parts of the products with pathogens, such as *Escherichia coli* O157:H7, either through transfer of the pathogen from the contaminated meat surface to the interior during needle injection, or from the use of brines that have become contaminated. Subsequent undercooking (due to consumer preference, or accidentally) may result in survival of the internalized pathogen, and possibly lead to human illness. Also, there are concerns that brine ingredients may make the pathogen more difficult to kill during cooking. Studies evaluated the effect of individual ingredients or combinations of ingredients used for brining (traditional and novel) on destruction of *E. coli* O157:H7, in a meat model system and a beef extract, during storage and simulated (in a water bath) cooking; survival/growth of the pathogen during frozen, refrigerated, or retail-type storage of moisture-enhanced beef steaks and roasts and subsequent destruction during cooking by pan-broiling, double pan-broiling and/or roasting; and survival of *E. coli* O157:H7 during storage of freshly prepared and recirculated brine solutions containing one or more antimicrobial ingredients. Selected findings indicated that moisture enhancement of beef products with brining formulations that contained the antimicrobial, cetylpyridinium chloride (0.2% or 0.5%), reduced *E. coli* O157:H7 contamination during product storage; the essential oils, thyme oil and grapefruit seed extract, alone or in combination with other antimicrobials, caused immediate inactivation of *E. coli* O157:H7 in a beef extract that contained the common brine ingredients, salt and phosphate; efficiency of destruction of internalized *E. coli* O157:H7 in moisture-enhanced products depended on the cooking method (i.e., pan-broiling, double pan-broiling, or roasting) used; more destruction of the pathogen was obtained in thicker (4.0 cm) than thinner steaks (1.5 or 2.5 cm); and contamination of freshly prepared or recirculated brines can be controlled with the addition of antimicrobials to the solutions, such as AvGard[®] XP (2.2%) or cetylpyridinium chloride (5.5%). The findings of these studies should be useful for development and/or improvement of brines for enhancing the safety of moisture-enhanced meat products.

II. TECHNICAL ABSTRACT

Moisture enhancement of beef products via the physical process of needle injection of brine solutions into the tissue, is an important public health concern as it may result in internalization of *Escherichia coli* O157:H7 into the muscle tissue. This can occur either by translocation of the pathogen from the meat surface to the interior during needle injection, or from contaminated brines. Intentional (consumer preference) or unintentional (accidental) undercooking of such products may result in survival of the internalized pathogen, and possibly lead to human illness. Additionally, there are concerns that ingredients of brining formulations may increase the heat resistance of *E. coli* O157:H7, thus interfering with inactivation of the pathogen during cooking. The studies described in this report were designed to identify ingredients used in brining solutions that may have antimicrobial effects during storage and/or enhance thermal inactivation of *E. coli* O157:H7 during cooking of moisture-enhanced beef products. Specifically, the main objectives of this work were to: (i) evaluate the effect of individual ingredients or combinations of ingredients used for brining on thermal inactivation of *E. coli* O157:H7 in a model system (laboratory media or ground beef) (Objective 1), (ii) evaluate survival/growth during frozen, refrigerated, or retail-type storage, and thermal resistance following storage of *E. coli* O157:H7-inoculated beef products moisture-enhanced with ingredients shown to enhance thermal destruction (Objective 2), and (iii) evaluate survival of *E. coli* O157:H7 in brine solutions containing one or more antimicrobial ingredients (Objective 3). Additional studies investigated quantitative transfer of *E. coli* O157:H7 during moisture enhancement of beef under two contamination scenarios, and thermal inactivation of *E. coli* O157:H7 at different depths of contamination of nonintact steaks. Under Objective 1, brining formulations, comprised of sodium chloride (NaCl, 0.5%) + sodium tripolyphosphate (STP, 0.25%), without or with single or combinations of antimicrobials, were tested for their potential effect on inactivation of *E. coli* O157:H7 in a meat model system comprised of two fat levels (approximately 5% and 15%), following storage at 4°C (24 or 96 h) and subsequent simulated cooking (in a circulating water bath) to 65°C. Results showed that moisture enhancement (110% of initial weight) of meat samples with NaCl + STP + cetylpyridinium chloride (CPC, 0.5%) reduced *E. coli* O157:H7 counts by approximately 1 log CFU/g during storage (4°C, 24 h). The same treatment also enhanced ($P < 0.05$) thermal destruction of the pathogen, compared to the NaCl + STP (without antimicrobials) treatment, when samples were cooked to 65°C (simulating medium-rare doneness of beef). NaCl + STP combined with potassium lactate (PL, 2%), sodium diacetate (SD, 0.15%), PL + SD, lactic acid (LA, 0.3%), acetic acid (AA, 0.3%), citric acid (CA, 0.3%), Nisaplin[®] (0.06%) or pediocin (ALTA[™] 2341; 0.5%) + EDTA (20 mM), AvGard[®] XP (0.2%), hops beta acids (0.00055%), Nisaplin + ALTA 2341 + EDTA, CPC (0.05%) + LA, or PL + SD + CPC (0.05%) did not ($P \geq 0.05$) enhance or protect the pathogen from heat inactivation, compared to a water control treatment. In an additional study, the antimicrobial effects of essential oils (thyme oil [TO], grapefruit seed extract [GSE], basil oil), alone or in combination with other antimicrobials, were evaluated against *E. coli* O157:H7 in a beef extract containing NaCl (0.5%) + STP (0.25%). Results showed immediate bactericidal effects (> 3.5 log CFU/ml reductions) of the pathogen in beef extract containing TO (0.25% and 0.5%) or GSE (0.5% and 1.0%), alone or in combination with CPC (0.04%) or SD (0.25%), and counts remained below the detection limit (0.0 log CFU/ml) even after 48 h incubation at 15°C. Under Objective 2, *E. coli* O157:H7-inoculated beef steaks (2.5 cm thickness) and roasts (2 kg) were prepared and moisture-enhanced (110% of initial weight) with NaCl (0.5%) + STP (0.25%) alone, or combined with CPC (0.2%),

LA (0.3%), or AvGard XP (0.2%). The meat products were packaged (steaks: vacuum and aerobically; roasts: vacuum) and were stored at -20°C (steaks and roasts), 4°C (steaks) or 12°C (steaks). On predetermined days of storage (depending on the storage temperature), steaks and roasts were evaluated for survival/growth of bacterial populations, and additionally, products were cooked to internal temperatures of 55°C (roasts) or 60°C (steaks and roasts) by pan-broiling (steaks), double pan-broiling (steaks), and roasting (steaks and roasts). In general, *E. coli* O157:H7 counts for steaks and roasts moisture-enhanced with NaCl + STP + CPC were lower (by 0.7-1.2 [steaks] or 0.7-2.4 [roasts] log CFU/g) than those of the other brining treatments. Overall, heat inactivation of the pathogen in steaks increased in order of: pan-broiling ≤ roasting < double pan-broiling. *E. coli* O157:H7 populations in moisture-enhanced roasts cooked to internal temperatures of 60°C or 55°C were reduced by >2.8 to >6.5 log CFU/g (i.e., depending on initial levels in uncooked roasts). Recovery of high numbers (≥2.1 log CFU/g) of the pathogen from some of the cooked roasts appeared to be correlated with a short cooking time combined with a low final temperature (i.e., internal temperature of roasts after a 20 min resting period). In an additional study carried out under Objective 2, thermal (internal temperature of 65°C) destruction of *E. coli* O157:H7 was investigated in moisture-enhanced (NaCl [0.45%] + STP [0.23%]) beef steaks of 1.5, 2.5 and 4.0 cm thickness, cooked by pan-broiling, double pan-broiling, and roasting using different cooking equipment. Major findings of this study were that, in general, greater inactivation of the pathogen was obtained in 4.0 cm than 1.5 or 2.5 cm thick steaks, regardless of cooking method or equipment. Additionally, thermal destruction of *E. coli* O157:H7 increased in order of: double pan-broiling ≤ pan-broiling < roasting, with no differences obtained between the different cooking equipment used for the same cooking method. Under Objective 3, survival of *E. coli* O157:H7 was evaluated in brines, prepared in a 3% meat homogenate (simulating recirculated brine) or water (simulating freshly prepared brine), and formulated without or with antimicrobials, during storage at 4°C or 15°C (up to 24 or 48 h). Pathogen populations did not increase or decrease, during the entire storage period, in the basic brine solution (i.e., NaCl [5.5%] + STP [2.75%]), and also when it was combined with PL (22%) and/or SD (1.65%). However, addition of AvGard XP (2.2%) or CPC (5.5%) to the brine resulted in immediate pathogen reductions of 1.4 to >2.6 log CFU/ml, and non-detectable (<1.3 log CFU/ml) levels during storage (4°C or 15°C). Additionally, depending on the presence or absence of meat residues in the brine solutions, and storage temperature, reductions of *E. coli* O157:H7 were obtained in NaCl + STP solutions containing LA (3.3%), AA (3.3%), CA (3.3%), Nisaplin (0.66%) or ALTA 2341 (5.5%) + EDTA (200 mM), or hops beta acids (0.0055%) during storage. Findings of the additional studies that were performed over and above the stated main objectives of the proposal were: (i) *E. coli* O157:H7 contamination was transferred to the interior of beef tissue following moisture enhancement (via needle injection), when the surface of the meat, or brine solution, were contaminated with the pathogen, and (ii) numbers of *E. coli* O157:H7 survivors increased ($P < 0.05$) as the depth of contamination (0-12 mm of a 24 mm steak) of nonintact steaks increased, when samples were cooked (60°C internal temperature) by pan-broiling, whereas, no differences ($P \geq 0.05$) in survivors were obtained at the different depths of contamination when steaks were cooked by roasting. Overall, the data presented in this report should be useful for development and/or improvement of brines for control of *E. coli* O157:H7 in moisture-enhanced meat products, during product storage and cooking. Furthermore, the data should be useful in updating risk assessments on nonintact beef products.

III. INTRODUCTION

Consumers consider tenderness to be the most important attribute associated with palatability of beef (Boleman et al., 1995; Miller et al., 1995). The most valued cuts from beef carcasses come from the loin and rib areas (Savell and Smith, 2000). However, these areas represent 26% of the total carcass indicating that the remainder of the carcass (74%) consists of lower valued cuts (Molina et al., 2005). Since the chuck and round primal cuts make up more than two-thirds of the total carcass, significant research efforts are focused at looking for ways to add value to these cuts (Molina et al., 2005; Savell and Smith, 2000). Solution enhancement technology is one alternative used by the beef industry to improve tenderness and flavor. This technology involves multi-needle injection of a brine solution comprised traditionally of salt, one or more of the sodium phosphates, and water, into the meat (Knock et al., 2006; Uttaro and Aalhus, 2007; Vote et al., 2000; Wicklund et al., 2006). Other ingredients that may be included in brining solutions are sodium or potassium lactate, sodium acetate or diacetate and varying flavoring agents (Knock et al., 2006; Miller, 1998; Vote et al., 2000; Wicklund et al., 2006).

Moisture enhancement of beef products via the physical process of needle injection of brine solutions into the product is an important public health concern as it may translocate pathogens, such as *Escherichia coli* O157:H7, from: (i) the surface of intact beef cuts to below the surface, and (ii) contaminated meat to previously non-contaminated pieces of meat (e.g., cross-contamination via needle injection and/or recycling of brines). It is very likely that consumers perceive nonintact (USDA-FSIS, 2002) and intact beef products the same, and in fact, may not know that the beef product has been moisture-enhanced or mechanically tenderized, and hence will cook them to the same degree of doneness, without considering the potential presence of microbial contamination in the interior of the product. Subsequently, *E. coli* O157:H7 in the interior of nonintact products may survive cooking and cause illness among consumers, and especially if the injected ingredients interfere with thermal inactivation or increase the heat resistance of the pathogen (Sofos et al., 2008). The public health risk associated with nonintact beef products in the U.S. is supported by outbreaks of *E. coli* O157:H7 illness linked to the consumption of these products: (i) August, 2000 - two cases in Michigan were linked to consumption of needle-tenderized sirloin steaks; (ii) June, 2003 - 11 cases in five states were linked to consumption of a tenderized beef steak product, specifically a boneless beef filet bacon-wrapped steak product injected with a marinade, and (iii) August, 2004 - four cases in Colorado were linked to consumption of a tenderized, marinated beef steak product (USDA-FSIS, 2005). Additionally, in 2007, a recall notice by the U.S. Department of Agriculture Food Safety and Inspection Service indicated that several cases of *E. coli* O157:H7 infection had been linked to consumption of steak products injected with tenderizers and flavor-enhancing solutions (USDA-FSIS, 2007). Thus, even though reports indicate that *E. coli* O157:H7 contamination on the surface of beef subprimals is low (BIFSCO, 2006), translocation of surface contamination to the interior of beef products appears to be likely.

Researchers have almost exclusively studied the effect of moisture enhancement and mechanical tenderization on sensory qualities of meat products, and little work has been done on the microbiological aspects related to these processes (Phebus et al., 1999). The limited work related to the microbiology of nonintact beef products pertains mainly to mechanical tenderization. A study by Sporing (1999) determined that 3×10^3 CFU/g of surface contamination (10^6 CFU/g) of *E. coli* O157:H7 was transferred to the center of beef top sirloin subprimals as a result of blade tenderization; this may pose a public health risk due to the low

infective dose of this pathogen (≥ 10 cells) (Doyle et al., 1997; Sporing, 1999). Gill and McGinnis (2004) reported deep tissue microbial contamination in retail cuts of mechanically tenderized beef obtained from three out of four retail stores sampled in Canada. The same group of researchers (Gill and McGinnis, 2005) found that beef prime cuts exposed to 1 or 8 incising events with a mechanical tenderizing machine had surface contamination levels, with aerobic microorganisms, of 2.98 and 3.55 log CFU/cm², respectively, and internal (76-100 mm deep) contamination levels of 0.14 and 0.76 log CFU/g, respectively. It was concluded that higher levels of deep tissue contamination were obtained with higher levels of surface contamination (Gill and McGinnis, 2005). In an additional study (Gill et al., 2005b), aerobic microorganisms were recovered from deep tissues of 8% of mechanically tenderized striploins cooked to medium-rare and well-done conditions where maximum temperatures reached at the center of the steaks were 64.2°C and 72.6°C, respectively.

A few relatively recent studies have started addressing the microbiological aspects of moisture enhancement. Bohaychuk and Greer (2003) reported that initial counts of psychrotrophic bacteria of moisture-enhanced pork loins were approximately 2 log units higher than those of noninjected boneless loins. Furthermore, the bacterial levels of brine-injected loins generally remained higher than those of the control (noninjected) product during a 5-week storage period at 2°C or 5°C in vacuum packages (Bohaychuk and Greer, 2003). An obvious source of the higher initial contamination levels of the moisture-enhanced products could be recirculating injection brines (Gill et al., 2005a; Greer et al., 2004). Indeed, counts of various bacteria types in recirculating brine increased after 2.5 h of moisture-enhanced pork production (Greer et al., 2004). Gill et al. (2005a) reported that the deep tissues of moisture-enhanced boneless pork loins were contaminated with 2.1 log CFU/g aerobic bacteria compared to no detectable contamination in noninjected product. Following cooking of the injected meat to 61°C, 1.0 log CFU/25 g of aerobic bacteria were recovered from the deep tissues, while cooking to 70°C resulted in no recovery of bacteria. The authors concluded that cooking moisture-enhanced pork to a medium-rare condition of doneness should be adequate for safety (Gill et al., 2005a). Wicklund et al. (2006) investigated the effect of addition of sodium lactate, and sodium lactate plus sodium diacetate to a salt-phosphate brine solution on survival of *E. coli* K12 during storage of beef strip steaks and in purge. The enhancement solutions were prepared to provide 0.3% salt, 0.3% phosphate, 3.0% sodium lactate and/or 0.25% sodium diacetate in the finished product enhanced to 110% of the initial weight (Wicklund et al., 2006). The enhancement solutions were inoculated with 3 or 6 log CFU/ml of *E. coli* K12, and were then used to inject the beef product, followed by vacuum-packaging and storage at 4°C for up to 14 days. At the higher inoculum level, addition of sodium lactate, and sodium lactate plus sodium diacetate to the brine resulted in initial reductions (0.8 log CFU/g) of *E. coli* K12 in enhanced beef steaks compared to steaks enhanced with salt and phosphate only. Storage for 7 days at 4°C reduced levels by another 0.5-0.7 log units in lactate- and lactate/diacetate-enhanced steaks, with no additional reductions after 14 days of storage. When the lower inoculum level was used, survivors were only detected in the purge of steaks enhanced with salt and phosphate (without lactate- and lactate/diacetate) (Wicklund et al., 2006). Thus, these results indicate that addition of ingredients with antimicrobial properties to enhancement solutions could minimize the risk of pathogen contamination of moisture-enhanced products. Additional work is needed to determine the effect of these ingredients on the subsequent thermotolerance of potential pathogens (i.e., *E. coli* O157:H7) during cooking.

IV. OVERALL PROJECT GOAL

The overall project goal was to identify ingredients used in brining solutions that may have antimicrobial effects during storage and/or enhance thermal inactivation of *E. coli* O157:H7 during cooking of moisture-enhanced beef products.

V. SUPPORTING OBJECTIVES

The above project goal is supported by the following objectives:

1. Evaluate the effect of individual ingredients or combinations of ingredients used for brining on thermal inactivation of *E. coli* O157:H7 in a model system (laboratory media or ground beef).
2. Evaluate survival/growth during frozen, refrigerated, or retail-type storage, and thermal resistance following storage of *E. coli* O157:H7-inoculated beef products moisture-enhanced with ingredients shown to enhance thermal destruction (identified in Objective 1).
3. Evaluate survival of *E. coli* O157:H7 in brine solutions containing one or more antimicrobial ingredients.

VI. MATERIALS AND METHODS

A. Preliminary Studies

Study 1: Heat inactivation kinetics of E. coli O157:H7 strains in a broth culture medium

Prior to the start of experiments, *E. coli* O157:H7 strains from the culture collection of the Pathogen Reduction Laboratory, Department of Animal Sciences, Colorado State University, were screened for their heat resistance to aid in the selection of strains for inclusion in the inoculum for subsequent studies.

Objective: To evaluate the thermotolerance of *E. coli* O157:H7 strains at 60°C, in tryptic soy broth.

Materials and Methods:

E. coli O157:H7 strains and inoculum preparation. A total of 37 *E. coli* O157:H7 strains were included in this study; 7 ATCC strains, 1 strain isolated from jerky (kindly provided by Dr. M. P. Doyle, University of Georgia, Griffin, GA), and 29 strains previously (Carlson et al., 2006; Woerner et al., 2006) isolated from cattle feces. The strains were activated and subcultured in 10 ml tryptic soy broth (TSB; Difco, Becton Dickinson, Sparks, MD) for 22 h at 35°C. Each culture (9 ml) was harvested individually by centrifugation (Eppendorf model 5810 R, Brinkman Instruments Inc., Westbury, NY; 4,629×g, 15 min, 4°C) and the cell pellet was resuspended in 0.9 ml of fresh TSB.

Inoculation, heat challenge and microbiological analysis. Prior to the heat challenge, 29.7 ml of TSB in oakridge tubes (Nalgene, Nalge Nunc, Rochester, NY) was equilibrated to 60°C in a shaking water bath (80 strokes/min) for approximately 20 min. The temperature of the water in

the water bath, and a second tube of TSB (30 ml), immediately adjacent to the tube that would be inoculated for the heat challenge, was continuously monitored with thermocouples (Pico Technology Ltd., Cambridge, UK) and recorded with real-time data recording software (PicoLog, Pico Technology Ltd.). The water level in the water bath was maintained at approximately 2 cm above the level of TSB in the tubes.

The tube containing 29.7 ml of pre-heated TSB was inoculated with 0.3 ml of the culture, achieving an inoculation level of approximately 8 log CFU/ml. Aliquots were removed from the tubes at 30, 60, 90, 120, 150, 180, and 240 s, and were immediately placed in an ice-water slush. The samples were spiral-plated (Spiral Plater Model D; Spiral Biotech, Bethesda, MD) on tryptic soy agar (Acumedia, Lansing, MI) supplemented with 0.1% sodium pyruvate (TSAP). Inoculated, non-preheated TSB was also plated and constituted the time-0 sample (i.e., inoculation level prior to heat challenge). Plates were incubated at 35°C for 48 h. Colonies were enumerated (Colony Image Analysis CASBA™ 4 scanner and software system; Spiral Biotech) and bacterial counts were expressed as log CFU/ml. The detection limit of the analysis was 1.3 log CFU/ml.

Data analysis. The heat challenge was repeated twice for each strain. Data were analyzed with MATLAB® software (version 7.6; <http://www.mathworks.com/products/matlab/>) using the equation developed by Juneja and Marks (2005). This equation was devised using data collected from beef gravy inoculated with *E. coli* O157:H7 and heated at 60°C. In our study, the MATLAB software was used to estimate the time required to achieve 1-, 2-, 3-, 4-, 5- and 6-log CFU/ml reductions at 60°C, for each tested strain.

Study 2: Selection of rifampicin-resistant *E. coli* O157:H7 variants and their thermotolerance as compared to the parental strains

Use of *E. coli* O157:H7 strains with a selective marker, like antibiotic resistance, allows tracking of the inoculum in meat products and brines without interference from the natural meat microflora. It also permits differentiation of the inoculum from the meat microflora on non-selective media, to detect cells injured by heat or brine ingredients and/or antimicrobials. Thus, it was decided to develop rifampicin-resistant variants of several *E. coli* O157:H7 strains for use in subsequent studies.

Objective: To develop rifampicin-resistant variants of 15 *E. coli* O157:H7 strains, and to compare their thermotolerance to that of the parental (wild-type) strains.

Materials and Methods:

Selection of strains and heat challenge. The criteria used for selection of *E. coli* O157:H7 strains for development of rifampicin-resistant variants included their heat resistance at 60°C (*Study 1*), source and genotype (data not shown). Genotype information for the strains isolated from cattle feces was obtained from previous work by Carlson et al. (2006), and included their PFGE profile and the absence or presence of one or both *stx* genes (data not shown). An additional strain (i.e., ATCC 700728), not included in the set of strains tested in *Study 1*, was included in the current study. Isolation of rifampicin (100 µg/ml)-resistant variants of the 15 selected *E. coli* O157:H7 strains, was carried out as described by Kaspar and Tamplin (1993).

The thermotolerance, at 60°C, of the rifampicin-resistant and corresponding parental strains was evaluated using the method described in *Study 1*. Survivors were enumerated on TSAP, and

in the case of the rifampicin-resistant strains, survivors were also enumerated on TSAP supplemented with rifampicin (100 µg/ml, Sigma-Aldrich, St. Louis, MO; TSAP+rif). Statistical analysis. The heat challenge was repeated two or three times for each rifampicin-resistant and corresponding parental strain. The time required to achieve 3-, 4-, and 5-log CFU/ml reductions was estimated using the MATLAB software. Data were statistically analyzed (ANOVA test using the JMP SAS program v7.0.2, SAS Institute, Cary, NC) to determine whether there were differences ($P < 0.05$) in heat resistance between the rifampicin-resistant and corresponding parental strains.

B. Studies Addressing Objective 1

Study 3: Effect of brining ingredients on thermal inactivation of E. coli O157:H7 in a meat model system

Objective: To evaluate brining ingredients for their potential effect on heat inactivation of *E. coli* O157:H7 during simulated cooking to 65°C in a meat model system comprised of two fat levels.

Materials and Methods:

E. coli O157:H7 strains and inoculum preparation. Rifampicin-resistant strains of *E. coli* O157:H7 (ATCC 43888, ATCC 43895, ATCC 43895/ISEHGFP, C1-057, C1-072, C1-109, C1-154, and C1-158) were activated and subcultured individually in TSB supplemented with 100 µg/ml rifampicin (22 h at 35°C). Each culture (30 ml) was centrifuged (as described in *Study 1*), washed with phosphate buffered saline (PBS; pH 7.4; 0.2 g KH₂PO₄, 1.5 g of Na₂HPO₄·7H₂O, 8.0 g of NaCl, and 0.2 g of KCl in 1 liter of distilled water) and cell pellets were resuspended in 30 ml PBS. The eight strains were combined and used to inoculate the ground beef model system.

Inoculation, treatment, storage, and cooking of samples. Fresh (approximately 72 h after animal slaughter) beef knuckles (approximately 5% fat) or beef shoulder (approximately 15% fat) were obtained from a meat packing plant in Northern Colorado. Each type of meat was individually ground with 0.95-cm and then 0.16-cm diameter plates in the Meat Science Laboratory, Department of Animal Sciences at Colorado State University. The meat was then transferred to the Pathogen Reduction Laboratory for inoculation, treatment and analyses. Meat (700 g per batch) was mixed in a bowl-lift stand mixer (KitchenAid[®], Professional 600, St. Joseph, MI) for 2 min with 7 ml of the inoculum (approximately 9 log CFU/ml) to achieve an initial inoculation level of 7 log CFU/g. Brining solutions (63 ml) were then added to the inoculated meat to simulate a product moisture-enhanced to 110% of initial weight. After addition, brining treatments were mixed with the meat for an additional 2 min. The brining ingredient combinations and their concentrations (on a finished product basis; wt/wt) were selected based on concentrations utilized by the industry, maximum allowable levels, and/or on published research. The treatments of this study included:

1. Control (uninoculated)
2. Control (inoculated)
3. Distilled water
4. Sodium chloride (NaCl, 0.5%; Fisher Scientific, Fair Lawn, NJ)
5. Sodium tripolyphosphate (STP, 0.25%; BK Giulini Corporation, Semi Valley, CA)

6. Sodium pyrophosphate (SPP, 0.25%; BK Giulini Corporation)
7. NaCl (0.5%) + STP (0.25%)
8. NaCl (0.5%) + SPP (0.25%)
9. NaCl (0.5%) + STP (0.25%) + Potassium lactate (PL, 2%; PURAC America Inc., Lincolnshire, IL)
10. NaCl (0.5%) + STP (0.25%) + Sodium diacetate (SD, 0.15%; Niacet Corporation, Niagara Falls, NY)
11. NaCl (0.5%) + STP (0.25%) + PL (2%) + SD (0.15%)
12. NaCl (0.5%) + STP (0.25%) + Lactic acid (LA, 0.3%; PURAC America Inc.)
13. NaCl (0.5%) + STP (0.25%) + Acetic acid (AA, 0.3%; EMD Chemicals Inc., Gibbstown, NJ)
14. NaCl (0.5%) + STP (0.25%) + Citric acid (CA, 0.3%; Fisher Scientific)
15. NaCl (0.5%) + STP (0.25%) + Nisaplin[®] (0.06%; Danisco USA Inc., New Century, KS) + EDTA (20 mM; Fisher Scientific)
16. NaCl (0.5%) + STP (0.25%) + ALTA[™] 2341 (0.5% [1,000 AU of pediocin per g of meat]; Kerry Bio Science, Rochester, NY) + EDTA (20 mM)
17. NaCl (0.5%) + STP (0.25%) + AvGard[®] XP (0.2%; Danisco USA Inc.)
18. NaCl (0.5%) + STP (0.25%) + Cetylpyridinium chloride (CPC, 0.5%; Safe Foods Corporation, North Little Rock, AR)
19. NaCl (0.5%) + STP (0.25%) + Hops beta acids (0.00055%; S.S. Steiner Inc., New York, NY)

Inoculated and treated samples (30 g) were extruded into sterile plastic tubes (2.5×10 cm; Nalgene) with a stainless steel caulking gun (Facilities Maintenance, Colorado State University) and air pockets (when present) were removed by pressing the meat with a spatula. Samples were either analyzed immediately after treatment, to determine any immediate effects on bacterial populations, or were covered with aluminum foil and stored at 4°C for 24 h, to simulate brining. After storage, samples were cooked in a circulating water bath (maintained at 75°C) to an internal temperature of 65°C, simulating medium-rare degree doneness of beef. The temperature of the water in the water bath, and the internal temperature of samples (intended for proximate analysis; Figure 1) was monitored and recorded using thermocouples and PicoLog software (Pico Technology Ltd.). The water level in the water bath was maintained approximately 2 cm above the level of meat in the tubes.

Microbiological analyses. Samples were analyzed immediately after inoculation, after the 24-h storage period at 4°C, and after cooking. Samples were transferred into filter bags (24 oz, WhirlPak[®], Nasco, Modesto, CA) containing 70 ml of maximum recovery diluent (MRD; 0.85% NaCl, 0.1% peptone) and pummeled (Masticator, IUL Instruments, Barcelona, Spain) for 2 min. Serial dilutions of the homogenate were prepared in 0.1% buffered peptone water (BPW, Difco) and appropriate dilutions were surface-plated on TSAP and TSAP+rif for enumeration of total bacterial and *E. coli* O157:H7 (inoculum) populations, respectively. Plates of both media were incubated at 35°C for 48 h. The detection limit of the analysis was 0.5 log CFU/g.

Physicochemical analyses. The pH, water activity, fat and moisture content, and weight losses due to cooking, of the meat samples were determined to serve as indicators of product acceptability in terms of technological and quality characteristics. The pH of plated samples was measured immediately after microbial analysis, with a Denver Instruments (Arvada, CO) pH meter and glass electrode. Fat and moisture contents (AOAC International official methods [960.39 and 950.46.B, respectively]; AOAC, 2000) and water activities (AquaLab, model series 3, Decagon Devices Inc., Pullman, WA) were determined for samples before storage at 4°C (i.e.,

after inoculation and treatment) and after cooking. Cooking losses were determined by weighing the separated fluid (purge) present in sample tubes of cooked meat samples, before microbial analysis of the entire (purge and meat) sample.

Statistical analysis. The study was repeated two times for each type of meat (i.e., two fat levels), with three samples analyzed per replication. Bacterial counts were converted to log CFU/g before statistical analysis. Data were analyzed as a randomized complete block design using Tukey's Honestly Significant Differences (HSD) test in conjunction with the PROC GLM procedure of SAS v9.2 (SAS Institute, Cary, NC). Means were considered significantly different when P-values were less than 0.05.

Study 4: Effect of selected brining ingredients on *E. coli* O157:H7 in a meat model system following a 96 h storage period at 4°C and subsequent cooking to 65°C

Results of *Study 3* (see RESULTS section) showed that the brining formulation containing 0.5% CPC was the only treatment that reduced *E. coli* O157:H7 populations (by approximately 1-log cycle) after storage (4°C, 24 h) of inoculated and treated samples (i.e., prior to simulated cooking). Therefore, in this study, we evaluated the potential antimicrobial effects of combinations of antimicrobials in the brining formulations during an extended 96 h (4 days) storage period prior to simulated cooking. Combinations of antimicrobials were selected based on results from *Study 3* and published reports showing the potential antimicrobial effects of such combinations. As there was no clear effect of fat level on bacterial inactivation in *Study 3*, only ground beef knuckle was used as the meat model system.

Objective: To evaluate combinations of antimicrobials in brining formulations for their potential effect on inactivation of *E. coli* O157:H7 in a meat model system, following a 96 h storage period at 4°C and subsequent simulated cooking to 65°C.

Materials and Methods:

E. coli O157:H7 strains and inoculum preparation. As described in *Study 3*.

Inoculation, treatment, storage, and cooking of samples. Meat samples were prepared, inoculated and treated as described in *Study 3*. The treatments of this study included:

1. Control (uninoculated)
2. Control (inoculated)
3. Distilled water
4. NaCl (0.5%)
5. NaCl (0.5%) + STP (0.25%) + Nisaplin (0.06%) + EDTA (20 mM)
6. NaCl (0.5%) + STP (0.25%) + ALTA 2341 (0.5%) + EDTA (20 mM)
7. NaCl (0.5%) + STP (0.25%) + Nisaplin + ALTA 2341 + EDTA (20 mM)
8. NaCl (0.5%) + STP (0.25%) + CPC (0.05%) + LA (0.3%)
9. NaCl (0.5%) + STP (0.25%) + PL (2%) + SD (0.15%) + CPC (0.05%)

Inoculated and treated samples were stored at 4°C for 96 h, and were then cooked to an internal temperature of 65°C (as described in *Study 3*).

Microbiological and pH analyses, and cooking and fat loss determinations. Meat samples were analyzed for bacterial (total bacteria and *E. coli* O157:H7) populations, and sample pH measurements were taken, as described in *Study 3*. Cooking losses were determined as described

by Mukherjee et al. (2008). In addition, the percentage of fat in the purge (i.e., fat loss), present in sample tubes of cooked meat samples, was estimated by measuring the volume of fat in the total volume of the purge.

Statistical analysis. The study was repeated two times with three samples analyzed per replication. Bacterial counts were converted to log CFU/g and were statistically analyzed as described in *Study 3*.

Study 5: Antimicrobial activity of essential oils, alone or in combination with other antimicrobials, against E. coli O157:H7 in a beef extract containing sodium chloride and sodium tripolyphosphate

Objective: To evaluate the antimicrobial effects of essential oils, alone or in combination with other antimicrobials, against *E. coli* O157:H7 in a beef extract containing the basic brining ingredients, salt and phosphate.

Materials and Methods:

E. coli O157:H7 strains and inoculum preparation. The inoculum was comprised of the eight rifampicin-resistant *E. coli* O157:H7 strains used in *Study 3*. The strains were activated, subcultured and washed (*Study 3*), and the cell pellet of the combined strains was resuspended and diluted to 5 log CFU/ml in PBS.

Treatments and inoculation. Frozen beef knuckles (approximately 5% fat) were thawed at 4°C for 24 h. After removing excess fat, the meat was cut into smaller pieces to facilitate blending. Beef knuckles (100 g) were blended (Waring Commercial Laboratory Blender 7012G, Torrington, CT) with sterile distilled water (200 ml), and filtered through cheese cloth. NaCl (0.5%) and STP (0.25%), commonly used as meat enhancement ingredients, were added to the beef extract, along with other ingredients, alone or in combinations. The treatments of this study included:

Uninoculated samples;

1. NaCl (0.5%)
2. STP (0.25%)
3. NaCl (0.5%) + STP (0.25%)

Inoculated samples;

1. NaCl (0.5%)
2. STP (0.25%)
3. NaCl (0.5%) + STP (0.25%)
4. NaCl (0.5%) + STP (0.25%) + Thyme oil (TO, 0.01, 0.05%, 0.1%, 0.25%, 0.5%; Now Foods, Bloomingdale, IL)
5. NaCl (0.5%) + STP (0.25%) + Grapefruit seed extract (GSE, 0.01, 0.1, 0.25, 0.5%, 1.0%; NutriBiotic®, Lakeport, CA)
6. NaCl (0.5%) + STP (0.25%) + Basil essential oil (BO, 0.1, 0.25, 0.5%; WFMED, Burke, VA)
7. NaCl (0.5%) + STP (0.25%) + CPC (0.02%, 0.04%)
8. NaCl (0.5%) + STP (0.25%) + CPC (0.02%) + TO (0.05%)
9. NaCl (0.5%) + STP (0.25%) + CPC (0.02%) + TO (0.1%)
10. NaCl (0.5%) + STP (0.25%) + CPC (0.02%) + GSE (0.25%)
11. NaCl (0.5%) + STP (0.25%) + CPC (0.02%) + BO (0.1%)

12. NaCl (0.5%) + STP (0.25%) + CPC (0.02%) + BO (0.25%)
13. NaCl (0.5%) + STP (0.25%) + CPC (0.04%) + TO (0.25%)
14. NaCl (0.5%) + STP (0.25%) + CPC (0.04%) + TO (0.5%)
15. NaCl (0.5%) + STP (0.25%) + CPC (0.04%) + GSE (0.5%)
16. NaCl (0.5%) + STP (0.25%) + CPC (0.04%) + GSE (1.0%)
17. NaCl (0.5%) + STP (0.25%) + CPC (0.04%) + BO (0.25%)
18. NaCl (0.5%) + STP (0.25%) + CPC (0.04%) + BO (0.5%)
19. NaCl (0.5%) + STP (0.25%) + SD (0.25%; Niacet Corporation, Niagara Falls, NY)
20. NaCl (0.5%) + STP (0.25%) + SD (0.25%) + TO (0.05%)
21. NaCl (0.5%) + STP (0.25%) + SD (0.25%) + TO (0.1%)
22. NaCl (0.5%) + STP (0.25%) + SD (0.25%) + TO (0.25%)
23. NaCl (0.5%) + STP (0.25%) + SD (0.25%) + TO (0.5%)
24. NaCl (0.5%) + STP (0.25%) + SD (0.25%) + GSE (0.25%)
25. NaCl (0.5%) + STP (0.25%) + SD (0.25%) + GSE (0.5%)
26. NaCl (0.5%) + STP (0.25%) + SD (0.25%) + GSE (1%)
27. NaCl (0.5%) + STP (0.25%) + SD (0.25%) + BO (0.1%)
28. NaCl (0.5%) + STP (0.25%) + SD (0.25%) + BO (0.25%)
29. NaCl (0.5%) + STP (0.25%) + SD (0.25%) + BO (0.5%)
30. NaCl (0.5%) + STP (0.25%) + LA (0.3%)
31. NaCl (0.5%) + STP (0.25%) + LA (0.3%) + TO (0.05%)
32. NaCl (0.5%) + STP (0.25%) + LA (0.3%) + TO (0.1%)
33. NaCl (0.5%) + STP (0.25%) + LA (0.3%) + GSE (0.25%)
34. NaCl (0.5%) + STP (0.25%) + LA (0.3%) + BO (0.25%)
35. NaCl (0.5%) + STP (0.25%) + LA (0.3%) + BO (0.1%)

Beef extract, prepared with the above ingredients, was aseptically dispensed (10 ml) into sterile test tubes and inoculated with 0.1 ml of the inoculum (except uninoculated treatments) to achieve an initial inoculation level of 3 log CFU/ml. The inoculated and uninoculated samples were incubated at 15°C for 48 h.

Microbiological and pH analyses. Samples were microbiologically analyzed at 0 and 48 h of incubation. Samples were appropriately diluted in 0.1% BPW and surface-plated on tryptic soy agar (TSA), for enumeration of total bacterial populations, and TSA supplemented with rifampicin (100 µg/ml; TSA+rif), for enumeration of *E. coli* O157:H7 populations. Plates were incubated at 35°C for 48 h. The detection limit of the analysis was 0.0 log CFU/ml. The pH of each sample, after being plated, was measured using a digital pH meter and glass electrode (Denver Instruments).

Data analysis. Two or three replications (two samples per replication) were performed for each of the treatments. Bacterial counts were converted into log CFU/ml and means and standard deviations were calculated.

C. Studies Addressing Objective 2

Study 6: Survival/growth of *E. coli* O157:H7 during frozen, refrigerated or retail-abusive storage conditions, and subsequent thermal inactivation during cooking of moisture-enhanced beef steaks

Objectives:

1. To evaluate the effects of different antimicrobials in brining formulations on survival/growth of *E. coli* O157:H7 in moisture-enhanced beef steaks stored under different packaging and temperature storage conditions.
2. To investigate thermal inactivation of *E. coli* O157:H7 following cooking (by pan-broiling, double pan-broiling, and roasting), in beef steaks moisture-enhanced with different brining formulations containing antimicrobials and stored under different packaging and temperature conditions.

Materials and Methods:

E. coli O157:H7 strains and inoculum preparation. The inoculum was comprised of the eight rifampicin-resistant *E. coli* O157:H7 strains used in *Study 3*. The strains were activated, subcultured and washed (*Study 3*), and the cell pellet of the combined strains was resuspended and diluted to 8 log CFU/ml in PBS.

Inoculation, treatment, and storage of steaks. Frozen (-23°C) beef knuckles (approximately 5% fat) were thawed at 3°C for 48 h prior to the start of the experiment. Excess seam fat was removed, and the knuckles were cut into smaller pieces to facilitate grinding through a 0.95-cm diameter plate (Hobart Mfg. Co., Troy, OH). The coarse-ground meat was then transferred to the Pathogen Reduction Laboratory for inoculation and treatment. Meat (1 kg per batch) was mixed in a bowl-lift stand mixer (KitchenAid) for 2 min (speed setting of "stir") with 10 ml of the inoculum to achieve an initial inoculation level of 6 log CFU/g. Brining solutions (90 ml) were then added to the inoculated meat to simulate a product moisture-enhanced to 110% of initial weight. The brining ingredient combinations and their concentrations (on a finished product basis; wt/wt) were selected based on results of *Study 3*. The four brining treatments of this study were:

1. NaCl (0.5%) + STP (0.25%)
2. NaCl (0.5%) + STP (0.25%) + CPC (0.2%)
3. NaCl (0.5%) + STP (0.25%) + LA (0.3%)
4. NaCl (0.5%) + STP (0.25%) + AvGard XP (0.2%)

After addition, brining solutions were mixed with the meat for an additional 2 min. The inoculated and treated meat was stuffed into polyethylene bags (2.3 kg, 15.2×45.7 cm, Koch; Figure 2), tied and placed in the freezer (-20°C) for 6 h to facilitate cutting of the product into steaks. The semi-frozen meat was cut into 2.5-cm steaks, and individual steaks (2.5×15.2 cm) were placed into vacuum bags (20.3×25.4 cm, 3 mil standard barrier, nylon/PE vacuum pouch; Koch), and vacuum-packaged (Hollymatic Corp., Countryside, IL) (Figure 2). Additionally, another set of steaks were individually placed on retail foam trays (20.3×25.4 cm; Pactiv, Lake Forest, IL) and covered with air-permeable film (Omni-film, Pliant Corporation, Uniontown, OH) (Figure 2). Vacuum- and aerobically-packaged steaks were kept at 4°C overnight (12-18 h) to simulate brining, and were then stored as follows:

- Vacuum-packaged steaks
 - -20°C, up to 30 days
 - 4°C, up to 28 days
 - 12°C, up to 21 days
- Aerobically-packaged steaks
 - 4°C, up to 7 days

→ 12°C, up to 7 days

Microbiological analyses. Steaks were analyzed on predetermined days of storage to evaluate survival/growth of bacterial populations. The day that the samples were placed into the freezer or incubators (i.e., 12-18 h after inoculation) was considered to be day-0 of storage. The sampling days for each packaging condition and storage temperature were:

- Vacuum-packaged steaks
 - -20°C; 0 and 30 days
 - 4°C; 0, 4, 7, 14, 21 and 28 days
 - 12°C; 0, 4, 7, 14 and 21 days
- Aerobically-packaged steaks
 - 4°C; 0, 4 and 7 days
 - 12°C; 0, 4 and 7 days

On each sampling day, individual beef steaks were placed into filter bags (55-oz, WhirlPak) and weighed. Samples stored at -20°C were thawed at 4°C for 24 h prior to the designated sampling day. MRD was added at a ratio of 1:1 (sample weight: volume [g] of MRD) to the samples and homogenized (Masticator, IUL Instruments) for 2 min. Serial dilutions of sample homogenates were prepared in 0.1% BPW and appropriate dilutions were surface-plated on TSAP, to enumerate total bacterial populations, and TSAP+rif to enumerate *E. coli* O157:H7 populations. TSAP and TSAP+rif plates were incubated at 25°C (72 h) and 35°C (48 h), respectively.

Cooking. On days corresponding to analysis of moisture-enhanced steaks for survival/growth of bacterial populations during storage, samples were cooked to an internal temperature of 60°C (simulating rare doneness of beef), by:

1. Pan-broiling (Presto® 16-inch electric skillet, National Presto Industries Inc., Eau Claire, WI)
2. Double pan-broiling (George Foreman® grill, Salton, Inc., Lake Forest, IL)
3. Roasting in a conventional kitchen oven (Magic Chef® Kitchen Oven, Maytag Corp., Newton, IA)

All cooking equipment were preheated before use. The electric skillet was preheated and maintained at 140°C (284°F) during pan-broiling, the conventional kitchen oven was preheated and maintained at 176.7°C (350°F) during roasting, whereas the George Foreman grill had no temperature settings. The temperature of the geometric center of the steaks was continuously monitored and recorded with thermocouples and PicoLog software (Pico Technology Ltd.) during cooking. Steaks that were pan-broiled were flipped over when the internal temperature reached approximately 40°C; samples that were double pan-broiled or roasted were not flipped over. When the target internal temperature was reached, samples were analyzed for surviving bacterial populations, as described above for the uncooked samples.

Physicochemical analyses. The pH of homogenized uncooked and cooked steaks was measured (Denver Instruments) after microbiological analysis. Water activity measurements (AquaLab), and fat and moisture analyses were also performed. Specifically, 1/8th of each steak destined for microbiological analysis was excised, and half was used to measure water activity, and the other half was used for fat and moisture analysis (details are given in *Study 3*). Fat and moisture analyses were only performed for samples on day-0 of storage. Cooking losses were determined by measuring the weight of samples before and after cooking.

Statistical analysis. The study was performed twice, with three samples analyzed per replication. Counts of total bacterial and *E. coli* O157:H7 populations were converted to log CFU/g. Data for

total bacterial and *E. coli* O157:H7 populations, pH, water activity and cooking losses from both replicates were combined to investigate main effects (brining treatment, storage day, cooking method) and their interactions (brining treatment×storage day, brining treatment×cooking method, storage day×cooking method, brining treatment×storage day×cooking method) using the GLIMMIX Model procedure of SAS v9.2. Moisture and fat content data were statistically analyzed only for brining treatment and cooking method, and their interaction (brining treatment×cooking method). Interaction terms that were not ($P \geq 0.05$) significant were removed from the model and data were reanalyzed using the GLM procedure of SAS. Means for main effects were generated and separated using Duncan's test ($P < 0.05$). Where interaction terms were significant ($P < 0.05$), least significance means for those factors were generated and separated with Fisher's *F*-protected test at $P < 0.05$.

Study 7: Effect of different brining formulations on survival during frozen storage, and thermal inactivation during cooking, of *E. coli* O157:H7 in moisture-enhanced beef roasts

Objectives:

1. To evaluate survival of *E. coli* O157:H7 in vacuum-packaged beef roasts, moisture-enhanced with different brining formulations, and stored under frozen (-20°C) conditions for 30 days.
2. To investigate the thermal inactivation profile of *E. coli* O157:H7 in different sections of beef roasts moisture-enhanced with different brining formulations, when cooked to internal temperatures of 60°C or 55°C on day-0 and -30 of frozen storage.

Materials and Methods:

E. coli O157:H7 strains and inoculum preparation. The inoculum was comprised of the eight rifampicin-resistant *E. coli* O157:H7 strains used in *Study 3*. The strains were activated, subcultured and washed (*Study 3*), and the cell pellet of the combined strains was resuspended and diluted to 8 log CFU/ml in PBS.

Inoculation, treatment, and storage of roasts. Beef knuckles (approximately 5% fat) were used to prepare the roasts. The meat was cut into pieces (approximately 50 g), using a band electric saw (AEW Thurne Ltd., 400M, Norwich, England), and was then transferred to the Pathogen Reduction Laboratory for inoculation, treatment and analyses. Meat (2 kg per batch) was mixed (KitchenAid bowl-lift stand mixer) with the inoculum (20 ml) to achieve a target inoculation level of 6-7 log CFU/g, and was then mixed with the same brining formulations (180 ml) tested in *Study 6* (the methods for inoculation and treatment application are described in detail in *Study 6*). The brining treatments were:

1. NaCl (0.5%) + STP (0.25%)
2. NaCl (0.5%) + STP (0.25%) + CPC (0.2%)
3. NaCl (0.5%) + STP (0.25%) + LA (0.3%)
4. NaCl (0.5%) + STP (0.25%) + AvGard XP (0.2%)

Inoculated and treated meat (2 kg) was stuffed into elastic cotton netting (24 squares, 8.9 cm flat width, 19.1 cm stuffing diameter, 71.1 cm stuffing circumference; Koch), tied, placed into vacuum bags (30.5×40.6 cm, 3 mil standard barrier, nylon/PE vacuum pouch; Koch) and vacuum-packaged. Vacuum-packaged roasts were kept at 4°C for 24 h, to simulate brining,

before being placed in the freezer (-20°C). The day that the roasts were transferred to the freezer was considered to be day-0 of storage, and samples were stored at -20°C for 30 days.

Cooking of roasts. Roasts were cooked in a conventional kitchen oven (Magic Chef Kitchen Oven), on day-0 and after 30 days of frozen storage. Frozen roasts were thawed at 4°C for approximately 48-72 h before the designated cooking day. For cooking, each roast sample was individually placed on an oven tray. Thermocouples were positioned at five locations (one thermocouple in the geometric center, one in each of the two sides, one below the surface on the top, and, one below the surface at the bottom) within the roast, as shown in Figure 3, for monitoring (PicoLog software) of the temperature during cooking. The roast was then covered with aluminum foil and placed in the center of the preheated (176.7°C) oven. The roasts were cooked to internal (geometric center) temperatures of 60°C or 55°C (simulating rare, and very rare degrees of doneness, respectively). The "cooking time" was considered to be the time it took for the internal (geometric center) temperature of the roast to reach 60°C or 55°C.

Sampling of roasts. Roasts were immediately removed from the oven when the target internal temperature was reached. The aluminum foil was removed and the roast was kept at room temperature (25±2°C) for 20 min, simulating the resting period. The temperature of the roast, with all thermocouples, continued to be recorded during the 20-min period. The internal (geometric center) temperature of the roast after the resting period was considered to be the "final temperature". After the resting period, each roast was divided into three sections (two sides and one center) with two vertical cuts. One side and the center section were again cut into three sub-sections (top, middle and bottom) with two horizontal cuts (Figure 4). The knife was dipped into ethanol and flame-sterilized between each cut to prevent cross-contamination between sections and sub-sections. One sub-sample was cored (2.3 cm diameter) out from each sub-section for microbiological analysis. Thus, a total of six sub-samples (center top, center middle, center bottom, side top, side middle and side bottom) were obtained from each cooked roast sample (Figure 4).

Microbiological analyses. Cooked sub-samples were transferred to individual WhirlPak filter bags (24 oz) and weighed. MRD was added at a ratio of 1:2 (sample weight: volume [g] of MRD) to the samples and homogenized by pummeling in a Masticator (IUL Instruments) for 2 min. Serial dilutions in 0.1% BPW were prepared and appropriate dilutions were surface-plated on TSAP (for enumeration of total bacterial populations) and TSAP+rif (for enumeration of *E. coli* O157:H7 populations). TSAP and TSAP+rif plates were incubated at 25°C (72 h) and 35°C (48 h), respectively. On the same day that roasts were cooked, four sub-samples of one uncooked roast per brining treatment were also analyzed for total bacterial and *E. coli* O157:H7 populations, as described above. The detection limit of the analysis was 0.5 log CFU/g.

Physicochemical analyses and cooking losses. Physicochemical analyses of meat samples included pH, water activity, and moisture and fat content (for day-0 samples only), as described in *Studies 3 and 6*. Also, cooking losses were calculated by determining the difference in weights of moisture-enhanced roasts before and after cooking.

Data analysis. One uncooked and two cooked moisture-enhanced roasts were analyzed for each brining treatment on day-0 and day-30 of vacuum-packaged frozen storage, and for each cooking temperature (60°C or 55°C). Total bacterial and *E. coli* O157:H7 counts were converted to log CFU/g, and means and standard deviations were calculated. Means and standard deviations were also calculated for pH and water activity data for both uncooked and cooked (60°C or 55°C) moisture-enhanced roasts from day-0 and -30. Surviving bacterial populations (i.e., total bacteria and *E. coli* O157:H7), cooking times, final cooking temperatures (i.e., internal temperature after

the resting period) and cooking losses were found to be different for individual roasts, regardless of brining treatment, storage day or cooking temperature. Therefore, data for surviving bacterial populations, cooking times, final cooking temperatures and cooking losses are presented for each roast sample individually.

Study 8: Inactivation of E. coli O157:H7 in moisture-enhanced beef steaks of different thickness by pan-broiling, double pan-broiling or roasting using different cooking equipment

Objective: To determine the effect of various cooking methods and equipment on thermal inactivation of *E. coli* O157:H7 in moisture-enhanced beef steaks of different thickness.

Materials and Methods:

E. coli O157:H7 strains and inoculum preparation. The inoculum was comprised of the eight rifampicin-resistant *E. coli* O157:H7 strains used in *Study 3*, and they were activated, subcultured and washed, as previously described. The cell pellet of the combined strains was resuspended in 80 ml of PBS, and further serially diluted in PBS to obtain a target inoculation level of 6 log CFU/g when 50 ml of inoculum was added to 1 kg of coarse-ground beef.

Preparation and inoculation of moisture-enhanced beef steaks. Coarse-ground beef knuckles (approximately 5% fat), prepared as described in *Study 6*, were used to prepare the beef steaks. The meat (1 kg per batch) was inoculated with 50 ml of the diluted rifampicin-resistant *E. coli* O157:H7 inoculum in a bowl-lift stand mixer (KitchenAid) and was mixed for 2 min (*Study 6*). The inoculated beef was then mixed for an additional 2 min with 50 ml of a solution containing 10% NaCl and 5% STP. The final concentration (wt/wt) of NaCl and STP in the product was 0.45% and 0.23%, respectively. The moisture-enhanced (110% of initial weight) beef was then extruded into polyethylene bags, tied, and placed in the freezer (-20°C) for 6 h (as described in *Study 6*). Semi-frozen beef was cut into 1.5, 2.5 and 4.0 cm thick steaks, and individual steaks were placed into vacuum bags (15×22 cm, 3 mil std barrier, nylon/polyethylene vacuum pouch, Koch), vacuum-packaged and returned to the freezer. After 48 h, the steaks were tempered at 4°C for 2.5-3.0 h, and were cooked as described below.

Cooking methods. The 1.5, 2.5 and 4.0 cm thick beef steaks were cooked to an internal temperature of 65°C. Samples were cooked by three cooking methods (pan-broiling, double pan-broiling, and roasting) using one or two cooking equipment per cooking method:

1. Pan-broiling with a Presto 16-inch electric skillet (National Presto Industries, Inc.)
2. Pan-broiling with a Sanyo® indoor barbecue grill (SANYO Fisher, Inc., Chatsworth, CA)
3. Double pan-broiling with a George Foreman grill (Salton, Inc.)
4. Roasting with a Oster® stainless steel toaster oven (Sunbeam Products, Inc., Boca Raton, FL)
5. Roasting with a conventional kitchen oven (Magic Chef, Maytag Corp.)

All cooking equipment with temperature settings (i.e., all but the George Foreman grill) were set to 176.7°C, and all equipment were preheated before use. Three or four thermocouples per steak were used to continuously monitor and record (PicoLog software) the temperature, at different locations of the sample, during cooking. Thermocouples were inserted close to one or both surfaces (i.e., 5 mm below the surface of the steak), edge (5 mm from the edge of the steak), and geometric center of the samples. Also, temperatures of cooking equipment surfaces were monitored during cooking. For steaks cooked by pan-broiling, samples were flipped over when

the center internal temperature reached 42°C.

Microbiological analyses. Uncooked and cooked samples were analyzed for total bacterial and *E. coli* O157:H7 populations. Individual samples were placed in a WhirlPak filter bag (55 oz) with 100 ml of MRD and homogenized (Masticator, IUL Instruments) for 2 min. Serial tenfold dilutions (0.1% BPW) of each sample were prepared and appropriate dilutions were surface-plated on TSAP and TSAP+rif, for enumeration of total bacterial and inoculated *E. coli* O157:H7 populations, respectively. Colonies were counted manually after incubation of the plates at 35°C for 48 h.

Physicochemical analyses and cooking losses. Physicochemical analyses of meat samples included pH, water activity, and moisture and fat content, as described previously (*Studies 3 and 6*). Also, cooking losses were calculated by determining the difference in weights of moisture-enhanced steaks before and after cooking.

Statistical analysis. The study was performed twice, and each replication included three individual samples per treatment. The pH, water activity, cooking losses, moisture and fat contents, and microbiological data (converted to log CFU/g) were analyzed using the Mixed Procedure of SAS v9.2, with independent variables including steak thickness, cooking equipment, and the interaction of steak thickness×cooking equipment. Means and standard deviations for microbiological data were calculated, and the mean differences among interactions were separated with the least significant difference procedure at the significance level of $\alpha=0.05$.

D. Studies Addressing Objective 3

Study 9: Survival of E. coli O157:H7 in brining solutions, prepared in a meat homogenate, during storage at 4 or 15°C

Objective: To evaluate survival of *E. coli* O157:H7 in brining solutions prepared in a 3% meat homogenate, simulating recirculating brine, stored for up to 48 h at 4 or 15°C.

Materials and Methods:

E. coli O157:H7 strains and inoculum preparation. The inoculum was comprised of the eight rifampicin-resistant *E. coli* O157:H7 strains described in *Study 3*. Activated and subcultured strains were harvested and washed (*Study 3*), and the cell pellet was resuspended and diluted in PBS to obtain a concentration of 4.5 log CFU/ml.

Preparation of brining solutions and inoculation. Brining solutions were prepared in a 3% meat homogenate, simulating recirculating brine. The meat homogenate was prepared by homogenizing (Masticator; 2 min) 250 g of ground beef in 250 ml of sterile distilled water in a filter bag (WhirlPak). The filtered portion of the homogenate was mixed with the brining ingredients to give a final meat homogenate concentration of 3%.

The brining ingredients evaluated in *Study 3* were also tested in this study; however, two additional treatments (i.e., NaCl + CPC, STP + CPC) were included. Sterile stock solutions of salt (27.5%) and phosphate (13.5%) were used in the preparation of the brining solutions. The concentrations of the ingredients in the brining solutions are based on the assumption that meat would be enhanced to 110% over initial weight (i.e., 10% pump rate). The treatments and controls, and the concentrations tested were:

1. Control (inoculated)
2. NaCl (5.5%)

3. STP (2.75%)
4. SPP (2.75%)
5. NaCl (5.5%) + STP (2.75%)
6. NaCl (5.5%) + SPP (2.75%)
7. NaCl (5.5%) + STP (2.75%) + PL (22%)
8. NaCl (5.5%) + STP (2.75%) + SD (1.65%)
9. NaCl (5.5%) + STP (2.75%) + PL (22%) + SD (1.65%)
10. NaCl (5.5%) + STP (2.75%) + LA (3.3%)
11. NaCl (5.5%) + STP (2.75%) + AA (3.3%)
12. NaCl (5.5%) + STP (2.75%) + CA (3.3%)
13. NaCl (5.5%) + STP (2.75%) + Nisaplin (0.66%) + EDTA (200 mM)
14. NaCl (5.5%) + STP (2.75%) + ALTA 2341 (5.5%) + EDTA (200 mM)
15. NaCl (5.5%) + STP (2.75%) + AvGard XP (2.2%)
16. NaCl (5.5%) + STP (2.75%) + CPC (5.5%)
17. NaCl (5.5%) + CPC (5.5%)
18. STP (2.75%) + CPC (5.5%)
19. NaCl (5.5%) + STP (2.75%) + Hops beta acids (0.0055%)

The solutions (i.e., meat homogenate + brine ingredients) were dispensed (48.5 ml) into 85-ml oakridge tubes (Nalgene) and were inoculated (target inoculation level of 3.5 log CFU/ml) by adding 1.5 ml of the inoculum. Immediately after inoculation, a 2 ml sub-sample was taken for enumeration and pH measurement. Brines were then incubated statically at 4°C or 15°C for up to 48 h to simulate conditions found in processing environments.

Microbiological and pH analyses. At 0, 4, 8, 12, 24 and 48 h (a 12 h sample was not analyzed for brining solutions stored at 4°C) of storage, aliquots were removed and analyzed for total bacterial and *E. coli* O157:H7 populations by spiral plating (Autoplate 4000, Spiral Biotech, Inc.) the samples on TSA and TSA+rif, respectively. The plates of both media were incubated at 35°C for 48 h, following which, colonies were enumerated (Colony Image Analysis CASBA™ 4 scanner and software system; Spiral Biotech). The detection limit of the analysis was 1.3 log CFU/ml. The pH (Denver Instruments) of samples was also measured at each sampling interval.

Statistical analysis. The study was repeated two times for each storage temperature, with three samples analyzed at each sampling interval. Cell counts were converted to log CFU/ml and analyzed in a two factor ANOVA (sampling time×brining solution) using PROC Mixed in SAS v9.2. Means were separated using an *F*-protected pairwise *t*-test with a significance level of alpha=0.05.

Study 10: Survival of *E. coli* O157:H7 in fresh, non-recirculated brining solutions stored at 4 or 15°C

Objective: To evaluate survival of *E. coli* O157:H7 in fresh (non-recirculated) brining solutions (i.e., in the absence of meat particles and natural meat microflora) stored for up to 24 h at 4 or 15°C.

Materials and Methods:

E. coli O157:H7 strains and inoculum preparation. As described in *Study 9*.

Preparation of brining solutions and inoculation. Brining solutions were prepared from concentrated stock solutions and inoculated as described in *Study 9*; however, this time they were prepared in sterile distilled water instead of the 3% meat homogenate. Also, the treatments, NaCl + CPC and STP + CPC, were omitted from this study.

The treatments tested included:

1. Control (inoculated)
2. NaCl (5.5%)
3. STP (2.75%)
4. SPP (2.75%)
5. NaCl (5.5%) + STP (2.75%)
6. NaCl (5.5%) + SPP (2.75%)
7. NaCl (5.5%) + STP (2.75%) + PL (22%)
8. NaCl (5.5%) + STP (2.75%) + SD (1.65%)
9. NaCl (5.5%) + STP (2.75%) + PL (22%) + SD (1.65%)
10. NaCl (5.5%) + STP (2.75%) + LA (3.3%)
11. NaCl (5.5%) + STP (2.75%) + AA (3.3%)
12. NaCl (5.5%) + STP (2.75%) + CA (3.3%)
13. NaCl (5.5%) + STP (2.75%) + Nisaplin (0.66%) + EDTA (200 mM)
14. NaCl (5.5%) + STP (2.75%) + ALTA 2341 (5.5%) + EDTA (200 mM)
15. NaCl (5.5%) + STP (2.75%) + AvGard XP (2.2%)
16. NaCl (5.5%) + STP (2.75%) + CPC (5.5%)
17. NaCl (5.5%) + STP (2.75%) + Hops beta acids (0.0055%)

Inoculated (approximately 3.5 log CFU/ml) brining solutions and control treatments were incubated statically at 4°C or 15°C for up to 24 h.

Microbiological and pH analyses. Samples were analyzed for surviving populations (total bacterial and *E. coli* O157:H7), and pH measurements were taken, at 0, 4, 8, and 24 h of storage at 4°C and 15°C, as described in *Study 9*.

Statistical analysis. The experiment was repeated two times for each storage temperature, with three samples analyzed at each sampling interval. Cell counts were converted to log CFU/ml, and statistically analyzed as described in *Study 9*.

E. Additional Studies

Study 11: Translocation of E. coli O157:H7 during needle injection for moisture enhancement of beef

Objective: To evaluate quantitative transfer of *E. coli* O157:H7 during moisture enhancement of beef under two contamination scenarios.

Materials and Methods:

E. coli O157:H7 strains and inoculum preparation. The non-pathogenic rifampicin-resistant *E. coli* O157:H7 mixture of strains (*Study 2*) was used for this experiment. The four strains included ATCC 700728, ATCC 43888 and two strains previously isolated from cattle feces (C1-057 and C1-158). The strains were activated, subcultured and washed (*Study 3*), and then resuspended in PBS to achieve a concentration of 9 log CFU/ml.

Contamination scenario-1: Surface inoculation of beef knuckles and moisture enhancement.

Contamination scenario-1 consisted of inoculating the surface of a whole beef knuckle, followed by moisture enhancement of the product. Specifically, an aliquot (1 ml) of the inoculum was spread onto the surface of the meat, using a sterile glass rod, to achieve an inoculation level of 4.7 ± 0.3 log CFU/g. The cells were allowed to attach to the surface for 30 min before moisture enhancement. A hand-operated, single-needle brine injector (Dick companies, Postfach, Deizisau, Germany) was used to inject either (i) sterile distilled water or (ii) a brine solution comprised of 5.5% NaCl and 2.75% STP. The water or brine was injected at seven different locations around the knuckle. The injector needle was sterilized between each injection by dipping it into ethanol and passing it through a flame. Knuckles were weighed before and after injection to determine the percent increase in weight.

Contamination scenario-2: Moisture enhancement solution inoculation. In this contamination scenario, the solutions (i.e., distilled water and brine) were inoculated (3.8 ± 0.1 and 3.4 ± 0.1 log CFU/ml, respectively) and then used to moisture enhance an uninoculated knuckle. For inoculation of the water and brine, the 4-strain rifampicin-resistant *E. coli* O157:H7 composite was diluted to 7 log CFU/ml and then 1-ml of this was used to inoculate 1 liter of water or brine solution (NaCl + STP). The inoculated solutions were then used to moisture-enhance the meat, as described above.

Sampling of knuckles, and microbiological and pH analyses. One core (8 cm in diameter) sample per knuckle was excised parallel to the direction of needle injection (Figure 5) using a coring device. Core samples were surface-decontaminated in boiling water (92-94°C, 60 s), and were then cooled (4°C, 15 min), and cut into six sections (1 through 6) of 1-cm (sections 1 through 3), 2-cm (sections 4 and 5), and 3- to 10-cm (section 6) thickness (Figure 5), while keeping the knife and cutting board sterile between each cut to avoid cross-contamination.

The weight of each section was determined and MRD was added at a ratio of 1:2 (sample weight: volume [g] of MRD). Samples were blended (Waring Commercial Laboratory Blender) for 1-2 min, and then pummeled (Masticator) for 2 min. The homogenate was serially diluted and surface-plated on TSA (to enumerate total bacterial populations) and TSA+rif (to enumerate inoculated *E. coli* O157:H7 populations). Colonies were counted after incubation at 35°C for 24 h. The pH of the meat homogenate was measured (Denver Instruments) after plating.

To determine the inoculation level of the surface of knuckles in contamination scenario-1, only the topmost 1-cm thick section of the core sample (that was not boiled) was analyzed. The inoculated water and brine solutions used for contamination scenario-2 were also microbiologically analyzed, and pH measurements were taken. Additionally, the purge generated after moisture enhancement of beef knuckles was collected and analyzed for bacterial counts.

Data analysis. For contamination scenario-1 and -2, the experiment was repeated three and two times, respectively, with one knuckle (i.e., one core sample with 6 sections; Figure 5) analyzed per treatment (moisture enhancement with water or brine) per replication. Bacterial counts (i.e., total bacteria and *E. coli* O157:H7) were converted to log CFU/g, and means and standard deviations were calculated. Means and standard deviations were also determined for the pH data.

Study 12: Thermal inactivation of *E. coli* O157:H7 at different depths of nonintact steaks cooked to a sublethal temperature

Objective: To evaluate thermal inactivation of *E. coli* O157:H7 at different depths of nonintact steaks cooked to an internal temperature of 60°C.

Materials and Methods:

E. coli O157:H7 strains and inoculum preparation. The inoculum was comprised of the eight rifampicin-resistant *E. coli* O157:H7 strains described in *Study 3*. Activated and subcultured strains were harvested and washed (*Study 3*), and the cell pellet was resuspended and diluted in PBS to obtain a concentration of 6.5 log CFU/ml.

Preparation of steaks, inoculation, and storage of samples. Beef eye of rounds (*Semitendinosus*, NAMP 171C) were purchased from a local packing plant and were stored at -23°C in the Meat Science Laboratory, Department of Animal Sciences, for no longer than 2 weeks. Whole muscle was tempered at 4°C for 24 h, the external fat, connective tissue and silver skin (epimysium) was removed, and placed at -23°C for 1 h for crust-freezing, to facilitate slicing. The eye of round was sliced (Hobart 2712 12" Semi Automatic Slicer, Hobart Mfg. Co., Troy, OH) perpendicular to the muscle fiber (longitudinal axis) into 3 mm slices. Eight slices were stacked on top of each other to form a 24 mm-thick nonintact steak, which was then tenderized using a Supertendermatic™ handheld tenderizer (Jaccard Corporation, Orchard Park, NY). Steaks were transferred to the Pathogen Reduction Laboratory for inoculation, storage, cooking and analysis.

Steaks were inoculated (initial level of 3.7 log CFU/cm²) with *E. coli* O157:H7 on the surface (0 mm) or between the slices at depths of 3, 6, 9 or 12 mm. Inoculum (200 µl) was spread over the surface at each depth of inoculation using a sterile bent glass rod. Nonintact steaks (24 mm-thick) were individually vacuum-packaged and stored at 4°C for 5 days.

Cooking. On day-5 of storage, packaged steaks and bag purge were weighed before cooking. Thermocouples (Pico Technology Ltd.) were inserted at the geometric center (12 mm) of all steaks, and also at the depth of inoculation (i.e., 3, 6 or 9 mm). Samples were cooked to a geometric center temperature of 60°C by: (i) pan-broiling (Toastermaster Cool-Touch Griddle, Toastermaster Inc.®, Columbia, MI) or (ii) roasting in a conventional kitchen oven (Magic Chef; Maytag Corp.). The griddle and oven were set to 149°C (300°F) and were preheated before cooking of samples. Steaks that were pan-broiled were turned every 2 min (Anonymous, 1995) until the cooking endpoint temperature was reached, whereas steaks cooked in the oven were not turned over. The temperature of the steaks, at the geometric center and/or depth of inoculation was monitored and recorded (PicoLog software) every 10 s during cooking. After the target temperature was reached, samples were reweighed to determine cooking loss and analyzed for total bacterial and *E. coli* O157:H7 populations.

Microbiological and pH analyses. Immediately after cooking, a 1.61cm²×24 mm core sample was excised from the center of each steak, placed in a WhirlPak filter bag (24 oz) and weighed. MRD (10 ml) was added, and samples were homogenized by pummeling (Masticator, IUL Instruments) for 2 min. The homogenate was serially diluted (0.1% BPW) and appropriate dilutions were spread-plated on TSAP and TSAP+rif, for enumeration of total bacterial and *E. coli* O157:H7 populations, respectively. Plates were incubated at 35°C for 48 h before enumeration of bacterial colonies. The detection limit of the analysis was 0.3 log CFU/cm². After plating, pH measurements (Denver Instruments) of meat samples were taken.

Statistical analysis. Experiments were repeated two times with three samples analyzed in each replicate. Bacterial counts were converted to log CFU/cm² before statistical analysis. For each cooking method (pan-broiling and roasting), cell counts, storage loss (purge in bag), pre- and post-cooking weights, cooking time, and core weight at each depth of inoculation (0, 3, 6, 9 and 12 mm) were analyzed as a one-way ANOVA using PROC MIXED of SAS v9.2. Based on significance in the ANOVA (F<0.05), means were separated using a pairwise *t*-test comparison

with significance set at the $P < 0.05$. If there were no significant ($P \geq 0.05$) differences between the depths of inoculation, values were combined and averaged for the cooking method.

Time-temperature curves for each depth of inoculation were normalized to the average time it took to reach the endpoint temperature for each cooking method. Each time point was multiplied by the average time to reach endpoint temperature for all steaks within cooking method divided by the observed time to reach endpoint temperature of each steak (average time to reach endpoint temperature/observed time to reach endpoint temperature). For each depth of inoculation, all normalized cooking curves were analyzed using PROG REG in SAS for a best fit equation. Model selection was conducted with the adjusted R^2 function in SAS and confirmed with Mallow's test statistic ($C(p)$).

VII. RESULTS

A. Preliminary Studies

Study 1: Heat inactivation kinetics of *E. coli* O157:H7 strains in a broth culture medium

- Heat inactivation curves of the 37 *E. coli* O157:H7 strains are shown in Figure 6. The strains are presented in groups of six or seven strains per figure so as to better see the inactivation curves for each strain.
- The time (in seconds) estimated by the MATLAB software to inactivate 1- through 6-log CFU/ml of each strain (data not shown), as well as other criteria (i.e., source of strain, and genotype; data not shown), were used for further selection of a smaller number of strains for development of rifampicin-resistant variants (*Study 2*).

Study 2: Selection of rifampicin-resistant *E. coli* O157:H7 variants and their thermotolerance as compared to the parental strains

- Overall, the data showed that for almost all cases, thermotolerance of the rifampicin-resistant *E. coli* O157:H7 strains was not ($P \geq 0.05$) different to that of the corresponding parental (wild-type) strains. Exceptions included a lower ($P < 0.05$) heat resistance for rifampicin-resistant strains C1-010 and C1-158, as compared to the parental strains, but only related to the 3-log reduction results. Also, the rifampicin-resistant variant of strain C1-094 had a lower ($P < 0.05$) thermotolerance (3- and 4-log reduction data) than the wild-type strain.
- From these data, two composite sets of rifampicin-resistant *E. coli* O157:H7 strains were selected for use in subsequent studies:
 - Composite 1 was comprised of eight strains: *E. coli* O157:H7 ATCC 43888, ATCC 43895, ATCC 43895/ISEHGFP (Noah et al., 2005), and five strains (C1-057, C1-072, C1-109, C1-154, C1-158) isolated from cattle feces in a previous study (Carlson et al., 2006). This composite was used for the majority of subsequent studies.
 - Composite 2 was comprised of four non-pathogenic strains: *E. coli* O157:H7 ATCC 43888, ATCC 700728, C1-057 and C1-158. This composite was used for *Study 11*.
- For the purpose of this report, results pertaining to changes (due to treatments, storage and/or cooking) in inoculum populations are referred to as "*E. coli* O157:H7", instead of "rifampicin-resistant *E. coli* O157:H7", populations.

B. Studies Addressing Objective 1

Study 3: Effect of brining ingredients on thermal inactivation of *E. coli* O157:H7 in a meat model system

- The average time for initial sample (for both types of meat) temperatures of $8\pm 2^{\circ}\text{C}$ to reach an internal temperature of 65°C (in a 75°C water bath) was 12.5 ± 0.4 min. The temperature cooking profile, averaged across both types of meat and all treatments, is shown in Figure 7.
- Total bacterial counts of uninoculated ground beef knuckle and shoulder samples were 3.3 ± 0.3 and 3.4 ± 0.2 log CFU/g, respectively. Rifampicin-resistant populations (including *E. coli* O157:H7) were not detected (detection limit, 0.5 log CFU/g) in uninoculated samples.
- In general, bacterial (total and *E. coli* O157:H7) counts in untreated or treated (with brining ingredients without/with antimicrobials) samples did not appear to be affected by the fat content of the meat (or type of meat). Furthermore, fat levels (approximately 5 or 15%) of the meat (or type of meat) did not appear to affect heat inactivation of the pathogen.
- No immediate (i.e., after inoculation and treatment) reductions in bacterial populations were obtained for any of the treatments.
- In general, total bacterial counts in inoculated samples were similar to *E. coli* O157:H7 counts, regardless of type of meat, sampling time, or brining/heat treatment.
- Pathogen counts remained relatively unchanged after 24 h storage at 4°C in all treatments, except for the treatment containing CPC, which reduced ($P<0.05$) pathogen and total bacterial counts by approximately 1 log CFU/g.
- Cooking of samples to 65°C resulted in pathogen reductions of 1.5-2.5 log CFU/g. Cooked samples treated with CPC had the lowest ($P<0.05$) bacterial counts compared to all the other treatments. NaCl, STP, SPP, NaCl + STP/SPP, and NaCl + STP combined with PL, SD, PL + SD, LA, AA, CA, Nisaplin + EDTA, ALTA 2341 + EDTA, AvGard XP, or hops beta acids did not ($P\geq 0.05$) enhance or protect *E. coli* O157:H7 from thermal destruction compared to the water control treatment. However, Nisaplin + EDTA- and ALTA 2341 + EDTA-treated samples had 0.6-0.7 log CFU/g lower ($P<0.05$) counts compared to the NaCl + STP treatment (without antimicrobials).
- The pH values of uninoculated ground beef knuckle and shoulder samples were 5.75 ± 0.03 and 5.98 ± 0.04 units, respectively.
- Brining treatments that contained LA, AA or CA had lower ($P<0.05$) initial pH values (5.23-5.57) than samples comprised of only NaCl + STP (5.94 and 6.19 for beef knuckle and beef shoulder, respectively). Samples containing AvGard XP had the highest ($P<0.05$) pH values (6.49 and 6.83) compared to all other tested treatments.
- In general, the pH of samples did not change during storage at 4°C (24 h), but increased after cooking.
- The water activities of uninoculated ground beef knuckle and shoulder samples were 0.985 ± 0.002 and 0.986 ± 0.003 , respectively. Uncooked and cooked samples containing PL had lower ($P<0.05$) water activities (0.970-0.973) compared to all the other treatments (0.977-0.988).
- Fat and moisture contents were $5.3\pm 2.4\%$ and $72.3\pm 2.3\%$, respectively, for uninoculated ground beef knuckle samples, and $15.3\pm 2.2\%$ and $67.5\pm 4.0\%$, respectively, for uninoculated ground beef shoulder samples. None of the treatments tested in the study affected ($P\geq 0.05$) the fat and moisture contents of the meat samples.

- Cooking losses for NaCl + STP/SPP treatments (without antimicrobials) were 13.1-16.1%. When organic acids were added to the NaCl + STP treatment, cooking losses increased to 20.7-23.6%, whereas when PL or AvGard XP were added, cooking losses decreased ($P < 0.05$) to 0.7-2.9%.

Study 4: Effect of selected brining ingredients on *E. coli* O157:H7 in a meat model system following a 96 h storage period at 4°C and subsequent cooking to 65°C

- The total bacterial count of uninoculated ground beef knuckle was 3.2 ± 0.6 log CFU/g. Rifampicin-resistant populations (including *E. coli* O157:H7) were not detected (detection limit, 0.5 log CFU/g) in uninoculated samples.
- No immediate (i.e., after inoculation and treatment) or after storage (4°C, 96 h) reductions in bacterial populations were obtained for any of the treatments.
- In general, total bacterial counts in inoculated samples were similar to *E. coli* O157:H7 counts, regardless of sampling time or brining/heat treatment.
- Cooking of samples to 65°C resulted in pathogen reductions of 1.9-2.7 log CFU/g. All tested brining formulations (with/without antimicrobial combinations) had surviving *E. coli* O157:H7 counts that were not ($P \geq 0.05$) different to those obtained for the water control samples. However, samples treated with ALTA 2341 had lower (by 0.9 log CFU/g; $P < 0.05$) bacterial counts than NaCl + STP + PL + SD + CPC-treated samples.
- The pH of uninoculated ground beef knuckle was 5.77. The NaCl + STP + CPC + LA treatment, analyzed before and after storage (4°C, 96 h) had the lowest ($P < 0.05$) pH (5.37 and 5.36, respectively) compared to all the other treatments containing NaCl + STP (5.68-5.85).
- Storage of samples at 4°C for 96 h did not ($P \geq 0.05$) affect the initial (before storage) pH of any of the treatments. Samples treated with NaCl + STP + Nisaplin + EDTA or NaCl + STP + ALTA 2341 + EDTA and cooked to 65°C had a higher ($P < 0.05$) pH than corresponding uncooked samples.
- Cooking moisture and fat losses for inoculated control samples were 14.3 and 0.5%, respectively. All samples treated with NaCl + STP had lower ($P < 0.05$) moisture losses compared to the water control. The highest and lowest ($P < 0.05$) moisture losses, compared to the water control, were obtained for samples treated with NaCl + STP + CPC + LA (15.5%) and NaCl + STP + PL + SD + CPC (2.1%), respectively.
- Fat losses, during cooking, for all treatments were not different ($P \geq 0.05$) from that of the inoculated control treatment.

Study 5: Antimicrobial activity of essential oils, alone or in combination with other antimicrobials, against *E. coli* O157:H7 in a beef extract containing sodium chloride and sodium triphosphate

- Total bacterial counts of uninoculated beef extract (no additives) and other uninoculated control treatments were 3.0-3.6 log CFU/ml at 0 h, and increased to 7.8-8.2 log CFU/ml after 48 h at 15°C. *E. coli* O157:H7 counts for these treatments were < 0.0 -0.2 log CFU/ml during the entire incubation period.

- *E. coli* O157:H7 counts in inoculated beef extract (no additives) and other control treatments (i.e., NaCl, STP, NaCl + STP) increased from 3.3-3.4 (0 h) to 5.1-5.6 (48 h) log CFU/ml. Corresponding total bacterial counts increased from 3.5-4.0 to 7.9-8.0 log CFU/ml.
- The NaCl + STP + TO (0.25% and 0.5%) and NaCl + STP + GSE (0.5% and 1.0%) treatments, alone or in combination with CPC (0.04%) or SD (0.25%), had immediate bactericidal effects on *E. coli* O157:H7. Pathogen counts were reduced from the inoculation level of 3.4 log CFU/ml to below the detection limit (0.0 log CFU/ml) in 0 h samples. Furthermore, *E. coli* O157:H7 counts in these treatments remained below the detection limit even after 48 h incubation.
- Immediately after inoculation, NaCl + STP + CPC (0.04%), alone or in combination with BO (0.25% and 0.5%) reduced pathogen populations by 2.8-3.3 log CFU/ml, and after 48 h, counts were below the detection limit.
- NaCl + STP + BO (0.5%), alone or in combination with SD, did not cause immediate (0 h) reductions of pathogen counts; however, after 48 h incubation, *E. coli* O157:H7 populations were 1.4 log CFU/ml lower than at the time of inoculation.
- NaCl + STP + TO (0.1%), NaCl + STP + BO (0.1 and 0.25%), NaCl + STP + SD (0.25%), or NaCl + STP + LA (0.3%), alone or in combination with other ingredients, inhibited the growth of *E. coli* O157:H7 at 15°C for 48 h.
- The background flora was more resistant to the antimicrobials and/or grew faster than *E. coli* O157:H7. However, NaCl + STP + TO (0.25% and 0.5%)/GSE (0.5% and 1.0%)/BO (0.5%), alone or in combination with other ingredients such as CPC (0.04%) or SD (0.25%), reduced initial levels of natural contamination and/or inhibited subsequent growth during incubation, as compared to the control (NaCl + STP).
- Treatments containing NaCl + STP + TO (0.25% and 0.5%)/GSE (0.5% and 1.0%)/BO (0.5%), and NaCl + STP + CPC (0.04%), alone or in combination with TO (0.25% and 0.5%), GSE (0.5% and 1.0%), or BO (0.25% and 0.5%), had higher pH values (6.35-6.44) than that of NaCl + STP alone (5.92). On the other hand, treatments containing LA had pH values that were approximately 1.4 pH units lower than that of the NaCl + STP control treatment.

C. Studies Addressing Objective 2

Study 6: Survival/growth of E. coli O157:H7 during frozen, refrigerated or retail-abusive storage conditions, and subsequent thermal inactivation during cooking of moisture-enhanced beef steaks

Due to the large number of tested variables of this study, the results have been separated as follows:

- I. Vacuum-packaged moisture-enhanced steaks stored at -20°C
- II. Vacuum-packaged moisture-enhanced steaks stored at 4°C or 12°C
- III. Aerobically-packaged moisture-enhanced steaks stored at 4°C or 12°C

I. Vacuum-packaged moisture-enhanced steaks stored at -20°C

- Statistical analysis of the data for vacuum-packaged moisture-enhanced beef steaks stored at -20°C for 30 days indicated that the three factors of this study (i.e., brining treatment, storage day, cooking method) had significant ($P < 0.05$) effects on the following response variables:

total bacterial counts, *E. coli* O157:H7 counts, pH, and cooking loss. No ($P \geq 0.05$) significant effects were obtained for the water activity data. Significant ($P < 0.05$) effects for fat and moisture contents of moisture-enhanced beef steaks (for day-0 samples only) included brining treatment and cooking method. All 2-way and 3-way interaction terms (i.e., brining treatment \times storage day, brining treatment \times cooking method, storage day \times cooking method, brining treatment \times storage day \times cooking method) were not ($P \geq 0.05$) significant.

- In general, the cooking time required for steak samples to reach an internal temperature of 60°C decreased in order of: roasting (23.3-27.5 min) > pan-broiling (14.5-25.0 min) > double pan-broiling (4.2-6.4 min), regardless of brining treatment or storage day.
- As indicated, statistical analysis of total bacterial and *E. coli* O157:H7 counts of moisture-enhanced steaks showed significant ($P < 0.05$) effects for brining treatment, storage day, and cooking method.
- Steak samples, moisture-enhanced with NaCl + STP + CPC, had lower ($P < 0.05$) *E. coli* O157:H7 and total bacterial populations compared to steak samples enhanced with NaCl + STP, NaCl + STP + LA, or NaCl + STP + AvGard XP.
- *E. coli* O157:H7 and total bacterial counts of vacuum-packaged beef steaks stored at -20°C for 30 days were 0.6 and 0.5 log CFU/g, respectively, lower ($P < 0.05$) than corresponding counts of day-0 samples.
- Cooking of moisture-enhanced steaks reduced total bacterial and *E. coli* O157:H7 counts by 1.4-3.3 and 1.4-3.4 log CFU/g, respectively, with reductions depending on cooking method.
- The greatest thermal inactivation of *E. coli* O157:H7 was obtained for samples that were double pan-broiled, and pathogen counts were 1.8-2.0 log CFU/g lower ($P < 0.05$) than those of pan-broiled or roasted samples. No differences ($P \geq 0.05$) were obtained between surviving bacterial populations of samples that were pan-broiled or roasted.
- As indicated, statistical analysis of pH data of moisture-enhanced steaks showed significant ($P < 0.05$) effects for brining treatment, storage day, and cooking method.
- The pH values of steak samples moisture-enhanced with NaCl + STP (6.04), NaCl + STP + CPC (6.03) or NaCl + STP + LA (6.04) were not ($P \geq 0.05$) different, whereas the pH of the NaCl + STP + AvGard XP treatment (6.40) was higher ($P < 0.05$) than that of the other three treatments.
- The pH of steaks stored for 30 days at -20°C was 0.06 pH units lower ($P < 0.05$) than that of day-0 samples.
- Cooked steak samples (pH 6.17-6.19) had a higher ($P < 0.05$) pH than uncooked samples (pH 5.99).
- None of the tested factors of the study were found to have significant ($P < 0.05$) effects on the water activity of uncooked and cooked moisture-enhanced steaks. Ranges of water activity values were 0.981 to 0.988, regardless of brining treatment, storage day or cooking method.
- As indicated, statistical analysis of fat and moisture contents of moisture-enhanced beef steaks, for day-0 samples only, showed significant ($P < 0.05$) effects for brining treatment and cooking method.
- The fat (4.9-5.1%) and moisture (68.9-69.7%) contents of steak samples enhanced with NaCl + STP or NaCl + STP + AvGard XP were similar ($P \geq 0.05$). Additionally, fat (7.3%) and moisture (65.8-66.3%) contents of samples treated with NaCl + STP + CPC/LA were similar ($P \geq 0.05$).
- Fat (5.8-6.9%) and moisture (63.9-67.4%) contents of cooked samples, regardless of cooking method, were lower than those of uncooked samples (5.0 and 72.9%, respectively).

- As indicated, statistical analysis of cooking losses for moisture-enhanced steaks showed significant ($P < 0.05$) effects for brining treatment, storage day, and cooking method.
- Percentage cooking losses for steak samples enhanced with NaCl + STP + AvGard XP (13.4%) were the smallest ($P < 0.05$) compared to the other brining formulations (20.7-22.2%).
- Higher ($P < 0.05$) cooking losses were obtained for samples stored for 30 days at -20°C (21.1%) than for day-0 samples (18.0%).
- Smaller ($P < 0.05$) cooking losses were found for samples cooked by roasting (15.6%) than by pan-broiling (21.1%) or double pan-broiling (21.8%).

II. Vacuum-packaged moisture-enhanced steaks stored at 4°C or 12°C

- Statistical analysis of the data for vacuum-packaged moisture-enhanced beef steaks stored at 4°C for 28 days showed significant ($P < 0.05$) effects for tested factors and interactions (shown in parentheses) for response variables as follows: total bacterial counts (brining treatment, cooking method, storage day \times cooking method); *E. coli* O157:H7 counts (brining treatment, storage day, cooking method); pH (brining treatment, storage day, cooking method); water activity (no significant effects); cooking loss (brining treatment, storage day, cooking method). Factors and interactions (2-way or 3-way) not listed for each of the response variables were not ($P \geq 0.05$) significant.
- Statistical analysis of the data for vacuum-packaged moisture-enhanced beef steaks stored at 12°C for 21 days showed significant ($P < 0.05$) effects for tested factors and interactions (shown in parentheses) for response variables as follows: total bacterial counts (brining treatment, cooking method, storage day \times cooking method); *E. coli* O157:H7 counts (cooking method); pH (brining treatment, storage day, cooking method); water activity (no significant effects); cooking loss (brining treatment, storage day, cooking method). Factors and interactions (2-way or 3-way) not listed for each of the response variables were not ($P \geq 0.05$) significant.
- Fat and moisture content analyses were performed only on day-0 samples, and in fact, were the same day-0 samples as for Part I of this study (vacuum-packaged samples stored at -20°C for 30 days); therefore, these results will not be described again here.
- Overall, beef steaks cooked by roasting took the longest time (17.5-29.7 min) to reach the internal temperature of 60°C , followed by pan-broiled (11.8-18.3 min) and double pan-broiled (3.9-6.1 min) samples, regardless of brining treatment, storage day or storage temperature.
- As indicated, statistical analysis of bacterial counts for vacuum-packaged, moisture-enhanced steaks stored at 4°C showed significant ($P < 0.05$) effects for brining treatment (total bacterial and *E. coli* O157:H7 counts), storage day (*E. coli* O157:H7 counts), cooking method (total bacterial and *E. coli* O157:H7 counts), and storage day \times cooking method (total bacterial counts).
- Samples moisture-enhanced with NaCl + STP + CPC or NaCl + STP + AvGard XP had lower ($P < 0.05$) total bacterial and *E. coli* O157:H7 counts than the control treatment (NaCl + STP); however, the treatment with the lowest ($P < 0.05$) counts was NaCl + STP + CPC.
- As expected, *E. coli* O157:H7 did not grow during the 28-day storage period at 4°C . Pathogen counts of samples on day-21 and -28 were 1 log CFU/g lower ($P < 0.05$) than those of day-0 samples.

- In contrast to the pathogen counts, increases of total bacterial populations were obtained during 28 days of vacuum-packaged storage at 4°C, increasing from 5.8 log CFU/g in day-0 samples to 7.5 log CFU/g in samples stored for ≥14 days.
- Cooking of moisture-enhanced steaks reduced total bacterial and *E. coli* O157:H7 counts by 2.4-4.5 and 1.6-3.6 log CFU/g, respectively, with reductions depending on cooking method.
- Survivors of total bacterial and *E. coli* O157:H7 populations were the lowest ($P<0.05$) for steaks that were cooked by double pan-broiling (2.3 and 1.8 log CFU/g, respectively), followed by samples cooked by roasting (3.2 and 2.9 log CFU/g, respectively) and pan-broiling (4.4 and 3.8 log CFU/g, respectively).
- As indicated, statistical analysis of bacterial counts for moisture-enhanced steaks stored at 12°C showed significant ($P<0.05$) effects for brining treatment (total bacterial counts), cooking method (total bacterial and *E. coli* O157:H7 counts), and storage day×cooking method (total bacterial counts).
- Total bacterial counts of the NaCl + STP + CPC, NaCl + STP + LA, and NaCl + STP + AvGard XP treatments were 0.4-0.7 log CFU/g lower ($P<0.05$) than the control brining treatment.
- As seen for the samples stored at 4°C, survivors of total bacterial and *E. coli* O157:H7 populations were the lowest ($P<0.05$) for steaks cooked by double pan-broiling (2.7 and 2.3 log CFU/g, respectively), followed by samples cooked by roasting (4.0 and 3.5 log CFU/g, respectively) and pan-broiling (5.0 and 4.5 log CFU/g, respectively). Overall, cooking reduced total bacterial and *E. coli* O157:H7 populations by 2.0-4.3 and 1.7-3.9 log CFU/g, respectively, regardless of cooking method.
- For samples stored at 12°C, total bacterial populations reached 7.4 log CFU/g by day-7 of storage.
- As indicated, statistical analysis of pH data of moisture-enhanced steaks showed significant ($P<0.05$) effects for brining treatment, storage day, and cooking method, within each storage temperature (4 or 12°C).
- The pH values of steaks moisture-enhanced with NaCl + STP + AvGard XP (6.07-6.19) were the highest ($P<0.05$), and those containing LA (5.70-5.76) the lowest, for samples stored at 4°C and 12°C.
- As storage time at 4°C or 12°C progressed, pH values of samples decreased ($P<0.05$) from 6.16 (day-0) to 5.68 (day-28), and 6.16 (day-0) to 5.85 (day-21), respectively.
- No differences ($P\geq 0.05$) in pH values were observed among 4°C or 12°C samples cooked by pan-broiling, double pan-broiling and roasting.
- The tested factors of the study did not ($P\geq 0.05$) have a significant effect on the water activity of uncooked and cooked moisture-enhanced steaks stored at 4°C or 12°C. Water activity values ranged from 0.974 to 0.991, regardless of brining treatment, storage day, storage temperature or cooking method.
- As indicated, statistical analysis of cooking losses for moisture-enhanced steaks showed significant ($P<0.05$) effects for brining treatment, storage day, and cooking method, within each storage temperatures.
- Steaks moisture-enhanced with NaCl + STP + AvGard XP (17.4-18.3%) had the smallest ($P<0.05$) percentage cooking losses compared to the other brining treatments (21.7-23.9%), for both storage temperatures.
- Cooking losses for day-0 samples (17.9%) were lower ($P<0.05$) than for samples that were stored at 4°C or 12°C (21.3-23.2%).

- Cooking losses for samples that were cooked by roasting (17.6-17.7%) were lower ($P<0.05$) than for samples cooked by pan-broiling or double pan-broiling (23.0-23.9%).

III. Aerobically-packaged moisture-enhanced steaks stored at 4°C or 12°C

- Statistical analysis of the data for aerobically-packaged moisture-enhanced beef steaks stored at 4°C for 7 days showed significant ($P<0.05$) effects for tested factors (shown in parentheses) for response variables as follows: total bacterial counts (brining treatment, cooking method); *E. coli* O157:H7 counts (brining treatment, storage day, cooking method); pH (brining treatment, storage day, cooking method); water activity (no significant effects); cooking loss (brining treatment, cooking method). Factors not listed for each of the response variables, and all 2-way and 3-way interactions were not ($P\geq 0.05$) significant.
- Statistical analysis of the data for aerobically-packaged moisture-enhanced beef steaks stored at 12°C for 7 days showed significant ($P<0.05$) effects for tested factors (shown in parentheses) for response variables as follows: total bacterial counts (brining treatment, storage day, cooking method); *E. coli* O157:H7 counts (cooking method); pH (brining treatment, storage day); water activity (no significant effects); cooking loss (brining treatment, cooking method). Factors not listed for each of the response variables, and all 2-way and 3-way interactions were not ($P\geq 0.05$) significant.
- Fat and moisture content analyses were performed only on day-0 samples, and these data were common for steak samples stored at 4 and 12°C. Statistical analysis of the fat and moisture content data for moisture-enhanced beef steaks indicated that only cooking method was significant ($P<0.05$).
- As seen for the previous two parts (i.e., I and II) of this study, the time required for the steaks to reach the target internal temperature of 60°C was the shortest for samples cooked by double pan-broiling (3.8-6.8 min), followed by pan-broiling (11.6-15.4 min) and roasting (21.0-29.7 min), regardless of sample storage temperature.
- As indicated, statistical analysis of bacterial counts for moisture-enhanced steaks stored at 4°C showed significant ($P<0.05$) effects for brining treatment (total bacterial and *E. coli* O157:H7 counts), storage day (*E. coli* O157:H7 counts), and cooking method (total bacterial and *E. coli* O157:H7 counts).
- *E. coli* O157:H7 counts for steaks moisture-enhanced with NaCl + STP + CPC (3.5 log CFU/g) or NaCl + STP + AvGard XP (4.0 log CFU/g) were lower ($P<0.05$) than those of the control treatment (4.5 log CFU/g). The brining treatment containing LA had pathogen counts that were not ($P\geq 0.05$) different to those of the control.
- As expected, and as seen for vacuum-packaged samples stored at 4°C, *E. coli* O157:H7 did not grow during storage at 4°C under aerobic conditions, and in fact, counts decreased ($P<0.05$) from 4.4 (day-0) to 3.8 (day-7) log CFU/g.
- Regardless of cooking method, total bacterial and *E. coli* O157:H7 counts of cooked samples were 1.5-3.8 and 1.1-3.1 log CFU/g, respectively, lower ($P<0.05$) than those of uncooked samples.
- As seen in Part II of this study, survivors of total bacterial and *E. coli* O157:H7 populations were the lowest ($P<0.05$) for steaks that were cooked by double pan-broiling (2.5 and 2.4 log CFU/g, respectively), followed by samples cooked by roasting (4.1 and 3.9 log CFU/g, respectively) and pan-broiling (4.8 and 4.4 log CFU/g, respectively).
- As indicated, statistical analysis of bacterial counts for moisture-enhanced steaks stored at 12°C showed significant ($P<0.05$) effects for brining treatment (total bacterial counts),

storage day (total bacterial counts), and cooking method (total bacterial and *E. coli* O157:H7 counts).

- Total bacterial counts of steaks moisture-enhanced with NaCl + STP + CPC/LA were not ($P \geq 0.05$) different (4.3 and 4.5 log CFU/g, respectively), but were 0.5-0.9 log CFU/g lower ($P < 0.05$) than the NaCl + STP and NaCl + STP + AvGard XP treatments. *E. coli* O157:H7 populations for samples stored at 12°C were not ($P \geq 0.05$) affected by the brining ingredients.
- *E. coli* O157:H7 counts were also not ($P \geq 0.05$) affected by storage day. For total bacterial populations, however, counts increased ($P < 0.05$) from 4.3 (day-0) to 5.0 (day-7) log CFU/g.
- Cooking of moisture-enhanced steaks reduced bacterial populations by 2.3-4.4 (total bacterial counts) and 1.5-3.7 (*E. coli* O157:H7 counts) log CFU/g, regardless of cooking method.
- Samples that were double pan-broiled (2.2 log CFU/g) had the lowest ($P < 0.05$) pathogen counts, followed by those that were roasted (4.0 log CFU/g) and pan-broiled (4.4 log CFU/g).
- As indicated, statistical analysis of pH data of moisture-enhanced steaks stored at 4°C or 12°C showed significant ($P < 0.05$) effects for brining treatment, storage day, and cooking method (4°C samples only).
- The pH values of control (NaCl + STP) steaks were 5.85 and 5.71 for samples stored at 4°C and 12°C, respectively. Samples moisture-enhanced with AvGard XP (6.11-6.21) had the highest ($P < 0.05$) pH values and those treated with LA (5.60-5.64) had the lowest pH values.
- The pH values of steaks stored at 4°C or 12°C were lower ($P < 0.05$) than those of day-0 samples.
- No differences ($P \geq 0.05$) in pH values were observed among samples stored at 4°C and cooked by pan-broiling, double pan-broiling and roasting; however, they were 0.12-0.23 pH units higher than uncooked samples.
- Tested factors of the study did not ($P \geq 0.05$) have a significant effect on the water activity of uncooked and cooked moisture-enhanced steaks stored at 4°C or 12°C. Water activity values ranged from 0.984 to 0.994, regardless of brining treatment, storage day, storage temperature or cooking method.
- As indicated, statistical analysis of fat and moisture contents of moisture-enhanced steaks (day-0 samples only), showed a significant ($P < 0.05$) effect for cooking method.
- The fat and moisture contents of uncooked steaks were 3.1% and 75.0%, respectively, and 4.5-6.0% and 65.4-69.3% for cooked samples, respectively.
- As indicated, statistical analysis of cooking losses for moisture-enhanced steaks showed significant ($P < 0.05$) effects for brining treatment, and cooking method, for samples stored at both storage temperatures.
- Samples moisture-enhanced with NaCl + STP + AvGard XP (15.0-16.8%) had the smallest ($P < 0.05$) percentage cooking losses compared to the control (20.0-21.3%) and other antimicrobial-containing brining treatments (19.5-23.6%).
- Percentage cooking losses of samples stored at 4°C or 12°C increased in the order of roasting (14.5% and 14.2%, respectively) > pan-broiling (20.9% and 21.3%, respectively) > double pan-broiling (23.1% and 24.6%, respectively).

Study 7: Effect of different brining formulations on survival during frozen storage, and thermal inactivation during cooking, of *E. coli* O157:H7 in moisture-enhanced beef roasts

- The time taken for roasts, that were moisture-enhanced with different brining formulations, to reach internal (geometric center) temperatures of 60°C or 55°C (i.e., cooking time), in an

oven set at 176.7°C, ranged from 112 to 167 min (average: 142±20 min, day-0; 140±15min, day-30), and 88 to 152 min (average: 132±12 min, day-0; 130±21min, day-30), respectively, regardless of brining treatment or day of storage. Thus, there was large variation in the cooking time of the 2-kg roasts. There was no apparent relationship between brining treatment and cooking time.

- The final temperature of the roasts after the 20 min resting period also varied among the individual roasts. The final temperature of samples cooked to internal temperatures of 60°C or 55°C ranged from 46°C to 69°C (average: 62±7°C, day-0; 64±2°C, day-30), and 46°C to 64°C (average: 59±5°C, day-0; 60±6°C, day-30), respectively.
- Initial (day-0; i.e., approximately 24 h after inoculation) levels of *E. coli* O157:H7 in the control brining treatment (NaCl + STP) were 7.0 and 5.8 log CFU/g for roasts destined to be cooked to internal temperatures of 60°C and 55°C, respectively. Roasts moisture-enhanced with NaCl + STP + CPC had 0.7 and 2.0 log CFU/g lower pathogen counts than the control on day-0. Pathogen counts of the roasts treated with NaCl + STP + LA or NaCl + STP + AvGard XP were not different to those of the control.
- *E. coli* O157:H7 populations of roasts after 30 days storage at -20°C and 48-72 h of thawing were similar to those of day-0 samples.
- Overall, the effect of the brining treatments on total bacterial populations was the same as that for *E. coli* O157:H7. However, for roasts destined to be cooked to 60°C, total bacterial counts on day-30 of storage for roasts treated with NaCl + STP, NaCl + STP + LA, or NaCl + STP + AvGard XP were 1.2-1.5 log CFU/g higher than corresponding counts for day-0 samples. This increase is most likely due to growth of the natural flora during the 48-72 h thawing period prior to sampling. An increase in total bacterial populations was not obtained for the NaCl + STP + CPC treatment.
- *E. coli* O157:H7 populations in moisture-enhanced roasts cooked to internal temperatures of 60°C or 55°C were reduced to non-detectable (<0.5 log CFU/g) levels in 75.0% and 65.6%, respectively, of the total number of sub-samples analyzed per roast, with reductions ranging from >2.8 to >6.5 log CFU/g (i.e., depending on initial levels in uncooked roasts).
- For roasts cooked to an internal temperature of 60°C, non-detectable *E. coli* O157:H7 counts were obtained for 72.9 and 77.1% of the total number of sub-samples analyzed from the center and side sections of the roasts, respectively, regardless of brining treatment or storage day.
- For roasts cooked to an internal temperature of 55°C, non-detectable *E. coli* O157:H7 counts were obtained for 64.6 and 66.7% of the total number of sub-samples analyzed from the center and side sections of the roasts, respectively, regardless of brining treatment or storage day.
- When the sub-sample location was considered, the percentage of samples with non-detectable *E. coli* O157:H7 counts was 56.3% (center top), 81.3% (center middle), 81.3% (center bottom), 62.3% (side top), 81.3% (side middle) and 87.5% (side bottom) for roasts cooked to 60°C. Corresponding percentages for roasts cooked to an internal temperature of 55°C, were 56.3% (center top), 68.8% (center middle), 68.8% (center bottom), 68.8% (side top), 68.8% (side middle) and 62.3% (side bottom).
- For roasts cooked to 60°C, counts of sub-samples with detectable levels of *E. coli* O157:H7 ranged between 0.5 and 1.8 log CFU/g, with one exception. Roast sample #2 of the NaCl + STP + AvGard XP treatment cooked on day-0 of storage had survivors in each of the six sub-samples ranging between 3.6 and 5.4 log CFU/g. The high counts are most likely due to a

combination of a short cooking time (112 min) and low final temperature (46°C). The temperature profile of this roast is shown in Figure 8.

- For samples cooked to 55°C, there were four roasts (day-0: NaCl + STP roast sample #1, NaCl + STP + LA roast samples #1 and #2; day-30: NaCl + STP + LA roast sample #1) that had *E. coli* O157:H7 survivors in each of the six sub-samples, and counts were between 2.1 and 5.2 log CFU/g. Again, these roasts were noted to have a short cooking time (88-129 min) and low final temperatures (46-54°C).
- In general, total bacterial counts of the sub-samples from cooked roasts were higher (most likely due to the presence of natural contamination at levels higher than that of the inoculum), or similar to pathogen counts.
- Temperature profiles of representative roasts are shown in Figures 9-11. In most cases, the temperature profile of the two sides, top and bottom of roasts was higher than the temperature in the center, during cooking and during the resting period.
- Both uncooked and cooked (60°C or 55°C) roasts enhanced with NaCl + STP + AvGard XP (6.19-6.58 pH units) had higher pH values compared to roast samples enhanced with the other brining formulations (5.15-6.22 pH units), on both storage days.
- The water activities of uncooked and cooked (60°C or 55°C) roast samples were 0.981-0.992 and 0.980-0.991, respectively.
- The moisture content of roasts enhanced with the different brining formulations on day-0 of storage was 67.0-76.3% for uncooked samples, and 63.7-76.0% for cooked (60°C or 55°C) samples. Corresponding fat contents were 0.7-8.5%, and 0.8-4.8%, respectively.
- Percentage cooking losses for the individual roasts did not have an apparent trend by brining treatment, storage day or cooking temperature. Overall, cooking losses ranged from 9.5 to 33.1%, and 3.2 to 31.5% for roasts cooked to internal temperatures of 60°C or 55°C.

Study 8: Inactivation of *E. coli* O157:H7 in moisture-enhanced beef steaks of different thickness by pan-broiling, double pan-broiling or roasting using different cooking equipment

- Temperatures of the cooking equipment were 176±16°C for the Presto electric skillet and Sanyo grill (used for pan-broiling), 176±15°C for the George Foreman grill (used for double pan-broiling), and 176±20°C for the Oster toaster oven and kitchen oven (used for roasting).
- For samples cooked by pan-broiling using the Presto electric skillet (Figure 12A) and Sanyo grill (Figure 13A), one thermocouple each was inserted at the bottom (closest to the heat source) and top (away from the heat source) of each sample. The temperature of the sample surface closest to the heat source reached 83.7-107.5°C and 70.9-110.8°C before steaks were flipped over (when the internal temperature reached 42°C), respectively. Immediately after flipping over, temperatures of the same surface (now on the top, and no longer in direct contact with the heat source) decreased to 49.5-65.0°C and 54.6-62.9°C, respectively. The temperature of the sample surface that was not in direct contact with the heat source at the beginning of cooking increased slowly before the samples were flipped over, and then increased quickly and reached approximately 80-90°C by the end of cooking (Figures 12B and 13B).
- For samples cooked by pan-broiling, the final temperature of the edge of steaks ranged from 65 to 74°C (Presto electric skillet; Figure 12C) and 51.8 to 76.5°C (Sanyo grill, Figure 13C).

In general, lower temperatures were attained at the edge of the steaks as the thickness of the samples increased.

- For steaks cooked by double pan-broiling (George Foreman grill), final sample surface and edge temperatures ranged from 91.4 to 117.7 °C, and 81.8 to 90.0°C, respectively (Figures 14A and 14B).
- The final sample surface and edge temperatures of steaks cooked by roasting ranged from 77.8 to 93.2°C and 70.2 to 84.9°C, respectively (Oster toaster oven, Figures 15A and 15B), and 82.2-92.6°C and 76.0-83.9°C, respectively (Magic Chef kitchen oven, Figures 16A and 16B).
- The initial internal center temperatures of steaks were -2 ± 1 °C. Cooking of steaks by double pan-broiling, pan-broiling, and roasting required 3.9-19.8 min (Figure 14C), 14.3-55 min (Figures 12D and 13D) and 11.3-63 min (Figures 15C and 16C), respectively, to reach an internal center temperature of 65°C. The time to reach the target internal temperature followed the order of George Foreman grill (3.9-19.8 min)<Oster toaster oven (11.3-45 min)<Presto electric skillet (16.3-55 min)<Sanyo grill (14.3-65 min)<kitchen oven (20-63 min), with variation in cooking time within each type of equipment related to steak thickness (Figures 12D, 13D, 14C, 15C, and 16C).
- The initial level of inoculated *E. coli* O157:H7 in uncooked samples was 6.4 ± 0.1 log CFU/g. Total bacterial counts on TSAP were similar ($P\geq 0.05$) to those on TSAP+rif agar for all treatments.
- Cooking of steak samples to an internal temperature of 65°C (medium-rare degree of doneness) caused overall reductions of pathogen counts ranging from 1.1 to 2.3, 1.6 to 2.8 and 2.0 to 4.2 log CFU/g by double pan-broiling, pan-broiling, and roasting, respectively.
- Bacterial counts of steaks cooked by the same cooking method but by using different cooking equipment were not ($P\geq 0.05$) different. For example, pathogen counts of samples cooked by pan-broiling using the Presto electric skillet and Sanyo grill were 3.6-4.7 and 3.6-4.8 log CFU/g, respectively. For samples cooked by roasting in the Oster toaster oven and kitchen oven, pathogen counts were 2.7-4.2 and 2.2-4.4 log CFU/g, respectively.
- In general, larger reductions of bacterial counts were obtained in the 4.0 cm thick steaks than the 1.5 and 2.5 cm thick steaks, regardless of cooking method or equipment. Cooking of 4.0 cm thick samples by the different methods resulted in 0.5-2.2 log CFU/g higher reductions than those for the 1.5 and 2.5 cm samples. The highest reduction (4.2 log CFU/g) was achieved in the 4.0 cm thick samples cooked by roasting in the conventional kitchen oven.
- The pH of uncooked beef steaks ranged from 5.67 to 5.76. Samples that were cooked by pan-broiling, double pan-broiling, and roasting had higher ($P<0.05$) pH values than uncooked samples, ranging from 6.03 to 6.14, 6.02 to 6.04 and 5.98 to 6.04, respectively.
- Water activities of cooked (0.974-0.987) steaks were lower ($P<0.05$) than those of uncooked samples (0.990-0.993).
- The moisture content of uncooked samples was 76.1%, and after cooking decreased ($P<0.05$) to 64.2-72.1%, regardless of steak thickness. Corresponding fat contents were 2.4-2.5% (uncooked samples) and 3.6-7.9% (cooked samples).
- Cooking resulted in weight losses of 27.2-32.3%, 23.1-31.1% and 21.3-27.2% for samples cooked by pan-broiling, double pan-broiling, and roasting, respectively. Overall, cooking losses increased as thickness of steaks increased, but no differences were found between different cooking equipment using the same heat transfer method.

D. Studies Addressing Objective 3

Study 9: Survival of *E. coli* O157:H7 in brining solutions, prepared in a meat homogenate, during storage at 4 or 15°C

- The natural contamination level of the meat homogenate was approximately 4 log CFU/ml, and no rifampicin-resistant bacterial (including *E. coli* O157:H7) populations were detected in the uninoculated control samples during the entire storage period at 4 or 15°C.
- Total bacterial populations increased ($P < 0.05$) by approximately 1.0 and 2.5 log CFU/ml in control (uninoculated, inoculated) samples stored at 4°C or 15°C for 48 h, respectively.
- As indicated, the initial inoculation level was approximately 3.5 log CFU/ml. Immediate (0 h) reductions in bacterial (total bacteria and pathogen) populations were observed in brining solutions containing AvGard XP or CPC, and in general, *E. coli* O157:H7 was not recovered from these solutions after the initial sampling point (0 h) for both storage temperatures.
- Bactericidal effects ($P < 0.05$) of *E. coli* O157:H7 to/or below the detection limit (1.3 log CFU/ml) by the end of storage at 4°C were also obtained in NaCl + STP solutions combined with LA, AA or Nisaplin + EDTA. For all the remaining treatments stored at 4°C, *E. coli* O157:H7 counts remained largely unchanged during the 48 h storage period.
- *E. coli* O157:H7 counts in control (inoculated) samples stored at 15°C for 48 h increased ($P < 0.05$) by 0.3 log CFU/ml, whereas no changes ($P \geq 0.05$) were obtained in solutions containing NaCl, STP, SPP, NaCl + STP/SPP, or NaCl + STP combined with PL and/or SD.
- Pathogen counts decreased ($P < 0.05$) to below detectable levels by 8 and 24 h incubation at 15°C in treatments containing LA or AA, respectively. For solutions containing Nisaplin + EDTA or ALTA 2341 + EDTA, *E. coli* O157:H7 populations decreased ($P < 0.05$) by 1.7 and 1.9 log CFU/ml, respectively, by the end of storage, whereas in solutions containing CA or hops beta acids, counts decreased ($P < 0.05$) by 0.9 and 0.8 log CFU/ml, respectively.
- At 15°C, total bacterial populations increased only in the controls (uninoculated, inoculated) and solutions containing the single ingredients of NaCl, STP or SPP.
- Initial pH values of control samples (uninoculated and inoculated meat homogenate) were 5.73-5.94.
- Addition of NaCl + STP to the meat homogenate increased the pH to 7.19 and 7.92. Addition of sodium diacetate, Nisaplin + EDTA, ALTA 2341 + EDTA or any of the organic acids lowered (pH values ranged from 3.13-6.00) the initial pH of the NaCl + STP solution, whereas AvGard XP increased (pH values of 12.18 and 12.41) the pH of the solution.
- Overall, the pH values of the solutions at the end of storage at 4 or 15°C were similar to the initial (0 h) values. One exception included the control (uninoculated, inoculated) samples stored at 15°C, where pH increases of 0.69-0.79 units were obtained, and is most likely due to growth of the total bacterial populations to levels > 7.0 log CFU/ml.

Study 10: Survival of *E. coli* O157:H7 in fresh, non-recirculated brining solutions stored at 4 or 15°C

- Total bacterial counts on TSA, and *E. coli* O157:H7 counts on TSA+rif, were similar at all sampling points indicating the absence of extraneous contamination of the brining solutions and control treatments.

- Immediate (0 h) and sustained (24 h at 4°C or 15°C) reductions to below the detection limit (1.3 log CFU/ml) of bacterial (total bacteria and *E. coli* O157:H7) populations were observed with the addition of AvGard XP or CPC to the NaCl + STP brining treatment.
- As expected, no growth of bacterial populations was obtained in the control (inoculated, no added additives) or any other treatment during storage, due to the absence of sufficient nutrients in the solutions. Interestingly, pathogen counts in general, remained unchanged ($P \geq 0.05$) during the 24 h storage period at 4°C or 15°C in the control, NaCl, STP, SPP, NaCl + STP/SPP and NaCl + STP + PL and/or SD treatments.
- Addition of LA, AA (15°C only) or CA to the brines decreased ($P < 0.05$) pathogen populations to below the detection limit by 4-24 h, at both storage temperatures.
- Pathogen counts of NaCl + STP treatments with added Nisaplin + EDTA, ALTA 2341 + EDTA, or hops beta acids and stored at 15°C for 24 h, were 1.3, 1.1 and 2.1 log CFU/ml lower ($P < 0.05$) than initial counts.
- The pH values of the solutions, and trends, were similar to those obtained in *Study 9*.
- Overall, the fate of *E. coli* O157:H7 at 4°C or 15°C was similar in brines prepared only with water and those prepared in a 3% meat homogenate (*Study 9*). Specifically, pathogen populations remained viable and unchanged in NaCl + STP/SPP solutions, alone or in combination with PL and/or SD, stored at 4 or 15°C for 24-48 h. However, addition of AvGard XP or CPC to the brine resulted in bactericidal effects to non-detectable levels.

E. Additional Studies

Study 11: Translocation of E. coli O157:H7 during needle injection for moisture enhancement of beef

- The percentage gain in product weight following injection with water or brine solution was 6.3 ± 0.7 and $9.7 \pm 0.5\%$, respectively, under contamination scenario-1. The corresponding percentage gain in product weight under contamination scenario-2 was 3.9 ± 1.5 and $4.5 \pm 0.4\%$, respectively.
- Meat surface contamination levels of total bacteria and *E. coli* O157:H7 before moisture enhancement were 5.2 and 4.7 log CFU/g, respectively, under contamination scenario-1.
- The inoculation level of *E. coli* O157:H7 in the water and brine solution used for contamination scenario-2 was 3.8 and 3.4 log CFU/ml, respectively.
- *E. coli* O157:H7 and total bacterial populations were recovered from all sections of water- and brine-injected samples under both contamination scenarios.
- Higher counts of the pathogen were obtained in all sections under scenario-1 (1.9-3.6 log CFU/g) than scenario-2 (0.6-1.6 log CFU/g).
- *E. coli* O157:H7 (2.3-3.6 log CFU/g) and total bacterial (2.4-4.0 log CFU/g) counts were similar within all sections after injection with water or brine under contamination scenario-1, except section-3 which had lower counts for *E. coli* O157:H7 (1.9 log CFU/g) after injection with water.
- Sections-3, -4, -5 and -6 had slightly higher counts of *E. coli* O157:H7 than sections -1 and -2 after injection with water or brine, under contamination scenario-2.
- The purge released following injection with water or brine solution had higher *E. coli* O157:H7 and total bacterial populations for scenario-1 than for scenario-2.

- The pH of uninoculated knuckles without moisture enhancement ranged from 5.52 to 5.60, whereas pH values of the different sections from moisture-enhanced knuckles ranged from 5.48 to 6.12.

Study 12: Thermal inactivation of *E. coli* O157:H7 at different depths of nonintact steaks cooked to a sublethal temperature

- Cooking times to reach a geometric center temperature of 60°C by pan-broiling and roasting were 11.5±1.2 and 26.2±4.3 min, respectively.
- Steak weight, core weight and meat pH were similar between the cooking methods.
- Surviving pathogen populations increased ($P < 0.05$) from 0.3±0.6 log CFU/cm² at 0 mm to 2.5±0.6 log CFU/cm² at 12 mm (center of the steak) depth of inoculation in steaks cooked by pan-broiling.
- For samples cooked by roasting, a similar increasing trend in surviving pathogen populations was obtained; 1.5 log CFU/cm² for surface (0 mm)-inoculated steaks and 2.9 log CFU/cm² for steaks inoculated in the center (12 mm).
- Thermal destruction of *E. coli* O157:H7 in steaks that were cooked by roasting was less, and differences in pathogen populations were not significant ($P \geq 0.05$) at each depth of inoculation, compared to steaks cooked by pan-broiling.
- Higher internal temperatures were observed during cooking by pan-broiling than roasting at 3 (88.0±7.3 vs 63.4±4.8°C), 6 (73.7±8.9 vs 59.6±10.0°C) and 9 (64.5±4.8 vs 60.5±2.1°C) mm depths of inoculation (Figures 17 and 18).

VIII. CONCLUSIONS

Under the conditions of the above studies, the obtained results showed that:

A. Preliminary Studies

- Overall, the thermotolerance (60°C, in tryptic soy broth) of the nine rifampicin-resistant *E. coli* O157:H7 strains selected to be used in one or both of the inoculum composites for contamination of meat or brine solutions, was not different to that of the corresponding parental (wild-type) strains. Rifampicin-resistant variants of the *E. coli* O157:H7 strains were used in all studies to allow tracking of the inoculum in meat products and brines without interference from the microflora naturally associated with meat products or other growth substrates. Furthermore, their use allowed for differentiation of the inoculum from the natural microflora on non-selective media (i.e., tryptic soy agar with/without 0.1% sodium pyruvate and supplemented with rifampicin), which permitted detection of cells injured by brine ingredients and/or antimicrobials, or heat during cooking (*Studies 1 and 2*).

B. Studies Addressing Objective 1

- Moisture enhancement of a meat model system, comprised of approximately 5 or 15% fat, with NaCl (0.5%) + STP (0.25%) + CPC (0.5%) reduced *E. coli* O157:H7 counts by approximately 1 log CFU/g after 24 h storage at 4°C, whereas pathogen counts for all other tested treatments (i.e., NaCl, STP, SPP [0.25%], NaCl + STP/SPP, NaCl + STP combined with potassium lactate [PL, 2%], sodium diacetate [SD, 0.15%], PL + SD, lactic acid [LA, 0.3%], acetic acid [AA, 0.3%], citric acid [CA, 0.3%], Nisaplin [0.06%] or pediocin

[ALTA™ 2341; 0.5%] + EDTA [20 mM], AvGard XP [0.2%], hops beta acids [0.00055%]) remained unchanged (*Study 3*).

- Overall, cooking of meat samples to 65°C (simulating medium-rare doneness of beef) reduced *E. coli* O157:H7 counts by 1.5-2.5 log CFU/g, with the highest reductions obtained for CPC-treated samples (reductions of 2.4 and 2.5 log CFU/g) (*Study 3*).
- Enhanced thermal (65°C) destruction of *E. coli* O157:H7 was obtained for NaCl + STP + CPC-treated samples compared to the water control treatment, whereas treatment of meat with NaCl, STP, SPP, NaCl + STP/SPP, and NaCl + STP combined with PL, SD, PL + SD, LA, AA, CA, Nisaplin or ALTA 2341 + EDTA, AvGard XP, or hops beta acids did not ($P \geq 0.05$) enhance or protect the pathogen from heat inactivation. However, Nisaplin + EDTA- and ALTA 2341 + EDTA-treated samples had lower ($P < 0.05$) counts compared to the NaCl + STP treatment (*Study 3*).
- Overall, the contribution of fat content (approximately 5 or 15%) of the meat model system used to evaluate the potential effects of brining ingredients on heat inactivation of *E. coli* O157:H7, was negligible (*Study 3*).
- No immediate (i.e., after inoculation and treatment) or after storage (4°C, 96 h) reductions in pathogen populations were obtained in meat samples moisture-enhanced with NaCl (0.5%) + STP (0.25%) and combinations of antimicrobials (i.e., Nisaplin [0.06%] + ALTA 2341 [0.5%] + EDTA [20 mM], CPC [0.05%] + LA [0.3%], or PL [2%] + SD [0.15%] + CPC [0.05%]). Furthermore, none of the tested brining formulations enhanced or protected *E. coli* O157:H7 from heat (65°C) inactivation, compared to the water control treatment (*Study 4*).
- Immediate (0 h) bactericidal (> 3.5 log CFU/ml reductions) effects of *E. coli* O157:H7 were obtained in a beef extract containing NaCl (0.5%) + STP (0.25%) and TO (0.25% and 0.5%) or GSE (0.5% and 1.0%), alone or in combination with CPC (0.04%) or SD (0.25%). Pathogen counts in these treatments remained below the detection limit (0.0 log CFU/ml) even after 48 h incubation at 15°C (*Study 5*).
- Growth of *E. coli* O157:H7 was inhibited for 48 h at 15°C in beef extract containing NaCl + STP + TO (0.1%), NaCl + STP + BO (0.1 and 0.25%), NaCl + STP + SD (0.25%), or NaCl + STP + LA (0.3%), alone or in combination with other ingredients (*Study 5*).

C. Studies Addressing Objective 2

- In general, beef steaks moisture-enhanced with NaCl (0.5%) + STP (0.25%) + CPC (0.2%) had lower (0.7-1.2 log CFU/g) *E. coli* O157:H7 counts compared to samples enhanced with NaCl + STP, NaCl + STP + LA (0.3%), or NaCl + STP + AvGard XP (0.2%), regardless of storage temperature, storage day, packaging condition, or cooking method (*Study 6*).
- Cooking (60°C) of moisture-enhanced steaks reduced *E. coli* O157:H7 populations by 1.1-3.9 log CFU/g, with reductions depending on cooking method (i.e., pan-broiling, double pan-broiling or roasting) (*Study 6*).
- Overall, thermal (60°C) destruction of *E. coli* O157:H7 increased in order of: pan-broiling \leq roasting $<$ double pan-broiling (*Study 6*).
- In general, the cooking time required for 2.5 cm thick beef steaks to reach an internal temperature of 60°C increased in order of double pan-broiling $<$ pan-broiling $<$ roasting (*Study 6*).
- *E. coli* O157:H7 counts of roasts moisture-enhanced with NaCl + STP + CPC were 0.7 and 2.0 log CFU/g lower than those of the control treatment (NaCl + STP) on day-0 of storage (i.e., approximately 24 h after inoculation), whereas counts of roasts treated with NaCl + STP

+ LA or NaCl + STP + AvGard XP were not different to those of the control. Similar results were obtained for roasts that were frozen (-20°C) for 30 days and subsequently thawed for 48-72 h (*Study 7*).

- Surviving *E. coli* O157:H7 populations after cooking of moisture-enhanced roasts to internal temperatures of 60°C or 55°C, cooking times to reach the target internal temperature, and final temperatures (i.e., internal temperature after a 20 min resting period) were found to vary between individual roasts, regardless of brining treatment, storage day or cooking temperature.
- *E. coli* O157:H7 populations in moisture-enhanced roasts cooked to internal temperatures of 60°C or 55°C were reduced to non-detectable (<0.5 log CFU/g) levels in 75.0% and 65.6%, respectively, of the total number of sub-samples analyzed per roast, with reductions ranging from >2.8 to >6.5 log CFU/g (i.e., depending on initial levels in uncooked roasts) (*Study 7*).
- Based on the data of individual roasts (regardless of brining treatment), a short cooking time combined with a low final temperature was associated with the presence of high numbers (roasts cooked to 60°C: 3.6-5.4 log CFU/g; roasts cooked to 55°C: 2.1-5.2 log CFU/g) of survivors in the six sub-samples analyzed from each roast (*Study 7*).
- Heat inactivation of *E. coli* O157:H7 in moisture-enhanced (NaCl [0.45%] + STP [0.23%]) beef steaks of 1.5, 2.5 and 4.0 cm thickness cooked to an internal temperature of 65°C increased in order of: double pan-broiling ≤ pan-broiling < roasting, with no ($P \geq 0.05$) differences obtained by using different cooking equipment for the same cooking method. Overall reductions of the pathogen were 1.1-2.3, 1.6-2.8 and 2.0-4.2 log CFU/g when steaks were cooked by double pan-broiling, pan-broiling, and roasting, respectively (*Study 8*).
- In general, greater inactivation of the internalized pathogen was obtained in 4.0 cm than 1.5 or 2.5 cm steaks, regardless of cooking method or equipment (*Study 8*).
- The time to reach the target internal temperature of 65°C followed the order of: George Foreman grill (3.9-19.8 min) < Oster toaster oven (11.3-45 min) < Presto electric skillet (16.3-55 min) < Sanyo grill (14.3-65 min) < kitchen oven (20-63 min), with variation in cooking time within each type of equipment related to steak thickness (*Study 8*).

D. Studies Addressing Objective 3

- *E. coli* O157:H7 populations remained viable and unchanged in brine solutions, comprised of NaCl (5.5%) + STP/SPP (2.75%) and prepared in a 3% meat homogenate (simulating recirculated brine) or water (simulating freshly prepared brine), during a 24 or 48 h storage period at 4°C or 15°C. Similarly, no changes in pathogen populations were obtained when PL (22%) and/or SD (1.65%) were added to the NaCl + STP solution (*Studies 9 and 10*).
- Brines formulated with NaCl + STP + AvGard XP (2.2%) or NaCl + STP + CPC (5.5%) resulted in immediate pathogen reductions of 1.4 to >2.6 log CFU/ml, and non-detectable levels during storage (4°C or 15°C, up to 24 or 48 h) (*Studies 9 and 10*).
- Depending on the presence or absence of meat residues in the brine solutions, and storage temperature, reductions of *E. coli* O157:H7 were obtained in NaCl + STP solutions supplemented with LA (3.3%), AA (3.3%), CA (3.3%), Nisaplin (0.66%) + EDTA (200 mM), ALTA 2341 (5.5%) + EDTA, or hops beta acids (0.0055%) during storage at 4°C or 15°C (*Studies 9 and 10*).

E. Additional Studies

- *E. coli* O157:H7 contamination was translocated to the interior of beef tissue following moisture enhancement via needle injection, either when the surface of the meat, or brine solution, were contaminated with the pathogen (*Study 11*).
- Numbers of *E. coli* O157:H7 survivors increased ($P < 0.05$) as the depth of contamination (0-12 mm of a 24 mm steak) of nonintact steaks increased, in samples cooked (60°C internal temperature) by pan-broiling. In contrast, for steaks cooked by roasting, no significant differences ($P \geq 0.05$) in survivors were obtained at the different depths of contamination.

IX. Recommendations for Future Research

Recommendations for future research related to this project include:

- Compare thermal inactivation of *E. coli* O157:H7 in beef products moisture-enhanced to different levels (e.g., 10% vs 12% pump rate).
- Evaluate survival of stress-hardened (e.g., heat, acid, starved) *E. coli* O157:H7 cultures during storage and subsequent cooking of moisture-enhanced products.
- Based on findings of *Study 5*, which was performed in a beef extract, evaluate survival/growth of *E. coli* O157:H7 in stored, and subsequently cooked, beef steaks or roasts moisture-enhanced with brines containing essential oils alone or in combination with other antimicrobials.
- Investigate whether the starting temperature of cooking equipment (i.e., kitchen ovens, electric skillets, barbecue grills, etc.) will affect the extent of thermal destruction of *E. coli* O157:H7 in moisture-enhanced products.
- Determine the extent of cross-contamination of moisture-enhanced products from contaminated equipment (i.e., needle injectors).
- Investigate sanitation protocols for brine injection needles to reduce cross-contamination of moisture-enhanced products.

X. Presentations and Publications

At this time, six abstracts (presented in the Appendix) have been submitted and accepted for presentation at upcoming scientific meetings.

- One abstract has been accepted for oral presentation at the 64th Annual Meeting of the Institute of Food Technologists (June 6-9, 2009; Anaheim, CA).
- Two abstracts have been accepted for oral presentation, and three for poster presentation, at the 96th Annual Meeting of the International Association for Food Protection (July 12-15, 2009; Grapevine, TX).

The accepted abstracts present the majority of data obtained under Objective 1, parts of Objectives 2 and 3, and the two studies that were conducted over and above the stated objectives of the submitted proposal. The remaining data of Objectives 2 and 3 will be presented in the near future. Additionally, all data will be prepared for publication in scientific journals.

XI. REFERENCES

Anonymous. 1995. Research guidelines for cookery, sensory evaluation and instrumental tenderness measurements of fresh meat. Published by American Meat Science Association in cooperation with National Live Stock and Meat Board, Chicago, IL.

AOAC (Association for Official Analytical Chemists). 2000. Official Methods of Analysis of AOAC International, 17th Ed. AOAC International, Gaithersburg, MD.

BIFSCO (Beef Industry Food Safety Council). 2006. Beef industry addresses the safety of non-intact beef products: summary report of the non-intact products processing workshop (December 13, 2005; Dallas TX). Available at: http://www.bifSCO.org/uDocs/03_26_06Non-IntactWhitePaper.pdf Accessed 26 April, 2009.

Bohaychuk, V. M., and G. G. Greer. 2003. Bacteriology and storage life of moisture-enhanced pork. *J. Food Prot.* 66:293-299.

Boleman, S. J., S. L. Boleman, T. D. Bidner, K. W. McMillin, and C. J. Monlezun. 1995. Effects of postmortem time on calcium chloride injection on beef tenderness and drip, cooking, and total loss. *Meat Sci.* 39:35-41.

Carlson, B. A., K. K. Nightingale, J. A. Scanga, J. D. Tatum, J. N. Sofos, G. C. Smith, and K. E. Belk. 2006. Identification and evaluation of cattle persistently shedding vs. cattle non-persistently shedding *Escherichia coli* O157:H7. Final Research Report submitted to the National Cattlemen's Beef Association by the Center for Red Meat Safety, Department of Animal Sciences, Colorado State University, Fort Collins, CO. 33p.

Doyle, M. P., T. Zhao, J. Meng, and S. Zhao. 1997. *Escherichia coli* O157:H7. pp. 171-191. In: Food microbiology – fundamentals and frontiers. M. Doyle, L. Beuchat, and T. Montville (Eds.). ASM Press, Washington, DC.

Gill, C. O., and J. C. McGinnis. 2004. Microbiological conditions of mechanically tenderized beef cuts prepared at four retail stores. *Int. J. Food Microbiol.* 95:95-102.

Gill, C. O., and J. C. McGinnis. 2005. Factors affecting the microbiological condition of the deep tissues of mechanically tenderized beef. *J. Food Prot.* 68:796-800.

Gill, C. O., C. J. McGinnis, A. Houde, L. Lamoureux, and D. Leblanc. 2005a. Microbiological conditions of moisture-enhanced pork before and after cooking. *Food Microbiol.* 22:321-327.

Gill, C. O., J. C. McGinnis, K. Rahn, D. Young, N. Lee, and S. Barbut. 2005b. Microbiological condition of beef mechanically tenderized at a packing plant. *Meat Sci.* 69:811-816.

Greer, G. G., F. Nattress, B. Dilts, and L. Baker. 2004. Bacterial contamination of recirculating brine used in the commercial production of moisture-enhance pork. *J. Food Prot.* 67:185-188.

- Juneja, V. K., and H. M. Marks. 2005. Heat resistance kinetics among various isolates of *Escherichia coli*. *Inn. Food Sci. Emerg. Technol.* 6:155-161.
- Kaspar, C. W., and M. L. Tamplin. 1993. Effects of temperature and salinity on the survival of *Vibrio vulnificus* in seawater and shellfish. *Appl. Environ. Microbiol.* 59:2425-2429.
- Knock, R. C., M. Seyfert, M. C. Hunt, M. E. Dikeman, R. A. Mancini, J. A. Unruh, J. J. Higgins, and R. A. Monderen. 2006. Effects of potassium lactate, sodium chloride, and sodium acetate on surface shininess/gloss and sensory properties of injection-enhanced beef strip-loin steaks. *Meat Sci.* 74:319-326.
- Miller, M. F., K. L. Huffman, S. Y. Gilbert, L. L. Hamman, and C. B. Ramsey. 1995. Retail consumer acceptance of beef tenderized with calcium chloride. *J. Anim. Sci.* 73:2308-2314.
- Miller, R. 1998. Functionality of non-meat ingredients used in enhanced pork. *Pork Quality Facts*. Des Moines, IA: National Pork Board. Available at: <http://www.pork.org/PorkScience/documents/functionalitynonmeat.pdf> Accessed 26 April, 2009.
- Molina, M. E., D. D. Johnson, R. L. West, and B. L. Gwartney. 2005. Enhancing palatability in beef chuck muscles. *Meat Sci.* 71:52-61.
- Mukherjee, A., Y. Yoon, K. E. Belk, J. A. Scanga, G. C. Smith, and J. N. Sofos. 2008. Thermal inactivation of *Escherichia coli* O157:H7 in beef treated with marination and tenderization ingredients. *J. Food Prot.* 71:1349-1356.
- Noah, C. W., C. I. Shaw, J. S. Ikeda, K. S. Kreuzer, and J. N. Sofos. 2005. Development of green fluorescent protein-expressing bacterial strains and evaluation for potential use as positive controls in sample analyses. *J. Food Prot.* 68:680-686.
- Phebus, R. K., J. L. Marsden, T. Harshavardhan, S. Spring, and C. Kastner. 1999. Blade tenderization and food safety. *Recip. Meat Conf. Proc.* 52:71-72.
- Savell, J. W., and G. C. Smith. 2000. Laboratory manual for meat science (7th ed.). American Press, Boston.
- Sofos, J. N., I. Geornaras, K. E. Belk, and G. C. Smith. 2008. Nonintact whole muscle food safety: The problem and research needs. Proceedings of the 61st Reciprocal Meat Conference, American Meat Science Association, June 22-25, Gainesville, FL. 7p.
- Spring, S. B. 1999. *Escherichia coli* O157:H7 risk assessment for production and cooking of blade tenderized beef steaks. Master of Science thesis, Kansas State University, Manhattan, KS. Professor: Randall K. Phebus.
- USDA-FSIS (United States Department of Agriculture Food Safety and Inspection Service). 2002. Technical report: Comparative risk assessment for intact (nontenderized) and non-intact (tenderized) beef. Food Safety and Inspection Service, U.S. Department of Agriculture, Washington, D.C. Available at:

http://www.fsis.usda.gov/PDF/Beef_Risk_Assess_Report_Mar2002.pdf Accessed 26 April, 2009.

USDA-FSIS (United States Department of Agriculture Food Safety and Inspection Service). 2005. HACCP plan reassessment for mechanically tenderized beef products. *Fed. Regist.* 70:30331-30334.

USDA-FSIS (United States Department of Agriculture Food Safety and Inspection Service). 2007. Pennsylvania firm recalls beef products for possible *E. coli* O157:H7. Available at: http://www.fsis.usda.gov/PDF/Recall_019_2007_Release.pdf Accessed 26 April, 2009.

Uttaro, B., and J. L. Aalhus. 2007. Effect of thawing rate on distribution of an injected salt and phosphate brine in beef. *Meat Sci.* 75:480-486.

Vote, D. J., W. J. Platter, J. D. Tatum, G. R. Schmidt, K. E. Belk, G. C. Smith, and N. C. Speer. 2000. Injection of beef strip loins with solutions containing sodium tripolyphosphate, sodium lactate, and sodium chloride to enhance palatability. *J. Anim. Sci.* 78:952-957.

Wicklund, R. A., D. D. Paulson, M. C. Rojas, and M. S. Brewer. 2006. Effects of shelf-life enhancers on *E. coli* K12 survival in solutions used to enhance beef strips steaks. *J. Food Sci.* 71:M190-195.

Woerner, D. R., J. R. Ransom, J. N. Sofos, J. A. Scanga, G. C. Smith, and K. E. Belk. 2006. Preharvest processes for microbial control in cattle. *Food Prot. Trends* 26:393-400.

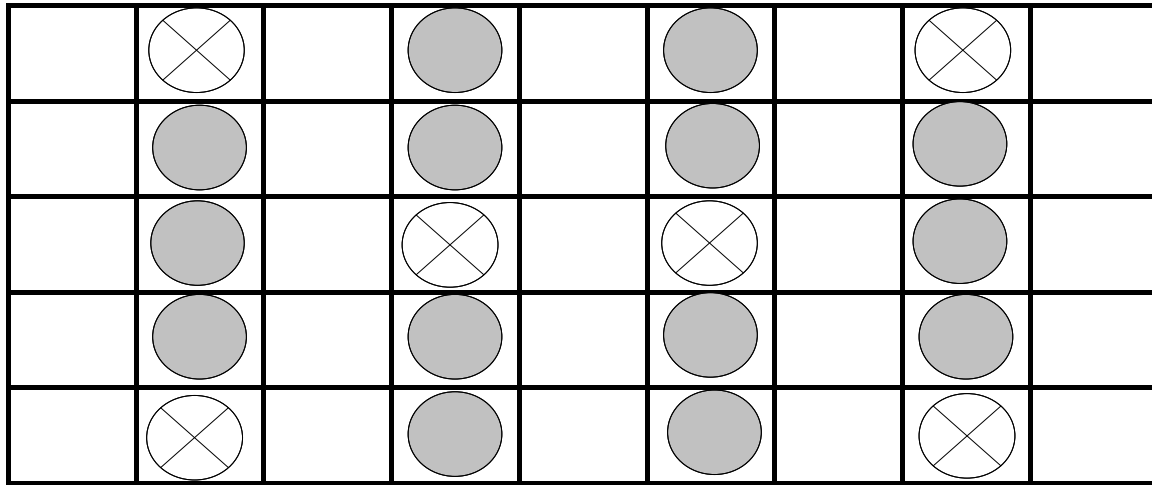


FIGURE 1. Schematic representation of the position of meat samples (indicated as circles) in the water bath during simulated cooking to an internal temperature of 65°C. Thermocouples were placed in meat samples intended for proximate analysis (circles with an X)



A



B



C

FIGURE 2: Inoculated and treated course-ground beef stuffed into polyethylene bags (A) was semi-frozen (-20°C , 6 h) and then cut into 2.5 cm thick steaks. Individual steaks were vacuum-packaged (B) or were placed on foam trays and over-wrapped with air-permeable plastic film (C)

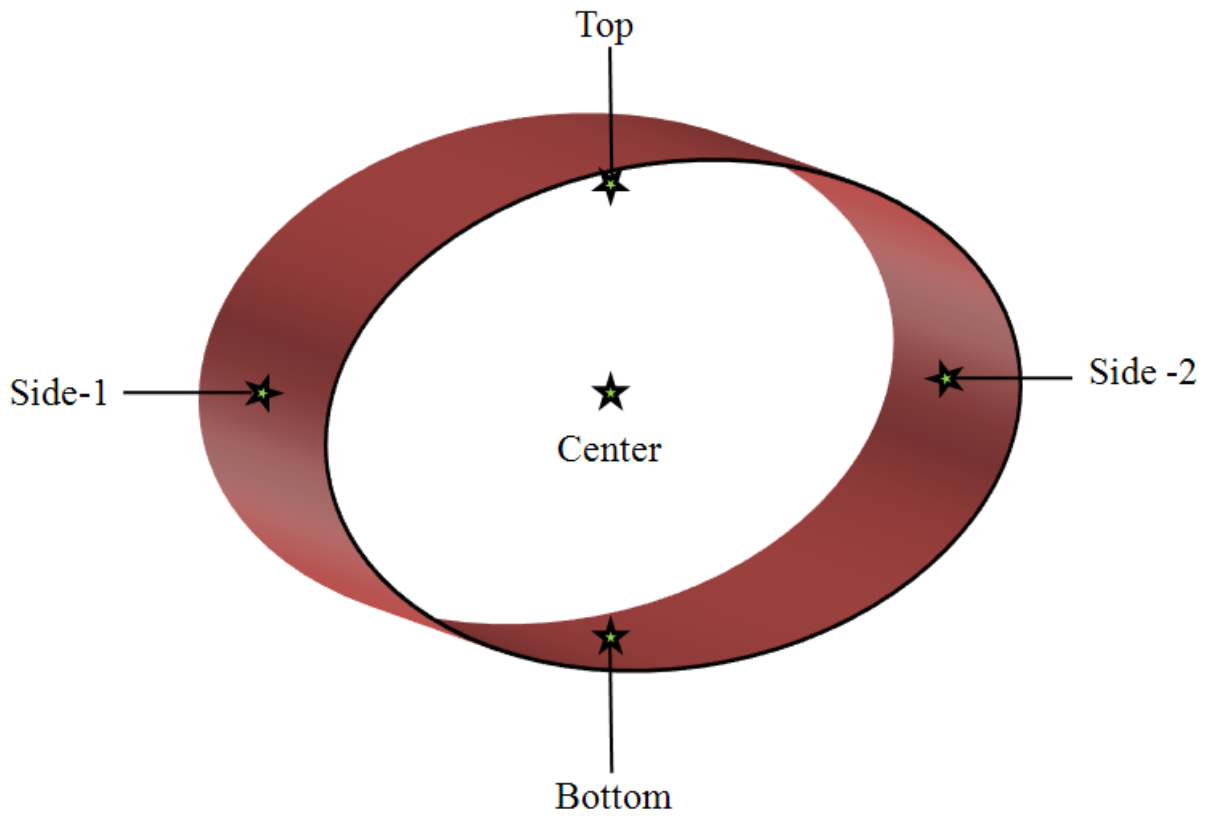


FIGURE 3. Placement of five thermocouples (one in the geometric center, one in each of the two sides, one below the surface on the top, and, one below the surface at the bottom) in moisture-enhanced roasts, for monitoring of temperature during cooking to internal (geometric center) temperatures of 60 or 55°C

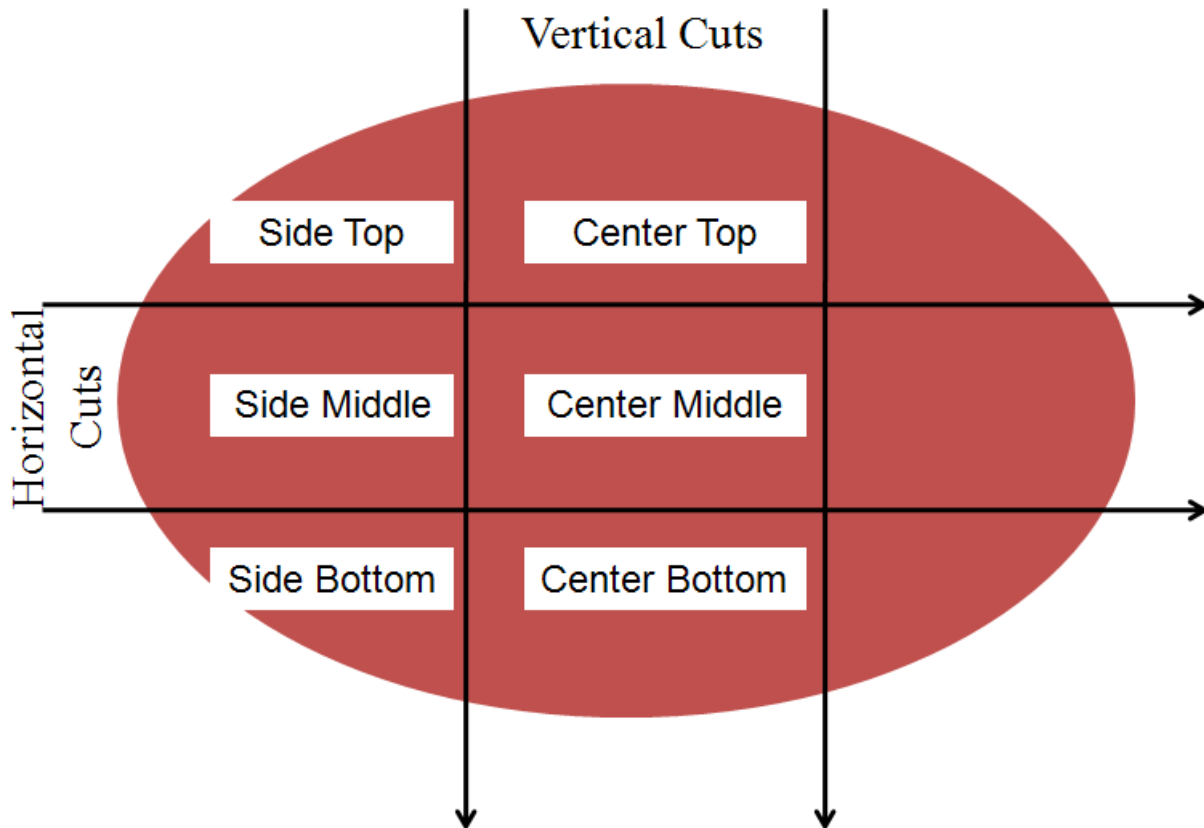


FIGURE 4. Six sub-sections were sampled from each cooked, moisture-enhanced roast: center top, center middle, center bottom, side top, side middle and side bottom

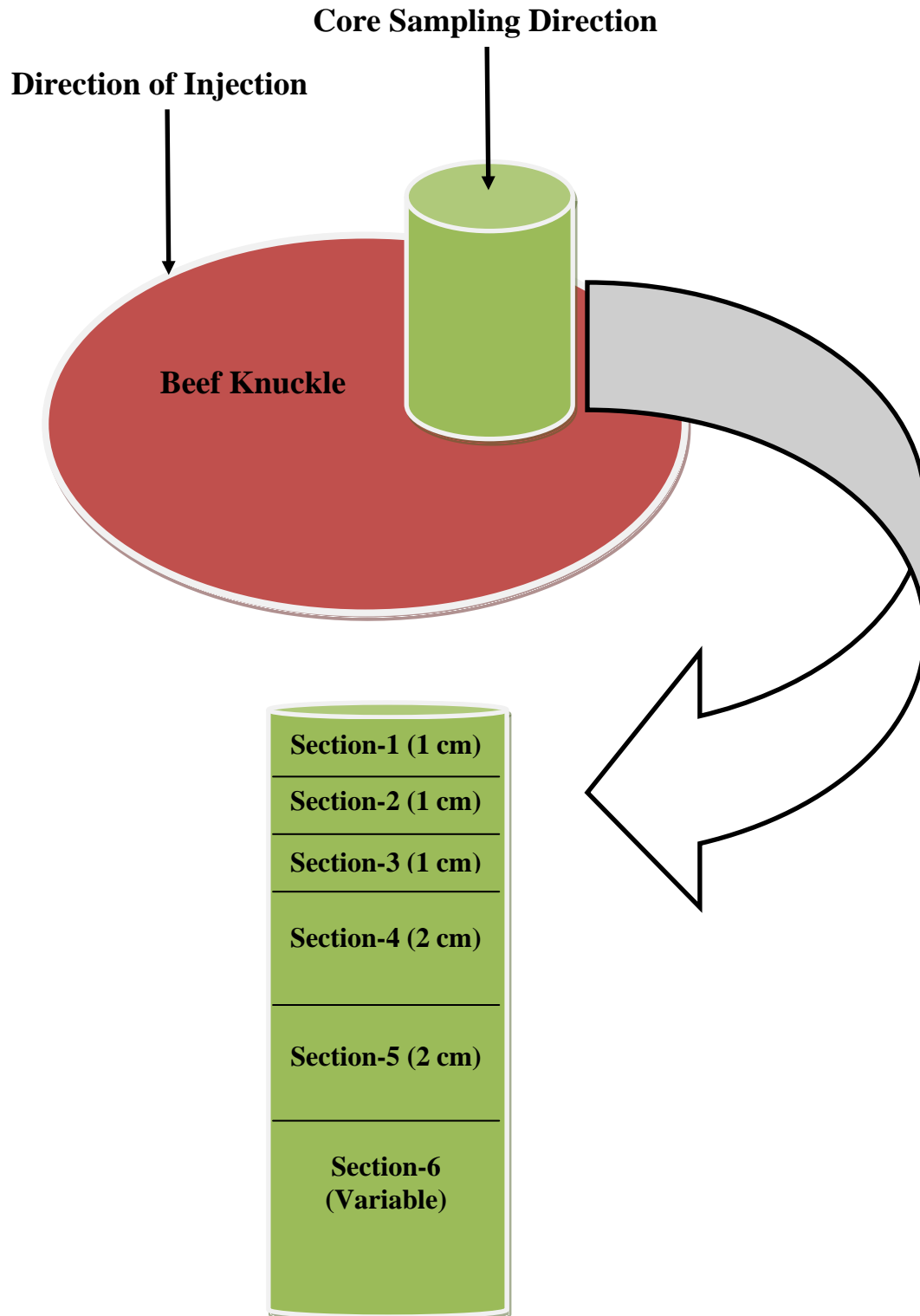


FIGURE 5. One core (8 cm in diameter) sample per moisture-enhanced knuckle was excised parallel to the direction of needle injection. Core samples were surface-decontaminated in boiling water (92-94°C, 60 s), and then cooled (4°C, 15 min), and cut into six sections (1 through 6); the sections were individually analyzed for bacterial counts

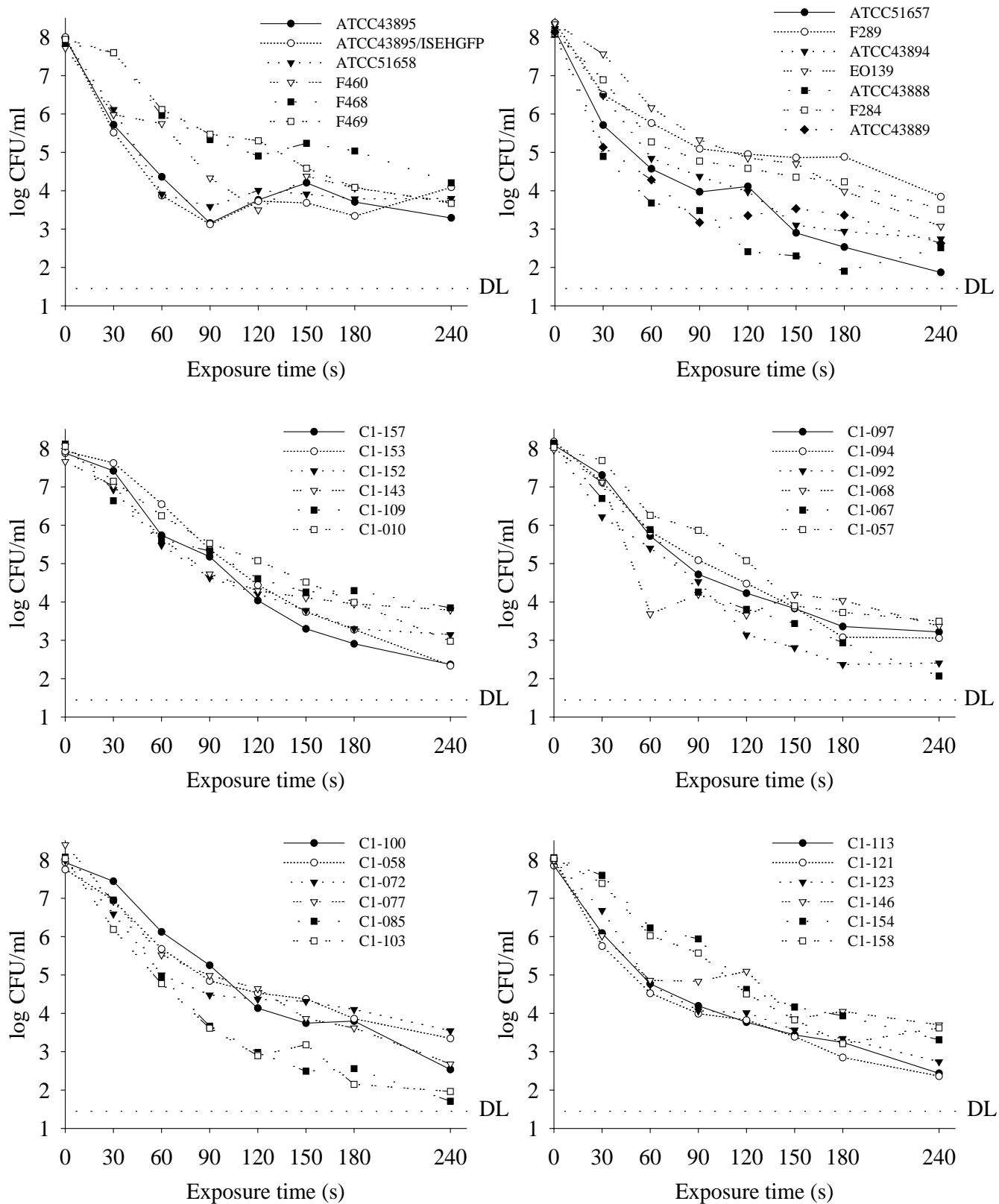


FIGURE 6. Inactivation of *E. coli* O157:H7 strains in tryptic soy broth at 60°C (DL: detection limit; 1.3 log CFU/ml)

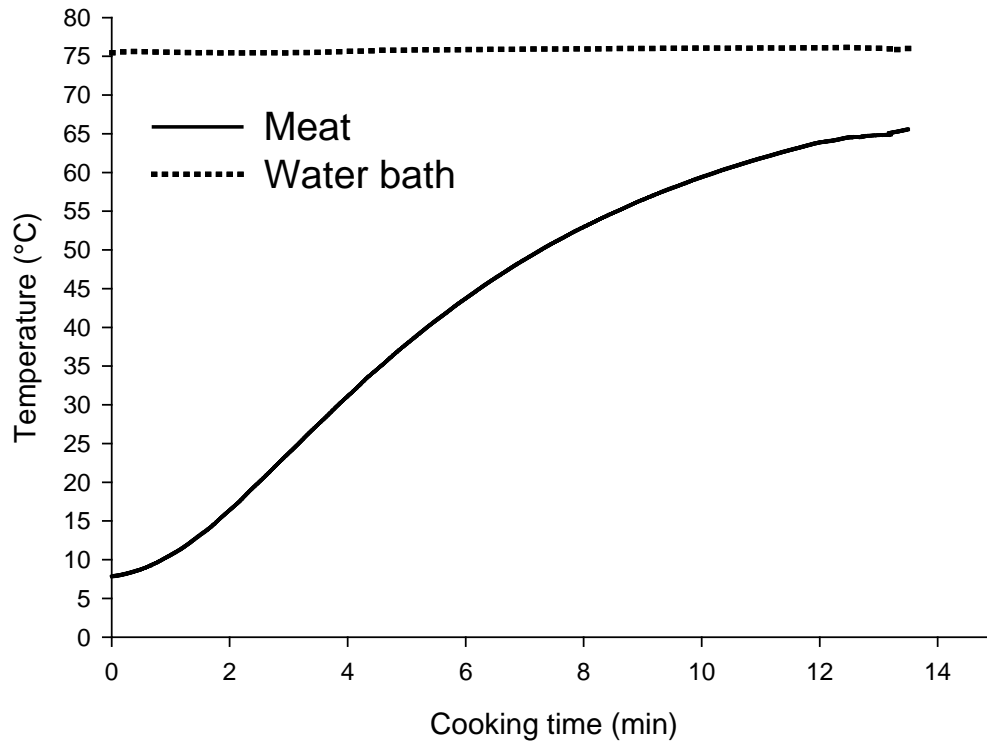


FIGURE 7. Temperature profile (averaged across both types of meat and all treatments) of meat samples cooked to an internal temperature of 65°C, and of the water in the water bath

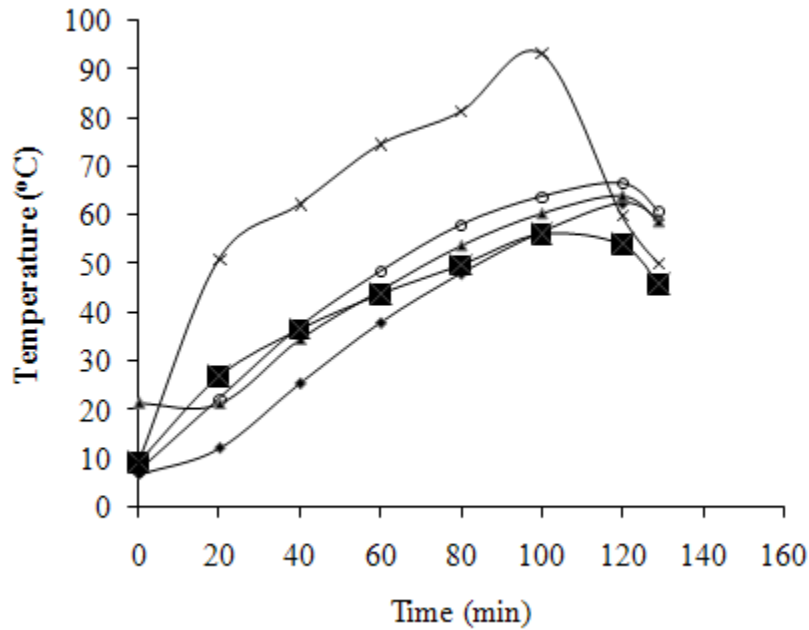


FIGURE 8. Temperature profile of different locations (see Figure 3) of a roast moisture-enhanced with NaCl + STP + AvGard XP (day-0 of vacuum-packaged frozen storage; sample 2), and cooked to an internal temperature of 60°C: Side-1 (◆), Side-2 (○), Top (▲), Center (■) and Bottom (×)

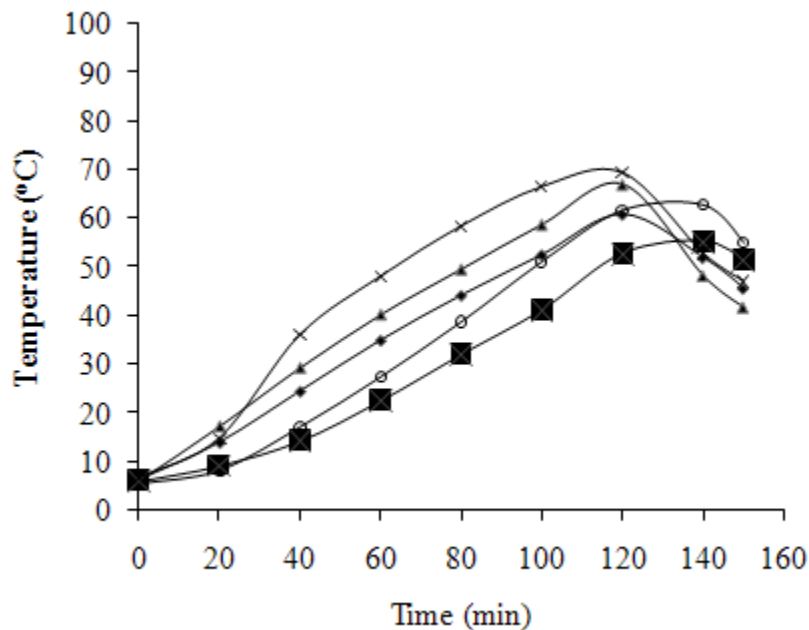


FIGURE 9. Temperature profile of different locations (see Figure 3) of a roast moisture-enhanced with NaCl + STP (day-0 of vacuum-packaged frozen storage; sample 1), and cooked to an internal temperature of 55°C: Side-1 (◆), Side-2 (○), Top (▲), Center (■) and Bottom (×)

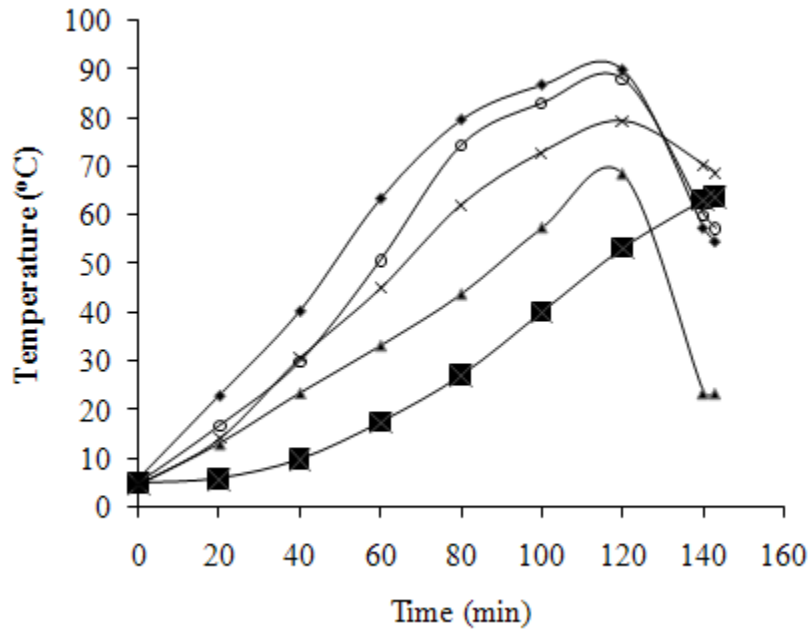


FIGURE 10. Temperature profile of different locations (see Figure 3) of a roast moisture-enhanced with NaCl + STP (day-30 of vacuum-packaged frozen storage; sample 2), and cooked to an internal temperature of 55°C: Side-1 (◆), Side-2 (○), Top (▲), Center (■) and Bottom (×)

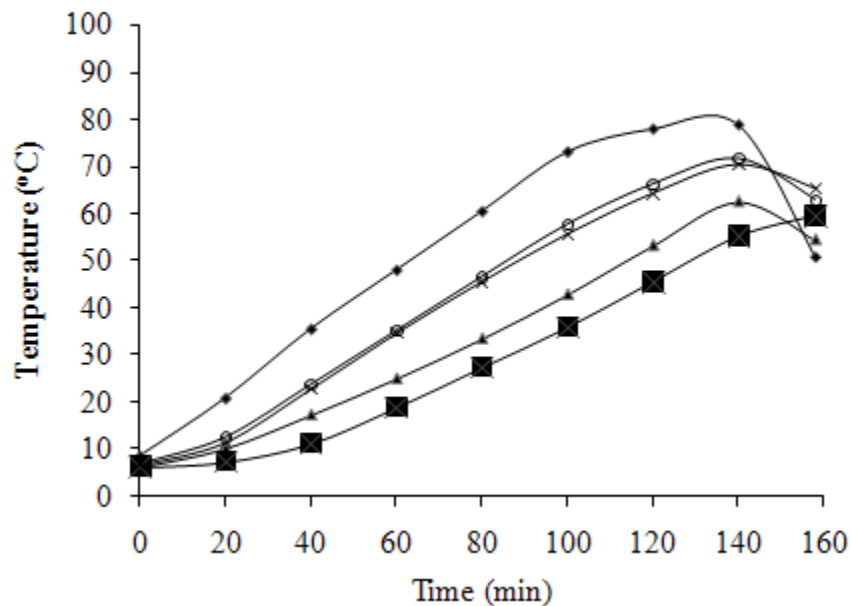


FIGURE 11. Temperature profile of different locations (see Figure 3) of a roast moisture-enhanced with NaCl + STP + AvGard XP (day-0 of vacuum-packaged frozen storage; sample 2), and cooked to an internal temperature of 55°C: Side-1 (◆), Side-2 (○), Top (▲), Center (■) and Bottom (×)

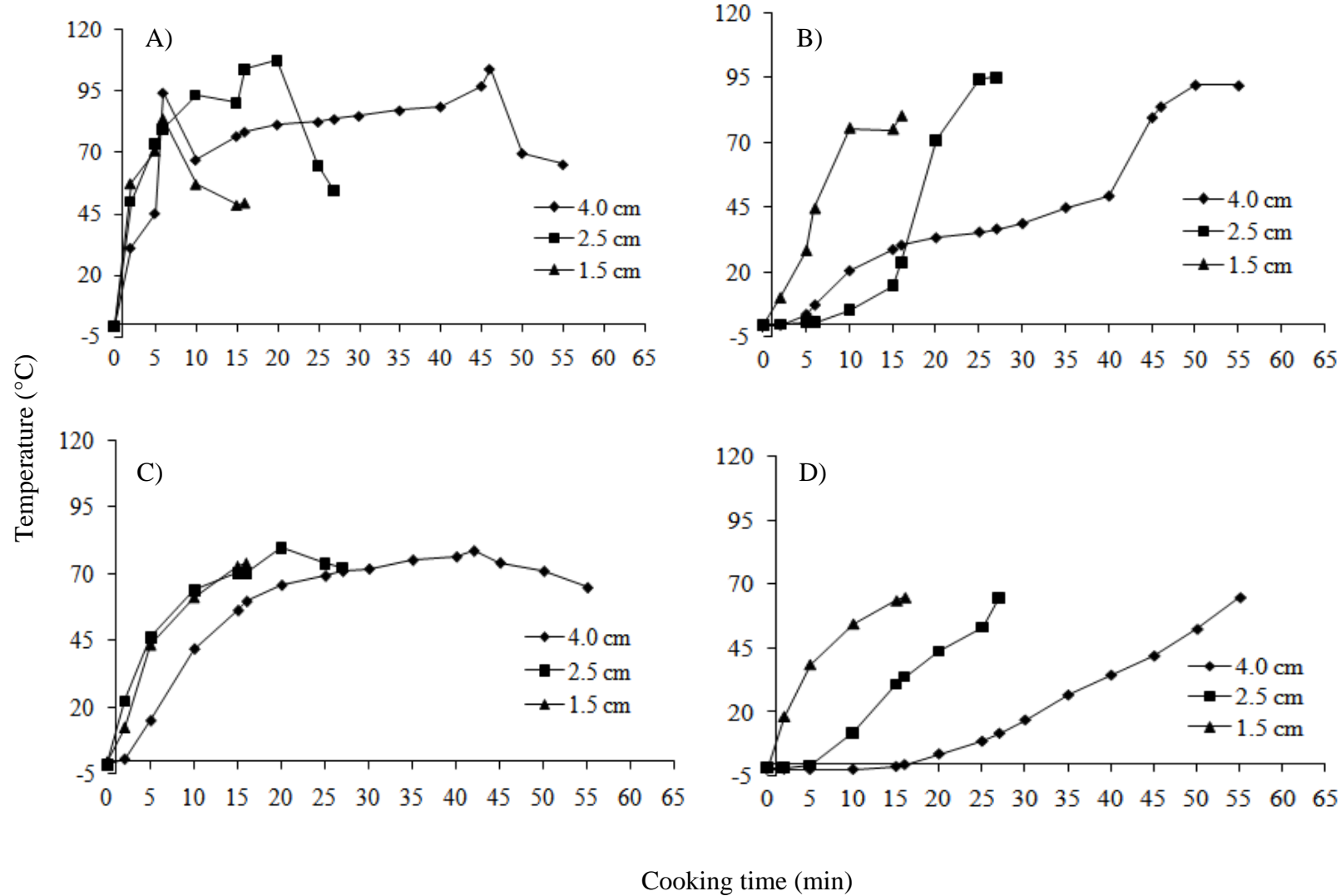


FIGURE 12. Cooking time and temperature curves for beef steaks cooked by pan-broiling using the Presto electric skillet. A) Lower surface of steaks before flipping over; B) Upper surface of steaks after flipping over; C) Edge of steaks; D) Center of steaks. Each point is the average of four determinants

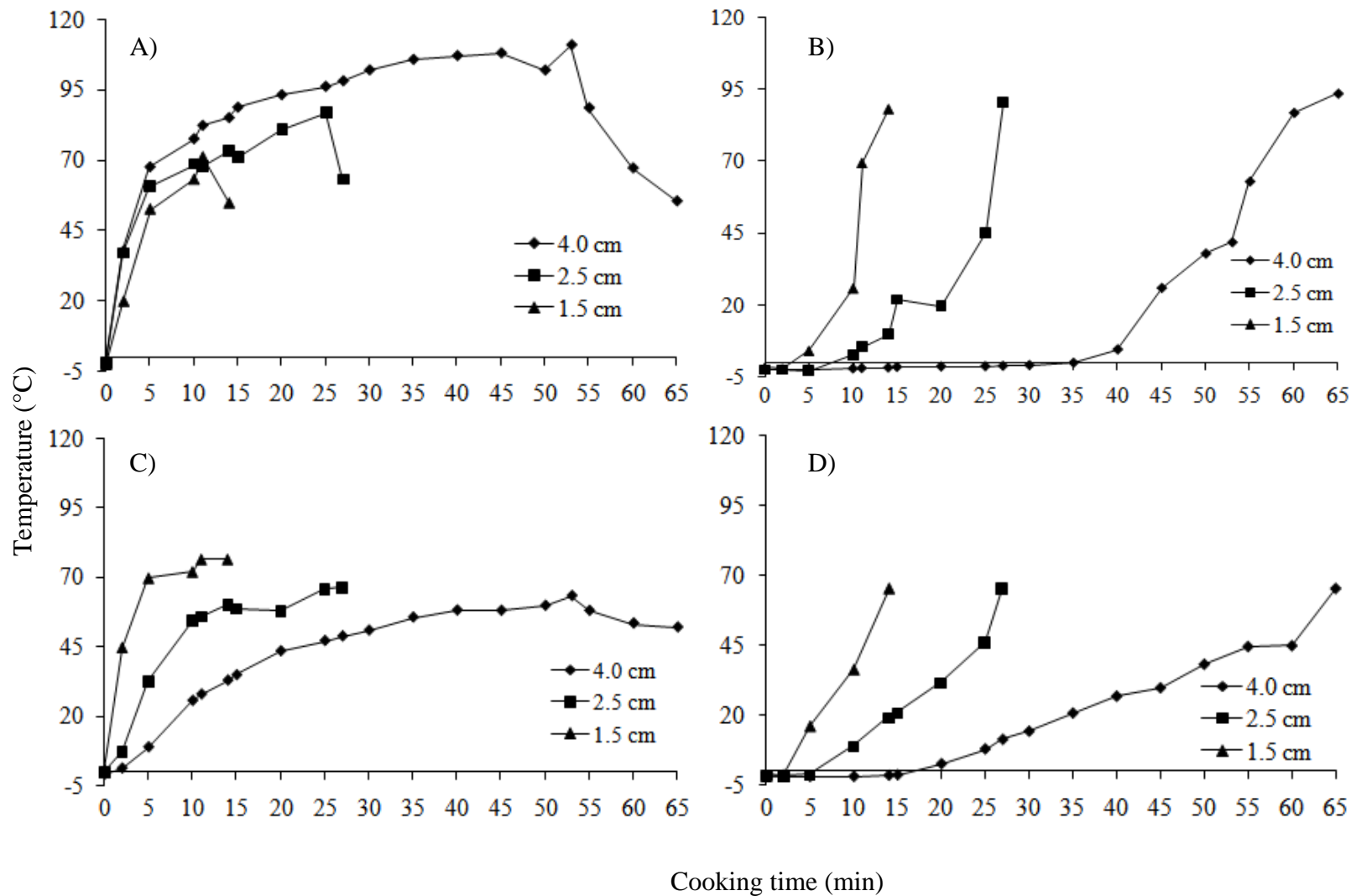


FIGURE 13. Cooking time and temperature curves for beef steaks cooked by pan-broiling using the Sanyo grill. A) Lower surface of steaks before flipping over; B) Upper surface of steaks after flipping over; C) Edge of steaks; D) Center of steaks. Each point is the average of four determinants

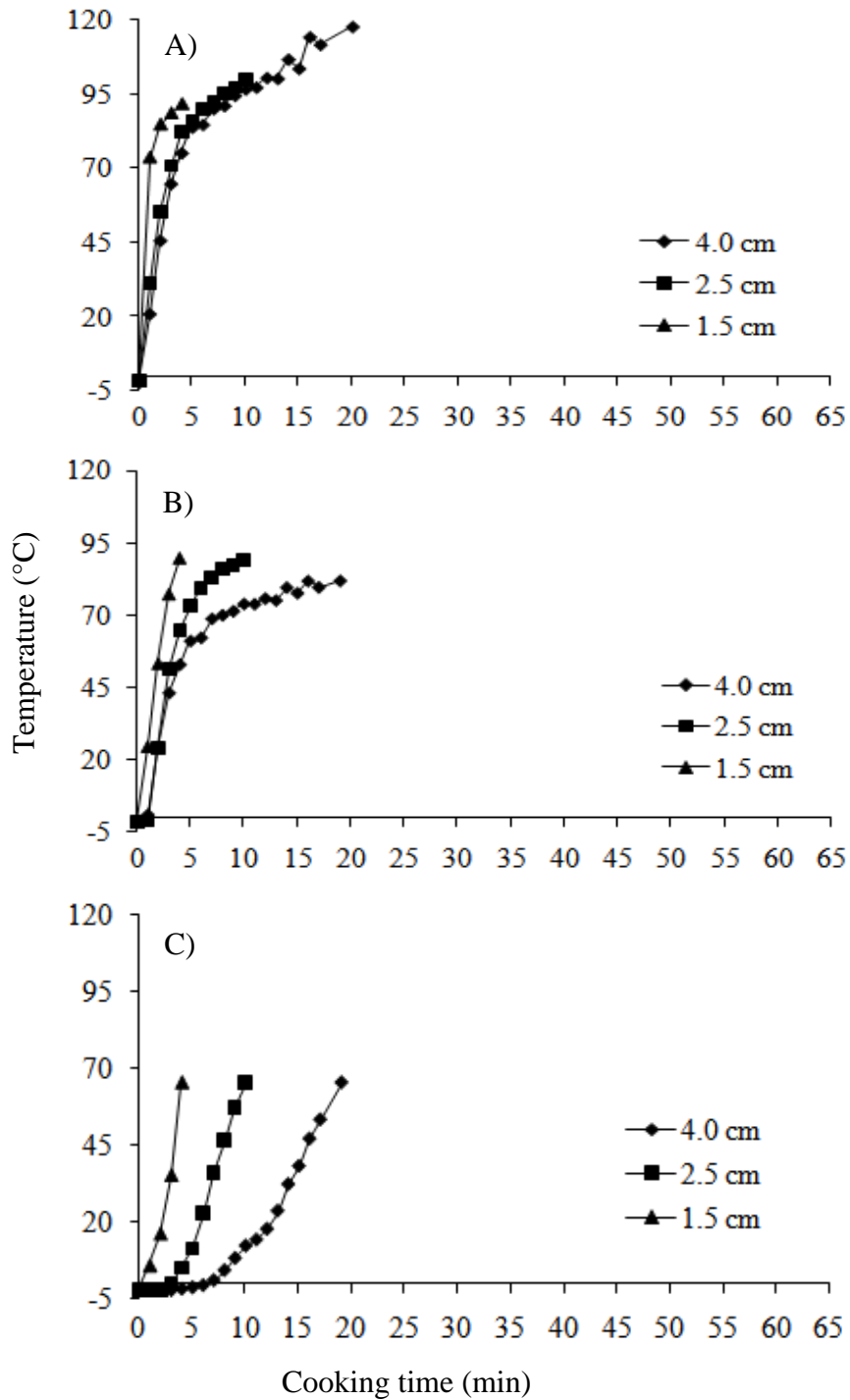


FIGURE 14. Cooking time and temperature curves for beef steaks cooked by double pan-broiling using the George Foreman grill. A) Surface of steaks; B) Edge of steaks; D) Center of steaks. Each point is the average of four determinants

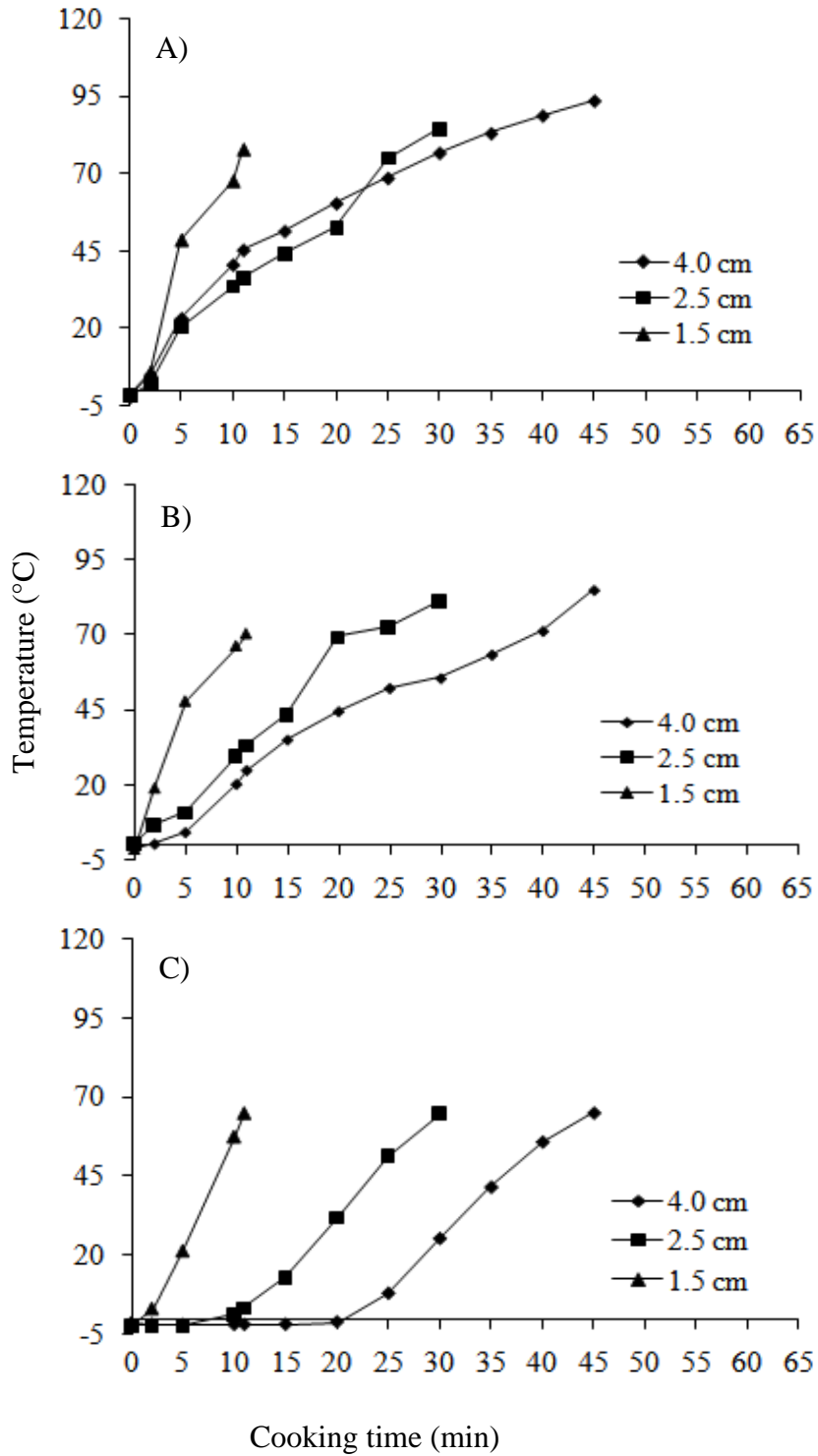


FIGURE 15. Cooking time and temperature curves for beef steaks cooked by roasting using the Oster toaster oven. A) Surface of steaks; B) Edge of steaks; D) Center of steaks. Each point is the average of four determinants

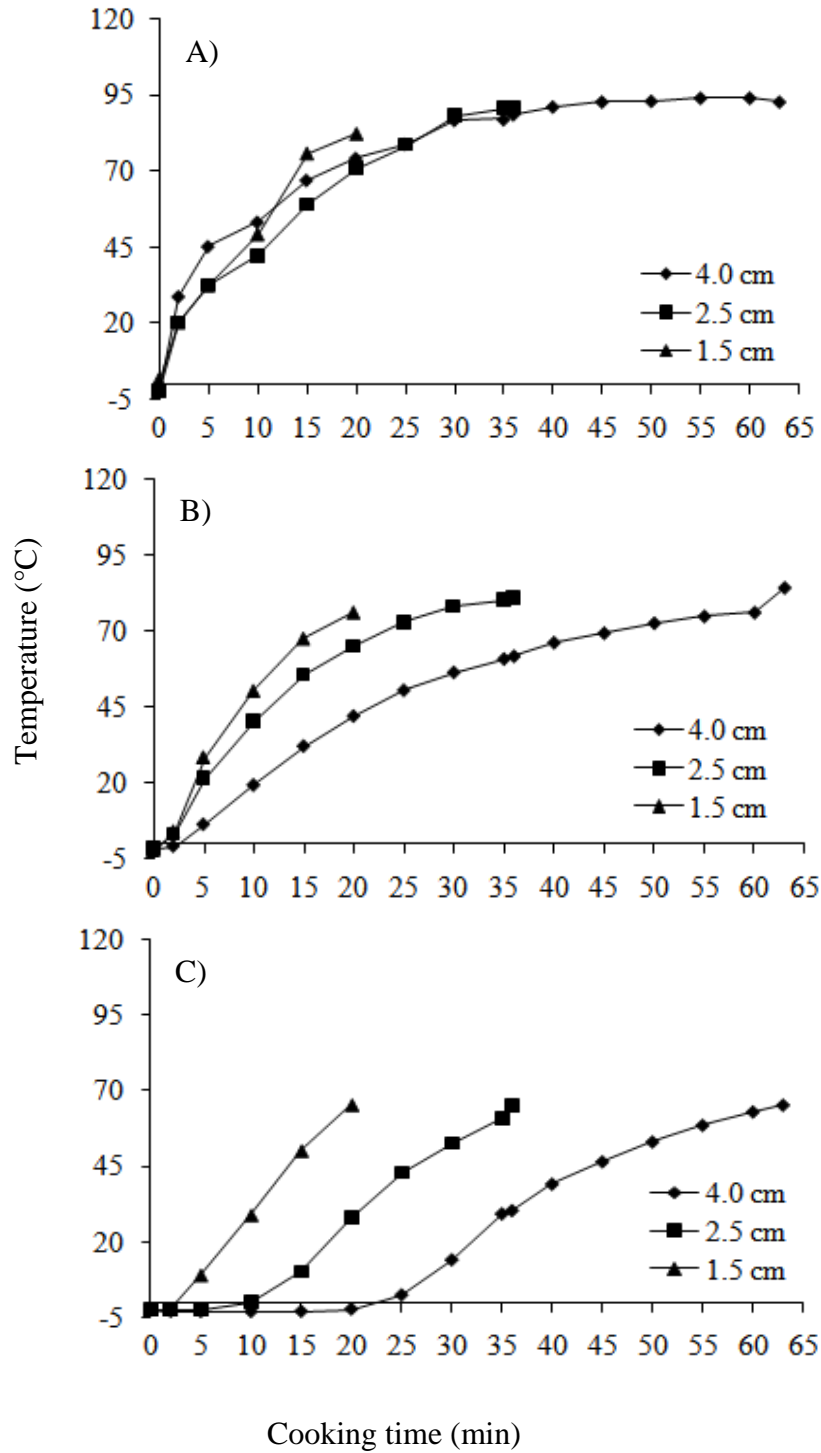


FIGURE 16. Cooking time and temperature curves for beef steaks cooked by roasting using the Magic Chef kitchen oven. A) Surface of steaks; B) Edge of steaks; D) Center of steaks. Each point is the average of four determinants

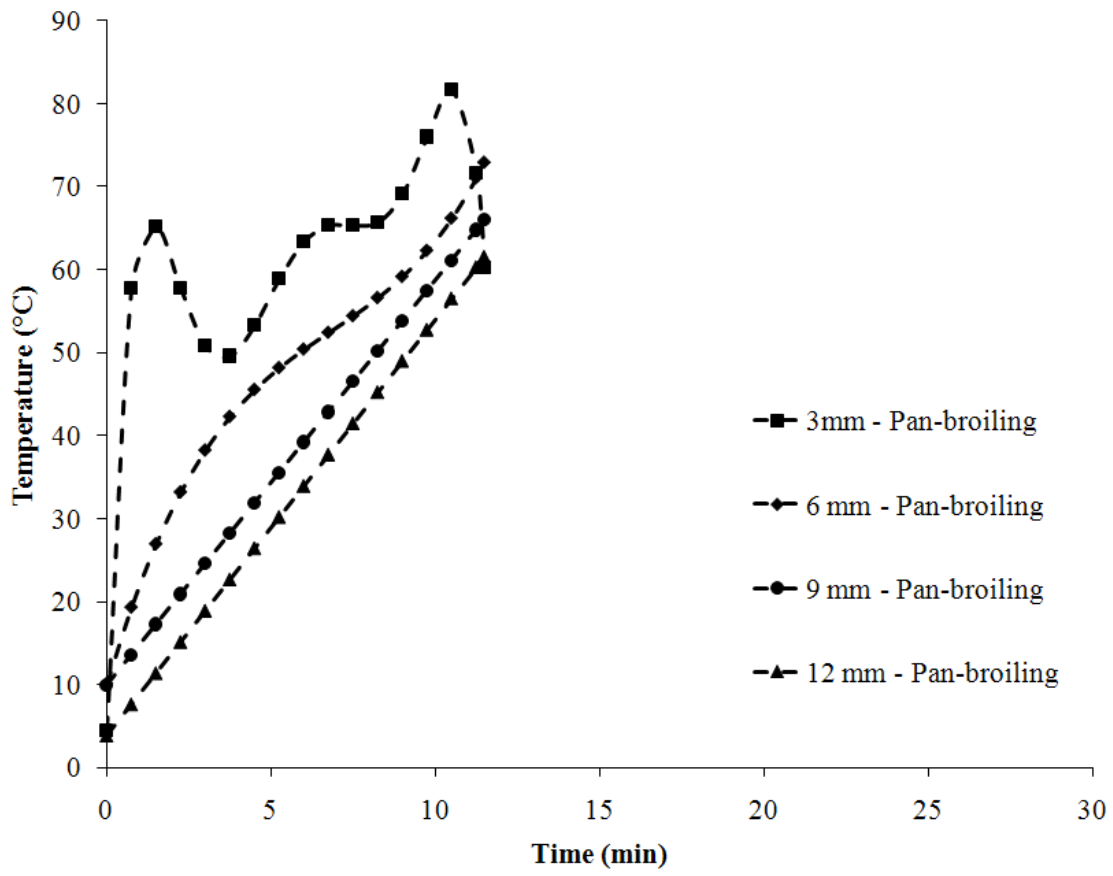


FIGURE 17. Cooking curves of nonintact steaks cooked by pan-broiling to an internal temperature of 60°C

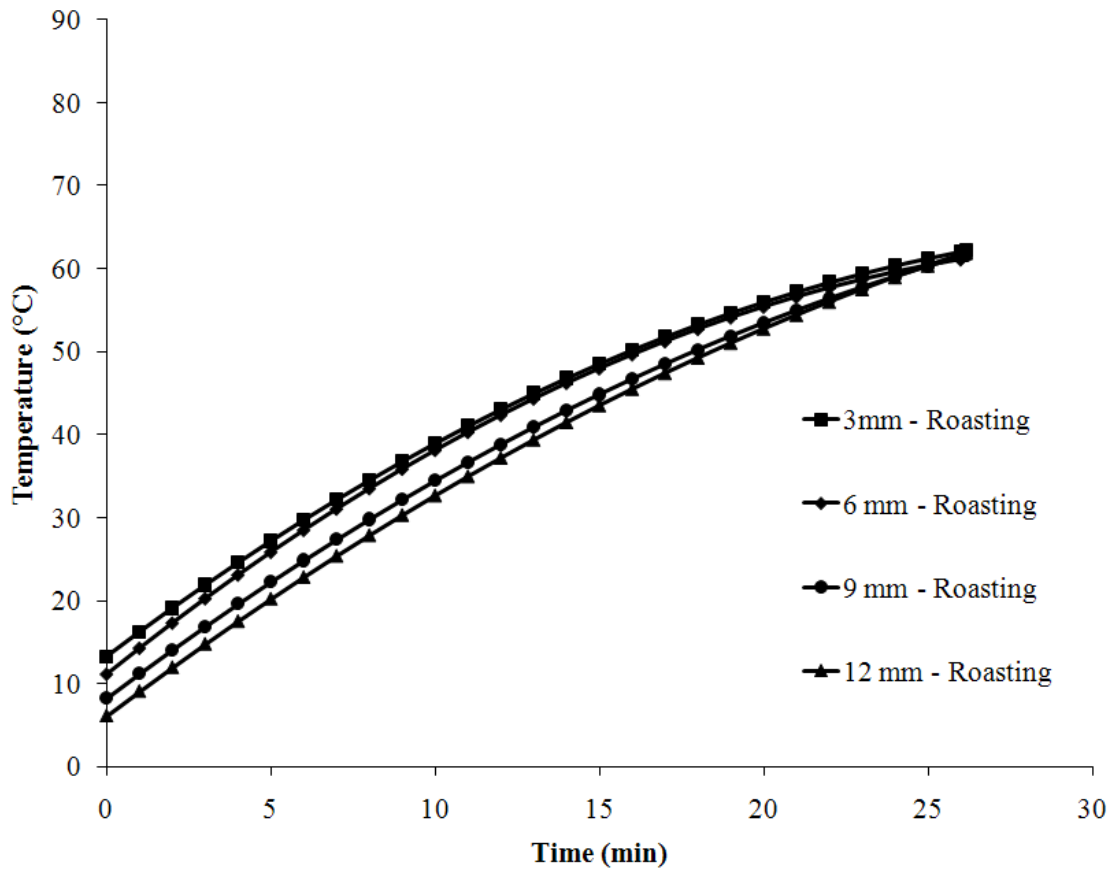


FIGURE 18. Cooking curves of nonintact steaks cooked by roasting to an internal temperature of 60°C

APPENDICES

ABSTRACTS

ABSTRACT ACCEPTED FOR ORAL PRESENTATION AT THE 64TH ANNUAL MEETING OF THE
INSTITUTE OF FOOD TECHNOLOGISTS
(JUNE 6-9, 2009; ANAHEIM, CA)

Title: *Escherichia coli* O157:H7 Survival in Meat Brining Solutions Containing Antimicrobials

Authors: Jeremy M. Adler, Ifigenia Geornaras, Oleksandr A. Byelashov, Keith E. Belk, Gary C. Smith, and John N. Sofos

Justification: Illness has been associated with consumption of *Escherichia coli* O157:H7-contaminated non-intact beef products. Brine injection of meat cuts for moisture enhancement may lead to entrapment of *E. coli* O157:H7 within the tissue if brining solutions used are contaminated with the pathogen.

Objective: This study evaluated the survival of *E. coli* O157:H7 in meat injection brines containing antimicrobials.

Methods: Brining treatments comprised of sodium chloride (NaCl; 5.5%), sodium tripolyphosphate (STP; 2.75%), sodium pyrophosphate (SPP; 2.75%), NaCl+STP, NaCl+SPP, NaCl+STP+potassium lactate (PL; 22%), NaCl+STP+sodium diacetate (SD; 1.65%), NaCl+STP+PL+SD, NaCl+STP+lactic acid (3.3%), NaCl+STP+acetic acid (3.3%), NaCl+STP+citric acid (3.3%), NaCl+STP+Nisaplin[®] (0.66%)+EDTA (200 mM), NaCl+STP+ALTA[™] 2341 (pediocin; 5.5%)+EDTA, NaCl+STP+AvGard[®] XP (sodium metasilicate; 2.2%), NaCl+STP+cetylpyridinium chloride (5.5%), or NaCl+STP+hops beta acids (0.0055%) were formulated (two replications/three samples each) in a 3% unsterile meat homogenate at concentrations used to enhance meat to 110% upon injection. The solutions were inoculated (3.5 log CFU/ml) with rifampicin-resistant *E. coli* O157:H7 (8-strain composite) and stored (48 h) at 4 or 15°C. Total bacterial and *E. coli* O157:H7 populations were enumerated periodically during incubation. Data were analyzed as a three factor (brine ingredients×incubation temperature×incubation time) ANOVA; least squared means were separated using a protected t-test.

Results: Natural flora increased (1.2-3.0 log CFU/ml) in the control (no ingredients) and single treatments of NaCl, STP and SPP at 15°C, and in the control and NaCl treatment (0.5-1.2 log CFU/ml) at 4°C. Pathogen levels decreased to below the detection limit (1.3 log CFU/ml) immediately (0 h) in brines containing cetylpyridinium chloride or AvGard[®] XP at both temperatures, and by 4-12 h in Nisaplin[®]+EDTA (4°C), and lactic and acetic acid (4 and 15°C) treatments. For all other treatments and the control, pathogen levels remained unchanged.

Significance: While several tested antimicrobials reduced pathogen populations, those immediately bactericidal, such as cetylpyridinium chloride and AvGard[®] XP, would best minimize the probability of interior muscle transfer through recirculated brine injection.

ABSTRACT ACCEPTED FOR POSTER PRESENTATION AT THE 96TH ANNUAL MEETING OF THE
INTERNATIONAL ASSOCIATION FOR FOOD PROTECTION
(JULY 12-15, 2009; GRAPEVINE, TX)

Title: Evaluation of Brining Ingredients and Antimicrobials for Effects on Thermal Destruction of *Escherichia coli* O157:H7 in a Meat Model System

Authors: Oleksandr A. Byelashov, Jeremy M. Adler, Ifigenia Geornaras, Kyung Yuk Ko, Keith E. Belk, Gary C. Smith, and John N. Sofos

Introduction: Brine injection is used to increase palatability of lower value meat cuts.

Escherichia coli O157:H7 may become internalized during this process, and may result in foodborne illness if the product is undercooked.

Purpose: This study evaluated the potential effect of brining ingredients, antimicrobials and fat content on *E. coli* O157:H7 in a ground beef model system after simulated brining, storage, and cooking.

Methods: Fresh beef knuckles ($5.3 \pm 2.4\%$ fat) or beef shoulder ($15.3 \pm 2.2\%$ fat) were ground individually, inoculated (7.2 ± 0.1 log CFU/g) with an 8-strain composite of rifampicin-resistant *E. coli* O157:H7, and mixed with brining solutions (to simulate a 10% pump rate). Treatments (700 g batches) included no brining, distilled water (DW), sodium chloride (NaCl, 0.5%), sodium tripolyphosphate (STP, 0.25%), NaCl + STP, and the NaCl + STP combination with added potassium lactate (PL, 2%), sodium diacetate (SD, 0.15%), PL + SD, lactic acid (0.3%), acetic acid (0.3%), citric acid (0.3%), Nisaplin[®] (0.06%) or pediocin (ALTA[™] 2341; 0.5%) + EDTA (20 mM), AvGard[®] XP (0.2%), cetylpyridinium chloride (CPC, 0.5%), and hops beta acids (0.00055%). Also, sodium pyrophosphate (0.25%) was tested singly and in combination with NaCl. Samples (30 g in test tubes) were analyzed for the pathogen (tryptic soy agar plus 0.1% sodium pyruvate and 100 µg/ml rifampicin) immediately after mixing, storage (24 h at 4°C), and cooking (65°C). Data (2 replications, 3 samples/treatment/replication) were analyzed as a randomized block factorial design using the General Linear Model and Tukey's Honestly Significant Differences procedures of SAS.

Results: The effect of the fat level of the meat on microbial counts was negligible. Following 24 h of storage at 4°C, pathogen numbers in CPC-treated samples were reduced by approximately 1 log-cycle, whereas for all other treatments counts remained unchanged ($P > 0.05$). Cooking of stored samples reduced counts by 1.5-2.5 log CFU/g. Surviving populations of *E. coli* O157:H7 in cooked samples were the lowest ($P < 0.05$) in those treated with CPC (3.7-3.8 log CFU/g), whereas for all other treatments, pathogen survivors (4.7-5.7 log CFU/g) were similar ($P > 0.05$) to the DW-control (5.1-5.3 log CFU/g). *E. coli* O157:H7 populations in cooked samples treated with Nisaplin[®] or ALTA[™] 2341 were lower ($P < 0.05$) than in those treated with AvGard[®] XP, and PL and/or SD.

Significance: These data should be useful in development/improvement of brines for control of *E. coli* O157:H7 in moisture-enhanced meat products.

**ABSTRACT ACCEPTED FOR POSTER PRESENTATION AT THE 96TH ANNUAL MEETING OF THE
INTERNATIONAL ASSOCIATION FOR FOOD PROTECTION
(JULY 12-15, 2009; GRAPEVINE, TX)**

Title: Antimicrobial Activity of Various Natural Compounds against *Escherichia coli* O157:H7
Cultured in Ground Beef Extract

Authors: Kyung Yuk Ko, Keith E. Belk, Gary C. Smith, and John N. Sofos

Introduction: Moisture-enhanced beef products contaminated with *Escherichia coli* O157:H7 have been involved in human illness. Considering consumer preference for natural antimicrobials, their potential for use in meat enhancement solutions should be evaluated.

Purpose: This study screened various natural ingredients, alone or in combinations, for their potential to control *E. coli* O157:H7 in meat extract, in combination with common salt and phosphate.

Methods: Beef knuckles (100 g) were blended with sterile distilled water (200 ml), filtered through cheese cloth, and then the extract was dispensed in test tubes (10 ml). Sodium chloride (NaCl; 0.5%) and sodium tripolyphosphate (STP; 0.25%), commonly present in meat enhancement solutions, were added along with each of the following, alone or in combinations: cetylpyridinium chloride (CPC; 0.02% and 0.04%), sodium diacetate (SD; 0.25%), lactic acid (LA; 0.3%), thyme oil (TO; 0.01%, 0.05%, 0.1%, 0.25%, and 0.5%), grapefruit seed extract (GSE; 0.01%, 0.1%, 0.25%, 0.5%, and 1.0%), and basil essential oil (BO; 0.01%, 0.25%, and 0.5%). The samples were inoculated (3 log CFU/ml) with an 8-strain composite of rifampicin-resistant *E. coli* O157:H7 and incubated at 15°C for 48 h (2 replicates, 3 samples/treatment/replication). Counts were determined with tryptic soy agar (TSA) and TSA with rifampicin (100 µg/ml).

Results: At day-0, CPC (0.04%), TO (0.25% and 0.5%) and GSE (0.5% and 1.0%), alone or in combination with other ingredients, reduced *E. coli* O157:H7 by approximately 3 log CFU/ml, whereas BO (0.5%) singly or BO (0.5%) with SA reduced pathogen counts by 1 log-cycle after 48 h incubation. Additionally, SD, LA, GSE (0.25%), BO (0.1 and 0.25%), and TO (0.1%), alone or in combination with other ingredients, showed bacteriostatic effects against *E. coli* O157:H7 after the 48 h incubation period. The background flora was more resistant to the antimicrobials and/or grew faster than *E. coli* O157:H7; however it was found to be susceptible to TO (0.25% and 0.5%), GSE (0.5% and 1.0%), and BO (0.5%) alone or their combination with other ingredients, and CPC (0.04%) with BO (0.25%).

Significance: Ingredients such as grapefruit seed extract, basil essential oil, thyme oil, and cetylpyridinium chloride were effective against *E. coli* O157:H7 in meat brine solutions, and may be considered for potential use in non-intact meat products.

ABSTRACT ACCEPTED FOR ORAL PRESENTATION AT THE 96TH ANNUAL MEETING OF THE
INTERNATIONAL ASSOCIATION FOR FOOD PROTECTION
(JULY 12-15, 2009; GRAPEVINE, TX)

Title: Thermal Inactivation of *Escherichia coli* O157:H7 in Nonintact Beef Steaks of Different Thickness by Different Cooking Methods and Equipment

Authors: Cangliang Shen, Jeremy M. Adler, Ifigenia Geornaras, Keith E. Belk, Gary C. Smith and John N. Sofos

Introduction: Outbreaks of *Escherichia coli* O157:H7 from nonintact beef products have been attributed to inadequate cooking or cooking from the frozen state. Evidence has indicated that when cooking to a given internal temperature, pathogen lethality increases with steak thickness.

Purpose: This study compared thermal inactivation of *E. coli* O157:H7 in nonintact beef steaks of different thickness, cooked to an internal temperature of 65°C by three cooking methods and with different cooking equipment.

Methods: Fresh beef (5% fat) was coarse-ground and batches (1 kg) were mixed with a composite (50 ml) of rifampicin-resistant *E. coli* O157:H7 (8-strain, 6.4 ± 0.1 log CFU/g) and sodium chloride (0.45%) plus sodium tripolyphosphate (0.23%) solution (50 ml), extruded into casings (10 cm diameter), and placed at -20°C for 6 h. Semi-frozen beef was cut into 1.5, 2.5 and 4.0 cm thick steaks, vacuum-packaged, frozen (-20°C, 48 h), and tempered (4°C, 2.5 h) before cooking. Steaks were double pan-broiled (George Foreman[®] grill), pan-broiled (Presto[®] electric skillet and Sanyo[®] grill) or roasted (Oster[®] toaster and Magic Chef[®] kitchen oven) to 65°C. Temperatures of cooking equipment surfaces and meat samples were monitored with thermocouples. Samples were analyzed for survivors on tryptic soy agar plus sodium pyruvate (0.1%, TSAP) and TSAP plus rifampicin (100 µg/ml). Data (two replicates/three samples each) were analyzed using the mixed model of SAS including independent variables and interactions.

Results: The order of pathogen inactivation was roasting (2.0-4.2 log CFU/g) > pan-broiling (1.6-2.8 log CFU/g) ≥ double pan-broiling (1.2-2.3 log CFU/g). Cooking of 4.0 cm thick steaks required a longer time (20-65 min; variation due to different cooking equipment) and caused greater ($P < 0.05$) reductions in counts (2.3-4.2 log CFU/g) than in thinner samples. The time to reach the target temperature increased in order of George Foreman[®] grill (3.9-19.8 min) < Oster[®] toaster (11.3-45.0 min) < Presto[®] electric skillet (16.3-55.0 min) < Sanyo[®] grill (14.3-65 min) < Magic Chef[®] kitchen oven (20.0-63.0 min), with time differences within each equipment related to steak thickness.

Significance: Increased steak thickness allowed greater inactivation of *E. coli* O157:H7, as time to reach the target internal temperature increased. Roasting in a kitchen oven was most effective for pathogen inactivation.

ABSTRACT ACCEPTED FOR ORAL PRESENTATION AT THE 96TH ANNUAL MEETING OF THE
INTERNATIONAL ASSOCIATION FOR FOOD PROTECTION
(JULY 12-15, 2009; GRAPEVINE, TX)

Title: Translocation of *Escherichia coli* O157:H7 during Needle Injection for Moisture Enhancement of Meat

Authors: Shivani Gupta, Ifigenia Geornaras, Lawrence D. Goodridge, Kendra K. Nightingale, Keith E. Belk, Gary C. Smith, and John N. Sofos

Introduction: Outbreaks linked to consumption of nonintact meat products contaminated with *Escherichia coli* O157:H7 indicate that processing may introduce pathogens into the interior of the meat. Such contamination may survive if the product is undercooked.

Purpose: This study evaluated quantitative transfer of *E. coli* O157:H7 during moisture enhancement of beef under two contamination scenarios.

Methods: In the first contamination scenario, beef knuckles (approximately 4 kg) were surface-inoculated (5 log CFU/g) with nonpathogenic rifampicin-resistant *E. coli* O157:H7 (5-strain mixture). The meat was then injected, with a hand operated single-needle brine injector, either with sterile distilled water (control) or a brine solution (NaCl [5.5%] and sodium tripolyphosphate [2.75%]) at seven locations per knuckle. In the second contamination scenario, the water and brine solution were inoculated (3-4 log CFU/ml) with *E. coli* O157:H7 and these were used for needle injection. Knuckles were weighed before and after injection and percent increase in weight was determined. One core (8 cm diameter) sample per knuckle was excised parallel to the direction of needle injection using a coring device. Core samples were surface-decontaminated with hot water (80°C, 60 s), cooled (4°C, 15 min), and cut into six sections (1 through 6) of 1-cm (sections 1 through 3), 2-cm (sections 4 and 5), and 3- to 10-cm (section 6) thickness, while keeping the knife and cutting board sterile between different cuts. Sections were analyzed (2-3 replicates) for *E. coli* O157:H7 by plating on tryptic soy agar with rifampicin (100 µg/ml). The purge generated following injection of each knuckle was also analyzed for the pathogen.

Results: The percentage gain in product weight following injection with water or brine solution was 3-10%. The purge released following injection with water or brine solution had *E. coli* O157:H7 levels of 6.2 log CFU/ml (scenario 1) and 3.1-3.6 log CFU/ml (scenario 2). *E. coli* O157:H7 was recovered from all sections of water- and brine-injected samples under both contamination scenarios, with similar counts obtained for the water and brine treatments. Higher counts of the pathogen were obtained in all sections under scenario 1 (1.9-3.6 log CFU/g) than scenario 2 (0.6-1.6 log CFU/g). Within each contamination scenario, similar counts were obtained between all sampled sections.

Significance: Moisture enhancement of beef via needle injection can transfer *E. coli* O157:H7 to the interior of whole muscle cuts when either the meat surface or brine solution is contaminated with the pathogen. The data may be useful in risk assessments for nonintact meat products.

ABSTRACT ACCEPTED FOR ORAL PRESENTATION AT THE 96TH ANNUAL MEETING OF THE
INTERNATIONAL ASSOCIATION FOR FOOD PROTECTION
(JULY 12-15, 2009; GRAPEVINE, TX)

Thermal Inactivation of *Escherichia coli* O157:H7 at Different Depths of Panbroiled and
Roasted Non-intact Steaks

Jeremy M. Adler, Ifigenia Geornaras, Keith E. Belk, Gary C. Smith, and John N. Sofos

Introduction: *Escherichia coli* O157:H7 transferred to internal muscle tissue during tenderization is a potential cause of foodborne illness if the meat is undercooked.

Purpose: This study evaluated thermal inactivation of *E. coli* O157:H7 at different depths in non-intact steaks cooked by panbroiling or roasting to a sublethal temperature.

Methods: Fresh beef (*Semiteminosus*; 3% fat) was cut (3 mm thickness) and eight slices were stacked on each other and tenderized (4.8 blades/cm²) using a Supertendermatic™ hand-held tenderizer to form 50×60×24 mm non-intact steaks. Steaks were inoculated with rifampicin-resistant *E. coli* O157:H7 (8 strains; 3.7 log CFU/cm²) on the surface (0 mm) or between the slices at a depth of 3, 6, 9 or 12 mm. Each steak was vacuum-packaged, stored (4°C, 12 h) and cooked by panbroiling, turning every 2 min, or roasting at 149°C, to a geometric center temperature of 60°C. The temperature of the geometric center and depth of inoculation (3, 6, 9 or 12 mm) of each steak was recorded with thermocouples. After cooking, a core sample (1.61 cm²×24 mm) was excised from the center of each steak and analyzed for *E. coli* O157:H7 on tryptic soy agar containing 0.1% sodium pyruvate and 100 µg/ml rifampicin. The experiment was repeated twice with six samples per replicate. Data were analyzed by cooking method as a single factor (inoculation depth) ANOVA in Proc Mixed of SAS. Means were separated using a *F*-protected t-test

Results: Cooking times to reach a geometric center temperature of 60°C by panbroiling and roasting were 11.5±1.2 and 26.2±4.3 min, respectively. Higher internal temperatures were observed during cooking by panbroiling than roasting at 3 (88.0±7.3 vs 63.4±4.8°C), 6 (73.7±8.9 vs 59.6±10.0°C) and 9 (64.5±4.8 vs 60.5±2.1°C) mm depths of inoculation. Surviving pathogen populations increased (*P*<0.05) from 0.3±0.6 log CFU/cm² at 0 mm to 2.5±0.6 log CFU/cm² at 12 mm depth of inoculation in steaks cooked by panbroiling. For samples cooked by roasting, although a similar increasing trend in pathogen populations was observed, thermal destruction of *E. coli* O157:H7 was less and differences in pathogen populations were not significant (*P*≥0.05) at each depth of inoculation.

Significance: There was more thermal destruction throughout, higher internal cooking temperatures and shorter cooking times when undercooking non-intact steaks by panbroiling than roasting to the same internal temperature.