DISSERTATION

ESCHERICHIA COLI O157:H7 CONTROL IN NONINTACT MEAT PRODUCTS AND INHIBITION OF *LISTERIA MONOCYTOGENES* BIOFILMS ON KITCHEN SURFACES

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ABSTRACT

ESCHERICHIA COLI O157:H7 CONTROL IN NONINTACT MEAT PRODUCTS AND INHIBITION OF *LISTERIA MONOCYTOGENES* BIOFILMS ON KITCHEN SURFACES

The objectives of this dissertation included examination of quantitative internalization of *Escherichia coli* O157:H7 cells during moisture enhancement processing of beef under two contamination scenarios, extent of *E. coli* O157:H7 contamination transfer from surface-inoculated beef steaks to interior during blade tenderization and to a subsequently processed uninoculated steak, effectiveness of blade tenderizer sanitation to prevent cross-contamination during mechanical tenderization of beef steaks, survival of *E. coli* O157:H7 in beef steaks and roasts moisture-enhanced with brine formulations containing antimicrobials during frozen (-20°C) storage, survival of *E. coli* O157:H7 in moisture-enhanced beef steaks when cooked with three different consumer-style cooking methods to 60°C, and thermal inactivation of *E. coli* O157:H7 in moisture-enhanced beef roasts cooked to rare (60°C) and very rare degree (55°C) of doneness, and as well as evaluation of survival of *Listeria monocytogenes* on laminate kitchen countertop surfaces without/with nutrition supplementation (ham extract), on laminate and corian surface with nutrient supplementation, and comparison of the efficacy of four wiping materials (handi-wipes®, heavy-wipes®, kitchen-cloth®, and

paper-towel) in removal of *L. monocytogenes* from laminate surface under, dry (50% relative humidity) and humid (90% RH) environmental conditions.

In the first study, beef knuckles (approximately 4-5 kg) were surface-inoculated (4.7±0.3 log CFU/g) with nonpathogenic rifampicin-resistant *E. coli* O157:H7 (4-strain composite) in the first contamination scenario, and were injected with a hand-operated single needle brine injector, either with sterile distilled water (control) or brine solution of sodium chloride [NaCl; 5.5%] and sodium tripolyphosphate [STP; 2.75%] at seven locations per knuckle. In the second contamination scenario, the enhancement solutions (i. e., water and brine) inoculated (3.7±0.1 and 3.4±0.2 log CFU/ml, respectively) with the 4-strain *E. coli* O157:H7 composite were used for needle injection. The results of this study indicated that *E. coli* O157:H7 levels were translocated to the entire depth of beef knuckles after injection with water and brine injection solutions under both contamination scenarios. Higher pathogen levels were transferred to topmost 1-cm depth (3.0 and 3.6 log CFU/g) compared to levels transferred to deeper tissue (i. e., from 2-cm depth to the entire depth of an average total 14.5 cm depth) when beef knuckles were surface-inoculated (contamination scenario-1); however, pathogen levels (1.5 log CFU/g) recovered from deeper tissues (average total 13-cm depth) were higher by 0.9 -1.0 log CFU/g compared to topmost 1-cm depth when inoculated solutions were used (contamination scenario-2), irrespective of enhancement solution (water or brine).

In the second study, surface-inoculated 3-cm thick beef steaks (8-strain rifampicin-resistant *E. coli* O157:H7 composite, at contamination levels; high: 7.0 log CFU/g and low: 4.2 log CFU/g) were subjected to single-pass blade tenderization (hand-operated tenderizer with 48 blades) without or with prior tenderizer sanitation. The

sanitizer treatments (30 s exposure) evaluated included: (i) no treatment (control), ii) water at 25-30°C, (iii) water at 70°C, (iv) water at 94°C, (v) peroxyacetic acid (PAA)/hydrogen peroxide (HP)/octanoic acid (OA) (Vortexx™; 2500 ppm), (vi) PAA/HP (Oxonia Active®; 2500 ppm), and (vii) a sequential treatment (30 s each) of 94°C water followed by PAA/HP/OA. The results of this study showed that single-pass tenderization vertically transferred surface contamination of *E. coli* O157:H7 throughout the 3.0 cm thick inoculated steak (high: 3.5-5.4 log CFU/g and low: 1.3-2.9 log CFU/g), and horizontally transferred to the surface (high: 5.8 log CFU/g and low: 2.9 log CFU/g) and interior (high: 2.2-4.2 log CFU/g and low: 0.7-1.5 log CFU/g) of the subsequently tenderized uninoculated steak. The effectiveness of sanitation treatments to reduce transfer of contamination for high (7.0 log CFU/g) surface contamination level increased in the order: water at 25-30°C < PAA/HP/OA = PAA/HP < 70°C water = 94°C water = 94°C water followed by PAA/HP/OA.

In the third study, restructured beef steaks (2.5-cm thick) were inoculated (6 log CFU/g) with rifampicin-resistant *E. coli* O157:H7 (8-strain composite) and moisture-enhanced (110%, wt/wt) with four brine formulations: sodium chloride (NaCl, 0.5%)+sodium tripolyphosphate (STP, 0.25%), NaCl+STP+cetylpyridinium chloride (CPC, 0.2%), NaCl+STP+lactic acid (0.3%), or NaCl+STP+sodium metasilicate (0.2%). Beef steaks were stored in vacuum-packaged frozen (-20°C) for 30 days. Beef steaks were cooked to internal temperatures of 60°C by pan-broiling (Presto®electric skillet), double pan-broiling (George Foreman®grill) or roasting (Magic Chef ®standard kitchen oven) on day-0 and day-30. The results of this study indicated that only cetylpyridinium chloride was effective as an antimicrobial agent when included in the brining formulation

at 0.2% concentration (wt/wt in final product) along with salt and phosphate, reducing *E. coli* O157:H7 populations by 0.5 logs in beef steaks stored under frozen conditions for 30 days. Double-panbroiling was more the effective compared to panbroiling and roasting, with *E. coli* O157:H7 reductions ranging from 2.5 to 4.5, 1.3 to 1.9 and 0.8 to 2.0 log CFU/g, respectively, in 2.5 cm beef steaks when cooked to the internal temperature of 60°C. However, cooking of 2.5 cm beef steaks with roasting (23.3-27.5 min) took the longest time to reach the target internal temperature followed by pan-broiling (14.5-25.0 min), and then double pan-broiling (4.2-6.4 min) which took least time.

In the fourth study, beef roasts (2 kg) were inoculated (6 -7 log CFU/g), moisture-enhanced with four brine formulations, and stored under frozen conditions, as described for the beef steaks study. Uncooked beef roasts were analyzed microbiologically on day-0 and -30 to determine effect of antimicrobials on *E. coli* O157:H7 population during storage under frozen conditions. Thawed roasts (4°C, 48-72 h) were cooked in a conventional kitchen oven (at 176.7°C) to the internal temperatures of 60°C (rare) or 55°C (very-rare) on day-0 (approximately 24 h after preparation), and on day-30 after frozen (-20°C) storage and 48-72 h of thawing. A total six subsamples from six locations (center top, center middle, center bottom, side top, side middle, and side bottom) from each cooked roasts were analyzed to determine thermal inactivation of *E. coli* O157:H7 at these specific locations. *E. coli* O157:H7 counts in uncooked roasts treated with CPC were 0.7-2.4 log CFU/g lower than those of the roast samples treated with control (i e., NaCl+STP) brining formulation. *E. coli* O157:H7 counts were undetectable (<0.5 log CFU/g) in 50.0 and 40.6 % of samples, from a total 32 roasts cooked to 60 or 55°C,

respectively. Survivors of <0.5-5.4 log CFU/g and <0.5-5.2 log CFU/g were obtained from subsamples of the remaining roasts cooked to 60 or 55°C, respectively.

In the last study, samples of laminate (2.5×4 cm) and corian (4.5×4.5 cm) kitchen countertop top surfaces were inoculated (5 log CFU/cm^2) with a 5-strain mixture of *L. monocytogenes* and incubated (25±2°C; 96 h) at 50 and 90% relative humidity (RH). Coupon surfaces received ham homogenate (0.1 ml) on inoculated areas every morning and evening simulating exposure to nutrients during food preparation. Surviving *L. monocytogenes* cells were recovered with kimwipes from laminate and corian surfaces, and laminate surfaces were first cleaned with handi-wipes®, heavy-wipes®, kitchen-cloth® or paper-towel, and then leftover cells were recovered with kimwipes, at 0, 6, 24, 48, 72, and 96 h sampling points. *L. monocytogenes* cells without nutrient supplementation were not detected (< below detection limit; i. e., 0.3 log CFU cm^2) from laminate surfaces after 3 days (72 h) and 4 days (96 h) under dry (50%) and humid (90%) environmental conditions respectively, indicating total reductions of 5.4 log CFU/cm^2. *L. monocytogenes* cells survived on laminate and corian kitchen counter surfaces (0.5-1.1 and 0.1-0.9 log CFU/cm^2) for 4 days (96 h) under both environmental (dry 50% and humid 90% RH) conditions. All four wipes were able to remove *L. monocytogenes* cells from the laminate surfaces for the entire length of storage (96 h), and were most efficient (5.0-6.2 log CFU/cm^2) for pathogen removal immediately after contamination (0 h), under both environmental conditions. Highest levels of left over *L. monocytogenes* (2.7-3.8 log CFU/cm^2) cells were recovered from these surfaces after cleaning with each wipe type, immediately after contamination, under both environmental conditions

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DEDICATION

I am really honored to have wonderful family specially my father who gave me the moral support and encouragement through my studies. I dedicate this dissertation to my parents for instilling the importance of hard work and higher education.

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CHAPTER 1

INTRODUCTION

Nonintact meat products such as moisture-enhanced/mechanically tenderized steaks (CFP, 2008), roasts (Rodrigue et al., 1995) and donair (a mixture of ground beef and spices, Currie et al., 2007), contaminated with *Escherichia coli* O157:H7, a foodborne pathogen, have been linked with several outbreaks and recalls (CFP, 2008; Currie et al., 2007; Rodrigue et al., 1995). Following three outbreaks in August 2000, June 2003, and August 2004 due to *E. coli* O157:H7 associated with mechanically tenderized and/or moisture enhanced steaks (Lain et. al., 2005; USDA-FSIS 2005). The Food safety and Inspection Services (FSIS) published a notice requiring beef processors to reassess their Hazard Analysis Critical Control plans (HACCP) for raw and cooked mechanically tenderized beef products, including products that are injected with a marinade (or "enhanced" products)" (USDA-FSIS, 2005). *E. coli* O157:H7, present on the external surface of meat cuts, may be internalized during mechanical tenderization/enhancement of nonintact meat products. Translocation of *E. coli* O157:H7 into sterile deep tissue during mechanical tenderization is well documented and cells are usually transferred to the top 5 cm (Gill and McGinnis, 2005; Luchansky et al., 2008; Sporing, 1999).

The interior of moisture-enhanced meat cuts may be contaminated either by translocation of surface pathogens or from contaminated brine solutions injected into meat while processing. Uttaro and Aalhus (2007) found that pathogenic cells may spread

throughout a primal during the brine injection procedure. Brine solutions are either re-circulated, refrigerated or treated to ensure quality during meat processing in industry (Anonymous, 2005). Greer et al. (2004) reported that 2.34 log CFU/100 ml *L. monocytogenes* recovered from the brine after 2.5 h of recirculation during moisture-enhancement of pork. Gill et al. (2005) also reported increases in count of aerobic bacteria in brines after 30 or 60 min of pork processing. Thus, it would be interesting to evaluate transfer of *E. coli* O157:H7 to different depths of beef primal cuts during injection when the pathogen is present on the surface compared to the injection brine is contaminated.

Cross-contamination at different stages of meat processing is another important contributing factor to the contamination of nonintact meat products with pathogens (Reij and Aantrekker, 2004). Blades of the tenderizer, not only can translocate contamination from external steak surface, but can also transfer pathogen cells and cross-contaminate subsequent steaks during mechanical tenderization (Chorianopoulos et al., 2009). Gill and McGinnis (2005) isolated high levels of total bacterial counts from blades of a commercial blade tenderizer. Raccach and Henderson (1979) indicated that unsanitary conditions during processing of nonintact meat products can turn the tenderizer into an "inoculating machine," resulting in a contaminated product. Proper cleaning and sanitation of mechanical tenderizers is considered an important factor for prevention of contamination of nonintact meat products during processing (Sofos et al., 2008). Effectiveness of different commercially available sanitizers to kill/reduce *E. coli* O157:H7 on the blades of mechanical tenderizers to prevent cross-contamination during tenderizing processing needs to be studied.

The internalized pathogenic cells after mechanical tenderization and/or enhancement of nonintact meat products could survive cooking especially if products are undercooked intentionally or unintentionally (Sofos et al., 2008; Obuz et al., 2004). The Food Code (FDA, 2005) recommends cooking of nonintact beef products to an internal temperature of 68.3°C (155°F). According to USDA-FSIS (2006) cooking of nonintact beef products to internal temperature of 76.7°C can significantly decrease the risk from *E. coli* O157:H7 associated with these products. However, studies have indicated that consumers prefer their steaks cooked to a medium degree of doneness or internal temperatures of 60.0 to 62.8°C (140-145°F) (Cox et al., 1997; Schmidt et al., 2002).

Thickness of steaks affects the thermal inactivation of pathogens as higher thermal inactivation can be achieved in thicker steaks, when contaminated products of different thicknesses are cooked to the same internal temperatures (Sporing, 1999; Shen et al., 2010). Different types and sizes of nonintact meat products are sold in the North American market. Roasts are large cuts for multiple serving at big gatherings, while steaks are small cuts of single-serving for individual consumption. Roasts are much thicker cuts and usually cooked for longer times (USDA-FSIS, 2002b). Different extents of thermal inactivation of *E. coli* O157:H7 were achieved when nonintact meat products were cooked in different cooking appliances, even though cooked to the same internal temperatures (Sporing, 1999; Shen et al., 2010). This could be due to different temperature-time profiles achieved by each cooking method (Mukherjee et al., 2008). Broiling, grilling, and roasting are common methods for cooking of steaks with different commercially available cooking appliances and, roasts are usually cooked by braising and roasting in the kitchen oven (USDA-FSIS, 2002b).

Different kinds of tenderizing, marinating, and antimicrobial agents are included in brining formulations for specific purposes and these agents have been reported to influence thermal inactivation of internalized *E. coli* O157:H7 during cooking of moisture-enhanced nonintact meat products (Byelashov et al., 2010; Juneja et al., 1999; Mukherjee et al., 2008, 2009; Yoon et al., 2009). Based on the above, it is imperative to consider different factors, when estimating the extent of thermal inactivation of internalized *E. coli* O157:H7, during cooking of nonintact meat products in the domestic and commercial environment. Thus, it is needed to study the effect of brining formulations on survival/thermal inactivation of *E. coli* O157:H7, during storage and cooking (different cooking methods using different appliances), in two different moisture-enhanced beef products (roasts/steaks).

L. monocytogenes can enter into the domestic kitchen with contaminated food products including ready-to-eat (RTE) meat products. Certain RTE meat products are classified as high risk for listeriosis because these products support growth of this pathogen. Moreover, this pathogen has the potential to grow at refrigerator temperatures. Thus, it may grow to dangerous levels if contaminated RTE meat products are stored under refrigeration conditions for long periods of time. Studies have isolated *L. monocytogenes* from household dishcloths, washing-up brushes, drains and different parts of domestic refrigerator surfaces (Azevedo et al., 2005; Beumer et al., 1996; Cox et al., 1989; Duggan and Phillipis, 1998; Sergelidis et al., 1997). Kitchen countertops are used for different tasks in the domestic kitchen. These surfaces can become contaminated with pathogens either by direct contact with contaminated food products and various objects, or indirectly through airborne particles (Kusumaningrum et al., 2003a). Once

these food contact surfaces become contaminated with pathogens, cells can adhere, survive and grow especially in the presence of food residues and debris under soiled conditions, and become sources of cross-contamination. Laminate and Corian materials are inexpensive and most widely used for furnishing countertops in the domestic kitchen. Difference in attachment ability of *L. monocytogenes* to kitchen countertop surfaces furnished with different materials would be interesting to investigate. The relative humidity (RH) represents moisture content in air and varies by region and season. The relative humidity has been reported to affect attachment of cells to different surfaces (Kim et al., 2008). The average home in different regions of the United States could have different levels of relative humidity according to website records of City Rating (http://www.cityrating.com) (Yang et al., 2009a). Studies are thus, needed to compare survival of *L. monocytogenes* on different kitchen countertop surfaces (laminate and corian) under different environmental conditions (dry - 50% RH and humid - 90% RH), in the presence of nutrients.

Nutrient availability can affect survival of attached pathogen cells to different surfaces. Yang et al. (2009a) reported that supplementation of *L. monocytogenes* cells with 10-fold-diluted tryptic soy broth containing 0.6% yeast extract helped to maintain viability of cells on High Density Polyethylene (HDPE) surfaces for at least 6 days. In another study, Parikh et al. (2009a) found that repeated exposure of HDPE and Polyethylene (PE) surfaces to nutrients helped *L. monocytogenes* to survive in multi-species biofilms for, up to 21 days at 25°C. Thus, studies are needed on the survival of *L. monocytogenes* attached to laminate kitchen countertop surfaces, without and with nutrient supplementation, under different environmental conditions. Testing survival of *L.*

monocytogenes with nutrient supplementation will simulate a scenario in the domestic kitchen, where kitchen countertop surfaces left unclean after meal preparation and leftover food after meal preparation could provide nutrients and enhance survival of attached pathogen cells. Testing survival of *L. monocytogenes* without nutrient supplementation would simulate another scenario in the domestic kitchen, where unused kitchen countertop surfaces are left unclean for long periods of time.

Pathogen cells present on these contaminated surfaces can attach irreversibly and form biofilms in the presence of limited nutrient supply. Contaminated food contact surfaces in the domestic kitchen if not cleaned before subsequent use can lead to cross-contamination of clean food products. Prevention of cross-contamination of products and other food contact surfaces is critical for minimizing risk of foodborne illnesses in the domestic environment. The potential for cross-contamination in the domestic kitchen can be reduced with proper cleaning and sanitation (Yang et al., 2009a). It would be interesting to know the extent of contamination removal by wiping contaminated surfaces like kitchen countertops.

The "Fight BAC" program recommends using fresh paper towels to clean kitchen surfaces and if cloth towels are used, consumers should wash them often in the hot cycle of washing machines (Food Code, 2001; FDA, 2006). Different materials available in the market for wiping of kitchen countertops differ in texture. It would be interesting to investigate the in cleaning efficacy of these materials for removal of pathogen cells from food contact surfaces. The overall goal of the five studies in this dissertation was to evaluate internalization of *E. coli* O157:H7, blade tenderizer sanitation for cross-contamination prevention during tenderization, effects of antimicrobials and thermal

intervention for control of *E. coli* O157:H7 in moisture-enhanced beef products, and control of *L. monocytogenes* in the home environment. The following objectives were addressed in five different studies to achieve these goals:

- Study-1: Evaluate quantitative transfer of *E. coli* O157:H7 during moisture enhancement processing of beef when contamination present on the surface (contamination scenario-1) and/or when a contaminated brine solution is injected (contamination scenario-2).
- Study-2: Evaluate horizontal and vertical transfer of *E. coli* O157:H7 during tenderization processing and effectiveness of six sanitation treatments for prevention of cross-contamination of *E. coli* O157:H7 during mechanical tenderization of steaks.
- Study-3: Investigate survival of *E. coli* O157:H7 in restructured beef steaks moisture-enhanced with brine formulations containing antimicrobials (i.e., cetylpyridinium chloride, lactic acid or sodium metasilicate) during frozen (-20°C, 30 days) storage and potential effect of these brine formulation ingredients on thermal destruction of *E. coli* O157:H7 in moisture-enhanced beef steaks, when cooked with three cooking methods (i.e., pan-broiling, double pan-broiling, and roasting) to an internal temperature of 60°C
- Study-4: Investigate effectiveness of cetylpyridinium chloride, lactic acid or sodium metasilicate antimicrobials on survival of *E. coli* O157:H7 in moisture-enhanced beef roasts that were stored under vacuum-packaged frozen conditions (-20°C) for 30 days and thermal inactivation of *E. coli* O157:H7 at six different locations of two-kilogram beef roasts, moisture-enhanced with four different brining formulations, when cooked to two degrees of doneness (60°C: rare and 55°C: very-rare) in a conventional kitchen

oven with consumer-style cooking, on day-0 (approximately 24 h after preparation) and, on day-30 of storage under frozen (-20°C) conditions, and after 48-72 h thawing.

- Study-5: Survival of *L. monocytogenes* on Laminate and Corian kitchen countertop surfaces exposed to dry (50% RH) and humid (90% RH) environmental conditions, in the presence of nutrients; survival of *L. monocytogenes* on laminate coupon surfaces, without and with nutrients at two different environmental conditions (50 and 90% RH); and efficacy of wiping materials (handy wipes®, heavy wipes®, kitchen cloth® and paper towel) for *L. monocytogenes* removal from artificially inoculated laminated kitchen countertop surfaces under two environmental conditions (50 and 90% RH).

CHAPTER 2

LITERATURE REVIEW

2.1. Contamination and control of *E. coli* O157:H7 in nonintact meat products

Meat quality characteristics, such as tenderness, are important for palatability and consumer preference. Lower quality meat cuts are tenderized or moisture-enhanced for tenderness improvement.

2.1.1. Microbiological safety concerns for nonintact meat cuts

Nonintact beef products, as defined by USDA-FSIS (1999b), include ground beef, beef injected with solutions, beef that has been mechanically tenderized by needling, cubing, frenching, or pounding devices, and beef that has been reconstructed into formed entrees. Eighteen percent of beef products sold at the retail level in North America were either mechanically tenderized or enhanced or subjected to both treatments (Anonymous, 2005). *E. coli* O157:H7 was declared an adulterant in raw ground beef (USDA-FSIS, 1994). In 1999, USDA-FSIS announced that *E. coli* O157:H7 should also be considered an adulterant for intact raw beef products including trimmings further processed into nonintact products (USDA-FSIS, 1999b). An outbreak of *E. coli* O157:H7 (August, 2000) was linked to contaminated mechanically tenderized steaks sold at a local steakhouse restaurant in Michigan. Investigation carried out by the Michigan Department of Community Heath (MDHC) found that poor sanitation practices during processing

would have led to contamination of culprit steaks (USDA-FSIS, 2005). Another multistate outbreak from *E. coli* O157:H7 (June, 2003) was traced back to vacuum packed frozen steaks injected with marinades and sold by door-to-door vendors. This outbreak led to a recall of 739,000 lb (335,506 kg) of frozen beef products (Laine et al., 2005). The establishment producing this meat was cleaning and sanitizing the injectors once per week. Another outbreak due to *E. coli* O157:H7 in Denver, Colorado (2004), investigated by the Colorado Department of Public Health and Environment (CDPHE), was linked to tenderized marinated beef steak (USDA-FSIS, 2005). Insufficient sanitation of enhancing/tenderizing equipment during processing was considered as the most plausible cause of contamination of nonintact meat products and led to these three outbreaks (August 2000, June 2003, and August 2004) (Engeljohn, 2005). USDA-FSIS (2005) published a notice requiring beef processors to reassess their Hazard Analysis and Critical Control points (HACCP) plan for raw and cooked mechanically tenderized beef products, including products that are injected with a marinade (or "enhanced" products)".

Injected or mechanically tenderized nonintact meat contaminated with *E. coli* O157:H7 were linked to another three outbreaks in the year 2007 after implementation of changes in HACCP plans as requested by regulatory agencies (CFP, 2008; USDA-FSIS, 2007). These outbreaks again raised concerns about the microbiological safety of nonintact meat products. Two outbreaks due to *E. coli* O157:H7, in California and Michigan, were associated with needle injected or mechanically tenderized meat product, respectively (CFP, 2008). The third outbreak due to *E. coli* O157:H7 in Pennsylvania was associated with contaminated steaks served at a family chain restaurant, led to several

illnesses, and the recall of 259,230 pounds meat, produced at a South Claysburg, Penn., firm plant (USDA-FSIS, 2007).

2.1.2. Pathogenesis of *E. coli* O157:H7

E. coli O157:H7 is a virulent human pathogen and symptoms associated with illnesses due to this pathogen are abdominal pain, vomiting, bloody diarrhea, hemorrhagic colitis (HC), and hemolytic uremic syndrome (HUS) (Nataro and Kaper, 1998). Hemorrhagic colitis is a clinical syndrome consists of abdominal cramps; diarrhea that progresses to become bloody; colonic mucosal edema, erosion, or hemorrhage (Riley, 1987). Complications associated with HUS are thrombocytopenia, microangiopathic hemolytic anemia and acute renal failure due to toxin (Dorn, 1993). Healthy patients can recover from HUS symptoms with hospitalization and supportive care but these symptoms can be life threatening in infants, children, the elderly and immunocompromised people like AIDS and cancer patients (Paton et al., 1993). Cattle are considered as the reservoir of *E. coli* O157 (Al-Saigh et al., 2004; Chapman et al., 2001; Hancock et al., 1994) and nonO157 STEC (Barkocy-Gallagher et al., 2003; Geue et al., 2002; Schurman et al., 2000).

2.1.3. Processing of nonintact meat cuts

Intact meat is converted into nonintact meat products using blades, by pounding or injecting brine or marinade solutions. Mechanical tenderization is usually carried out with single or multiple pass blade tenderizers. Banks of sharp and double-edged blades penetrate meat, pierce muscle tissue and fibers, and break muscle integrity (Ross

Industries, 1998). Mechanical tenderization is usually carried out at central cutting or retail store facilities (Gill et al., 2005).

Lower valued muscle cuts are moisture-enhanced with brine solutions containing salt, sodium polyphosphates and flavoring agents to enhance water-holding capacity, yield and improve eating quality like juiciness and flavor (Zheng et al., 1999). Brine and marinade solutions are pumped into whole muscle with hollow-needles during moisture-enhancement. Brines are usually injected into meat with multi-needle automatic stitch-type injection equipment under pressure. Streaking or striping of moisture-enhanced meats after injection is a problem encountered by meat processors (Gooding et al., 2009; Knight and Parsons 1988; Voyle et al., 1986). Uttaro and Aalhus (2007) found that brine gets distributed parallel to muscle fiber and thawing rate did not influence the distribution path of brine solutions in muscles. In a continuation of this study, Gill et al. (2008) conducted a study to determine distribution pattern of *L. innocua* (10^9 CFU/g) when present in brine solution during moisture-enhancement. *L. innocua* was distributed along oriented muscle fibers if muscle is not blade tenderized before injection. However, bacteria get distributed in unoriented groups in damaged muscle fiber areas if muscle is blade tenderized before injection.

The distribution pattern of brine from the injection site, along muscle fiber long axes, has been reported in various studies (Gooding et al., 2009; Swatland, 2004; Uttaro and Aalhas, 2007; Voyle et al., 1986). Internal structures of meat, like connective tissue, could restrict the brine flow inside the meat. Freezing and thawing of meat could injure the internal muscular cellular structure, because of ice crystal formation, and possibly the uptake and brine distribution in muscles. Boles and Swan (2002) reported higher brine

uptake by thawed meat, as compared to fresh meat, when injection settings were kept constant. However, a study conducted by Uttaro and Aalhas (2007) did not find significant differences in brine distribution inside fresh, slowly and rapidly thawed muscles.

The effect of different brining components on the physiochemical properties of moisture-enhanced meat has been studied extensively (Bendall, 1954; Baublits et al., 2005, 2006; Eiler et al., 1994; Kerth et al., 1995; Morris et al., 1997; Offer and Trinick, 1983; Pringle et al., 1999; Scanga et al., 2000; Wheeler et al., 1993). Both salt and phosphate act synergistically in brine solutions and cause maximum myofibrillar swelling (Offer and Trinick, 1983). The effect of phosphates like sodium hexametaphosphate (SHMP), sodium tripolyphosphate (STPP), or tetrasodium pyrophosphate (TSPP) without or with sodium chloride on the physiochemical properties of moisture-enhanced beef muscles has been studied (Baublits et al., 2005, 2006). Examples of some chemicals included in brine solutions are: calcium chloride (Eiler et al., 1994; Kerth et al., 1995; Morris et al., 1997; Pringle et al., 1999; Scanga et al., 2000; Wheeler et al., 1993); calcium lactate (Lawrence et al., 2004); sodium acetate (Knock et al., 2006 a, b); sodium lactate (Vote et al., 2000); sodium chloride (Vote et al., 2000); potassium lactate (Knock et al., 2006 a, b; Vote et al., 2000); natural tenderizing enzymes - Ficin, Bomelain and Papain (Calkins and Sullivan, 2007).

2.1.4. Contamination of nonintact products with *E. coli* O157:H7 during processing

The external surface of meat cuts can be contaminated with *E. coli* O157:H7 during the slaughtering and the cutting process. Different interventions are implemented

on beef carcasses during the slaughtering process to reduce/eliminate *E. coli* O157:H7 contamination (Sofos and Smith, 1998). Microbiological samples collected from muscle cuts from different slaughtering facilities in North America were found to have very low prevalence of *E. coli* O157:H7 (Heller et al., 2007; Kennedy and Bodnaruk, 2004, 2005). Heller et al. (2007) found 0.2% samples positive with *E. coli* O157:H7 (levels 0.375CFU/cm^2) out of 1,014 subprimal cuts collected from six beef processing plants over a five-week period in the United States. Kennedy and Bodnaruk (2004, 2005) found that the overall incidence of *E. coli* O157:H7 on beef subprimals was less than 0.083% during the winter (January and February) and the late summer and fall (August into November) months from the Southern Midwest, Northern Midwest and the Southeast areas of the United States.

The tenderization process can break the integrity of muscle fibers and translocate surface contamination into deeper muscle tissue (Gill and McGinnis, 2004; Hajmeer et al., 2000; Luchansky et al., 2008; Sporing, 1999). Cells primarily transferred to the topmost 1 cm thick section of muscle tissue after blade tenderization process (Luchansky et al., 2008; Sporing, 1999). Sporing (1999) found that approximately 3 to 4% of surface inoculated *E. coli* O157:H7 can be transferred to the interior of muscle tissue during single-pass blade tenderization. The number of bacteria recovered from deep tissue was significantly ($P < 0.05$) affected by the number of times the surface was incised and the depth of tissue below the incised surface (Gill and McGinnis, 2005). Sporing (1999) also found that the concentration of cells translocated inside beef knuckle, decreased with increasing depth of blade penetration. Another study reported similar results and found that translocation of surface inoculated *Salmonella* to the interior of blade-tenderized and

needle-injected pork loins decreased progressively with depth of penetration of the blades or needles (Thippareddi et al., 2000). Luchansky et al. (2008) found that number of times the tenderization blade passed through meat did not affect ($P \geq 0.05$) the levels of the pathogen transferred into each of the six segments, whether tenderization process were done from the inoculated fat- or lean-side of beef subprimals. However, studies (Luchansky et al., 2008; Gill and McGinnis, 2005) have found that higher surface contamination levels led to higher deeper tissue contamination during mechanical tenderization. This could be expected because as more bacterial cells were available on the surface, higher numbers could be transferred into the deep tissue.

2.1.5. Control of *E. coli* O157:H7 in nonintact meat products

Proper cleaning and sanitation of mechanical tenderizers is considered one of the important factors to prevent cross-contamination of nonintact meat products during mechanical tenderization processing (Sofos et al., 2008). Different commercially available sanitizers that can be considered for sanitation of blade tenderizers are: water at room temperature (rinsing), hot water, sodium hypochlorite (SH), quaternary ammonium compound (Quat), peroxyacetic acid (PA), peroxyacetic acid/octanoic acid mixture (PA/OA), warm lactic acid, acetic acid, acidified sodium chlorite, acidified chlorine, acidified lactoferrin (King et al., 2005; Ransom et al., 2003; Stopforth et al., 2003; Sofos et al., 2008). Peroxyacetic (14%, active ingredients) at concentrations of 250 and 1000 mgl^{-1} (pH 3.6 and 3.2, respectively) significantly reduced (1.38-1.40 log CFU/cm^2) counts of *E. coli* O157:H7 attached to stainless steel (Farrell et al., 1998). Peroxyacetic acid/octanoic acid mixture (PA/OA, 2600 ppm) lowered populations of *E. coli* O157:H7

attached to stainless steel coupons to the detection limit (from 3 to 0.6 log CFU/cm^2) after 1 min exposure (Adler et al., 2009b). Peroxyacetic acid is effective against microorganisms on food contact surfaces at low temperatures and in presence of organic matter (Chmielewski and Frank, 2003). Inspexx™ 200 is a 0.02% peroxy acid solution (EcoLab) for red meat surfaces. Ransom et al. (2003) found that 0.02% (from 5% solution) of peroxyacetic acid solution reduced populations of *E. coli* O157:H7 by 1.0-1.4 log inoculated onto beef carcass tissue under laboratory conditions. However, Gill and Badoni (2004) found that a 0.02% solution of peroxyacetic acid was not effective against *E. coli* O157:H7 on meat surfaces in a commercial setting.

Salt and phosphate are the most common ingredients in brine solutions, at levels of 0.25 - 0.50% and 0.25 - 0.45% in the final product, respectively. Government regulations recommend not to exceed concentration of 0.50% phosphate in brine solutions (USDA-FSIS, 2002a). Cetylpyridinium chloride (CPC) is a quaternary ammonium compound, used as an antimicrobial agent in mouthwashes (Ashley et al., 1984). Aqueous solution of CPC, as a fine mist spray, is approved by the government to be applied on poultry carcasses prior to immersion in chilling solutions (USDA-FSIS, 2002a). CPC has been found to be effective in reducing the microbial load on beef carcass surfaces (Bosilevac et al., 2004; Cutter et al., 2000). AvGard®XP has an active ingredient sodium metasilicate and is approved to be used in marinades for raw meat and poultry products and/or can be used on raw beef carcasses, subprimals and trimmings in solutions not exceeding 6.0% concentration (USDA-FSIS, 2002a). Carlson et al. (2008 a, b) found that sodium metasilicate was effective in reducing populations of *E. coli* O157:H7 on beef hides. Heller et al. (2007) found that treatment of inoculated surfaces of

beef cuts with lactic acid before tenderization or moisture enhancement was effective in reducing levels of *E. coli* O157:H7. Levels decreased to below the detection limit (1.3 log CFU/ml) immediately (0 h) in fresh and recirculated brines containing CPC or AvGard®XP or lactic and acetic acid at 4 and 15°C treatments (Adler et al., 2009a).

Nonintact meat products are either cooked to rare (60°C or 145°F); medium rare (65°C or 160°F) or well done (70°C or 170°F) states (Obuz et al., 2004). Cox et al. (1997) conducted a study to find the effect of degree of doneness of beef steaks on consumer acceptability of meals in restaurants and reported that a higher percentage of people (53%) preferred their steaks cooked medium to medium rare whereas only 4.5% of consumers ordered steaks cooked rare. Schmidt et al. (2002) also reported that steaks cooked to medium rare degree of doneness, needed temperatures of 60.0 to 62.8°C (140 to 145°F), which is preferred by consumers compared to steaks cooked to rare or an internal temperature of 54.4 to 57.2°C (130 to 135°F) or very rare to an internal temperature of 48.8 to 51.6°C (120 to 125°F). Consumers regard nonintact meat products as intact and may cook only the surface tissue to recommended temperatures without raising the temperatures of all deep tissues to required levels (Kastner et al., 2001). Because, contamination is usually translocated into the interior of meat products during the tenderization process, time-temperature combinations usually considered optimum to kill pathogen cells present on the surface, may not be sufficient to kill pathogens present in the interior of meat products. This may compromise the safety of nonintact meat products (Sofos et al., 2008). Studies (Gill et al., 2005; Luchansky et al., 2009) have been conducted to provide recommendations for cooking of nonintact meat products sufficiently to kill contamination present in deeper tissues. Gill et al. (2005) found that

aerobic bacteria recovered from deeper tissue of moisture-enhanced pork were reduced to 1.2 log CFU/25g (initial levels 2.1 log CFU/25g) after cooking of moisture-enhanced pork to internal temperature of 61°C and no bacteria were recovered when cooked to 70°C. The findings under the conditions of this study suggested that moisture-enhanced pork cooked to a medium rare condition can be microbiologically safe. The final internal temperature that must be achieved for blade-tenderized steaks, comminuted and injected meats, which are all considered nonintact, is 155°F (68°C) for 15 seconds (Food Code, 2005).

Steaks are usually prepared using a variety of cooking methods like grilling, roasting, broiling or frying before consumption at the food service or household level. Cooking appliances include: electric skillet, conventional kitchen oven, forced-air convection oven, gas grilling, George Foreman Grill, electric broiler etc., are used for cooking of different steaks at the consumer level (Lawrence et al., 2001; Shen et al., 2010; Sporing 1999; USDA-FSIS 2002b). These appliances employ different principles of heating. Sporing (1999) investigated the difference in thermal inactivation of *E. coli* O157:H7 in blade-tenderized steaks cooked with three different appliances (oven broiling, electric skillet, and commercial gas grilling). Cooking of nonintact products with an electric skillet was found to be least effective in reducing pathogen levels, not achieving a 5-log reduction of *E. coli* O157:H7 even at 170°F (76.7°C). Oven broiling of contaminated blade-tenderized steaks to 140°F (60°C) achieved a 5-log reduction of *E. coli* O157:H7 and was found to be the most effective cooking method. A 5-log reduction of *E. coli* O157:H7 was also achieved with commercial gas grilling when nonintact products were cooked to the internal temperature of 150°F (65.6°C) (Sporing 1999). Shen

et al. (2010) compared thermal inactivation of *E. coli* O157:H7 in moisture-enhanced restructured beef steaks cooked to the internal temperature of 65°C with different methods: double pan-broiled (George Foreman® grill), pan-broiled (Presto® electric skillet and Sanyo® grill) or roasted (Oster® toaster and Magic Chef® kitchen oven). The order of pathogen inactivation was roasting (2.0 to 4.2 log CFU/g) > pan-broiling (1.6 to 2.8 log CFU/g) ≥ double pan-broiling (1.2 to 2.3 log CFU/g). The results of a study conducted by Mukherjee et al. (2007) were similar to those of the above studies and found that broiling achieved more thermal inactivation of *E. coli* O157:H7 in restructured beef products compared with grilling or frying when cooked to the same internal temperatures.

Different thermal inactivation profiles of pathogenic bacterial cells with different cooking methods could be due to different temperature-time profiles achieved by each cooking method (Mukherjee et al., 2008). There was considerable variability in the temperature delivered to the product when a skillet was used for cooking, and more even distribution of heat with oven broiling (Sporing, 1999). The higher thermal inactivation of pathogen cells with broiling as reported by many studies (Mukherjee et al., 2007; Oretga-Valenzuela et al., 2001; Shen, 2010; Sporing, 1999) could be due to longer times needed to reach internal temperatures and higher increases of product temperatures near the surface as compared to grilling and frying. Additionally, different heat transfer methods are used by each cooking method. Roasting is done in closed preheated ovens and heat transfer is usually carried out by convection. Heat is transferred through meat product and cooking surfaces with conduction during pan-broiling used for grilling and frying (AMSA, 1995). Thickness of steaks is another factor that influences inactivation of pathogen cells during cooking. Sporing (1999) compared thermal inactivation of *E. coli*

O157:H7 in steaks of different thicknesses (3.2 cm vs. 1.3 cm). Higher thermal inactivation of *E. coli* O157:H7 was achieved in thicker steaks (3.2 cm vs. 1.3 cm) when cooked to the same target internal temperature. Shen et al. (2010) also compared thermal inactivation of *E. coli* O157:H7 in steaks of three different thicknesses (1.0, 2.5 and 4.0 cm). They also found that thicker (4.0 cm) steaks had greater ($P < 0.05$) reductions in counts (2.3 to 4.2 log CFU/g) than thinner ones (1.0 and 2.5 cm). Possible reason for the higher thermal inactivation of pathogens in thicker steaks could be the longer cooking time it takes to reach the internal temperature than in thinner steaks. Shen et al. (2010) reported that cooking of 4.0 cm thick steaks required a longer time (20 to 65 min) compared to thinner steaks (1.0 and 2.5 cm). Higher thermal inactivation of pathogen cells on the surface of large cuts like roasts could be due to the higher temperature reached on the external layer as compared to the geometric center of the big cuts (USDA-FSIS, 2002b). Additional thermal inactivation is achieved during the resting period when meat products are kept at room temperature after removing from the cooking equipment when the specified temperature is attained in the center (Gill et al., 2009).

Besides cooking method and steak thickness, different restructuring, marination and antimicrobial agents may influence thermal inactivation of *E. coli* O157:H7 internalized in moisture-enhanced meat products. Juneja et al. (1999) reported that salt (6%) alone had a protective effect on thermal inactivation of *E. coli* O157:H7, but when combined with a 0.3 % sodium pyrophosphate (0.3%) increased the sensitivity of *E. coli* O157:H7 to heat, in beef gravy cooked in a water-bath. Mukherjee et al. (2008) found that addition of organic acids, like citric and acetic acid, in brine solutions caused greater ($P<0.05$) reduction (4 to 5 log CFU/g) of *E. coli* O157:H7 internalized in a moisture-

enhanced ground beef model system than control samples, when cooked to 65°C in a circulating water bath. In another study, Mukherjee et al. (2009) found that restructuring of beef with salt/phosphate, algin/calcium, Activa^{TM}RM, or Fibermex$^{®}$ did not affect inactivation of internalized *E. coli* O157:H7 when cooked to the internal temperature of 60 or 65°C, whereas inclusion of lactic acid (0.27%) in restructured nonintact beef products enhanced destruction of this pathogen when cooked to 65°C. Addition of tenderizing agents like calcium ascorbate and calcium chloride, along with sodium chloride did not significantly ($P \geq 0.05$) affect thermal inactivation of *E. coli* O157:H7 compared to control (water treatment) samples when cooked to 60 or 65°C in water bath. However, inclusion of organic (acetic and citric) acids in beef tenderizing recipes may help in thermal inactivation of *E. coli* O157:H7 transferred to the interior of nonintact products during their production (Yoon et al., 2009). Antimicrobials agents like, cetylpyridinium chloride (0.5%), reported to cause higher reduction in *E. coli* O157:H7 population, when added in brining formulation with sodium chloride (0.5%) and sodium tripolyphosphate (0.25%), in a moisture-enhanced ground beef model system cooked to the internal of temperature of 65°C in water bath (Byelashov et al., 2010).

2.2. Prevalence, cross-contamination and control of *L. monocytogenes* in the home environment

L. monocytogenes is a human pathogen that can cause non-invasive mild flu-like symptoms or invasive (listeriosis with mortality rates of 20-30%) life threatening symptoms depending on the number of organisms consumed, the host susceptibility, and strain virulence (WHO-FAO, 2004). The infection with *L. monocytogenes* can be fatal in

certain segments of the population including the elderly, neonates, pregnant women, human immunodeficiency virus-infected like AIDS, and individuals undergoing immunosuppressive therapy (Kathariou, 2002). The transmission of this pathogen through contaminated food was first conclusively demonstrated by epidemiological and laboratory investigations in 1983 (Schlech et al., 1983). This pathogen has been involved in both sporadic and epidemic forms of listeriosis.

Food products have been divided into high, medium and low risk categories based on whether they support growth of *L. monocytogenes*. Ingestion of contaminated ready-to-eat food (delicatessen products such as luncheon meats, turkey deli meats, cheese, coleslaw) fresh vegetables, prepared salads, poultry products, soft cheeses, pâté and other cooked meat products, and fish have been found to be transmission routes for listeriosis (McLauchlin, 1996). The high mortality rate associated with listeriosis has led regulatory agencies in the United States to implement a zero tolerance policy for this pathogen in processed foods (Shank et al., 1996). Zero tolerance policy for this pathogen states that a positive test for *L. monocytogenes* in 25 g of cooked RTE foods is unacceptable from a public health perspective and food is considered adulterated (FDA, 2003). The Food Safety and Inspection Services (FSIS) recommended the food processing industry to implement post-lethality alternatives interventions to control contamination in products either by killing and/or inhibiting growth of *L. monocytogenes* in these products (Code of Federal Regulations, 2003a).

Out of 13 serovars of *L. monocytogenes*, 4b, 1/2a and 1/2b, have been associated with most human illnesses (Schuchat et al., 1991; Gellin and Broome, 1989). Type 4b seems to be the one most often associated with large outbreaks of foodborne listeriosis

(McLauchlin, 1996). The high frequency of large outbreaks caused by this serovar could be due to a higher virulence, better adaptation and survival in foods, broader distribution in the environment, or increased ability to survive in food processing environment via biofilm formation (Chae et al., 2006).

Home is a central place that may receive foodborne pathogens from different sectors of the community, such as hospitals, schools, day care centers, retail food stores, residential care centers, work places, and leisure locations. Other ways pathogens can enter the home is through visiting people, water, food, pets, insects, and through air (Bloomfield, 2001). Foodborne illnesses originating from food consumed in private homes is three times more frequent than that arising from food consumed at retail places (Borneff et al., 1988). Kitchen is considered microbiologically the dirtiest place together with the bathroom, with potentially heavy loads of *Salmonella* and *Campylobacter* and fecal coliforms (Rusin et al., 1998).

2.2.1. Prevalence of *L. monocytogenes* in the domestic environment

L. monocytogenes has been isolated from different areas of the domestic kitchen. Studies (Azevedo et al., 2005; Beumer et al., 1996; Cox et al., 1989; Duggan and Phillips, 1998; Sergelidis et al., 1997) conducted in different countries found different *Listeria* species like *ivanovii* subspp *londiniesis, innocua, welshimeri, seeligeri, grayi,* and also pathogenic *L. monocytogenes* in different kitchen areas. Beumer et al. (1996) isolated different *Listeria* spp. from household dish clothes, washing-up brushes, kitchen sinks, tooth brushes and surfaces around drains. *L. innocua* and *L. monocytogenes* were the predominant species in the positive samples. Cox et al. (1989) isolated *Listeria* spp. from

the refrigerator, dish clothes and two dustbins, and hypothesized that dish clothes could be a source and spread these pathogens in different areas of the kitchen. Azevedo et al. (2005) sampled 86 domestic refrigerators in Portugal and isolated *L. monocytogenes* from cheese and meat shelves, and *L. grayi* and *L. innocua* from vegetable shelves. Another study, conducted in Greece, isolated *L. monocytogenes* from walls and shelves of domestic refrigerators (Sergelidis et al., 1997). Duggan and Phillips (1998) isolated *L. monocytogenes* from baskets of home refrigerators in the United Kingdom.

2.2.2. Attachment and survival of *L. monocytogenes* to food contact surfaces

Attachment of bacterial cells to any surface is a multifactorial phenomenon and depends on the physiochemical properties of the surface and the bacterial cells, and on environmental conditions (Chmielewski and Frank, 2003). Physiochemical properties of food contact surfaces, important for bacteria cell attachment, are: i) surface topography (smooth versus rough surface); ii) hydrophobic (glass, marble, polyethylene, polypropylene, stainless steel (304) versus hydrophilic (granite) surface properties (Blackman and Frank 1996; Smoot and Pierson, 1998); iii) preconditioning of the surface (Helke et al., 1993; Hood and Zottola, 1997); and iv) pH and temperature of the preconditioning medium (Jeong and Frank, 1994); and v) porosity of surface.

Materials found in the domestic kitchen are: i) Stainless steel which is extensively used for blades of knives, sink surfaces and utensils; ii) High density polyethylene (HDPE), polyethylene (PE), polypropylene (PP), glass and wood which are used for cutting boards, knives handles and utensils; and, iii) Tile, concrete, laminate, granite, silestone, ceramic, natural stone, engineered stone, quartz and soapstone, which are used

for furnishing kitchen countertop surfaces. Stainless steel is preferred over other materials in domestic kitchens because of its properties like mechanical strength, corrosion resistance, longevity and ease of fabrication (Holah and Thorpe, 1990). Studies, investigating survival of *Salmonella* spp., foodborne pathogens found in domestic kitchen, on materials found in domestic kitchen have been reported. Teixeira et al. (2007) reported that *Salmonella* Typhimurium colonized on marble and granite (two materials commonly used for bench cover in domestic kitchen) to the same extent, but less than stainless steel (SS). However, *Salmonella* Enteritiditis strains were less prone to colonization on two plastic materials (polyethylene from cutting board and polypropylene from basin) compared to granite (Oliveria et al., 2006). *Salmonella* Typhimurium was reported to survive on Formica and polypropylene up to 6 h without and with supplementation of protein (Moore et al., 2007). A study investigating adhesion ability of 10 different strains of *L. monocytogenes* on materials commonly found in domestic kitchen, reported that strains adhered most tightly to granite and marble, followed by the stainless steel 304, glass, silestone and finally polypropylene surfaces (Silva et al., 2008). Variation in adherence capabilities and biofilm formation among different strains of *L. monocytogenes* has been reported (Norwood and Gilmour, 2001; Borucki et al., 2003). Borucki et al. (2003) reported increased biofilm formation capabilities by Division II strains (serotypes 1/2a and 1/2c) on stainless surfaces out of eighty strains of *L. monocytogenes* tested in this study.

Bacterial surface properties like surface charge, cell density and presence of exopolysaccharides are other factors important for the adhesion process (Oliveira et al., 2006). Environmental conditions like temperature (Moltz and Martin, 2005) and relative

humidity (Kim et al., 2008) also influence attachment of cells to different surfaces. Nutrient availability for bacteria (Folsom et al., 2006; Kim and Frank, 1994) influences attachment and survival of this pathogen on surfaces. Substances like serum used for preconditioning of food contact surfaces can provide nutrients and allow better survival of pathogens on food contact surfaces (Helke et al., 1993).

2.2.3. Cross-contamination of *L. monocytogenes* between different food contact surfaces

Cross-contamination with pathogen cells depends on microbial contamination levels on the surfaces and the probability of these cells to be transferred to other surfaces and foods before consumption (Bloomfield and Scott, 1997). Residues of fluid ("juice") from raw meat or poultry after food preparation might remain on the work surfaces in the domestic kitchen and can transfer disease agents to raw vegetables or other foods that are not cooked further before being eaten (Ak et al., 1994). Cogan et al. (1999) found that handling of artificially contaminated chicken on cutting boards can cross-contaminate these surfaces at levels of up to 10^4 CFU/5 cm^2. Hydration levels of surfaces, surface contamination levels, structure of surfaces, attachment strength of the microbial cells and structure of biofilms have been reported to affect transfer of pathogen between different surfaces (Flores et al., 2006; Midelet and Carpentier, 2002; Rodriguez and Mclandsborough, 2007; Rodriguez et al., 2007 a and b; Sattar et al., 2001).

Sattar et al. (2001) reported higher bacterial transfer levels from moist donor fabrics compared to dry ones. However, Rodriguez et al. (2007b) did not find a statistical difference ($P \geq 0.05$) in the level of transfer between wet and dry food processing

surfaces (stainless steel and polyethylene). Rodriguez and Mclandsborough (2007) found that dry biofilms tended to transfer more *L. monocytogenes* to foods compared with attached wet cells. Weakening of cell-to-cell interactions in biofilms and cell-to-surface interactions of biofilms during drying could be a possible reason for increased transfer levels from surfaces to food products. Rodriguez et al. (2007a) found higher transfer of *L. monocytogenes* from food contact surfaces (stainless steel and high density polyethylene) to foods (bologna) with higher surface contamination levels (10^9 versus 10^5 CFU/cm^2). Rodriguez et al. (2007) also reported higher transfer of *Listeria* from stainless steel surfaces to bologna than high-density polyethylene to bologna, but, surface roughness did not affect transfer of bacteria from food contact surfaces.

2.2.4. Sampling methods for pathogen cell recovery from different food contact surfaces

The efficacy of any sampling method for recovery of cells depends on mechanical force to effectively dislodge the bacteria from the surface, and effectively release cells from sampling devices for microbiological analysis (Kang et al., 2007). Swabs, sponges, adhesive tapes, ATP bioluminescence, and direct contact method using solidified agar are examples of conventional recovery methods. These recovery methods are associated with their own advantage and disadvantages. Swabs are usually good for small surfaces and sponges are good for larger surfaces. However, both these devices are only able to recover small proportion of bacteria present on a surface, and technician-to-technician variability affects the outcome of the enumeration results (Kang et al., 2007). There is no consensus for an accepted standard method (Bredholt et al., 1999; Moore and Griffith,

2002). A recovery method developed at Michigan State University, used, kimwipes as the device to recover cells from stainless steel. This method was more efficient in recovering cells from surfaces as well as cheaper than other conventional recovery methods (Vorst et al., 2004). Kang et al. (2007) in their study developed three novel methods to recover cells from surfaces: i) Sonication in an ultrasonic water bath (40 kHz); (ii) Contact with the bristles of a sonicating brush head for 1 min; and, (iii) Indirect contact (2-4 mm distance) with a sonicating brush head for 1 min. The three sonication methods yielded higher recovery of *L. monocytogenes* from artificially contaminated stainless steel surfaces than the other three conventional methods (swabbing with a premoistened Dacron swab, rinsing with phosphate-buffered saline and direct contact onto tryptic soy agar containing 0.6% yeast extract (TSAYE) plates for 10 s) ($P \leq 0.05$) (Kang et al., 2007).

The physical characteristics of a food or food contact surface impact a sampling method's overall efficiency (Kang et al., 2007). Moore et al. (2003) recovered higher levels of *Salmonella* Typhimurium from formica and stainless steel than polypropylene and wood surfaces. Wood is intrinsically porous, which allows food juices and bacteria to enter the body of the wood unless a highly hydrophobic residue covers the surface. The moisture is drawn in by capillary action until there is no more free fluid on the surface, at which point migration ceases (Cliver, 2006). It is difficult to remove cells present inside these porous surfaces (Abrishami et al., 1994; Ak et al., 1994; Moore et al., 2007).

2.2.5. Sanitation of contaminated food contact surfaces in the domestic kitchen

Decontamination of contaminated food contact surfaces means removal/inactivation/killing of pathogen cells present on these surfaces. The commercially available clothes used for wiping food contact surfaces may be available without or with antimicrobial agents. Wipes without antimicrobial agents cause only physical removal of pathogen cells from the surfaces. Examples are: 1) Handy® wipes; 2) Heavy® wipes; 3) Kitchen cloth®; and 4) Paper towel; etc. These wipes can be combined with different commercially and home-made disinfectants to achieve better reduction of pathogen cells on food contact surfaces. Wipes with antimicrobial agents have a dual action, they not only remove, but also kill cells present on food contact surfaces. Examples are: 1) Disinfecting wipes Clorox®; 2) Laminate® floor wipes; 3) Antimicrobial Handy®; and, 4) Heavy® wipes; etc. Kusumaningrum et al. (2003b) found that commercially available disposable wetted napkins with anionic surfactants performed better in removing foodborne pathogen cells from stainless steel surfaces than reusable microfiber, viscose, and sponges without antimicrobial components.

Different types of sprays (sanitizers and disinfectants) are commercially available to be used for cleaning and sanitation purposes for different food contact surfaces in the domestic kitchen. Examples of some commercially available disinfectants include: 1) "Disinfecting kitchen cleaner" from Clorox®; 2) "Anywhere hard surface" from Clorox®; 3) "All purpose cleaner" from Lysol®; 4) "Antibacterial kitchen cleaner" from Lysol®; 5) "All purpose cleaner antibacterial" from 409®; 6) "All purpose cleaner antibacterial" from Fantastik®; 7) "Antibacterial kitchen cleaner" from Fabulous®; 8) "Mutli-surface antibacterial" from Windex®; 9) "Oasis 146 multi-quat sanitizer" from Ecolab®; etc

(Yang et al., 2009a). Application of sanitizers on food contact surfaces for decontamination includes three major steps according to manufacturer's instructions: rinsing with water, reaction with sanitizer for a certain amount of time, and rinsing with water (Yang et al., 2009a). However, some commercially available sprays and wipes with an antibacterial agent, do not advocate rinsing after their use despite evidence that rinsing is vital step in cleaning (Cogan et al., 1999 and 2002; Rusin et al., 1998; Kusumaningrum et al., 2002).

These commercially available sprays have various chemical components and concentrations of active ingredients. Basic chemical agents in these products might have antiseptic and disinfectant properties (McDonnell and Russell, 1999). Quaternary ammonium compounds, lactic acid and sodium hypochlorite are the most common active ingredients in these sanitizers (Yang et al., 2009a). Yang et al. (2009a) compared efficacy of 10 commercially available sanitizers (quaternary ammonium-, lactic acid- and sodium hypochlorite- based) against *L. monocytogenes* biofilms on high density polyethylene (HDPE) cutting board surfaces. Lactic acid-based sanitizers (pH 3.03) were the most effective and quaternary ammonium- based sanitizers (pH 6.24) were least effective against *L. monocytogenes* (Yang et al., 2009a).

Household products like Clorox bleach, baking soda, white vinegar, hydrogen peroxide, ammonia can be used as sanitizers for different food contact surfaces in the domestic environment. These products are inexpensive and convenient (Yang et al., 2009b). The solutions of four household products (Clorox bleach, baking soda, white vinegar, and hydrogen peroxide at different concentrations) were found to be effective against planktonic cells of *L. monocytogenes*. The decreasing order of efficacy of these

compounds was: 0.0314% sodium hypochlorite >3% hydrogen peroxide > undiluted vinegar > 5% acetic acid > 5% citric acid > baking soda (50% sodium bicarbonate) (Yang et al., 2009b). Parikh et al. (2009b) found that three home prepared sanitizers (300 ppm sodium hypochlorite, 5% acetic acid, and 3% hydrogen peroxide) were effective in reducing *L. monocytogenes* population in biofilms formed on high density polyethylene (HDPE) and polypropylene (PE) surfaces and were more effective against younger compared with older biofilms.

2.2.6. Consumer education about safe food handling practices in the domestic kitchen

Food safety enhancement requires cooperation among people at all stages in the food chain, from farm to table. Home is the end point of the food chain and the domestic kitchen is considered as "the final line of defense" against foodborne illnesses (Redmond and Griffith, 2003). Foods sold to public may be contaminated with pathogens (Griffith and Worsfold, 1994). Apart from government and food industry, it is the responsibility of consumers to follow sanitary practices at home to control foodborne illnesses (Griffith, 2001; ICMSF, 1988; WHO, 1997). Consumers not only purchase and receive products but also process food products in the domestic kitchen. The implementation of proper food handling practices at home can reduce risk of pathogen multiplication, cross-contamination to other products and eventually prevents foodborne illnesses (USEPA, 1997; Redmond and Griffith, 2003). Foodborne outbreaks that originate in the home typically involve small numbers of people and thus are less likely to be identified by public health authorities (Worsfold and Griffith, 1997). Therefore, the actual proportion

of foodborne outbreaks and individual cases originating in the home is likely to be much larger than it has been reported (Zhao et al., 1998). Many campaigns and education programs have been developed to educate consumers about risks associated with unhygienic practices during food preparation (Redmond and Griffith, 2005). “Fight Bac” was developed by partnerships from industry associations, professional societies in food science, nutrition and health consumer groups, the United States Department of Agriculture (USDA), the Environmental Protection Agency (EPA), the Department of Health and Human Services (DHHS), the Centers for Disease Control and Prevention (CDC), and the Food and Drug Administration (FDA), an important initiative for food safety education for consumers to prevent foodborne illnesses (FDA, 2006).

CHAPTER 3

TRANSLOCATION OF *ESCHERICHIA COLI* O157:H7 INTO INTERNAL TISSUES DURING MOISTURE ENHANCEMENT OF BEEF BY NEEDLE INJECTION

This study quantitated internalization of *Escherichia coli* O157:H7 cells in moisture enhancement beef under two contamination scenarios. In the first contamination scenario, beef knuckles (approximately 4-5 kg) were surface-inoculated (4.7±0.3 log CFU/g) with nonpathogenic rifampicin-resistant *E. coli* O157:H7 (4-strain composite). The meat was then injected, with a hand-operated single needle brine injector, either with sterile distilled water (control) or a brine solution of sodium chloride [NaCl; 5.5%] and sodium tripolyphosphate [STP; 2.75%] at seven locations per knuckle. In the second contamination scenario, the enhancement solutions were inoculated (3.7±0.1 and 3.4±0.2 log CFU/ml for water and brine, respectively) with the 4-strain *E. coli* O157:H7 composite and were then used for needle injection. One core sample (8-cm diameter and average length of 7-cm) per knuckle was excised parallel to the direction of needle injection using a coring device. Core samples were surface-decontaminated with hot water (90-95°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. Sections and purge collected after enhancement processing, were analyzed for total

bacterial and *E. coli* O157:H7 counts by plating on tryptic soy agar (TSA) and TSA with rifampicin (100 µg/ml). The study was repeated three times for both contamination scenarios, with one knuckle (i. e., one core sample with six sections representing six different depths) analyzed per treatment (moisture-enhanced with water and brine) per replication. The average percent gain in product weight ranged from 4.3±1.3 to 9.7±0.5% and, purge had high *E. coli* O157:H7 levels (6.2 and 5.3 log CFU/ml), after injection with both enhancement solutions and under both contamination scenarios. After enhancement processing *E. coli* O157:H7 levels (3.3 log CFU/g) translocated were higher in the topmost 1-cm than the 2.0-14.5 cm depth (2.4-2.8 log CFU/g) under sceanario-1; however, pathogen counts translocated to the deepest tissue (average total 14.5 -cm depth) were higher by 0.9 CFU/g compared to the topmost 1-cm under scenario-2. Overall, higher pathogen counts were recovered from all sections under scenario-1 (2.4-3.3 log CFU/g) than scenario-2 (0.6-1.5 log CFU/g).

The findings of this study could be helpful in development of risk assessment models that can quantify the translocation of pathogens under different contamination scenarios.

3.1. INTRODUCTION

Muscles from lower quality grade carcasses may be injected with brine solutions to improve juiciness, tenderness and palatability (Ray et al., 2010). At commercial settings, brines are injected into meat through hollow needles with brine injectors under pressure. Brine pressure must be at least high enough to overcome the pressure of the meat around the needles. The duration of injection of needles in the meat (also known as

'dwell time') and the speed of the conveyor belt are two factors that influence the percent moisture enhancement of muscle cuts (Uttaro and Aalhus, 2007).

Sodium chloride is the most common component of brine solutions along with one or more of phosphate salts (Uttaro and Aalhas, 2007). The common salt helps in water retention with chloride ions binding to thick and thin filaments, creating an area coated with similar charges, thus forcing adjacent regions away from one another (electrostatic repulsion) and causing the myofilament lattice to expand as much as possible within the constraints of actin/myosin cross-bridges (Bendall, 1954; Offer and Trinick, 1983). Phosphates most commonly used in the meat industry are alkaline or acidic types of di- and tripolyposphates and some cyclic phosphates (Dziezak, 1990; Sofos, 1986). Pyrophoshate and tripolyphosphate have been reported to improve water holding capacity of raw muscle with maximum hydration at pH 6 (Hamm and Grau, 1958; Hellendoorn, 1962). Most phosphates used in brines have a pH higher than meat so their introduction raises the pH of their environment, contributing to water retention in this manner. Phosphates also act by dissociating the actin/myosin complex starting at the edge of the A-band (Offer and Knight, 1988).

Moisture-enhanced beef products contaminated with *E. coli* O157:H7 have been associated with outbreaks and recalls in the past decade (CFP, 2008). Outbreaks of *E. coli* O157:H7 infections associated with non-intact beef steaks/roasts were responsible for more than 25 confirmed cases and product recalls of several hundred thousands of kilograms of products (CFP, 2008). Sterile deeper tissues of moisture-enhanced products may be contaminated during processing through two possible ways: i) internalization of surface contamination; or ii) introduction of pathogen cells with contaminated fresh or

recycled moisture-enhancing brine solution during processing. Enhancement solutions are recirculated in commercial settings and reportedly have increased bacterial loads over time during moisture enhancement processing (Anonymous, 2005; Greer et al., 2004). The internalized pathogenic cells have been of concern because pathogens could survive cooking, especially if products are undercooked intentionally or unintentionally (Sofos et al., 2008).

Several studies have evaluated and quantified transfer of pathogen cells into sterile internal tissues of meats at different depths during mechanical tenderization processing (Echeverry et al., 2009; Gill and McGinnis, 2004; Hajmeer et al., 2000; Johnston et al., 1978; Luchansky et al., 2008, 2009; Sporing, 1999; Thippareddi et al., 2000). These studies investigated factors that included shape and structure of the blades or needles, orientation of the surface fibers, surface contamination levels, and type and extent of manipulation of the muscle tissue, that could influence the rate of translocation of the bacterial cells. Additional studies have quantified transfer of pathogen cells during moisture enhancement processing with contamination present on the external surface of beef cuts (Bohaychuk and Greer, 2003; Echeverry et al., 2009; Thippareddi et al., 2000). However, no published studies have quantified transfer of pathogen cells when contaminated injection solutions are used for moisture enhancement processing. It would be interesting to determine quantitative transfer of *E. coli* O157:H7 into beef under different contamination scenarios during moisture enhancement processing. Thus, quantitative transfer of *E. coli* O157:H7 during moisture enhancement of beef injected with water and/or brine either when contamination is present on the surface

(contamination scenario-1) or when a contaminated brine solution is injected (contamination scenario-2) was investigated in this study.

3.2. MATERIALS AND METHODS

3.2.1. *E. coli* O157:H7 strains and inoculum preparation

Four strains of *E. coli* O157:H7 with out virulent shiga toxin producing genes, including two human isolates (ATCC 700728 and ATCC 43888) and two isolates from cattle feces (C1-057 and C1-058; Carlson et al., 2009) were selected for this study. All four strains were negative for stx-1 and stx-2 encoding genes virulence. Rifampicin (100 µg/ml) resistant variants of these four strains were isolated as described by Kaspar and Tamplin (1993). All rifampicin resistant variants of these four strains were maintained as frozen (-70°C) stock cultures in tryptic soy broth (TSB; Difco, Becton Dickinson, Sparks, MD) plus rifampicin (100 µg/ml; Sigma, St. Louis, MO) supplemented with 15% glycerol. Working cultures were maintained on tryptic soy agar (TSA; Difco, Becton Dickinson) supplemented with 100µg/ml rifampicin and transferred to new plates monthly. Each strain was activated and subcultured (35°C, 24 h) in 10 ml of tryptic soy broth plus 100 µg/ml rifampicin. The strains were subsequently combined and centrifuged (4,629×g, 15 min, 4°C; Eppendorf, model 5810 R, Brinkmann Instruments Inc., Westbury, NY), washed with phosphate buffered saline (PBS; pH 7.40, 0.2 g/L KH_2PO_4, 1.5 g/L $Na_2HPO_4.7H_2O$, 8.0 g/L NaCl and 0.2 g/L KCl), and cell pellets were resuspended to achieve a concentration of 9 log CFU/ml of *E. coli* O157:H7 in PBS. The mixture of these four non-pathogenic rifampicin resistant *E. coli* O157:H7 strains was

used to inoculate the surface of beef knuckles for contamination scenario-1 and the enhancement solutions for contamination scenario-2.

3.2.2. Beef knuckles for moisture enhancement

Beef knuckles (95% lean: *Vastus lateralis*, *Vastus intermedius*, *Vastus medialist* and *Rectus femoris* muscles), approximately 3-5 kg, were obtained from a local meat packing plant and stored vacuum packaged at -23°C in the Meat Science Laboratory, Center for Meat Safety & Quality, Department of Animal Sciences at Colorado State University, for not more than two weeks. Frozen beef knuckles were completely thawed in a walk-in cooler (4°C) for 72 h before moisture enhancement processing.

3.2.3. Moisture enhancement solutions

Brine solution used for moisture-enhancement processing was prepared by dissolving sodium chloride (NaCl 5.5%; Fisher Scientific, Fair Lawn, N.J., U.S.A) and sodium tripolyphosphate (STP 2.75%; FMC Corp., Philadelphia, Pa., U.S.A) in sterile distilled water. The concentration of each brining ingredients in the brining solution was selected based on concentrations utilized by the industry and/or published research (Byelashov et al., 2010; Shen et al., 2010). The brine solution was prepared on the day of the experiment. Sterile distilled water was selected as a control treatment.

3.2.4. Moisture enhancement equipment

A hand-operated, single needle brine injector (Dick Companies, Postfach, Deizisau, Germany) was used for enhancement solution injection. The needle of this brine injector was 18-cm long with a 2-cm long pointed tip that helped to pierce the muscle during the enhancement procedure. The needle surface area (5-cm long) just above the tip had sixteen holes (1-mm in diameter) to deliver injection solution into the

interior of the knuckles. The other end of the needle had a plastic knob that fitted with the plastic cylinder of the hand pump. White plastic tubing with filters at both ends carried the injection solutions through the plastic cylinder to the needle and then into the interior of the muscle (Figure 3.1).

3.2.5. Moisture enhancement of knuckles for contamination scenario-1

Whole beef knuckles were thawed and surface inoculated with 1 ml of the *E. coli* O157:H7 inoculum, 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. The topmost 1-cm thick section was excised from a core sample (that was not surface decontaminated) and this particular sample was cored out from surface-inoculated knuckle without any enhancement processing. This section was analyzed microbiologically to determine the surface inoculation levels of rifamipicin-resistant *E. coli* O157:H7 for this contamination scenario. The surface inoculated beef knuckles were injected either with sterile distilled water or the brine solution. The water or brine solution was injected at seven different locations around the knuckle. The injector needle was inserted completely to the bottom of the beef knuckle and solutions were injected while withdrawing the needle from the interior of the knuckle. The injector needle was sterilized between each injection with 70% ethanol.

3.2.6. Moisture enhancement of knuckles for contamination scenario-2

The enhancement solutions (i. e., water and brine) were inoculated with the four non-pathogenic rifampicin-resistant *E. coli* O157:H7 strains mixture, to achieve an inoculation level of 3.7±0.1 and 3.4±0.2 log CFU/ml, respectively. One ml of the inoculated water and brine solution was analyzed microbiologically, as described later, to

determine inoculated level of rifampicin-resistant *E. coli* O157:H7 population, in both enhancement solutions for this contamination scenario. Inoculated enhancement solutions were used for moisture enhancement processing of the beef knuckles, as described for contamination scenario-1.

3.2.7. Sampling of knuckles after moisture enhancement

All beef knuckles, after moisture enhancement processing, under both contamination scenarios, were given a rest time of 15 min at 4°C for dispersion of enhancement solutions. Knuckles under both contamination scenarios were weighed before and after enhancement processing to determine the percent moisture enhancement. One core (8-cm in diameter) sample per knuckle was excised parallel to the direction of middle needle injection using a coring device (Facilities Maintenance, Colorado State University, Fort Collins; Figure 3.2). Core samples were surface-decontaminated in boiling water (90-94°C, 60 s), cooled (4°C, 15 min), and cut (Figure 3.3) 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), while sterilizing the knife and cutting board with 70% ethanol between cuts to avoid cross-contamination. Beef knuckles used for different treatments were not of uniform shape and weight (3-5 kg) and this led to variable lengths (3-10 cm) of section-6. Two core samples (8-cm diameter) were excised from uninoculated unprocessed beef knuckles to determine background total plate and rifampicin-resistant *E. coli* O157:H7 microbial populations. One section (1-cm thick) from the topmost layer and one section (1-cm thick) from geometric center were cut from these core samples that were not surface decontaminated. Both of these sections were analyzed microbiologically as described later. Additionally, the purge generated during moisture enhancement

processing under both contamination scenarios was collected and also analyzed microbiologically for bacterial populations.

3.2.8. Microbiological analysis

The weight of each sample section was determined before blending (Waring Commercial Laboratory Blender, CT, U.S.A) for 1-2 min with double the weight of maximum recovery diluent (MRD; 0.85% NaCl and 0.1% peptone). Samples were transferred into sterile bags (Whirl-Pak, Nasco, Modesto, Calif., U.S.A.) and pummeled at 8 strokes/s (Masticator, IUL Instruments, Barcelona, Spain) for 2 min. The homogenate was serially diluted and surface-plated on tryptic soy agar (TSA; Acumedia, Lansing, Mich., U.S.A.), and TSA with 100 μg/mL rifampicin (Sigma Aldrich; TSA + rif) to enumerate total bacterial and inoculated *E. coli* O157:H7 populations, respectively. Colonies were counted after incubation at 35°C for 48 h.

3.2.9. Statistical analysis

Bacterial (total bacterial and *E. coli* O157:H7) counts were converted to log CFU/g before statistical analysis. The study was repeated three times for both contamination scenarios, with one knuckle (i. e., one core sample with six sections) analyzed per treatment (enhancement solutions: i. e., water and brine) per replication. The experimental design for this study was split-split plot. The independent variable, contamination scenarios, was split into two independent variables treatment (2: water and brine) and this variable was again split into independent variable sections (6). The effect of these three individual variables (contamination scenario, treatment, section) and their interactions (contamination scenario × treatment, contamination scenario × section,

treatment × section and contamination scenario × treatment × section) were analyzed on the dependent variable which was bacterial counts (log CFU/g).

During initial statistical analysis, it was found that the independent variable treatment did not have a significant ($P \leq 0.05$) effect on the response variable. Data from both treatments (i e., water and brine) was combined for both contamination scenarios and re-analyzed for the main effects of these two individual variables (contamination scenario and section) and their interaction (contamination scenario × section) on the dependent variable i. e., bacterial counts (log CFU/g).

Data were analyzed using Kenward-Roger mix model procedure of SAS® version 9.2 (SAS Institute Cary, NC). Means and standard deviations were calculated for *E. coli* O157:H7 and total microbial populations (log CFU/g) from all sections, purge generated, and, the percent gain in weight after enhancement processing, under both contamination scenarios and the least square means were separated at the significance level of $\alpha=0.05$.

3.3. RESULTS AND DISCUSSION

3.3.1. Extent of moisture enhancement

The average percent gain in knuckle original weight after moisture enhancement processing with water and brine injection solutions was 4.3±1.3 % and 4.8±0.7 %, and, 6.3±0.7 % and 9.7±0.5 % for contamination scenario -1 and -2, respectively (Table 3.1). The wide range in percent gain of knuckle weights, even though the number of times each knuckle was moisture-enhanced with the single-pass needle brine injector was constant between different treatments under two contamination scenarios, could be due to differences in size and compactness of different knuckles used for this study. Knuckles

that were less compact retained less enhancement solution compared to more densely compact knuckles. The muscle cuts are usually injected commercially to levels of 108-115% of the green weight (Uttaro and Aalhas, 2007). Freezing and thawing of meat could influence the brine uptake (Boles and Swan, 2002) and knuckles used for the present study were slowly thawed in a cooler at 4°C for 72 h before moisture enhancement.

3.3.2. Microbiological analysis of the purge

Purge, a mixture of enhancement solution, meat particles and blood, was collected as excess enhancement solution either run off from the external surface or purged from the knuckles immediately after processing. The bacterial cell load in the purge could originate either from the external surface of inoculated muscles under contamination scenario-1 or from the enhancement solutions for contamination scenario-2. Purge generated under contamination scenario-1 had higher, although not statistically significant ($P > 0.5$), total bacterial and *E. coli* O157:H7 counts compared to purge from contamination scenario-2, irrespective of enhancement solutions used (Table 3.1). Background flora naturally present and *E. coli* O157:H7 cells artificially inoculated, on the external surface of knuckles, could have concentrated in the purge with run off enhancement solutions during processing and led to higher total bacterial and *E. coli* O157:H7 counts in purge generated under contamination scenario-1.

High levels of contamination in purge generated after enhancement processing, as indicated by results of this study (Table 3.1), could cross-contaminate other muscle cuts during moisture enhancement processing if the contaminated purge was recycled and/or mixed with fresh brine solutions as practiced often by the industry. The contaminated purge can also transfer cells to processing equipment, like conveyor belts which stresses

the importance of sanitation for processing facilities and equipments, and use of fresh brines for moisture-enhancement (Echeverry et al., 2009).

3.3.3. Translocation of contamination during moisture enhancement

Total bacterial populations recovered from the topmost surface and center samples (8-cm diameter and 1-cm thickness) of uninoculated beef knuckles, without moisture enhancement processing, varied from 1.1±1.0 to 4.5±0.6 and 0.5±0.0 to 2.3±0.4 log CFU/g, respectively. However, rifamipicin resistant *E. coli* O157:H7 levels were below the detection limit (< 0.5 Log CFU/g) in these samples. Other studies have also reported very low prevalence of *E. coli* O157:H7 on muscle cuts collected from different slaughtering facilities in the North America (Heller et al., 2007; Kennedy and Bodnaruk, 2004 and 2005). This could be expected due to different interventions implemented on beef carcasses during the slaughtering process to reduce microbial populations (Sofos and Smith, 1998).

Total bacterial and *E. coli* O157:H7 counts were 5.2±1.0 and 4.7±0.3 log CFU/g, respectively, on the surface of knuckles under contamination scenario-1 (Table 3.2). A portion of 3.2 % (3.3±0.8 log CFU/g) of the surface inoculated *E. coli* O157:H7 cells were transferred to the topmost 1-cm thickness (section -1) after enhancement processing for this contamination scenario (Table 3.2). The pathogen levels transferred to the 2-cm (section-2) depth were 5-fold lower (0.7 log CFU/g) compared to the topmost 1-cm thickness (section-1) of moisture-enhanced beef knuckles when pathogen cells were inoculated on the surface (Table 3.2). Withstanding large standard deviation, pathogen levels recovered from 2- (section-2), 3- (section-3), 5- (section-4), 7- (section-5) cm and average total 14.5-cm (section -6) depth of moisture-enhanced knuckles inoculated on

surface were similar ($P \leq 0.5$) among each other (Table 3. 2). Translocation patterns of total microbial populations to different depths of beef knuckles after enhancement processing were similar to those of *E. coli* O157:H7 under contamination scenario-1 (Tablea 3.2 and 3.3).

Luchansky et al. (2008) conducted a study where knuckles were surface-inoculated with *E. coli* O157:H7 at four (3.2 log CFU/g, 2.5 log CFU/g, 1.5 log CFU/g, and 0.6 log CFU/g) levels and were processed mechanically by single pass from the lean side with a blade tenderizer. Similar to results of the present study, this study reported that 32-41 % of surface inoculated pathogen cells were transferred to the topmost 1-cm depth of knuckles and similarly levels transferred to section-2 (2-cm depth) were 7- to 34- fold lower than the levels transferred to section-1. However, pathogen levels (-0.8-0.61 log CFU/g) transferred to deeper tissues (section -3 to -5) were lower compared to levels transferred to these corresponding sections observed in the present study. These differences in translocation levels observed in these two studies could be due to differences in initial inoculation levels and in processing. Luchansky et al. (2008) used a commercial tenderizer (TC 700M, Ross industries) with a multiple set of steel blades for mechanical tenderization processing; however, a hand-operated single needle brine injector was used to inject enhancement solutions at seven different locations of knuckles in the present study. Thippareddi et al., (2000) reported similar levels (2.6 log CFU/g) of *Salmonella* Typimurium transferred to deepest tissues (4-cm depth) of moisture-enhanced pork lions when pork loins were surface-inoculated at levels of 5.2 log CFU/g.

The inoculation levels of *E. coli* O157:H7 were 3.7±0.1 and 3.4±0.2 log CFU/ml in the enhancement solutions of brine and water, respectively, for contamination

scenario-2 (Table 3.2). A portions of 0.1 (0.6±0.6 log CFU/g) % of inoculated *E. coli* O157:H7 levels in enhancement solutions were transferred to the topmost 1-cm thickness (section -1) of moisture-enhanced knuckles. There was a linear increase in pathogen levels transferred with increasing depth of knuckles after enhancement processing under this contamination scenario. Overall, pathogen counts recovered from section-6 representing the entire depth (14.5 cm) were higher by 0.9 CFU/g compared to the topmost 1-cm depth (section -1) of the knuckle after enhancement processing (Table 3.2). Gill et al. (2009) recovered higher levels (6.0 log CFU/g) of *E. coli* O157:H7 from 3-cm thick steaks after injection with three inoculated enhancement solutions (i. e., half strength Brain Heart Infusion (BHI), brine containing 2% NaCl+2% Sodium Tripolyphosphate (TPP), brine containing 5% NaCl+5% TPP) compared to levels observed in the present study. Total bacterial counts recovered from different depths of enhanced knuckles were higher compared to *E. coli* O157:H7 levels recovered from corresponding depths under this contamination scenario (Tables 3.2 and 3.3). This could be possibly due to translocation of background flora present on the external surface of knuckles to different depths of enhanced knuckles along with contaminated enhancement solutions.

Pathogen levels translocated to all different depths (topmost surface to deepest tissues) of moisture-enhanced knuckles when surface inoculated (contamination scenario-1) were higher ($p \geq 0.05$) than translocated when contaminated enhanced solutions injected (contamination scenario-2) (Table 3.2). Contrary to the results of the present study, Echeverry et al. (2009) recovered similar levels of *E. coli* O157:H7 from different depths of 7.6-cm thick surface inoculated **(**5.0 log CFU/g) steaks aged for 14 or 21 days,

when subjected to mechanical tenderization (2.9-4.0 log CFU/g) and brine-injection (3.5-4.0 log CFU/g) processing. During moisture enhancement processing, movement of the needles of the brine injector and injection solutions, carry the cells into the interior of the muscles, and translocate the contamination into the deeper tissues of beef cuts (Thippareddi et al., 2000). Large standard deviations for bacterial population means recovered from different depths under both contamination scenarios could be due to differences in amounts of enhancement solution uptakes by knuckles during enhancement processing. Differences in enhancement solution uptake could be attributed to differences in size and compactness of different knuckles as explained earlier.

Moreover, internal muscle structure could influence the distribution of brine interior of muscles and brine solutions usually are better distributed parallel than perpendicular to the direction of muscle fibers during enhancement processing (Uttaro and Aalhas, 2007). Bacterial cells are usually oriented along muscle fibers in the interior of muscle tissue (Uttaro and Aalhas, 2007) and have non-uniform deposition at varying depths of injected meat products.

3.4. CONCLUSIONS

The results of this study indicated that needle injection processing of muscle cuts at 103-110% enhancement levels can translocate *E. coli* O157:H7 cells into the deepest tissue of enhanced muscle cuts either when contamination is present on the external surface of meat cuts or in contaminated enhancement solutions, irrespective of composition of enhancement solution. Higher pathogen levels were vertically translocated to the topmost tissues close to the injection site when contamination was

present on the external surface of meat cuts and higher contamination levels were translocated to deepest tissues of enhanced knuckles when contaminated enhancement solutions were injected. Additionally, the high pathogen levels found in purge collected after enhancement processing should discourage the industry for recycling these solutions and/or mix them with fresh brining solutions. The findings of this study could be helpful in understanding translocation patterns of pathogen cells under different contamination scenarios during moisture enhancement processing that would be helpful in risk assessments of nonintact meat products.

Table 3.1. Percent moisture enhancement, and total bacterial and *E. coli* O157:H7 counts (mean±standard deviation) in purge collected after processing of beef knuckles with water or brine solution, when pathogen cells were inoculated on the knuckle surface (4.7±0.3 log CFU/g) (contamination scenarios-1) or inoculated enhancement solutions (i. e., water or brine; 3.7±0.1 and 3.4±0.2 log CFU/ml, respectively) were used (contamination scenario-2)

Parameter	Scenario-1		Scenario-2	
	Water	Brine	Water	Brine
Moisture Enhancement (%)	6.3±0.7[a]	9.7±0.5[a]	4.3±1.3[a]	4.8±0.7[a]
Total Bacterial Population (log CFU/g)	6.7±1.2[a]	6.6±0.5[a]	6.4±2.0[a]	5.6±1.0[a]
E. coli O157:H7 (log CFU/g)	6.2±0.3[a]	6.2±0.2 [a]	5.3±2.1[a]	5.3±1.1[a]

Mean values within each row with different letters are different (P<0.05).

Table 3.2 (Appendix Figure 1). *E. coli* O157:H7 counts (mean±standard deviation; log CFU/g) and percent of inoculated pathogen cells transferred to different depths of knuckles after injection when pathogen cells inoculated, on the knuckle surface (contamination scenario-1) and in enhancement solutions, i. e. water or brine solution (Contamination scenario-2)

Knuckle	Scenario-1		Scenario-2	
Sections (cm)	Log CFU/g	Cell Transfer (%)	Log CFU/g	Cell Transfer (%)
1 (1-cm)	3.3 ± 0.8 [aX]	3.2	0.6 ± 0.4 [aY]	0.1
2 (2-cm)	2.6 ± 0.6 [aX]	0.6	0.8 ± 0.7 [aY]	0.2
3 (3-cm)	2.4 ± 0.8 [aX]	0.4	1.1 ± 0.7 [aY]	0.3
4 (5-cm)	2.6 ± 0.5 [aX]	0.7	1.4 ± 0.3 [aY]	0.6
5 (7-cm)	2.8 ± 0.3 [aX]	1.0	1.5 ± 0.5 [aY]	0.8
6 (14.5-cm)	2.8 ± 0.2 [aX]	1.1	1.5 ± 0.4 [aY]	0.8

Surface inoculation level: 4.7±0.3 log CFU/g.
Water and brine solution inoculation levels: 3.7±0.1 and 3.4±0.2 log CFU/ml, respectively.
Mean values within each column with different letters are different ($P \leq 0.05$).
Mean values with different uppercase letters in the same row with in each section for both contamination scenarios are significantly different ($P \leq 0.05$).
The values in the parenthesis along with each section number represent the depth of knuckles.
*Percent cell transfer is calculated as [Pathogen count (log CFU/g) in each section from knuckles after moisture enhancement/Pathogen count (log CFU/g) in topmost section (1-cm depth) from knuckle without moisture enhancement] × 100.

Table 3.3 (Appendix Figure 2). Total bacterial populations (mean±standard deviation; log CFU/g) and percent of inoculated pathogen cells transferred to different depths of knuckles after injection when pathogen cells inoculated on the knuckle surface (contamination scenario-1) and in enhancement solutions, i. e. water or brine solution (Contamination scenario-2).

Knuckle	Scenario-1		Scenario-2	
Sections (cm)	Log CFU/g	Cell Transfer (%)	Log CFU/g	Cell Transfer (%)
1 (1-cm)	3.7 ± 1.3^{aX}	3.3	2.7 ± 1.7^{aX}	13.3
2 (2-cm)	2.8 ± 0.7^{aX}	0.4	2.6 ± 1.6^{aX}	9.0
3 (3-cm)	2.7 ± 0.8^{aX}	0.3	2.3 ± 0.8^{aX}	4.5
4 (5-cm)	2.7 ± 0.7^{aX}	0.3	2.0 ± 0.6^{aX}	2.4
5 (7-cm)	2.8 ± 0.3^{aX}	0.4	2.1 ± 0.9^{aX}	2.9
6 (14.5-cm)	3.2 ± 1.2^{aX}	1.0	3.2 ± 1.4^{aX}	43.0

Surface inoculation level: 4.7±0.3 log CFU/g.
Water and brine solution inoculation levels: 3.7±0.1 and 3.4±0.2 log CFU/ml, respectively.
Mean values within each column with different letters are different ($P \leq 0.05$).
Mean values with different uppercase letters in the same row with in each section for both contamination scenarios are significantly different ($P \leq 0.05$).
The values in the parenthesis along with each section number represent the depth of knuckles.
*Percent cell transfer is calculated as [Pathogen count (log CFU/g) in each section from knuckles after moisture enhancement/Pathogen count (log CFU/g) in topmost section (1-cm depth) from knuckle without moisture enhancement] × 100.

Figure 3.1. Hand-operated, single-needle brine injector (Dick Companies, Postfach, Deizisau, Germany.

Figure 3.2. Coring sampling device (Facilities Maintenance, Colorado State University, Fort Collins).

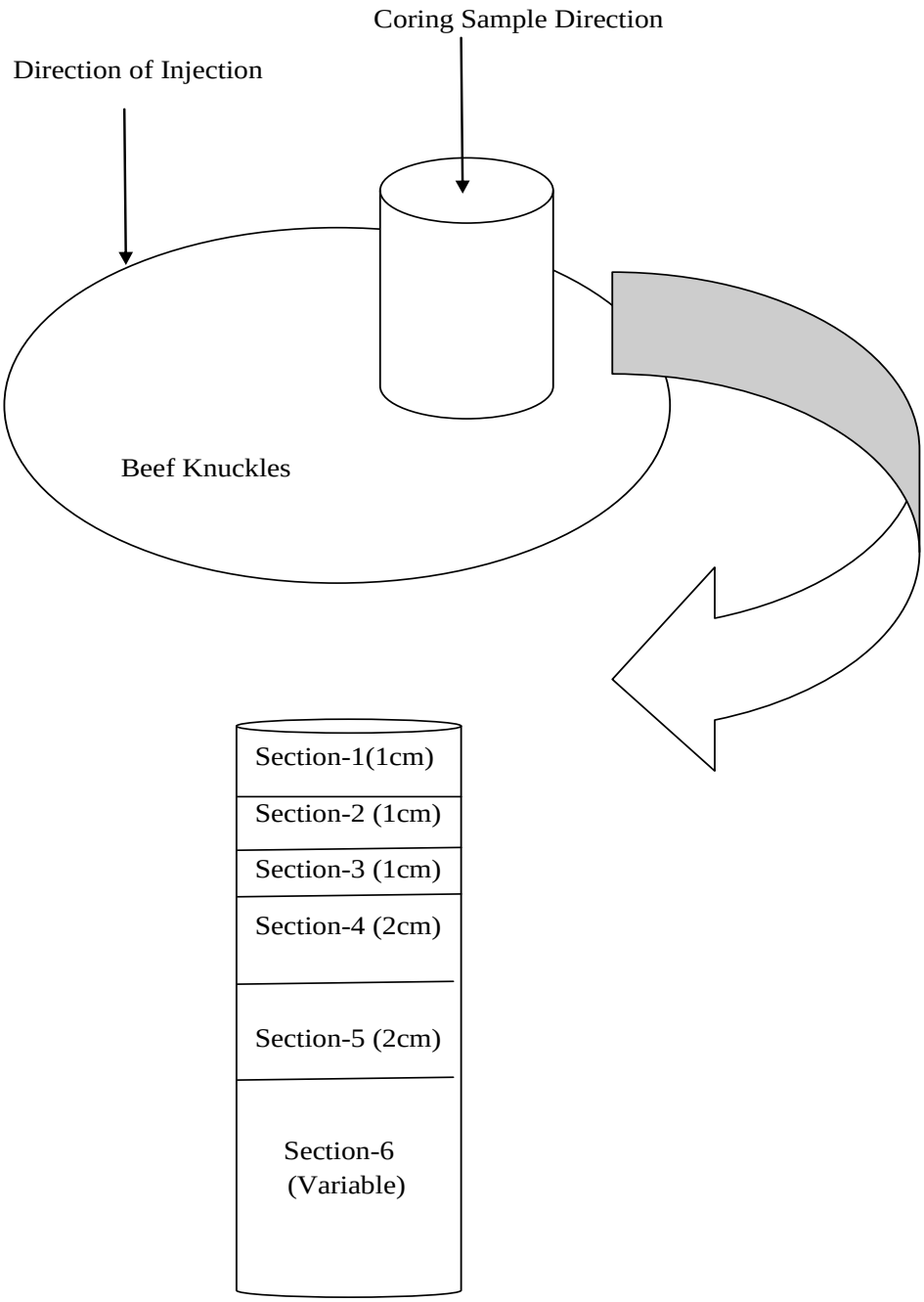

Figure 3.3. Brine solution enhancement and coring samples direction, and sectioning of core samples into six sections of different thicknesses, under both contamination scenarios.

CHAPTER 4

SANITATION OF TENDERIZER BLADES TO REDUCE VERTICAL TRANSLOCATION AND HORIZONTAL TRANSFER OF *ESCHERICHIA COLI* O157:H7 DURING BEEF PROCESSING

Blade tenderizers contaminated with *Escherichia coli* O157:H7 during meat tenderization may be a source of cross-contamination. This study evaluated the extent of *E. coli* O157:H7 contamination vertically translocated from surface-inoculated beef steaks to their interior during blade tenderization, and horizontally transferred to a subsequently processed uninoculated steak, without or with prior sanitation of the tenderizer. Thin slices (approximately 7×7×0.2 cm) of beef eye of round (*Semitendinosus* muscle) were placed on top of each other to form 3.0 cm-thick steaks. The topmost surface of the steaks was inoculated with rifampicin-resistant *E. coli* O157:H7 (8-strain composite; at two contamination levels; high: 7.0 log CFU/g and low: 4.2 log CFU/g) and the whole steak was then subjected to single-pass blade tenderization. The same tenderizer was then used to tenderize an uninoculated steak, without or with prior sanitation of the blades. Total bacterial and *E. coli* O157:H7 counts (six replicates) were determined for the topmost slice (0.2 cm) and for steak depths of 0.2-1.5 and 1.5-3.0 cm. Single-pass tenderization, without or with sanitation of blades, vertically translocated surface contamination throughout the 3.0 cm-thick surface inoculated steak (high: 3.5-5.4 log CFU/g and low: 1.3-2.9 log CFU/g), and also horizontally transferred contamination

to the surface (high: 5.8 log CFU/g and low: 2.9 log CFU/g) and interior (high: 2.2-4.2 log CFU/g and low: 0.7-1.5 log CFU/g) of the subsequently tenderized uninoculated steak; the extent of transferred contamination decreased with steak depth. Blade sanitation (30 sec exposure) treatments reduced transfer of contamination for the high surface contamination level (7.0 log CFU/g) in the order: water at 25-30°C < peroxyacetic acid (PAA)/hydrogen peroxide (HP)/octanic acid (OA) = PAA/HP < 70°C water = 94°C water = 94°C water followed by PAA/HP/OA.

4.1. INTRODUCTION

Blade tenderization processing helps to improve tenderness of lower quality meat cuts but it may vertically translocate surface contamination into deeper tissues of processed cuts, as well as horizontally transfer contamination to subsequently tenderized meat cuts (Chorianopoulos et al., 2009; Huang and Sheen., 2010; Sofos et al., 2008). Mechanically tenderized and/or moisture-enhanced beef steaks contaminated with *E. coli* O157:H7 has been linked to outbreaks reported (Lain et al., 2005; USDA-FSIS, 2005 and 2007). Contamination of enhanced/tenderized beef steaks linked to these outbreaks may be insufficient sanitation of tenderizing equipment during processing (Englejohn, 2005). Following these three outbreaks, the Food Safety and Inspection Service (FSIS) published a notice requiring beef processors to reassess their hazard analysis critical control point (HACCP) plans for raw and cooked mechanically tenderized beef products, including products that were injected with a marinade (or "enhanced" products)" (USDA-FSIS, 2005). Proper cleaning and sanitation of needle or blade tenderizing equipment could be an important control measure for reducing and/or preventing contamination of

tenderized beef products (Sofos et al., 2008), and blade tenderizer sanitation could be adopted by processors as one of the critical control points in their HACCP plans.

Several studies reported in the literature quantified vertical translocation of surface contaminated pathogen cells at different depths into sterile internal tissues of meat cuts during mechanical tenderization (Echeverry et al., 2009; Gill and McGinnis, 2004; Hajmeer et al., 2000; Johnston et al., 1978; Luchansky et al., 2008, 2009; Sporing, 1999; Thippareddi et al., 2000). However, there is a scarcity of studies in the scientific literature that quantified horizontal transfer of pathogen cells to subsequently tenderized meat during tenderization processing.

Blade tenderizer sanitation could reduce/prevent vertical translocation of surface contamination into the deeper tissues of processed cuts, but also can prevent horizontal transfer of contamination to subsequently tenderized meat cuts. Because of wide availability, economic feasibility and environmental friendliness, and effectiveness of water at different temperatures to reduce *E. coli* O157:H7 contamination on beef carcass surfaces (Sofos and Smith, 1998), water was selected as one of the treatments for blade tenderizer sanitization. Effectiveness of peroxyacetic acid-based sanitizers against *E. coli* O157:H7 under different conditions has been reported in the literature (Adler et al., 2009; Gill and Badoni 2004; Farrell et al., 1998; Ransom et al., 2003). An equilibrium mixture of peroxyacetic acid, octanoic acid, acetic acid, hydrogen peroxide, peroxyoctanoic acid, and 1-hydroxyethylidene-1, 1-diphosphonic acid has been approved by FSIS for beef carcasses decontamination (Code of Federal Regulations, 2003b). Two commercially available peroxyacetic acid-based sanitizers were selected as another treatment for blade tenderizer sanitation in the present study.

Therefore in the first objective of this study, extent of vertical translocation of *E. coli* O157:H7 contamination from beef steaks surface-inoculated at two contamination levels to the interior of the same steak, and horizontal transfer of contamination to a subsequently processed uninoculated steak during single-pass blade tenderization was evaluated. Another objective of this study, effectiveness of six sanitation treatments to reduce cross-contamination of beef steaks with *E. coli* O157:H7 during single-pass blade tenderization was evaluated.

4.2. MATERIALS AND METHODS

4.2.1. Bacterial strains and inoculum preparation

Eight rifampicin-resistant derivatives of *E. coli* O157:H7 strains ATCC 43888 (human isolate), ATCC 43895 (raw hamburger meat isolate), ATCC 43895/ISEHGFP (Noah et al., 2005), and C1-057, C1-072, C1-109, C1-154, and C1-158 (bovine fecal isolates, Carlson et al., 2009) were used in the present study. Rifampicin (100 µg/ml) resistant variants of these eight *E. coli* O157:H7 strains were isolated as described by Kaspar and Tamplin (1993). Strains were individually activated and subcultured (35°C, 24 h) in 10 ml of tryptic soy broth (TSB; Difco, Becton Dickinson, Sparks, MD) plus rifampicin (100 µg/ml; Sigma, St. Louis, MO). Cultures of strains were subsequently combined, centrifuged (4,629×*g*, 15 min, 4°C; Eppendorf, model 5810 R, Brinkmann Instruments Inc., Westbury, NY), washed with phosphate buffered saline (PBS; pH 7.40, 0.2 g/L KH_2PO_4, 1.5 g/L $Na_2HPO_4.7H_2O$, 8.0 g/L NaCl and 0.2 g/L KCl), and cell pellets were resuspended in PBS. The strain composite was used to inoculate the topmost surface of beef steaks at high (7.0 log CFU/g) contamination level. The culture composite was

diluted in PBS to 6 log CFU/ml before inoculation to achieve the lower (4.0 log CFU/g) contamination level.

4.2.2. Beef steak preparation and inoculation

Eye of round meat cuts (*Semitendinosus* muscle; 72 h postmortem and 95% lean) obtained from a meat packing plant in Northern Colorado were stored in frozen (-23°C) vacuum packages before use. Frozen meat was thawed at 4°C for 48-72 h and semi frozen meat was cut into thin slices (0.2 - 0.3 cm) with a deli slicer. Thirteen of these meat slices were placed on each other to form 3.0-cm thick beef steaks under biological cabinet to prevent contamination from air. Beef steaks were kept at 4°C for few hours before tenderization processing to completely thaw the meat. The topmost slice of each steak was surface-inoculated with one ml of the eight strain rifampicin-resistant *E. coli* O157:H7 composite at high (7 log CFU/g) and low (4 log CFU/g) contamination levels, and uniformly spread on the entire surface with a sterile glass rod. Surface-inoculated beef steaks were kept at 4ºC for 15 min for bacterial attachment.

4.2.3. Tenderization processing and sanitation treatment of tenderizer

The hand-operated blade tenderizer (Jaccard Super Meat Tenderizer Size: 146×38×102 mm, Orchard Park, NY) used in the present study had 48 blades on a surface area of 146×38 mm. Surface-inoculated beef steaks (3-cm thick) were tenderized with this hand-operated tenderizer with a single-pass and with pressure enough so that blades of tenderizer completely pierce through the deepest tissue of steaks. The entire surface area of steaks was tenderized with blade tenderizer using multiple strokes (3-4). Contaminated blades without prior sanitation were used to tenderize an additional uninoculated 3-cm thick beef steak similarly in order to accomplish the first objective of

present study. Contaminated blades were sanitized with each treatment selected before tenderization of additional uninoculated steaks in order to accomplish the second objective of this study. For sanitation treatment, contaminated blades were completely immersed in 1500 ml of a sanitizer solution in a sterilized stainless steel bowl and washed with a swinging motion for 30 sec. The blade tenderizer was decontaminated with bleach between treatments and rinsed three times with sterile distilled water to remove bleach residues before use.

4.2.4. Tenderizer sanitation treatments

Contaminated blades of the tenderizer after steak tenderization processing were sanitized by exposing for 30 sec to one of the following treatments: (i) unsanitized (no treatment); (ii) water at 25-30°C; (iii) warm water (WW; water at 70°C); (iv) boiling water (BW; water at 94°C); (v). a mixture of peroxyacetic acid (PAA; 5.8%) and hydrogen peroxide (HP; 27.5%) (PAA/HP, 2500ppm, commercially available as Oxonia Active® by Ecolab Inc., St. Paul, MN); (vi) a mixture of PAA (4.4%)/HP (6.9%)/octanic acid (OA; 3.3%) (PAA/HP/OA; 2500ppm, commercially available as Vortexx™ by Ecolab Inc., St. Paul, MN); and (vii) a sequential treatment (30 s each) of boiling water at 94°C followed by PAA/HP/OA. Commercially available peroxyacetic acid-based sanitizers (PAA/HP and PAA/HP/OA) selected for the present study are recommended by manufacture at concentrations of 0.13-0.26% and 0.20-0.28%, respectively, for food contact surfaces sanitation and no rinsing of food contact surfaces is recommended after sanitation treatment. Both of these peroxyacetic acid-based sanitizers were used at concentration levels of 2500 ppm (0.25%) for blade tenderizer sanitation. The final concentration (2500 ppm) was prepared by dissolving concentrated solutions of these

peroxyacetic acid-based sanitizers in 1500 ml of sterile distilled water and thoroughly mixed before use.

4.2.5. Microbiological analysis

Background flora and surface inoculated pathogen cells could be transferred to the deepest tissue of the 3-cm beef steak with run off purge from all sides during tenderization processing. Tenderized beef steaks were turned upside down and trimmed from all four sides to quantify bacterial cells translocated and/or transferred with blades of tenderizer cells during processing. Two sets of six slices, representing the 0.2-1.5 and 1.5-3.0 cm thicknesses of a steak, and the topmost slice (0.2 cm), from each processed steaks, were placed in a separate whirl pack bags (24-oz, 1,627 ml, 19 by 30 cm; Nasco, Modesto, CA). These slices in bags were homogenized (Masticator, IUL Instruments, Barcelona, Spain) for 2 min with maximum recovery diluent (0.85% NaCl and 0.1% peptone) at a ratio of 1:1 (sample weight: volume [g] of MRD). Serial 10-fold dilutions of each sample were prepared in 0.1% buffered peptone water (BPW) and appropriate dilutions were surface-plated onto tryptic soy agar (TSA; Acumedia, Lansing, MI) and TSA plus rifampin (100 mg/ml; TSA+rif) for enumeration of total bacterial and inoculated *E. coli* O157:H7 populations, respectively. TSA and TSA+rif plates were incubated and colonies were counted manually after incubation at 25°C (72 h) and 35°C (48 h), respectively. Each treatment solution after sanitation of contaminated blades was also analyzed microbiologically for total bacterial and inoculated *E. coli* O157:H7 populations as described earlier.

4.2.6. Statistical analysis

The study was repeated six times (n=6) for both surface contamination levels with one surface-inoculated/uninoculated tenderized beef steak (three samples from different depths from each steak) for each sanitation treatment. Bacterial counts (total bacterial and *E. coli* O157:H7) were converted into log CFU/g before analysis. The experimental design for this study was a split-split plot: the sanitation treatment (6) variable was split into the variable steak (2: surface-inoculated/uninoculated), and then steak was split section (3) variable. Main effects of these three individual variables (sanitation treatment, steak, section) and their interactions (sanitation treatment × steak, sanitation treatment × section, steak × section and sanitation treatment × steak × section) were analyzed on dependent variable (bacterial counts - log CFU/g). Data for total bacterial and *E. coli* O157:H7 (Log CFU/g) populations were analyzed for main effects and their interactions using the Kenward-Roger ProMIX model procedure of SAS v9.2. Least square means was generated and separated using Tukey-Kramer test at $P < 0.05$.

4.3. RESULTS AND DISCISSION

4.3.1. Vertical translocation and horizontal transfer of *E. coli* O157:H7 during tenderization

Total microbial populations ranged from 2.9±0.4 to 3.5±0.3 log CFU/g in uninoculated sliced eye of round samples that were destined to be mechanically tenderized. Rifampicin-resistant *E. coli* O157:H7 were below the detection limit (< 0.3 log CFU/g) in these samples. Initial surface inoculation levels of *E. coli* O157:H7 on the topmost slice (0.2 cm) of the 3.0-cm thick steaks were 7.0±0.4 (high) and 4.2±0.2 (low) log CFU/g (Tables 4.1 and 4.2).

Percent of surface contamination levels (high: 7.0 and low: 4.2 log CFU/g) vertically translocated after single-pass blade tenderization processing were 77% and 69% to 0.2-1.5 cm depth, and 50% and 31% to 1.5-3.0 cm depth of beef steaks, respectively (Tables 4.1 and 4.2). Decrease in pathogen translocation levels with increase in the depth of beef steaks during tenderization processing was also reported by others (Chorianopoulos et al., 2009; Gill and McGinnis, 2004; Hajmeer et al., 2000; Luchansky et al., 2008; Sporing, 1999). High (7.0 log CFU/g: 77% and 55%) surface contamination levels led to higher vertical translocation levels of *E. coli* O157:H7 to the interior (0.2-1.5 and 1.5-3.0 cm) of beef steaks compared to low (4.2 log CFU/g: 69% and 31%, respectively) surface contamination levels after single-pass blade tenderization (Tables 4.1 and 4.2). Chorianopoulos et al. (2009) and Luchansky et al. (2008) also reported higher vertical translocation levels of *E. coli* O157:H7 during blade tenderization processing of beef cuts when inoculated at high compared to low pathogen levels. Total bacterial populations followed vertical translocation patterns similar to those of *E. coli* O157:H7 during tenderization processing of beef steaks inoculated at high and low pathogen levels (Tables 4.1-4.4).

The horizontal transfer of *E. coli* O157:H7 contamination occurred when contaminated blades after tenderization processing of inoculated beef steak used to tenderize an additional uninoculated 3-cm thick beef steak. Contaminated blades after single-pass tenderization processing of beef steaks inoculated at high (7.0 log CFU/g; 5.8 log CFU/g and 4.2-2.2 log CFU/g) levels horizontally transferred higher levels of pathogens to the topmost surface (0.2 cm) and interior (0.2-3.0 cm) of subsequently processed uninoculated beef steaks compared to pathogens transferred by contaminated

blades from low (4.0 log CFU/g; 2.9 log CFU/g and 1.5-0.7 log CFU/g, respectively) inoculation levels (Table 4.1 and 4.2). Horizontal transfer of total bacterial cells followed patterns similar to those of *E. coli* O157:H7 during tenderization processing of beef steaks at high inoculation levels (Tables 4.1 and 4.3). However, total bacterial counts (2.7-2.8 log CFU/g) recovered from surface (0.2 cm) and interior (0.2-3.0 cm) of uninoculated beef steaks processed with contaminated blades from beef steaks at lower (4.2 log CFU/g) contamination levels were higher than those of *E. coli* O157:H7 counts. The levels of these bacteria were similar (2.9±0.4 log CFU/g) to levels of these bacteria recovered from uninoculated sliced eye of round samples and could have represented natural background flora in eye of round meat cuts used for the present study (Tables 4.2 and 4.4).

4.3.2. Effectiveness of sanitizers to reduce cross-contamination

As indicated earlier, contaminated blades without prior sanitation horizontally transferred *E. coli* O157:H7 cells to the surface and the entire 3.0-cm depth of the subsequently processed uninoculated steaks and all the six sanitation treatments tested reduced horizontal transfer of *E. coli* O157:H7 during single-pass blade tenderization (Tables 4.1 and 4.2).

Among all blade tenderizer sanitation treatments tested, water at 25-30°C for 30 sec was least effective in reducing horizontal transfer; and these reductions were 1.8, 1.0-1.7 and 0.3-0.8 log CFU/g to 0.2, 0.2-1.5, and 1.5-3.0 cm depth of subsequently processed steak, respectively, for high (7.0 log CFU/g) contamination level (Table 4.1). Penney et al. (2007) reported similar (1.25 and 1.31 log CFU/cm^2) levels of reductions in *E. coli* O157:H7 counts on veal and beef carcasses, respectively, after washing with

potable water for 5 sec at 20°C. Similar reductions in *E. coli* O157:H7 counts on carcass surfaces with plain water at room temperature were reported by others (Ellebracht et al., 2005; Lillard, 1988), and these small reductions could be due to the washing effect of plain water at room temperature (Bolder, 1997) and physical removal of microorganism from these surfaces.

Warm (70°C) or boiling (94°C) water for 30 sec was the most effective in reducing horizontal transfer among all blade tenderizer sanitation treatments tested; and these reductions were 4.0-4.2, 3.3-3.5 and 1.6 log CFU/g to 0.2, 0.2-1.5 and 1.5-3 cm depth of subsequently processed steak respectively, for high surface contamination level (Table 4.1). Higher reductions (2-4 logs units) in *E. coli* O157:H7 horizontal transfer with warm (70°C) and boiling (94°C) water when used as blade tenderizer sanitation treatment during processing compared to reductions with plain water at room temperature (1-2 logs units) could be due to an additional antimicrobial action of the heat of boiling and warm water (Rodriguez et al., 1996). Based on a literature survey conducted by Sofos and Smith (1998), an average of 2-3 log unit reductions in pathogen counts could be achieved with hot water when used for carcass decontamination. Reductions in *E. coli* O157:H7 horizontal transfer with warm (70°C) and boiling (94°C) water were not statistically ($P > 0.05$) different when these treatments used for blade tenderizer sanitation during tenderization processing in the present study (Table 4.1); however, Davey and Smith (1989) reported higher effectiveness of water at 80-85°C in reducing *E. coli* O157:H7 counts on carcass surfaces compared to water at 74°C. Water at room temperature could be an inexpensive and simple contamination reduction strategy for beef processors that

produce mechanically tenderized steaks and effectiveness of this treatment could be enhanced with elevation of water temperatures.

Commercially available peroxyacetic acid based sanitizers i. e., PAA/HP (Oxonia Active®) and PAA/HP/OA (Vortexx™) tested were equally ($P \geq 0.05$) effective in reducing *E. coli* O157:H7 horizontal transfer, even though the composition of both sanitizers was different (Table 4.1). The reductions in horizontal transfer with both peroxyacetic acid based sanitizers for high dose inoculated steaks were 2.7, 2.6-2.9 and 1.4-1.8 log CFU/g to 0.0-0.2, 0.2-1.5 and 1.5-3.0 cm depths of additional tenderized steaks, respectively (Table 4.1). Similar levels of reductions (2.4 log CFU/cm^2) for *E. coli* O157:H7 were observed when inoculated stainless steel coupons were treated with a PAA/OA mixture at 2600 ppm (Adler et al. 2009). Efficacy of commercially available peroxyacetic acid solutions for reduction of *E. coli* O157:H7 populations on carcass surfaces has been widely studied. Ransom et al. (2003) reported that 0.02% peroxyacetic acid (prepared from 5% peracetic acid solution, Birko Corporation, Denver, CO) was effective in reducing population of by 1.0-1.4 log *E. coli* O157:H7 inoculated onto beef carcass tissue. In another study, Ellebracht et al. (2005) achieved 0.5-0.7 log reductions in *E. coli* O157:H7 populations on fresh beef trim when treated with peroxyacetic acid (Inspexx 200, Ecolab Inc., St. Paul, MN) at concentrations at 200, 500 and 1000 ppm. However, Gill and Badoni (2004) reported that 0.02% solution of peroxyacetic acid was not effective against *E. coli* O157:H7 on meat surfaces in a commercial setting. All of the above mentioned studies (Ransom et al. 2003; Ellebracht et al. 2005; Gill and Badoni 2004) reported lower reductions (0.0-1.4 logs) with peroxyacetic acid compared to reductions (1.4-2.9 logs) in *E. coli* O157:H7 populations achieved in present study. The

difference in reductions of pathogens counts with peroxyacetic acids could be due to lower concentrations (0.02% to 0.1%) of these solutions used for carcass decontaminations in the above mentioned studies compared to concentration (0.25%) used in the present study for blade tenderizer sanitation, and also differences in composition of these commercially available peroxyacetic acid solutions.

Sequential application of boiling (94°C) water followed by PAA/HP/OA (SBP) was more effective (3.6 and 3.8 log CFU/g) than peroxyacetic acid based sanitizer (2.7 and 2.6-2.9 log CFU/g,) when used individually for blade tenderizer sanitizer (Table 4.1). It has been well established (Bacon et al., 2000; Edler et al., 2000; Hardin 1995) that decontamination interventions when used in combinations are more effective in reducing pathogen populations on carcass surfaces than when used individually. After blade tenderizer sanitation with SBP, pathogen horizontal transfer levels was reduced by 3.6 and 3.8 log CFU/g to 0.0-0.2 and 0.2-1.5 cm depth, respectively, of blade tenderized beef steaks (Table 4.1). Farrell et al. (1998) reported similar levels (total 2-3 log CFU/cm^2) of reductions in *E. coli* O157:H7 population when used water at 60°C and commercially available peroxyacetic acid based sanitizer (Oxonia Active® by Ecolab Inc., St. Paul, MN) in sequence for treatment of contaminated stainless steel grinders.

All the five (i. e., warm and boiling water, both peroxyacetic acid based sanitizers, and SBP) sanitation treatments reduced pathogen horizontal transfer to equal or below detection limit (<0.3 log CFU/g) for low inoculation levels; and reductions were >2.3-2.5, >1.5 and > 0.7 log CFU/g to 0.2, 0.2-1.5 and 1.5- 3.0 cm depths of steaks (Table 4.2). Total bacterial populations recovered from all the three depths of beef steaks processed with sanitized blades for both contamination levels and all sanitation

treatments were higher than *E. coli* O157:H7 counts. These total bacterial levels represented possibly combined inoculated *E. coli* O157:H7 and natural background flora from meat (Tables 4.3 and 4.4).

All treatments after sanitation treatment from both contamination levels had total bacterial and *E. coli* O157:H7 counts below detectable limit (<0.0 log CFU/g); except for water at 25-30°C which had 2.1±1.4 log CFU/ml levels of these bacteria at the high contamination levels. High contamination levels in water at room temperature could led to spread of contaminants over a greater proportion in the processing area (Castillo et al., 2003).

4.4. CONCLUSIONS

The results of this study indicated that single-pass blade tenderization processing could vertically translocate *E. coli* O157:H7 cells in the entire 3.0 cm depth of tenderized beef steaks. Contaminated blade tenderizer could also horizontally transfer *E. coli* O157:H7 cells to the surface and interior of subsequently processed meat. All sanitation treatments tested could reduce horizontal transfer of contamination and the increasing order of effectiveness of these treatments against high contamination levels was water at 25-30°C < PAA/HP/OA= PAA/HP < 70°C water = 94°C water = 94°C water followed by PAA/HP/OA. All sanitation treatments tested, except water at room temperature, were effective in total elimination of horizontal transfer of pathogens for low contamination levels. The high pathogen loads in water at 25-30°C after sanitation of contamination blades should discourage beef processors from recycling this sanitation treatment during tenderization of meat cuts. Sanitation of the blade tenderizer could be adopted as an

intervention step by beef processors in HACCP systems for prevention of cross-contamination during mechanical tenderization processing of beef steaks.

Table 4.1 (Appendix Figures 5 and 7). *E. coli* O157:H7 population (high contamination level: 7.0 log CFU/g) at three depths of 3.0 cm thick beef steaks after processing with blade tenderizer that was previously sanitized or unsanitized (control). Exposure time of blades tenderizer for each sanitation treatment was 30 sec.

Depth (cm)	Inoculated Tenderized	Uninoculated Tenderized						
		No T (Control)	Water 25-30°C	WW (70°C)	BW (94°C)	PAA/HP (Oxonia Active®)	PAA/HP/OA (Vortexx™)	WW and PAA/HP/OA
0.2	7.0±0.4 ^{X}	5.8±0.3 ^{aX}	4.0±0.5 ^{bX}	1.6±1.1 ^{dX}	1.8±1.2 ^{dX}	3.1±0.8 ^{bcX}	3.1±0.8 ^{bcX}	2.2±0.7 ^{cdX}
0.2-1.5	5.4±0.5 ^{Y}	4.2±0.5 ^{aY}	2.5±0.4 ^{bY}	0.7±0.6 ^{cX}	0.9±0.7 ^{cX}	1.6±0.6 ^{bcY}	1.3±0.7 ^{bcY}	0.4±0.2 ^{cY}
1.5-3.0	3.5±0.9 ^{Z}	2.2±0.7 ^{aZ}	1.4±0.8 ^{abY}	0.6±0.5 ^{bX}	0.6±0.7 ^{bX}	0.4±0.1 ^{bY}	0.8±0.8 ^{bY}	1.3±1.0 ^{bXY}

No T = no treatment (unsanitized blades); WW = warm water, water at 70°C; BW = boiling water, water at 94°C; PAA = peroxyacetic acid; HP = hydrogen peroxide; OC = octanic acid; PAA/HP (Oxonia Active®) and PAA/HP/OA (Vortexx™) = 2500 ppm. Mean values within each specific steak depth for different sanitation treatments, not followed by same lower case letter within the same row are significantly different ($P < 0.05$) from each other. Mean values within specific sanitation treatment for different beef steak depths, not followed by same upper case letter within the same column are significantly different ($P < 0.05$) from each other. Detection limit to detect *E. coli* O157:H7 at different depths of tenderized beef steak was below 0.3 log CFU/g.

Table 4.2 (Appendix Figures 5 and 8). *E. coli* O157:H7 population (low contamination level: 4.2 log CFU/g) at three depths of 3.0 cm thick beef steaks after processing with blade tenderizer that was previously sanitized or unsanitized (control). Exposure time for blades tenderizer for each sanitation treatment was 30sec.

Depth (cm)	Inoculated Tenderized	Uninoculated Tenderized						
		No T (Control)	Water 25-30°C	WW (70°C)	BW (94°C)	PAA/HP (Oxonia Active®)	PAA/HP/OA (Vortexx™)	BW and PAA/HP/OA
0.2	4.2±0.2 ^{X}	2.9±0.1 ^{aX}	1.1±0.5 ^{bX}	< 0.3 ^{cX}	0.3±0.0 ^{bX}	0.4±0.1 ^{bX}	0.5±0.3 ^{bX}	0.3±0.0 ^{bX}
0.2-1.5	2.9±0.4 ^{Y}	1.5±0.3 ^{aY}	0.5±0.4 ^{bX}	< 0.3 ^{cY}	< 0.3 ^{cY}	< 0.3 ^{cY}	< 0.3 ^{cY}	< 0.3 ^{cY}
1.5-3.0	1.3±0.7 ^{Z}	0.7±0.7 ^{aY}	0.4±0.1 ^{aX}	< 0.3 ^{bY}	< 0.3 ^{bY}	< 0.3 ^{bY}	< 0.3 ^{bY}	< 0.3 ^{bY}

No T = no treatment (unsanitized blades); WW = warm water, water at 70°C; BW = boiling water, water at 94°C; PAA = peroxyacetic acid; HP = hydrogen peroxide; OC = octanic acid; PAA/HP (Oxonia Active®) and PAA/HP/OA (Vortexx™) = 2500 ppm. Mean values within each specific steak depth for different sanitation treatments, not followed by same lower case letter within the same row are significantly different ($P < 0.05$) from each other. Mean values within specific sanitation treatment for different steak depths, not followed by same upper case letter within the same column are significantly different ($P < 0.05$) from each other. Detection limit to detect *E. coli* O157:H7 at different depths of tenderized beef steak was below 0.3 log CFU/g.

Table 4.3 (Appendix Figures 6 and 9). Total bacterial population (high contamination level: 7.0 log CFU/g) at three depths of 3.0 cm thick beef steaks after processing with blade tenderizer that was previously sanitized or unsanitized (control). Exposure time of blades tenderizer for each sanitation treatment was 30sec.

Depth (cm)	Inoculated Tenderized	Uninoculated Tenderized						
		No T (Control)	Water 25-30°C	WW (70°C)	BW (94°C)	PAA/HP (Oxonia Active®)	PAA/HP/OA (Vortexx™)	BW and PAA/HP/OA
0.2	7.0 ± 0.4^{X}	5.6 ± 0.6^{aX}	4.4 ± 0.8^{abX}	3.3 ± 0.7^{abX}	3.3 ± 0.6^{bX}	4.0 ± 0.6^{abX}	3.7 ± 0.6^{abX}	3.3 ± 0.5^{abX}
0.2-1.5	5.5 ± 0.5^{X}	3.9 ± 0.7^{axy}	3.1 ± 0.8^{aX}	2.9 ± 0.6^{aX}	2.7 ± 0.3^{aX}	2.9 ± 0.6^{aX}	3.0 ± 0.2^{aX}	3.2 ± 0.5^{aX}
1.5-3.0	3.7 ± 0.9^{Y}	2.8 ± 1.1^{aY}	3.0 ± 0.7^{aX}	2.6 ± 0.3^{aX}	2.6 ± 0.6^{aX}	2.7 ± 0.8^{aX}	3.0 ± 0.4^{aX}	3.1 ± 0.5^{aX}

No T = no treatment (unsanitized blades); WW = warm water, water at 70°C; BW = boiling water, water at 94°C; PAA = peroxyacetic acid; HP = hydrogen peroxide; OC = octanic acid; PAA/HP (Oxonia Active®) and PAA/HP/OA (Vortexx™) = 2500 ppm. Mean values within each specific steak depth for different sanitation treatments, not followed by same lower case letter within the same row are significantly different ($P < 0.05$) from each other. Mean values within specific sanitation treatment for different steak depths, not followed by same upper case letter within the same column are significantly different ($P < 0.05$) from each other. Detection limit to detect total bacterial population at different depths of tenderized beef steak was below 0.3 log CFU/g

Table 4.4 (Appendix Figures 6 and 10). Total bacterial population (low contamination level: 4.2 log CFU/g) at three depths of 3.0 cm beef steaks after processing with blade tenderizer that was previously sanitized or unsanitized (control). Exposure time of blades tenderizer for each sanitation treatment was 30sec.

Depth (cm)	Inoculated Tenderized	Uninoculated Tenderized						
		No T (Control)	Water 25-30°C	WW (70°C)	BW (94°C)	PAA/HP (Oxonia Active®)	PAA/HP/OA (Vortexx™)	BW and PAA/HP/OA
0.2	4.2±0.2 ^{X}	3.2±0.2 ^{aX}	2.8±0.3 ^{aX}	2.6±0.5 ^{aX}	2.9±0.4 ^{aX}	2.8±0.4 ^{aX}	2.8±0.2 ^{aX}	2.3±0.6 ^{aX}
0.2-1.5	3.1±0.3 ^{Y}	2.8±0.2 ^{aX}	2.5±0.3 ^{aX}	2.4±0.4 ^{aX}	2.4±0.6 ^{aX}	2.5±0.6 ^{aX}	2.4±0.2 ^{aX}	2.2±0.4 ^{aX}
1.5-3.0	2.4±0.6 ^{Y}	2.7±0.3 ^{aX}	2.5±0.3 ^{aX}	2.4±0.2 ^{aX}	2.3±0.3 ^{aX}	2.6±0.6 ^{aX}	2.4±0.4 ^{aX}	2.5±0.5 ^{aX}

No T = no treatment (unsanitized blades); WW = warm water, water at 70°C; BW = boiling water, water at 94°C; PAA = peroxyacetic acid; HP = hydrogen peroxide; OC = octanic acid; PAA/HP (Oxonia Active®) and PAA/HP/OA (Vortexx™) = 2500 ppm. Mean values within each specific steak depth for different sanitation treatments, not followed by same lower case letter within the same row are significantly different ($P < 0.05$) from each other. Mean values within specific sanitation treatment for different steak depths, not followed by same upper case letter within the same column are significantly different ($P < 0.05$) from each other. Detection limit to detect total bacterial population at different depths of tenderized beef steak was below 0.3 log CFU/g.

CHAPTER 5

SURVIVAL OF *ESCHERICHIA COLI* O157:H7 DURING FROZEN STORAGE AND SUBSEQUENT COOKING OF MOISTURE-ENHANCED BEEF STEAKS

Ingredients used in brining formulations may interfere with thermal inactivation of *Escherichia coli* O157:H7 internalized in beef products during moisture enhancement. This study evaluated survival of *E. coli* O157:H7 during frozen storage and subsequent cooking to 60°C, using three cooking methods, of restructured beef steaks moisture-enhanced with four different brining formulations. Coarse-ground beef (95% lean: 0.95 cm) was inoculated (6 log CFU/g) with rifampicin-resistant *E. coli* O157:H7 (8-strain composite) and then mixed with one of the following brine treatments, with a total of 10% water added: sodium chloride (NaCl, 0.5%)+sodium tripolyphosphate (STP, 0.25%), NaCl+STP+cetylpyridinium chloride (CPC, 0.2%), NaCl+STP+lactic acid (0.3%), or NaCl+STP+sodium metasilicate (0.2%). Inoculated and treated meat was formed into steaks (2.5 cm thick), vacuum-packaged and stored at -20°C (30 days). On day-0 and -30, steaks were cooked to 60°C by pan-broiling (Presto®electric skillet), double pan-broiling (George Foreman®grill), or roasting (Magic Chef ®standard kitchen oven). Uncooked/cooked steaks were analyzed (two replications, three samples/treatment/replication) for total bacterial and *E. coli* O157:H7 populations. No reductions in *E. coli* O157:H7 counts in moisture-enhanced beef steak samples were

observed immediately (day-0 samples) and after storage under frozen (-20°C) conditions for 30 days with all tested brining treatments except for day-30 CPC-treated steak samples that had 0.5 log CFU/g lower pathogen counts compared to day-0 samples. Extent of thermal inactivation of *E. coli* O157:H7, when cooked to the internal temperature of 60°C, decreased in order of: double pan-broiling > pan-broiling = roasting, with reductions were in range from 2.5-4.5, 1.3-1.9 and 0.8-2.0 log CFU/g, respectively. The time taken for steaks to reach the target internal temperature 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). Results of this study indicated that addition of cetylpyridinium chloride to brining formulations and subsequent cooking by double pan-broiling could help to enhance the safety of moisture-enhanced restructured beef steaks when cooked to rare degree of doneness.

5.1. INTRODUCTION

Enhancement of beef products with injecting brine solutions (107% to 115% of the initial weight) helps to improve organoleptic properties like juiciness and tenderness (Miller, 1998). At the same time, multi-needle injection processing disrupts the muscle structure and translocates bacterial cells into the meat interior where they could survive during cooking; the bacterial cells could be either present on the external surface of knuckles and/or introduced with brine solutions. Contaminated moisture-enhanced beef products have resulted in outbreaks with many confirmed cases of *E. coli* O157:H7 infections and hundreds and thousands of kilograms of product recalled (CFP, 2008). Addition of antimicrobials in the enhancement solutions, and cooking of enhanced beef

products to appropriate degree of doneness (internal geometric central temperatures) before consumption are the interventions (Sofos et al., 2008) that were investigated for *E. coli* O157:H7 control in moisture-enhanced beef products in the present study.

Basic brining solutions, currently used by many processors contain salt and phosphate, are injected (107% to 115% of the initial weight) into beef products for moisture enhancement (Miller, 1998; Wicklund et al., 2005). Three antimicrobials, cetylpyridinium chloride (CPC), lactic acid (LA) and sodium metasilicate (SM), that were selected to be included in the brining formulation to improve microbial safety of enhancement products, have shown antimicrobial activity against *E. coli* O157:H7 (Cutter et al,. 2000; Carlson et al., 2008; Huffman, 2002; Ransom et al., 2003). CPC, a quaternary ammonium compound, is approved as a surface decontamination intervention for raw poultry carcasses either prior to or after immersion in chilling water (USDA-FSIS, 2010, FSIS Directive 7120.1; Byelashov et al., 2010). Lactic acid is approved for food animal carcass decontamination prior to fabrication (i. e., pre and post chill), variety meats, and for beef and pork subprimals and trimmings (USDA-FSIS, 2010). Sodium metasilicate is currently accepted as generally recognized as safe (GRAS) when used as a processing aid to wash fruits, vegetables, and nuts (FDA, 2003; Weber, 2004), as a component of marinades for raw meat and poultry products, and also approved as a surface decontamination intervention for raw beef carcasses, subprimals and trimmings, and RTE meats (USDA-FSIS, 2010, FSIS Directive 7120.1; Byelashov et al., 2010). There is a need for evaluation of the effectiveness of these three antimicrobials against *E. coli* O157:H7 when included in brining formulations during storage and cooking of restructured moisture-enhanced beef steaks.

Consumers' preference for underdone or undercooked beef to maintain juiciness and flavor could result in survival of *E. coli* O157:H7 cells internalized in moisture-enhanced beef products. The cooking recommendations for ground beef patties provided by the U.S. Department of Agriculture Food Safety Inspective Services (USDA-FSIS, 1999a) that are currently in place specify that all parts of the these meat products should be heated to internal temperatures of 63, 65 or 68°C for 180, 60 or 15 s, or to the minimum temperature of 71.2°C without the need for holding time. Studies (Rhee et al., 2003; Shen et al., 2010; Sporing, 1999) have reported different *E. coli* O157:H7 thermal inactivation levels in meat products when prepared with different consumer-style cooking methods, even though cooked to the same internal temperatures. Also, studies have reported that components of brine formulations could influence thermal inactivation of *E. coli* O157:H7 in moisture-enhanced meat products (Byelashov et al., 2010; Juneja et al., 1999; Mukherjee et al., 2008, 2009; Yoon et al., 2009). Cooking recommendations for meat products from federal agencies did not take into account the variability in thermal inactivation levels of *E. coli* O157:H7 populations due to these factors. Additionally, the National Advisory Committee on Microbiological Criteria for Foods (NACMCF, 2002) concluded that there are no sufficient data available whether nonintact blade tenderized beef products present a greater risk to consumers from *E. coli* O157:H7 compared to intact beef products if prepared similarly. Survival of *E. coli* O157:H7 in restructured beef steaks, moisture-enhanced with brine formulations containing antimicrobials (i.e., cetylpyridinium chloride, lactic acid or sodium metasilicate) after frozen (-20°C, 30 days) storage was investigated in the first objective of the present study. The potential effect of these brine formulation ingredients on thermal destruction of *E. coli* O157:H7 in

moisture-enhanced beef steaks, when cooked with three cooking methods (i.e., pan-broiling, double pan-broiling, and roasting) to an internal temperature of 60°C was investigated in the second objective of the study.

5.2. MATERIALS AND METHODS

5.2.1. Bacterial strains and inoculum preparation

Eight *E. coli* O157:H7 strains [i. e., ATCC 43888 (human isolate), ATCC 43895 (raw hamburger meat isolate), ATCC 43895/ISEHGFP (Noah et al., 2005), and C1-057, C1-072, C1-109, C1-154, and C1-158 (bovine fecal isolates) (Carlson et al., 2009)] were selected for inoculation of beef steaks in the present study. Rifampicin (100 µg/ml) resistant derivatives of these strains were isolated as described by Kaspar and Tamplin (1993), and these variants allow selective isolation from natural background flora in the meat. The thermotolerance of rifampicin-resistant derivatives of these strains were tested in tryptic soy broth (TSB; Difco, Becton Dickinson, Sparks, Md., U.S.A.) at 60°C and found not to be different from the parental strains (Ko et al., 2010). Each of these eight strains was activated individually and subcultured (35°C, 24 h) in 10 ml of tryptic soy broth (TSB; Difco, Becton Dickinson, Sparks, MD) plus rifampicin (100 µg/ml; Sigma, St. Louis, MO). All strains were subsequently combined on the day of the experiment, centrifuged (4,629×*g*, 15 min, 4°C; Eppendorf, model 5810 R, Brinkmann Instruments Inc., Westbury, NY), and washed twice with phosphate buffered saline (PBS; pH 7.40, 0.2 g/L KH_2PO_4, 1.5 g/L $Na_2HPO_4.7H_2O$, 8.0 g/L NaCl and 0.2 g/L KCl). The washed cell pellet was resuspended in PBS and used to inoculate the steaks at levels of 6.0 log CFU/g.

5.2.2. Preparation of moisture-enhanced beef steaks

The procedure for inoculation and moisture enhancement of coarse ground beef to prepare steaks for the present study was adopted from Shen et al. (2010). The moisture-enhanced beef steaks simulated restructured beef products. Fresh beef knuckles (72 h postmortem, 95% lean) were obtained from a local meat packing plant in Northern Colorado. The thawed knuckles (3°C for 48 h) were cut into smaller pieces to facilitate grinding and passed through a 0.95 cm plate for coarse-grinding (Hobart Mfg. Co., Troy, OH). Coarsely ground meat (2 kg) was mixed with 20 ml of the eight strain rifampicin-resistant *E. coli* O157:H7 inoculum (10 ml/kg) to achieve an initial inoculation level of approximately 6 log CFU/g in a bowl-lift stand mixer (KitchenAid®, Professional 600, St. Joseph, MI) for 2 min at "stir" speed. The inoculated meat was mixed with 180 ml of each brining treatment (90 ml/kg) for an additional 2 min at moisture-enhancement level of 110% of initial weight.

The four brining treatments selected for moisture-enhancement of beef steaks were: (i) sodium chloride (NaCl, 0.5%; Fisher Scientific, Fair Lawn, NJ) + sodium tripolyphosphate (STP, 0.25%; BK Giulini Corporation, Semi Valley, CA); (ii) NaCl (0.5%) + STP (0.25%) + cetylpyridinium chloride (CPC, 0.2%; Safe Foods Corporation, North Little Rock, AR); (iii) NaCl (0.5%) + STP (0.25%) + lactic acid (LA, 0.3%; PURAC America Inc.); and (iv) NaCl (0.5%) + STP (0.25%) + sodium metasilicate (SM, 0.2%; Danisco USA Inc.). The concentration of each brining ingredient (wt/wt finished product) to be included in brining formulations for the present study was selected based on allowable levels of each ingredient by federal regulations in meat products (USDA-

FSIS, 2010, FSIS Directive 7120.1), published research (Byelashov et al., 2010 and Shen et al., 2010), and levels used by industry.

Inoculated and treated coarse-ground beef was then extruded into polyethylene bags (2.3 kg, 15.2×45.7 cm, Koch; Kansas City, MO), 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 thick steaks, and individual steaks (2.5×15.2 cm) were placed into vacuum bags (15.2×21.6 cm, 3 mil standard barrier, nylon/PE vacuum pouch, water vapor, and oxygen transmission rates of 9.3 g/m^2/24 h [97% relative humidity] and 54.3 cm^3/m^2/24h 21°C, [0% relative humidity], respectively, Koch; Kansas City, MO), and vacuum-packaged (Hollymatic Corp., Countryside, IL). The vacuum packaged beef steaks were stored under frozen conditions (-20°C) for 30 days.

5.2.3. Cooking of moisture-enhanced beef steaks

Day-0 and -30 (partially thawed at 4°C for 24 h) 2.5-cm beef steak samples were cooked to an internal geometric temperature of 60°C (simulating rare doneness of beef, AMSA 1995), with three cooking methods. The terminology for different cooking methods used in the present study was adopted from Shen et al. (2010). The methods and appliances selected for steak cooking in the present study were: (i) pan-broiling with a 16-in. (~ 41 -cm) Presto® electric skillet (National Presto Industries Inc., Eau Claire, WI); (ii) double pan-broiling with a George Foreman®grill (Salton, Inc., Lake Forest, IL); (iii) roasting in a Magic Chef ® Kitchen Oven (Maytag Corp., Newton, IA). All cooking appliances were preheated before use. The Presto® electric skillet and the Magic Chef ® kitchen oven were maintained at 176.7°C (350°F) during cooking. Beef steak samples were placed in center of each cooking appliance. Steak samples 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. Temperature in the geometric center of each beef steak was continuously monitored with type-K thermocouple and recorded at 5-s intervals with PicoLog data acquisition (Pico Technology Ltd., Cambridge, UK) while cooking. Total time required to reach internal temperature of 60°C in the geometric center of each beef steak when cooked with three different cooking method was considered as cooking time for each steak in the present study.

5.2.4. Physiochemical and microbiological analyses

On days 0 and 30, steak samples before (uncooked) and immediately after cooking were analyzed for surviving *E. coli* O157:H7 and total bacterial populations, pH and water activity. However, only day-0 uncooked/cooked samples were analyzed for fat and moisture. The weight of each sample was measured before and immediately after cooking to determine cooking losses.

On each sampling day, individual beef steaks were placed into filter bags (55-oz, 1,627 ml, 19 by 30 cm; Nasco, Modesto, CA), weighed, and maximum recovery diluent (0.85% NaCl and 0.1% peptone) was added at a ratio of 1:1 (sample weight: volume [g] of MRD) to the samples which were then homogenized (Masticator, IUL Instruments, Barcelona, Spain) for 2 min. Serial 10-fold dilutions of each sample were prepared in 0.1% BPW and appropriate dilutions were surface-plated on tryptic soy agar (Acumedia, Lansing, MI) supplemented with 0.1% sodium pyruvate (Fisher Scientific, FairLawn, NY) (TSAP) and TSAP plus rifampicin (100 mg/ml; TSAP+rif) for enumeration of total bacterial populations and inoculated *E. coli* O157:H7, respectively. TSAP and TSAP+rif

plates were incubated and colonies were counted manually after incubation at 25°C (72 h) and 35°C (48 h), respectively. The pH of the meat homogenate was measured after plating of the sample by using a digital pH meter with a glass electrode (Denver Instruments, Arvada, CO). The water activity was measured with an AquaLab (model series 3, Decagon Devices, Inc., Pullman, WA) water activity meter. Moisture and fat contents were measured with AOAC International official methods 950.46.B and 960.39, respectively (AOAC, 2000).

5.2.5. Statistical analysis

This study was repeated twice with three individual samples per brining treatment per cooking method for each storage day per replicate. Microbial counts (total bacterial and *E. coli* O157:H7) were converted into log CFU/g before statistical analysis. Data for microbial counts, pH, water activity and cooking losses were analyzed for main effects (brining treatment, cooking method and storage day) and interactions between main factors (brining treatment × storage day, brining treatment × cooking method, storage day × cooking method, brining treatment × storage day × cooking method) using the PROCMIXED Model procedure of SAS v9.2 (SAS, 2002). Moisture and fat content data were statistically analyzed only for brining treatment and cooking method, and their interaction (brining treatment × cooking method). Multiple pairwise comparisons of the means were done using Tukey's honestly significance difference testes. Means were considered significantly different when P-values were less than 0.05.

5.3. RESUTS AND DISCUSSION

5.3.1. Physiochemical characteristics

The average pH of uncooked samples moisture-enhanced with NaCl + STP was 5.81±0.17, and was higher than pH values of uncooked samples without any enhancement processing (5.45-5.52) reported by Mukherjee et al. (2008) (Table 5.1). Addition of alkaline phosphates in the brining solutions used for moisture-enhancement processing of meat products has been reported to increase pH of the enhanced meat products (Baublits et al., 2006; Byelashov et al., 2010) which was found in the present study. The pH of enhanced steak samples after frozen (-20°C) storage for 30 days (5.87-6.40) was lower ($P < 0.05$) than that of day-0 (5.81-6.53) samples. Cooked enhanced steak samples had higher ($P < 0.05$) pH values (pH 6.01-6.53) than uncooked samples (pH 5.81-6.37). Other studies (Berry, 1998; Byelashov et al., 2010; Mukherjee et al., 2008; Trout, 1989) have reported similar increases in pH values of beef products after cooking. The pH values of steak samples moisture-enhanced with NaCl + STP (5.81-6.19), NaCl + STP + CPC (5.87-6.16), and NaCl + STP + LA (5.87-6.19) were not significantly ($P > 0.05$) different from each other, whereas the pH values for samples enhanced with NaCl + STP + SM (6.28-6.53) were significantly ($P < 0.05$) higher than the other three treatments (Table 5.1). Sodium metasilicate is a strong alkali (Weber et al., 2004), and has been reported to increase the pH of brine enhanced beef products (Byelashov et al., 2010; Quilo et al., 2009).

The water activity values for moisture-enhanced beef steaks ranged from 0.981 to 0.988, which were within the expected range and similar to values that have been reported by others (Table 5.2) (Byelashov et al., 2010; Mukherjee et al., 2008, 2009;

Yoon et al., 2009). The brining treatment, cooking method and storage day did not have significant effects ($P > 0.05$) on water activity values.

Moisture content of cooked (61.7-70.0%) moisture-enhanced beef samples were significantly ($P < 0.05$) lower than those of uncooked samples (72.2-73.7%); in contrast, fat contents of cooked (4.3-9.2%) samples were significantly ($P < 0.05$) higher than uncooked (3.6-6.0%) samples (Table 5.3). Similar trends in reductions of moisture contents and increases of fat contents of enhanced beef samples after cooking were reported by others (Anderson and Berry, 2001; Byelashov et al., 2010; Mukherjee et al., 2008; Shen et al., 2010; Trout, 1989). Shen at al. (2010) explained that water of the beef tissues could have either evaporated and/or have been expelled when samples were cooked for long periods of time leading to lower moisture content in cooked samples. Beef steak samples had lower moisture and fat contents after cooking by double pan-broiling compared to samples cooked by pan-broiling and roasting (Table 5.3). Similar to the results of the present study, Shen et al. (2010) reported lower moisture contents for moisture-enhanced steaks (2.5-cm thick) cooked by double pan-broiling compared to samples cooked by pan-broiling and roasting. Beef samples enhanced with brining treatments NaCl + STP + CPC/LA had significantly ($P < 0.05$) lower moisture, and higher fat contents compared to samples enhanced with the other two brining treatments of present study (i. e., NaCl + STP/+SM) (Table 5.3).

Cooking losses were variable (9.8-28.1%) for moisture-enhanced beef steaks when cooked to the internal temperature of 60°C (Table 5.4). Similarly, Byelashov et al. (2010) reported a cooking loss range from 1.4 to 23.6% for enhanced ground beef knuckles when cooked to 65°C, and a cooking loss range of 8.0-26.1% was reported by

Mukherjee et al. (2008) for enhanced ground beef samples when cooked to 60°C. Percent cooking losses for roasted (9.8-20.7%) samples were significantly ($P < 0.05$) smaller than pan-broiled (14.7-28.1%) and double pan-broiled (14.6-27.8%) samples (Table 5.4). Shen et al. (2010) also reported lower percent cooking losses for roasted (23.2 ± 5.1%) steak (2.5-cm thick) samples compared to pan-broiled (30.5 ± 4.3%) and double pan-broiled (30.1 ± 2.5%), when cooked to 65°C. Steak samples enhanced with NaCl + STP + SM (9.8-16.2%) had significantly ($P < 0.05$) lower cooking losses than samples enhanced with other brining treatments (i. e., NaCl + STP; 14.9-28.1%, NaCl + STP + CPC; 14.6-23.4%, and NaCl + STP + LA; 14.6-23.4%) (Table 5.4). Similar to results of the present study, Byelashov et al. (2010) reported lower cooking losses for ground beef knuckle and shoulder samples treated with a brine containing sodium metasilicate. Brewer and Novakofski (1999) reported highest cooking losses for ground beef model system with pH values close to 5.3-5.9 (i. e., vicinity of meat protein isoelectric point). As discussed earlier, addition of sodium metasilicate in brining formulation could increase pH values (6.28-6.53) for enhanced meat products beyond meat proteins isoelectric point and that could lead to lower cooking losses for enhanced meat products.

5.3.2. Surviving bacterial population in uncooked/cooked beef steaks

Total bacterial counts was 4.2 ± 1.1 log CFU/g in uninoculated coarsely ground meat. Rifamipicin-resistant *E. coli* O157:H7 were below the detection limit (<0.3 log CFU/g) in these samples. The average *E. coli* O157:H7 and total bacterial counts in uncooked moisture-enhanced with NaCl + STP steaks were 5.8±0.2 and 5.9±0.2 log CFU/g (as recovered with TSA and TSA+rif, respectively) on day-0 (Table 5.5 and 5.6). *E. coli* O157:H7 counts in steak samples enhanced with brining treatments containing

antimicrobials (i. e., NaCl + STP + CPC, NaCl + STP + LA and NaCl + STP + SM) (5.6 - 5.7 log CFU/g) were similar to those of steaks enhanced with brining treatments only NaCl + STP (5.8 log CFU/g) on day-0 (i. e., 18-24 h after inoculation and preparation of moisture-enhanced steaks). Similar to result of this study, Byelashov et al. (2010) reported no immediate reductions in *E. coli* O157:H7 counts in a ground beef model system enhanced with NaCl+STP, NaCl+STP+CPC and NaCl+STP+SM. Mukherjee et al. (2009) also reported no reduction in *E. coli* O157:H7 and total bacterial counts after overnight exposure to 0.27% lactic acid in restructured ground beef. *E. coli* O157:H7 counts were relatively unchanged in steak samples enhanced with NaCl+STP, NaCl+STP+LA, and NaCl+STP+SM brining treatments after storage under frozen (-20°C) conditions for 30 days; however, day-30 samples enhanced with cetylpyridinium chloride (NaCl+STP+CPC) brining treatment and stored under these conditions had 0.5 log CFU/g lower counts than day-0 samples (Table 5.5). Cetylpyridinium chloride had previously shown antimicrobial activity against *E. coli* O157:H7 in enhanced meat products prepared in a way similar to the present study. Byelashov et al. (2010) reported one log reductions of *E. coli* O157:H7 counts in CPC-treated ground beef samples after 24 h of storage at 4°C. The antimicrobial compounds (CPC, LA and SM) tested in present study have demonstrated antimicrobial effects against a wide range of foodborne pathogens depending on concentration and application method (Cutter et al., 2000; Pohlam et al., 2002; Ransom et al., 2003; Singh et al., 2005; Weber, 2004). Loss of antimicrobial properties of these compounds under conditions of the present study could be due to entrapment of bacterial cells in meat particles (Byelashov et al., 2010) and the strong buffering capacity of meat (Kyla-Puhju et al., 2004).

Variable terminology has been used by studies in the literature for cooking methods and equipment used by consumers for meat products preparation. The terminology for cooking methods used in the present was adopted from Shen et al., (2010). Irrespective of brining treatment and storage day, *E. coli* O157:H7 reductions were in the ranges of 2.4-4.5, 1.3-1.9 and 0.9-2.0 log CFU/g in moisture-enhanced beef steaks when cooked with double pan-broiling, roasting, and pan-broiling, respectively, to endpoint temperature of 60°C (simulating rare degree of doneness) in the geometric center of 2.5-cm beef steaks (Table 5.5). Contrary to results of the present study, Shen et al. (2010) reported higher levels of *E. coli* O157:H7 inactivation (2.9 log CFU/g) in roasted compared to pan-broiled (2.1 log CFU/g) and double pan-broiled (1.9 log CFU/g) 2.5 cm thick beef steaks samples when cooked to end point temperatures of 65°C. Other studies (Mukherjee et al., 2007; Ortega-Valenzuela et al., 2001; Sporing 1999) also reported higher levels of *E. coli* O157:H7 inactivation with broiling, oven broiling and oven grilling, respectively (referred as roasting by Shen et al., 2010), compared to other cooking methods such as grilling, electric skillet, frying tested under these studies. Similar to results of present study, cooking of food service ground beef patties with rapid high-temperature double-sided grilling-broiling system resulted in higher destruction (5.7 log CFU/g and 5.4 log CFU/g) in *E. coli* O157:H7 and *L. monocytoegens* counts, respectively, than samples cooked with single-sided broiling (D'SA et al., 2000). Shen et al., (2010) also reported differences in extent of thermal inactivation of these pathogens in meat products when cooked with these methods and explained that these differences could be due to differences in methods of heat transmission (i. e., conduction for pan-broiling and double pan-broiling, and convection for roasting, broiling and oven broiling)

involved in these cooking methods. Shen et al. (2010) also explained that discrepancy in *E. coli* O157:H7 reductions levels attained with cooking methods used in in these studies could be due to different types of beef products, inoculation methods, thicknesses of meat cuts, starting cooking surface equipment temperatures and starting sample temperatures.

Cooking times (i. e., time required to reach endpoint temperature of 60°C in geometric center of 2.5-cm thick moisture-enhanced steaks) 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) (Table 5.6). Shen et al. (2010) observed similar trends in cooking times for moisture-enhanced steaks when cooked by these methods to 65°C. Kerth et al. (2003) also observed the longest cooking times with oven roasting (22.8 min) and shortest with grilling method (7.0 min; referred as double pan-broiling in the present study) for beef loin strip steaks when cooked to 71°C. Ortega-Valenzuela et al. (2001) explained that the grilling method cooked steaks from both sides simultaneously increasing internal temperatures in short times reducing cooking times and increasing cooking rates. Shorter cooking time and faster cooking rates for double-pan broiled steak samples could be the reason for higher level of *E. coli* O157:H7 reductions attained with this cooking method in the present study (Table 5.5; Figures 5.1 and 5.2).

Even though antimicrobials tested in present study did not enhance or protect thermal destruction of *E. coli* O157:H7 at statistically ($P > 0.05$) significant levels, overall, CPC-treated steaks cooked with double-pan broiling had highest reductions (3.9 - 4.5 log CFU/g) for *E. coli* O157:H7 when samples were cooked to 60°C (Table 5.6). Mukherjee et al. (2009) did not find protective effects of salt/phosphate + lactic acid brining treatment on *E. coli* O157:H7 in ground beef model system when cooked to

60°C. However, Byelashov et al. (2010) observed statistically ($P < 0.05$) higher reductions of *E. coli* O157:H7 in CPC-treated ground beef samples compared to samples enhanced with other brining treatments when cooked to 65°C.

Total bacterial counts recovered from TSAP plates were similar to *E. coli* O157:H7 counts from TSAP + rif plates for the majority of beef steak samples indicating no or minimal injury by heat or antimicrobials (Byelashov et al., 2010). Total bacterial population reduction trends were consistent with *E. coli* O157:H7 reduction trends in moisture-enhanced 2.5-cm thick beef steaks during storage under frozen conditions for 30 days and during cooking with three consumer-style methods to endpoint temperature of 60°C (Table 5.7).

5.4. CONCLUSIONS

The results of this study indicated that addition of cetylpyridinium chloride at 0.2% concentration (wt/wt in final product) in meat with salt and phosphate in the brining formulations could help control of *E. coli* O157:H7 in moisture-enhanced beef steaks. The more rapid cooking method i. e., double pan-broiling was more effective in destroying *E. coli* O157:H7 in 2.5-cm beef steaks compared to other two slow cooking methods i. e., roasting and pan-broiling. Results of this study should be useful for development of novel brining formulations for enhancement of restructured beef products, and time and temperature recommendations for cooking of enhanced meat products at consumer level, that could enhance microbiological safety of nonintact meat products.

Table 5.1. The pH values (mean ± standard deviation) of uncooked/cooked beef steaks (2.5-cm thickness), moisture-enhanced with four brining formulations, after frozen (-20°C) storage for 30 days and after cooking with three cooking methods to 60°C.

Cooking Method	Treatment	Day-0	Day-30
None (Uncooked)	NaCl + STP	5.81 ± 0.17^{aX}	5.94 ± 0.07^{abX}
	NaCl + STP + CPC	5.87 ± 0.13^{abX}	5.88 ± 0.06^{aX}
	NaCl + STP + LA	5.91 ± 0.11^{abX}	5.87 ± 0.05^{aX}
	NaCl + STP + SM	6.37 ± 0.13^{cX}	6.28 ± 0.13^{cX}
Pan broiling	NaCl + STP	6.06 ± 0.12^{bX}	6.09 ± 0.08^{abX}
	NaCl + STP + CPC	6.10 ± 0.08^{bX}	6.01 ± 0.02^{abX}
	NaCl + STP + LA	6.19 ± 0.11^{bcX}	6.03 ± 0.04^{abX}
	NaCl + STP + SM	6.53 ± 0.08^{cX}	6.33 ± 0.09^{cY}
Double Pan broiling	NaCl + STP	6.05 ± 0.10^{bX}	6.10 ± 0.08^{bcX}
	NaCl + STP + CPC	6.16 ± 0.05^{bX}	6.12 ± 0.07^{bcX}
	NaCl + STP + LA	6.10 ± 0.10^{bX}	6.08 ± 0.07^{bX}
	NaCl + STP + SM	6.49 ± 0.08^{cX}	6.40 ± 0.09^{cX}
Roasting	NaCl + STP	6.19 ± 0.05^{bcX}	6.05 ± 0.06^{abX}
	NaCl + STP + CPC	6.05 ± 0.08^{bX}	6.04 ± 0.05^{abX}
	NaCl + STP + LA	6.16 ± 0.10^{bX}	6.01 ± 0.04^{abX}
	NaCl + STP + SM	6.49 ± 0.08^{cX}	6.33 ± 0.10^{cX}

NaCl = Sodium Chloride, 0.5%; STP = Sodium Tripolyphosphate, 0.25%; CPC = Cetylpyridinium Chloride, 0.2%; LA = Lactic Acid, 0.3%, SM = Sodium Metasilicate, 0.2%. Mean values with different lower case letter in the same column are significantly different ($P<0.05$). Mean values with different upper case letter in the same row are significantly different ($P<0.05$).

Table 5.2. Water activity values (mean ± standard deviation) of uncooked/cooked beef steaks (2.5-cm thickness), moisture-enhanced with four brining formulations, after frozen (-20°C) storage for 30 days and after cooking with three cooking methods to 60°C.

Cooking Method	Treatment	Day-0	Day-30
None (Uncooked)	NaCl + STP	0.984 ± 0.001 ^{aX}	0.984 ± 0.007 ^{aX}
	NaCl + STP + CPC	0.987 ± 0.007 ^{aX}	0.984 ± 0.003 ^{aX}
	NaCl + STP + LA	0.984 ± 0.001 ^{aX}	0.987 ± 0.003 ^{aX}
	NaCl + STP + SM	0.986 ± 0.004 ^{aX}	0.988 ± 0.002 ^{aX}
Pan broiling	NaCl + STP	0.986 ± 0.006 ^{aX}	0.985 ± 0.003 ^{aX}
	NaCl + STP + CPC	0.982 ± 0.003 ^{aX}	0.983 ± 0.004 ^{aX}
	NaCl + STP + LA	0.981 ± 0.008 ^{aX}	0.984 ± 0.004 ^{aX}
	NaCl + STP + SM	0.986 ± 0.004 ^{aX}	0.985 ± 0.004 ^{aX}
Double Pan broiling	NaCl + STP	0.986 ± 0.002 ^{aX}	0.985 ± 0.002 ^{aX}
	NaCl + STP + CPC	0.983 ± 0.003 ^{aX}	0.984 ± 0.002 ^{aX}
	NaCl + STP + LA	0.982 ± 0.003 ^{aX}	0.984 ± 0.003 ^{aX}
	NaCl + STP + SM	0.983 ± 0.003 ^{aX}	0.984 ± 0.002 ^{aX}
Roasting	NaCl + STP	0.984 ± 0.004 ^{aX}	0.988 ± 0.004 ^{aX}
	NaCl + STP + CPC	0.984 ± 0.003 ^{aX}	0.988 ± 0.004 ^{aX}
	NaCl + STP + LA	0.986 ± 0.003 ^{aX}	0.988 ± 0.002 ^{aX}
	NaCl + STP + SM	0.985 ± 0.003 ^{aX}	0.986 ± 0.004 ^{aX}

NaCl = Sodium Chloride, 0.5%; STP = Sodium Tripolyphosphate, 0.25%; CPC = Cetylpyridinium Chloride, 0.2%; LA = Lactic Acid, 0.3%, SM = Sodium Metasilicate, 0.2%. Mean values with different lower case letter in the same column are significantly different ($P<0.05$). Mean values with different upper case letter in the same row are significantly different ($P<0.05$).

Table 5.3. Fat and moisture contents (mean ± standard deviation) of uncooked/cooked beef steaks (2.5-cm thickness), moisture-enhanced with four brining formulations and after cooking with three cooking methods to 60°C.

Cooking Method	Treatment	Moisture	Fat
None (Uncooked)	NaCl + STP	73.7±1.6 [c]	4.6±1.7 [ab]
	NaCl + STP + CPC	72.2±2.4 [bc]	6.0±2.9 [ab]
	NaCl + STP + LA	72.3±2.7 [bc]	6.0±3.4 [ab]
	NaCl + STP + SM	73.5±1.8 [c]	3.6±1.2 [a]
Pan broiling	NaCl + STP	68.4±1.9 [bc]	5.5±1.2 [ab]
	NaCl + STP + CPC	63.7±4.8 [ab]	7.7±2.6 [ab]
	NaCl + STP + LA	64.3±2.9 [ab]	9.2±2.7 [b]
	NaCl + STP + SM	70.0±1.7 [bc]	5.0±1.2 [ab]
Double Pan broiling	NaCl + STP	65.6±3.1 [ab]	5.1±2.6 [ab]
	NaCl + STP + CPC	61.7±1.8 [a]	7.7±1.8 [ab]
	NaCl + STP + LA	61.8±1.7 [a]	6.4±1.5 [ab]
	NaCl + STP + SM	66.9±2.6 [ab]	4.3±1.0 [a]
Roasting	NaCl + STP	68.3±2.8 [bc]	5.4±1.2 [ab]
	NaCl + STP + CPC	65.9±1.7 [ab]	7.8±2.4 [ab]
	NaCl + STP + LA	67.1±3.0 [b]	7.8±3.0 [ab]
	NaCl + STP + SM	68.7±3.0 [bc]	6.9±4.1 [a]

NaCl = Sodium Chloride, 0.5%; STP = Sodium Tripolyphosphate, 0.25%; CPC = Cetylpyridinium Chloride, 0.2%; LA = Lactic Acid, 0.3%, SM = Sodium Metasilicate, 0.2%. Mean values with different lower case letter in the same column are significantly different ($P<0.05$).

Table 5.4 (Appendix Figure 11). Cooking losses (mean ± standard deviation) of uncooked/cooked beef steaks (2.5-cm thickness), moisture-enhanced with four brining formulations, after frozen (-20°C) storage for 30 days and after cooking with three cooking methods to 60°C.

Cooking Method	Treatment	Day-0	Day-30
Pan broiling	NaCl + STP	20.1 ± 4.1 ^{abX}	28.1 ± 5.7 ^{bX}
	NaCl + STP + CPC	21.2 ± 3.2 ^{abX}	23.3 ± 5.3 ^{bX}
	NaCl + STP + LA	20.2 ± 3.1 ^{abX}	25.4 ± 5.6 ^{bX}
	NaCl + STP + SM	15.0 ± 2.9 ^{abX}	14.7 ± 3.5 ^{abX}
Double Pan broiling	NaCl + STP	21.3 ± 8.1 ^{abX}	27.8 ± 2.2 ^{bX}
	NaCl + STP + CPC	23.6 ± 2.8 ^{bX}	23.4 ± 3.6 ^{bX}
	NaCl + STP + LA	23.5 ± 7.4 ^{bX}	25.7 ± 4.0 ^{bX}
	NaCl + STP + SM	14.6 ± 3.8 ^{abX}	16.0 ± 5.1 ^{abX}
Roasting	NaCl + STP	14.9 ± 2.6 ^{ab}	18.5 ± 2.7 ^{ab}
	NaCl + STP + CPC	14.6 ± 3.4 ^{abX}	18.6 ± 1.3 ^{abX}
	NaCl + STP + LA	17.8 ± 1.7 ^{abX}	20.7 ±10.5 ^{abX}
	NaCl + STP + SM	10.4 ± 1.1 ^{aX}	9.8 ± 1.7 ^{aX}

NaCl = Sodium Chloride, 0.5%; STP = Sodium Tripolyphosphate, 0.25%; CPC = Cetylpyridinium Chloride, 0.2%; LA = Lactic Acid, 0.3%, SM = Sodium Metasilicate, 0.2%. Mean values with different lower case letter in the same column are significantly different ($P<0.05$). Mean values with different upper case letter in the same row are significantly different ($P<0.05$).

Table 5.5 (Appendix Figures 12 and 13). *E. coli* O157:H7 population (log CFU/g; mean ± standard deviation) of uncooked/cooked beef steaks (2.5-cm thickness), moisture-enhanced with four brining formulations, after frozen (-20°C) storage for 30 days and after cooking with three cooking methods to 60°C.

Cooking Method	Treatment	Day-0	Day-30
None (Unncooked)	NaCl + STP	5.8 ± 0.2^{bX}	5.8 ± 0.1^{cX}
	NaCl + STP + CPC	5.6 ± 0.3^{bX}	5.1 ± 0.7^{cX}
	NaCl + STP + LA	5.7 ± 0.2^{bX}	5.8 ± 0.1^{cX}
	NaCl + STP + SM	5.6 ± 0.1^{bX}	5.3 ± 0.1^{cX}
Pan broiling	NaCl + STP	4.9 ± 0.4^{bX}	4.1 ± 0.3^{bcX}
	NaCl + STP + CPC	4.1 ± 0.5^{bX}	3.1 ± 1.2^{bX}
	NaCl + STP + LA	4.9 ± 0.1^{bX}	4.3 ± 0.9^{bcX}
	NaCl + STP + SM	4.4 ± 0.3^{bX}	3.7 ± 0.6^{bcX}
Double Pan broiling	NaCl + STP	3.3 ± 2.1^{abX}	1.8 ± 1.5^{abX}
	NaCl + STP + CPC	1.7 ± 0.7^{aX}	0.6 ± 0.7^{aY}
	NaCl + STP + LA	2.6 ± 2.2^{bX}	2.2 ± 1.0^{abX}
	NaCl + STP + SM	3.2 ± 1.6^{abX}	2.7 ± 1.2^{bX}
Roasting	NaCl + STP	4.4 ± 0.2^{bX}	4.1 ± 0.7^{bcX}
	NaCl + STP + CPC	3.9 ± 0.7^{bX}	3.2 ± 0.8^{bX}
	NaCl + STP + LA	4.3 ± 0.3^{bX}	4.0 ± 0.8^{bcX}
	NaCl + STP + SM	3.9 ± 0.8^{bX}	4.0 ± 0.5^{bcX}

NaCl = Sodium Chloride, 0.5%; STP = Sodium Tripolyphosphate, 0.25%; CPC = Cetylpyridinium Chloride, 0.2%; LA = Lactic Acid, 0.3%, SM = Sodium Metasilicate, 0.2%. Mean values within different lower case letter in the same column are significantly different ($P<0.05$). Mean values within different upper case letter in the same row are significantly different ($P<0.05$).

Table 5.6 (Appendix Figures 14 and 15). Cooking times (mean ± standard deviation) of uncooked/cooked beef steaks (2.5-cm thickness), moisture-enhanced with four brining formulations, after frozen (-20°C) storage for 30 days and after cooking with three cooking methods to 60°C.

Cooking Method	Treatment	Day-0	Day-30
Pan broiling	NaCl + STP	14.9±4.0	25.0±8.5
	NaCl + STP + CPC	18.3±5.4	16.5±4.7
	NaCl + STP + LA	18.0±6.0	16.1±5.3
	NaCl + STP + SM	16.4±4.7	14.5±2.6
Double Pan broiling	NaCl + STP	4.2±1.4	6.0±0.8
	NaCl + STP + CPC	4.9±0.5	5.9±1.4
	NaCl + STP + LA	4.7±1.2	6.4±1.2
	NaCl + STP + SM	5.6±0.9	4.9±1.3
Roasting	NaCl + STP	27.5±1.6	27.1±3.8
	NaCl + STP + CPC	23.3±4.5	25.9±2.4
	NaCl + STP + LA	27.4±2.1	25.2±3.9
	NaCl + STP + SM	23.9±3.0	23.9±4.3

NaCl = Sodium Chloride, 0.5%; STP = Sodium Tripolyphosphate, 0.25%; CPC = Cetylpyridinium Chloride, 0.2%; LA = Lactic Acid, 0.3%, SM = Sodium Metasilicate, 0.2%.

Table 5.7 (Appendix Figures 16 and 17).Total bacterial population (log CFU/g; mean ± standard deviation) of uncooked/cooked beef steaks (2.5-cm thickness), moisture-enhanced with four brining formulations, after frozen (-20°C) storage for 30 days and after cooking with three cooking methods to 60°C.

Cooking Method	Treatment	Day-0	Day-30
None (Uncooked)	NaCl + STP	5.9 ± 0.2^{cX}	6.0 ± 0.3^{cX}
	NaCl + STP + CPC	5.6 ± 0.4^{bcX}	5.0 ± 0.7^{cX}
	NaCl + STP + LA	5.8 ± 0.2^{bcX}	6.2 ± 0.2^{cX}
	NaCl + STP + SM	5.8 ± 0.3^{bcX}	5.8 ± 0.5^{cX}
Pan broiling	NaCl + STP	5.0 ± 0.4^{bcX}	4.2 ± 0.4^{bcX}
	NaCl + STP + CPC	4.2 ± 0.6^{bcX}	3.1 ± 1.2^{bX}
	NaCl + STP + LA	5.3 ± 0.8^{bcX}	4.5 ± 0.6^{bcX}
	NaCl + STP + SM	4.3 ± 0.3^{bcX}	4.0 ± 0.8^{bcX}
Double Pan broiling	NaCl + STP	3.6 ± 1.8^{abX}	2.3 ± 0.9^{abX}
	NaCl + STP + CPC	1.7 ± 0.7^{aX}	1.2 ± 1.0^{aX}
	NaCl + STP + LA	2.8 ± 2.1^{abX}	2.2 ± 1.0^{abX}
	NaCl + STP + SM	3.4 ± 1.5^{abX}	2.3 ± 1.6^{abX}
Roasting	NaCl + STP	4.4 ± 0.3^{bcX}	4.1 ± 0.6^{bcX}
	NaCl + STP + CPC	3.9 ± 0.7^{bX}	3.2 ± 0.7^{bcX}
	NaCl + STP + LA	4.3 ± 0.3^{bcX}	3.9 ± 0.8^{bcX}
	NaCl + STP + SM	3.9 ± 0.9^{bcX}	3.9 ± 0.5^{bcX}

NaCl = Sodium Chloride, 0.5%; STP = Sodium Tripolyphosphate, 0.25%; CPC = Cetylpyridinium Chloride, 0.2%; LA = Lactic Acid, 0.3%, SM = Sodium Metasilicate, 0.2%. Mean values within different lower case letter in the same column are significantly different ($P<0.05$). Mean values within different upper case letter in the same row are significantly different ($P<0.05$).

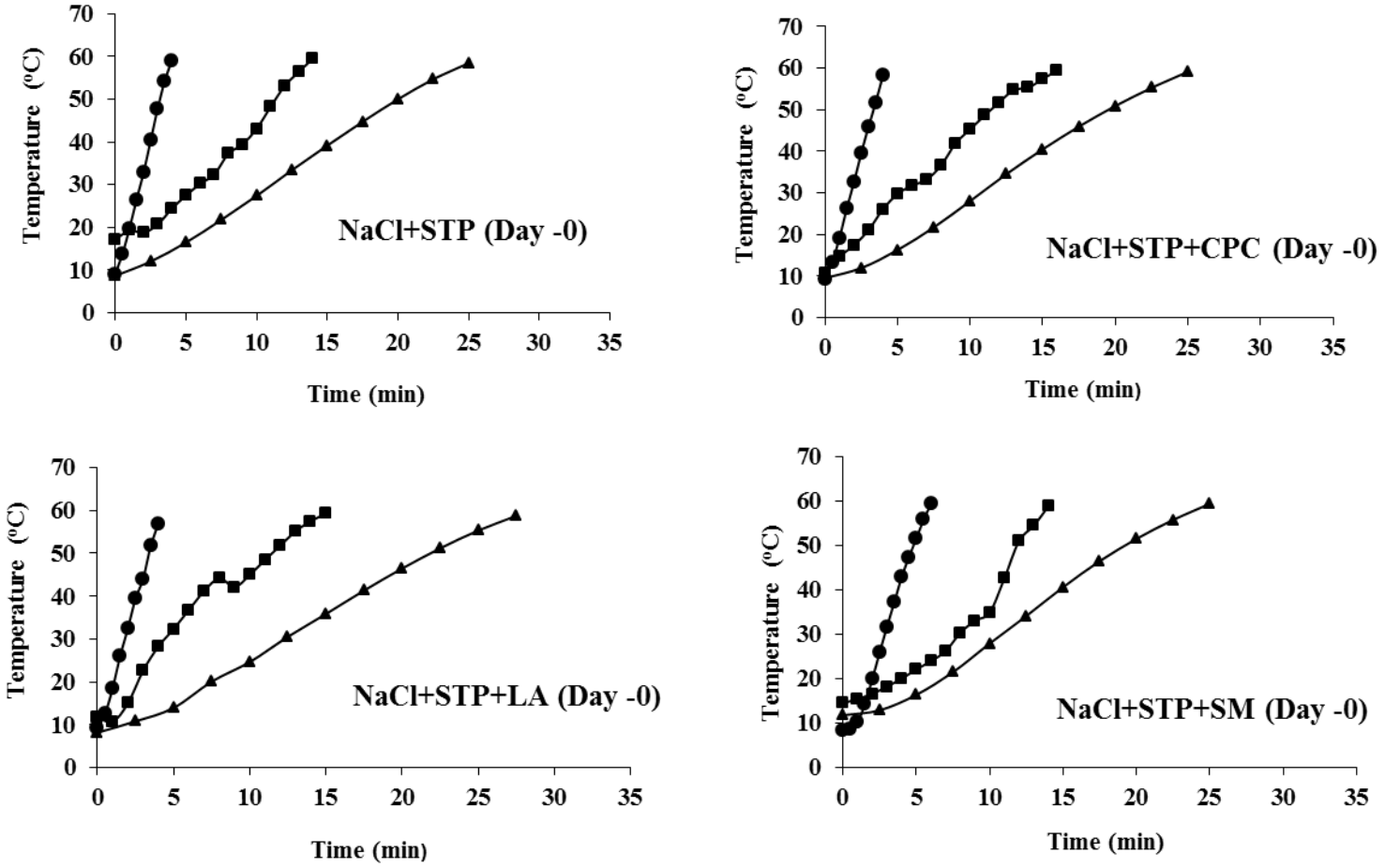

Figure 5.1. Cooking time and temperature curves for moisture-enhanced beef steaks (2.5-cm thickness) when cooked to internal temperature of 60°C in the geometric center with three cooking methods on day-0. Roasting (▲), Pan broiling (■) and Double pan broiling (●). Each point is the average of six determinants. NaCl = Sodium Chloride, 0.5%; STP = Sodium Tripolyphosphate, 0.25%; CPC = Cetylpyridinium Chloride, 0.2%; LA = Lactic Acid, 0.3%, SM = Sodium Metasilicate, 0.2%.

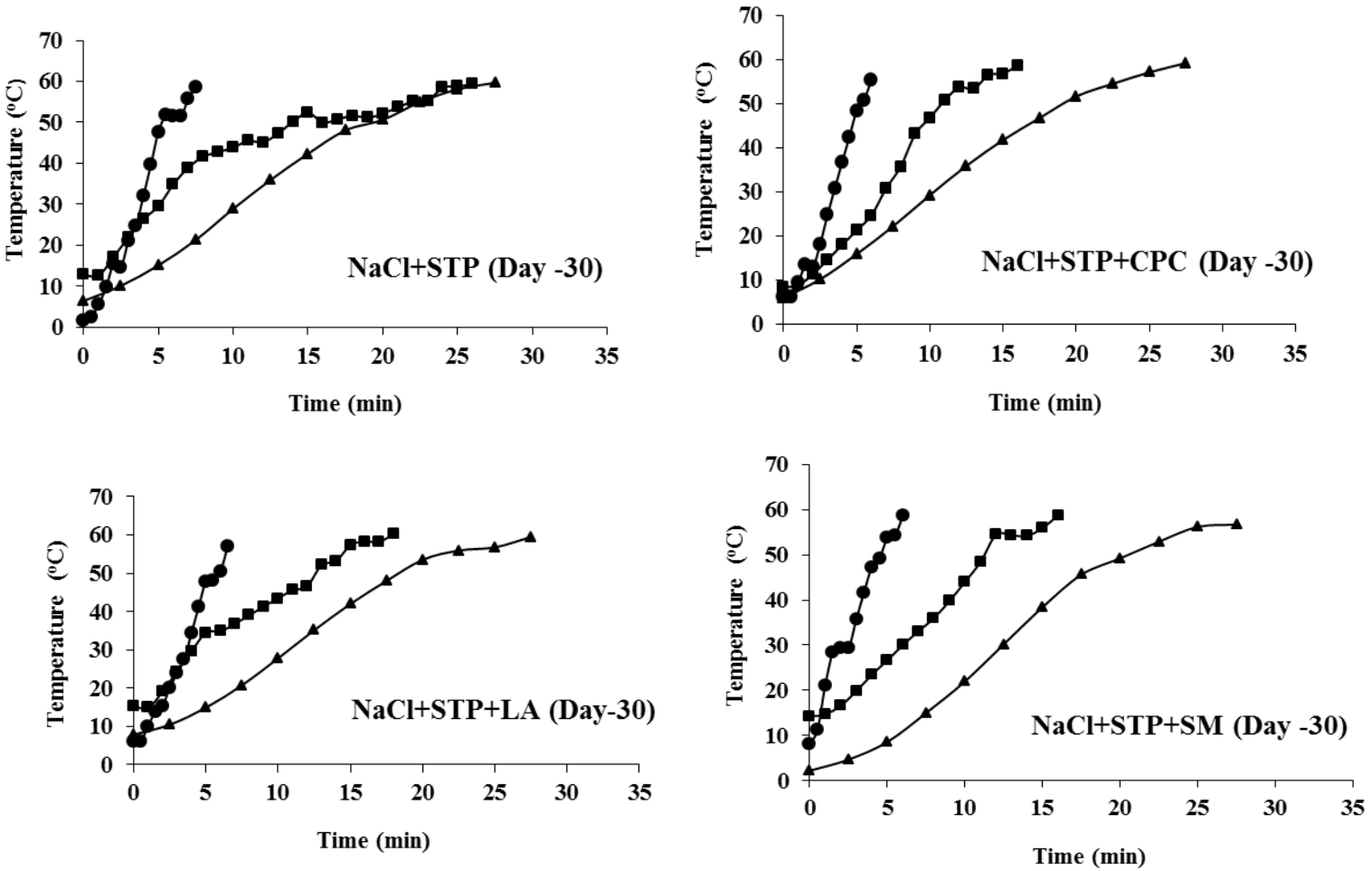

Figure 5.2. Cooking time and temperature curves for moisture-enhanced beef steaks (2.5-cm thickness) when cooked to internal temperature of 60°C in the geometric center with three cooking methods on day-30 after frozen (-20°C) storage. Roasting (▲), Pan broiling (■) and Double pan broiling (●). Each point is the average of six determinants. NaCl = Sodium Chloride, 0.5%; STP = Sodium Tripolyphosphate, 0.25%; CPC = Cetylpyridinium Chloride, 0.2%; LA = Lactic Acid, 0.3%, SM = Sodium Metasilicate, 0.2%.

CHAPTER 6

THERMAL INACTIVATION OF *ESCHERICHIA COLI* O157:H7 IN DIFFERENT PARTS OF BEEF ROASTS MOISTURE-ENHANCED WITH DIFFERENT BRINING FORMULATIONS AND COOKED TO RARE OR VERY-RARE DEGREES OF DONENESS

Escherichia coli O157:H7 internalized in moisture-enhanced large meat cuts (e. g., beef roasts) could survive undercooking of such products and may lead to foodborne illnesses. This study evaluated the effect of four brining formulations on survival of *E. coli* O157:H7 in roasts during frozen storage and thermal destruction when roasts cooked to two degrees of doneness. Batches (2 kg) of 50 g pieces of beef (95% lean) were mixed with an 8-strain composite (20 ml) of rifampicin-resistant *E. coli* O157:H7 (6-7 log CFU/g) and a brine solution (180 ml). Brine treatments included: sodium chloride (NaCl, 0.5%)+sodium tripolyphosphate (STP, 0.25%), NaCl+STP+cetylpyridinium chloride (CPC, 0.2%), NaCl+STP+lactic acid (0.3%), and NaCl+STP+sodium metasilicate (0.2%). Inoculated and treated meat (2 kg) was stuffed into elastic netting, vacuum-packaged, and frozen (-20°C, 30 days). On day-0 and day-30 roast samples thawed at 4°C for 48-72 h were cooked in a conventional kitchen oven (at 176.7°C) to the internal temperatures of 60°C (rare) or 55°C (very-rare). On day-0 and day-30, four subsamples from uncooked, and three (top, middle and bottom) core (2.3 cm diameter) subsamples

each from side and center subsections of cooked roasts were analyzed for total bacterial and *E. coli* O157:H7 populations. Data (log CFU/g) from two roasts/treatment/cooking temperatures are presented individually due to differences encountered in the time-temperature profile during cooking of each roast. Pathogen counts of uncooked roasts treated with CPC were 0.7-2.4 log CFU/g lower than those of the control (NaCl+STP). Out of the thirty two roasts that were cooked to 60 or 55°C, 50.0 and 40.6 % of samples, respectively, had undetectable (<0.5 log CFU/g) levels of the pathogen in all of the tested subsamples. Survivors of <0.5-5.4 log CFU/g and <0.5-5.2 log CFU/g were obtained in subsamples of the remaining roasts cooked to 60 or 55°C, respectively. Results of this study may be helpful to regulators and industry in selection of cooking schedules for moisture-enhanced roasts to reduce the risk of *E. coli* O157:H7.

6.1. INTRODUCTION

The integrity of non-intact muscle cuts could become compromised during tenderization and enhancement processing, and processing could also vertically translocate pathogen cells present on external surfaces into the sterile interior of these meat cuts. Recycled enhancement brines could also contaminate the interior or deeper tissue of moisture-enhanced meat products (Bohaychuk and Greer, 2003; Greer et al., 2004). Addition of antimicrobials in injected brines could be considered as a preventive measure for control of *E. coli* O157:H7 in enhanced meat products when stored for long periods of time (Sofos et al., 2008). Cetylpyridinium chloride (CPC) is nonvolatile, soluble in water, versatile ingredient with neutral pH (Anonymous, 2000) and could be included in meat products without adversely affecting flavor, texture, appearance or odor

of these products (Zdemir et al., 2006). Even though this particular antimicrobial is not approved for meat products; however has been found to be effective against diverse foodborne pathogens including *E. coli* O157:H7 (Byelashov et al., 2010; Cutter et al., 2000; Huffman, 2002), *Salmonella* (Breen et al., 1997; Kim and Slavik, 1996), and *L. monocytogenes* (Zdemir et al., 2006). Organic acids, like lactic acid, have been recognized as antimicrobials effective against foodborne pathogens for centuries, and have been currently accepted as cost effective carcass decontamination interventions (Koohmaraie et al., 2005). Sodium metasilicate (SM), a strong alkali, has been reported to increase pH of treated meat (Byelashov et al., 2010; Carlson et al., 2008 a and b; Quilo et al., 2009; Weber et al., 2004), and reduce *E. coli* O157:H7 counts on beef carcass surfaces (Byelashov et al., 2010; Carlson et al., 2008 a and b; Quilo et al., 2010; Weber et al., 2004), and in bacterial suspensions (Weber et al., 2004). Considering the effectiveness of these antimicrobials (CPC, LA and SM) against *E. coli* O157:H7, they (CPC, LA and SM) were selected as antimicrobials ingredients in the brining formulations of the present study.

Roasts contaminated with *E. coli* O157:H7 have been epidemiologically linked to outbreaks reported in North Dakota (CDC, 1991), Wisconsin (Rodrigue et al., 1995), and Nebraska (Olsen, 2008). Late July and early August in 1990 in North Dakota, 70 (3.5%) of the more than 2000 attendees in an agricultural threshing show developed gastroenteritis symptoms due to *E. coli* O157:H7 infection from contaminated roasts served at that party. Sixteen inside round roasts served at this dinner event were purchased from a local grocery store. Fourteen of these roasts were cooked to the internal temperature of 60°C (140°F) for approximately 10 hours on a noncommercial grade

metal spit rotated in a closed drum above a charcoal fire, and, two other roasts were cooked in enamel-lined electric roasting pans preset at 149°C (300°F), and internal temperatures of these cooked roasts was not recorded (CDC, 1991). Cooked roasts were sliced individually and served on the electric roasting pans. The second outbreak due to consumption of roasts contaminated with *E. coli* O157:H7 happened at a graduation banquet party at a university gathering in Wisconsin and sickened 61 (32%) of 193 banquet attendees (Rodrigue et al., 1995). The third outbreak due to *E. coli* O157:H7 from contaminated roasts sickened at least 14 people at a private event at the Sarpy County reception hall in Nebraska (Olsen, 2008). It was difficult, to conclude with the limited information available about these outbreaks, whether contaminated roasts implicated in these outbreaks were intact or nonintact. Nonintact meat cuts have gained importance from a public health perspective in recent years because nonintact meat cuts contaminated with *E. coli* O157:H7 have been associated with many outbreaks and recalls.

According to Sofos et al. (2008) internalized pathogen cells present in the deepest tissue of nonintact meat products could survive time and temperature that could be sufficient to kill cells if present on the surface of meat cuts. The majority of consumers prefer their meat products to be cooked to internal temperatures of 60.0 to 62.8°C (Cox et al., 1997; Schmidt et al., 2002). Large meat cuts like roasts have been processed with dry heat cooking methods, like roasting and broiling, or moist heat cooking methods like braising, at consumer and food service levels (NLSMB, 1977). The effects of different cooking rates, endpoint temperatures, oven types on physical characteristics of food service beef roasts like yield and composition (Belk et al., 1993a), and palatability (Belk

et al., 1993b) have been previously investigated. There has been a scarcity of studies that reported microbial inactivation of bacterial cells in roasts during consumer-style cooking. A study conducted by Belk et al. (1993c) reported mean aerobic plate counts below 1 log CFU/cm^2 in food service beef roasts after cooking in four oven types (conventional, forced sir convection and forced air/steam) to four end point temperatures (54.4°C, 62.8°C, 68.3°C and 73.9°C). Moreover, temperature variations within meat cuts at different locations during consumer-style cooking have been reported (Murphy et al., 2001; Rhee et al., 2003; Shen et al., 2010). Thus, it would be interesting to determine thermal inactivation levels of *E. coli* O157:H7 at different locations of moisture-enhanced large meat cuts during consumer-style cooking. Effectiveness of three antimicrobials on survival of *E. coli* O157:H7 in moisture-enhanced beef roasts stored under vacuum-packaged frozen conditions (-20°C) for 30 days was investigated under the first objective of the present study. Thermal inactivation of *E. coli* O157:H7 at six locations of moisture-enhanced beef roasts (2 kg) cooked to 60°C (rare) and 55°C (very- rare degrees of doneness) in a conventional kitchen oven, on day-0 (i e., approximately 24 h after preparation) and on day-30 of frozen (-20°C) storage after 48-72 h of thawing, was also investigated under the objectives of the present study.

6.2. MATERIALS AND METHODS

6.2.1. Bacterial strains and inoculum preparation

Eight *E. coli* O157:H7 strains [ATCC 43888 (human isolate), ATCC 43895 (raw hamburger meat isolate), ATCC 43895/ISEHGFP (Noah et al., 2005), and C1-057, C1-072, C1-109, C1-154, and C1-158 (bovine fecal isolates, Carlson et al., 2009)] were

selected for inoculation of beef roasts in the present study. Rifampicin (100 µg/ml; Sigma, St. Louis, MO) resistant derivatives of these strains were isolated as described by Kaspar and Tamplin (1993). These variants helped selective isolation of inoculated *E. coli* O157:H7 from the natural background flora present in meat. The thermotolerance of rifampicin-resistant derivatives of these strains were tested in tryptic soy broth (TSB; Difco, Becton Dickinson, Sparks, Md., U.S.A.) at 60°C and found not to be different from the parental strains (Ko et al., 2010). Each rifampicin-resistant derivative of these eight *E. coli* O157:H7 strains was activated individually and subcultured (35°C, 24 h) in 10 ml of tryptic soy broth (TSB; Difco, Becton Dickinson, Sparks, MD) plus rifampicin (100 µg/ml; Sigma, St. Louis, MO). The eight strains were subsequently combined on the day of each experiment, centrifuged (4,629×*g*, 15 min, 4°C; Eppendorf, model 5810 R, Brinkmann Instruments Inc., Westbury, NY) and washed twice with phosphate buffered saline (PBS; pH 7.40, 0.2 g/L KH_2PO_4, 1.5 g/L $Na_2HPO_4.7H_2O$, 8.0 g/L NaCl and 0.2 g/L KCl). The washed cell pellet was resuspended in PBS and was used to inoculate beef roasts at levels of 7 log CFU/g and 6 log CFU/g, which were destined to be cooked to rare (60°C) and very-rare (55°C) degree of doneness, respectively.

6.2.2. Brining formulation preparation

The four brining treatments selected for moisture enhancement of beef roasts were: (1) sodium chloride (NaCl, 0.5%; Fisher Scientific, Fair Lawn, NJ) + sodium tripolyphosphate (STP, 0.25%; BK Giulini Corporation, Semi Valley, CA); (2) NaCl (0.5%) + STP (0.25%) + cetylpyridinium chloride (CPC, 0.2%; added as Cecure™, kindly provided by Safe Foods Corporation, North Little Rock, AR); (3) NaCl (0.5%) + STP (0.25%) + lactic acid (LA, 0.3%; PURAC America Inc.); (4) NaCl (0.5%) + STP

(0.25%) + sodium metasilicate (SM, 0.2%; added as AvGard®XP, kindly provided by Danisco USA Inc.). The concentration of each brining ingredient (wt/wt finished product) was based on allowable levels by federal regulations in meat products (USDA-FSIS, 2010, FSIS directive 7120.1) published research (Byelashov et al. 2010; Shen et al., 2010), and levels used by industry. Each brining treatment was prepared by dissolving each brining ingredient in 2 liters of sterile distilled water with stirring and slight heat to dissolve ingredients. Brining treatments were prepared on the day of roast preparation.

6.2.3. Preparation of moisture-enhanced beef roasts

The inoculation and enhancement procedures of beef roasts for the present study were adopted from Shen et al. (2010). Fresh beef knuckles (72 h postmortem, 95% lean) used for preparation of roasts were obtained from a local meat packing plant and stored vacuum packaged and frozen (-20°C) for not more than 2 weeks. Frozen knuckles were thawed for 24 h at 3°C and cut into approximately 50 g pieces with an electric band saw (AEW Thurne Ltd., 400M, Norwich, England). The cut meat was thawed completely for an additional 4-6 h at 3°C before inoculation and processing. Two kilogram batches of beef pieces (50 kg) were mixed with 20 ml of eight strains the rifampicin-resistant *E. coli* O157:H7 composite at levels of 6-7 log CFU/g in a bowl-lift stand mixer (KitchenAid®, Professional 600, St. Joseph, MI) for 2 min at "stir" speed for uniform distribution of microbial cells in the final product. The inoculated meat was then mixed with 180 ml of each brining treatment (90 ml/kg) for an additional 2 min to achieve a final moisture-enhancement level of 110% of initial weight. Two kilogram batches of inoculated and treated meat pieces were extruded into elastic cotton netting (24 squares, 8.9 cm flat width, 19.1 cm stuffing diameter, 71.1 cm stuffing circumference; Koch, Kansas City,

Mo) and tied. The roasts were placed in vacuum bags (30.5×40.6 cm, 3 mil standard barrier, nylon/PE vacuum pouch, water vapor, and oxygen transmission rates of 9.3 $g/m^2/24$ h [97% relative humidity] and 54.3 $cm^3/m^2/24h$ 21°C, [0% relative humidity], respectively; Koch, Kansas City, Mo) and vacuum-packaged. The vacuum packaged beef roasts were stored under frozen conditions (-20°C) for 30 days.

6.2.4. Roast cooking to rare (60°C) and very-rare (55°C) degrees of doneness

The frozen roasts (-20°C) were thawed at 4°C for 48-72 h before cooking. On day -0 and -30, beef roasts were cooked to endpoint temperatures of 60°C or 55°C (simulating rare and very rare doneness, respectively) in a Magic Chef ® Kitchen Oven (Maytag Corp., Newton, IA) (AMSA 1995).

Each moisture-enhanced roast (2 kg) was weighed before cooking. Roasts were placed on the center of aluminum foil covered oven tray and five K- type thermocouples were positioned at five (two sides, geometric center, top, and, bottom of roast, as shown in Figure 5.1) locations. Thermocouples were inserted into beef pieces for temperature monitoring on these specific locations. Beef roasts were draped with a piece of aluminum foil down to oven tray to make a tent and placed in the center of the preheated (176.7°C) kitchen oven on racks. Roasts were removed immediately from the oven when the specified target endpoint internal temperature was reached in their geometric center. Total time required to reach the internal endpoint temperatures of 60°C or 55°C in the geometric center of the roast was considered "cooking time". The aluminum foil was removed from the cooked roasts which were kept at room temperature (25±2°C) for 20 min simulating a resting period. During cooking, the temperature of each roast was monitored with K-type thermocouples and recorded at 30 sec intervals with PicoLog data

acquisition (Pico Technology Ltd., Cambridge, UK) at five different locations during cooking and 20-min resting period. The internal temperature in the geometric center of the cooked roast after the resting period was considered as "final temperature". After the resting period, each cooked roast was weighed, sampled, and analyzed as described below.

6.2.5. Microbiological and physiochemical analyses

Uncooked and cooked roast samples were analyzed for surviving *E. coli* O157:H7 and total bacterial populations, pH, and water activity on day-0 (approximately 24 h after preparation), and day-30 of frozen storage (-20°C) and after 48-72 h of thawing. However, only day-0 uncooked and cooked roast samples were analyzed for fat and moisture contents. The weight of each cooked roast sample was measured before and immediately after cooking to determine cooking losses.

Each cooked roast sample was divided into three sections (two sides and one center) with two vertical cuts, and then, the side and center sections were again cut into three sub-sections (top, middle and bottom) with two horizontal cuts (Figure 5.2). The knife was dipped into ethanol and flame-sterilized between each cut to prevent cross-contamination. One sub-sample was cored (2.3 cm diameter) out from each sub-section for microbiological analysis. A total of six sub-samples (center top, center middle, center bottom, side top, side middle and side bottom as shown in Figure 5.2) from each cooked roast, and a total of four sub-samples from uncooked roast samples, were obtained. Each sub-sample was individually placed into a filter bag (55-oz, 1,627 ml, 19 by 30 cm; Nasco, Modesto, CA), weighed, and maximum recovery diluent (0.85% NaCl and 0.1% peptone) was added at a ratio of 1:1 (sample weight: volume [g] of MRD). Samples were

homogenized (Masticator, IUL Instruments, Barcelona, Spain) for 2 min and serial 10-fold dilutions were prepared in 0.1% BPW and were surface-plated on tryptic soy agar (Acumedia, Lansing, MI) supplemented with 0.1% sodium pyruvate (Fisher Scientific, FairLawn, NY) (TSAP) and TSAP plus rifampin (100 µg/ml; TSAP+rif). TSAP and TSAP+rif plates were incubated and colonies were counted manually after incubation at 25°C (72 h) and 35°C (48 h), for total bacterial populations and inoculated *E. coli* O157:H7 counts, respectively. The pH of the meat homogenate was measured after plating of uncooked and cooked sample by using a digital pH meter with a glass electrode (Denver Instruments, Arvada, CO). The water activities of uncooked and cooked samples were measured with an AquaLab (model series 3, Decagon Devices, Inc., Pullman, WA) water activity meter. Moisture and fat contents were measured with AOAC International official methods 950.46.B and 960.39, respectively (AOAC, 2000).

6.2.6. Statistical analysis

This study was repeated twice, with two cooked and one uncooked roast samples per brining treatment for each storage day for each endpoint cooking temperature (i e., 60°C or 55°C) per repetition. Total bacterial and *E. coli* O157:H7 counts were converted to log CFU/g. Microbial (total bacterial and *E. coli* O157:H7) counts from uncooked roast samples were analyzed for main effects (brining treatment and storage day) and interactions between these two main factors (brining treatment × storage day). The pH and water activity data from both uncooked and cooked roast samples were analyzed for main effects (brining treatment, storage day and cooking) and interactions between main factors (brining treatment×storage day, storage day×cooking, brining treatment×storage day×cooking), and fat and moisture from uncooked/cooked roast for day-0 samples only

were analyzed for brining treatment and cooking, and their interaction (brining treatment×cooking), for both endpoint cooking temperatures. Data from the above mentioned variables were analyzed using the PRO MIXED Model procedure of SAS v9.2 (SAS Institute 2003). Multiple pairwise comparisons of means were done using Tukey's honestly significant difference test, and means were considered significantly different when P-values were less than 0.05. Surviving bacterial populations (i. e., total bacterial and *E. coli* O157:H7), cooking times, final cooking temperatures (i. e., internal temperature after 20-min 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.

6.3. RESULTS AND DISCUSSION

6.3.1. Physiochemical properties of moisture-enhanced beef roasts

Uncooked and cooked roast samples enhanced with NaCl+STP+SM (6.26-6.47) had higher ($P < 0.05$) pH values compared to samples enhanced with NaCl+STP (5.49-6.15), NaCl+STP+CPC (5.87-6.10) and NaCl+STP+LA (5.45-5.85). This trend was observed for samples on day-0 and -30 for both (60°C and 55°C) cooking temperatures (Table 6.1). Sodium metasilicate (SM) is a strong alkali, and the 0.1% (wt/wt) aqueous solution of SM has a pH of approximately 11.3 (Weber et al., 2004). Increases in pH values for beef treated with sodium metasilicate similar to results of the present study have been reported previously in the literature (Byelashov et al., 2010; Quilo et al. 2009; Weber, 2004). The water activity values of moisture-enhanced roast samples ranged from

0.983 to 0.996 for both (60°C and 55°C) cooking temperatures (Table 6.2). The brining formulation and storage day did not have significant ($P < 0.05$) effects on the water activity values of roast samples. Similar to results of the present study, Byelashov et al., (2010) reported no reduction in water activity values of ground meat with addition of salt at 0.5% level. In contrast of results of the present study, Mukherjee et al. (2008) reported reductions in the water activity values of enhanced uncooked and cooked ground beef samples with addition of NaCl at 2.5 %. For samples from both cooking temperatures, cooked roasts (60°C: 2.5-5.8%, and 55°C: 2.2-8.4%) had higher fat content than uncooked (60°C: 0.9-7.2% and 55°C: 1.4-3.2%) roast samples, and in contrast, cooked (60°C: 63.3-69.9% and 55°C: 64.4-70.1%) roast samples had significantly ($P < 0.05$) lower moisture content than uncooked (60°C: 71.1-74.6% and 55°C: 71.5-74.8%) roast samples (Table 6.3). Increases in fat content and decreases in moisture content in enhanced beef samples after cooking observed in present study were also reported by Byelashov et al. (2010) and Mukherjee et al. (2008).

6.3.2. Bacterial populations in uncooked roast samples

Total bacterial populations were 4.4 ± 2.2 and 2.9 ± 1.5 log CFU/g in uninoculated beef pieces (50 g) used to prepare roasts that were destined to be cooked to 60°C and 55°C endpoint temperatures, respectively. *E. coli* O157:H7 counts in uncooked roast samples enhanced with a brining formulation containing lactic acid (NaCl+STP+LA) and sodium metasilicate (NaCl+STP+SM) were not ($P > 0.05$) different, however, CPC-treated uncooked roast samples had *E. coli* O157:H7 counts lower ($P < 0.05$) by 0.8 log CFU/g and 1.5 log CFU/g, from uncooked roast samples enhanced with NaCl+STP, destined to be cooked to 60°C and 55°C on day-0 (i. e.,

approximately 24 h after inoculation and enhancement at 4°C) (Table 6.4). One to two log reductions in *E. coli* O157:H7 counts in enhanced beef products with CPC after 24 h of storage at 4°C observed in the present study was also observed by Byelashov et al., (2010). However, Cutter et al., (2000) demonstrated higher (5 to 6 log CFU/g) reductions in *E. coli* O157:H7 counts when CPC applied as beef carcass decontamination intervention. Reduction and/or loss of antimicrobial properties of acidic and basic antimicrobials in enhanced meat products observe in the present study and by other researchers (Byelashov et. al., 2010; Mukherjee et. al., 2008) could be due to the strong buffering capacity of meat (kyla-Puhju et al., 2004). *E. coli* O157:H7 levels in uncooked roasts after frozen storage (-20°C) for 30 days and 48-72 h of thawing were lower by 0.5-0.6 log CFU/g compared to counts from day-0 samples enhanced with NaCl+STP+CPC; however, *E. coli* O157:H7 levels from day-30 uncooked beef roast samples enhanced with the other three brining formulations (NaCl+STP, NaCl+STP +LA, NaCl+STP +SM) of the present study were statistically ($P > 0.05$) similar to day-0 samples (Table 6.4). In the majority of cases, total bacterial populations (Table 6.5) from uncooked moisture-enahnced roasts recovered on nonselective media (TSAP) closely paralleled those of *E. coli* O157:H7 (Table 6.4) on selective media (TSAP+rif), indicating that the majority of colonies found on TSAP were inoculated *E. coli* O157:H7. However, higher total bacterial levels were observed on day-30 roast samples treated with NaCl+STP, NaCl+STP +LA, NaCl+STP +SM destined to be cooked to 60°C than *E. coli* O157:H7 counts in these samples, and most likely due to growth of the natural flora during the 48-72 h thawing period (Table 6.5).

6.3.3. Inactivation of *E. coli* O157:H7 during cooking of roasts

The cooking time was defined as the time required to reach designated endpoint internal temperatures i. e., 60°C (rare) and 55°C (very-rare degree of doneness) in the geometric center (Figure 6.1) of 2 kg moisture-enhanced beef roasts when cooked in conventional kitchen oven maintained at 176.7°C (350°F). Average cooking times were 131±17 and 122 ±16 min for roasts, when cooked to 60°C and 55°C, respectively (Table 6.6). Cooking time ranges for roasts cooked to 60°C (106-167 min) were slightly higher compared to roasts cooked to 55°C (88-151 min) (Table 6.6). The cooking time ranges for roasts cooked on day-0 (i. e., approximately 24 h after preparation; 60°C: 112-167 min and 55°C: 103-141 min) were similar to roasts cooked on day-30 after frozen storage (-20°C) and 48-72 h thawing (60°C: 106-163 min, and 55°C: 88-152 min); this trend was also observed for roasts enhanced with all brining formulations tested (Table 6.6).

Temperature at four locations (i. e., side-1, side-2, top and bottom) of roasts represented surface temperatures on the entire circumference of 2 kg beef roasts, and was also monitored during cooking (Figure 6.1). Endpoint temperature ranges at these four locations when target cooking temperature (i. e., 60°C and 55°C) reached in geometric center of roasts achieved were: side-1: 61-121°C and 63-92°C; side-2: 56-120°C and 63-92°C; top: 49-86°C and 46-86°C; bottom: 68-96°C and 58-92°C, respectively (Figure 6.1, Table 6.7 and 6.8). Temperatures in the geometric center of roasts were lower than at these four locations during entire the cooking period indicating that the geometric center was the slowest cooking point compared to the surface of the roasts (Appendix Figures 30-45; Table 6.7 and 6.8). Similarly, temperatures at the center of ground chicken patties were lower than surface temperatures when cooked in an air convection oven maintained

between 163-218°C (Murphy et al., 2001). According to Feyissa et al. (2009), heat is transferred from circulating air in the oven chamber to the meat surface through convection, while conduction is a method of heat transfer from the surface into center of meat products, during roasting process. A two step heat transfer during roasting could be the reason for delayed temperature increases in the center compared to the surface of meat products.

Final temperatures (°C) in the geometric center of roasts after a 20-min resting period was either increased (to 69°C and 66°C for 60°C and 55°C, respectively) or decreased (to 46°C for both cooking temperatures) beyond target internal temperatures (Table 6.9 and 6.10). Similar increases in internal temperatures of restructured steaks above target temperatures after gas grilling and oven broiling was observed by Ortega-Valenzuela et al. (2001). Gill et al. (2009) explained that rise in central temperature in the geometric center of large cuts during the resting period could be due to latent heat. Average final temperatures in the geometric center of roasts cooked to 60°C (64±5°C) was 3°C higher than samples cooked to 55°C (61±4°C) endpoint temperature (Table 6.9 and 6.10). Different sizes of post-cook temperature increases of meat cuts reported in the literature and in the present study could be due to differences, in degrees of doneness of meat products cooked, oven setting temperatures, and size and shape of meat cuts (NLSMB, 1977). Final temperatures at other four locations (i. e., side-1, side-2, top, and bottom) of roasts after 20-min the resting period decreased, and these temperature ranges were: side-1: 44-77°C and 28-73°C; side-2: 37-67°C and 44-74°C; top: 53-71°C and 41-64°C; bottom: 50-71°C and 53-72°C, for 60°C and 55°C cooking temperature, respectively (Table 6.9 and 6.10).

Overall cooking losses for roasts ranged from 9.5 to 38.4%, and 3.2 to 36.8% for 60°C or 55°C endpoint temperatures, respectively (Table 6.11). As shown earlier, large variations were observed in cooking times, endpoint and final temperatures at five locations of roasts and cooking losses; these parameters did not have any apparent trend by brining treatment, storage day or cooking temperature. Possible explanations of these variations are: (i) non uniform delivery of heat by conventional kitchen oven used for the present study (Marshall et al., 1960); (ii) displacement of thermocouples with movement of purge of roasts during cooking; and, (iii) uncertainty about placement of thermocouple in the geometric center, and could have placed in fat, meat or air pocket in the geometric center of roasts, and different heating rates could be expected for these three types of materials.

E. coli O157:H7 ranged from 6.1to 6.9 and 4.4 to 5.9 log CFU/g for day-0, and from 5.6 to 6.7 and 3.8 to 5.9 log CFU/g for day-30, in uncooked roast samples prior to cooking to 60°C and 55°C, respectively (Table 6.4). A total of sixteen moisture-enhanced roasts were cooked on day-0 (i. e., approximately 24 h after inoculation and enhancement) and on day-30 after frozen (-20°C) storage and 48-72 h thawing, for each cooking temperature (i. e., 60°C and 55°C). A total of six subsamples from six locations (i. e., center and side top, center and side middle, center and side bottom) from each cooked roast were analyzed for surviving *E. coli* O157:H7 populations. *E. coli* O157:H7 counts in these subsamples were either reduced to below detection limit (< 0.5 log CFU/g), or as high as 5.4 and 4.8 log CFU/g were recovered from these subsamples cooked to 60°C and 55°C, respectively (Table 6.12, 6.13, 6.14 and 6.15).

Number of cooked roasts with *E. coli* O157:H7 counts equal or below dectection limit (< 0.5 log CFU/g) at all six locations were eleven and five on day-0, and four and nine on day-30 when cooked to 60°C and 55°C, respectively, and corresponding cooking time and final temperature ranges for these roasts were 115-167 and 126-156 min on day-0 and 112-141 and 112-152 min on day-30, and 62-69 and 64-67°C on day-0 and 62-66 and 59-66°C on day-30, when cooked to 60°C and 55°C, respectively (Table 6.6, 6.12, 6.13, 6.14 and 6.15). Number of cooked roasts with survived *E. coli* O157:H7 at all six locations were two and two on day-0, and five and two on day-30 and corresponding cooking time and final temperature ranges were 112-113 and 112-121 min on day-0 and 106-129 and 88-98 min on day-30, and 46-56 and 63°C on day-0 and 54-58 and 46-63°C on day-30 when cooked to 60°C and 55°C, respectively (Table 6.6, 6.12, 6.13, 6.14 and 6.15). In majority of cases, roast samples without survived *E. coli* O157:H7 cells at any of six locations had longer cooking times and higher final temperatures compared to roasts with survived pathogen cells at these locations; and longer cooking times and higher final temperatures could be reason for higher thermal inactivation of *E. coli* O157:H7cells (Table 6.6, 6.12, 6.13, 6.14 and 6.15).

Shen et al., (2010) reported higher reductions of *E. coli* O157:H7 in thicker (2.3 to 4.2 log CFU/g) steaks than in thinner (1.1 to 2.9 log CFU/g) steaks and explained that longer cooking time for thicker (63.0 ± 2.8 min) steaks compared to thinner steaks (20.0 ± 2.3 min) could be reason for higher reductions. Ortega-Valenzuela et al. (2001) also reported higher thermal inactivation levels for pathogen cells in thicker steaks compared to thinner steaks could be due to longer cooking times and higher post-cook temperature rise for thicker steaks.

The decreasing order of survived *E. coli* O157:H7 cells at six locations of cooked roasts was; center and side top > center and side middle > center and side bottom. This trend was seen in majority of roasts with survived pathogen cells when cooked to 60°C and 55°C for both cooking days (Table 6.12, 6.13, 6.14 and 6.15). Lower *E. coli* O157:H7 survivors could be expected in the geometric center of roasts compared to surface because geometric center had slower cooking rate compared to surface of roasts during entire cooking process as discussed earlier (Appendix Figures 30-45).

Percent of subsamples with undetectable (< 0.5 log CFU/g) levels of *E. coli* O157:H7 at six locations ranged from 75.0-81.3% and 43.8-56.3% on day-0, and 43.7-75.0% and 43.7-87.5% on day-30, for roasts cooked to 60°C and 55°C, respectively; no apparent trend considering subsample location and cooking day has been observed (Table 6.20). Injury to *E. coli* O157:H7 cells could be expected during freezing (-20°C for 30 days) and thawing (48-72 h at 4°C) of roast samples, and higher thermal inactivation levels could be expected for injured *E. coli* O157:H7 cells from day-30 roast samples (Yamamoto and Harris, 2001).

Higher number of roast samples had surviving total bacterial population compared to roast samples with surviving *E. coli* O157:H7 cells for both cooking days (day-0 and day-30) and cooking temperatures (60°C and 55°C) (Table 6.16, 6.17, 6.18 and 6.19). It have been reported previously (Mukherjee et al., 2008 and 2009) that selective media (TSAP+rif as *E. coli* O157:H7) could be harsh for injured pathogen cells from heat and/or antimicrobials, and less recovery of cells on selective media (Table 6.16, 6.17, 6.18 and 6.19).

6.4. CONCLUSIONS

Results of this study indicated that one to two log reductions in *E. coli* O157:H7 population could be achieved with addition of cetylpyridinium chloride (0.2%) to brine (NaCl+STP) formulations in moisture-enhanced roast samples during frozen (-20°C) conditions. Average cooking times for 2 kg moisture-enhanced roasts in conventional kitchen oven maintained at 350°F, to rare (60°C) and very rare (55°C) degree of doneness was 2 h 21 minutes and 2 h one minute, respectively. Surface temperatures rose higher during cooking and post cooking temperatures declined rapidly during 20-min resting period compared to geometric center of roasts. No survivors of *E. coli* O157:H7 were detected at different locations of roast samples that have longer cooking times and higher post-cook temperatures rise after 20-min resting period. However, roasts with shorter cooking times and lower post-cooked temperatures rises had survived *E. coli* O157:H7 at some of these locations. The results of this study should be useful for development and/or improvement of brines for moisture enhancement of roasts, and to select cooking schedules for moisture-enhanced meat products cooked in conventional kitchen oven that would help *E. coli* O157:H7 control in these meat products.

Table 6.1. The pH values (mean ± standard deviation) of uncooked and cooked beef roasts samples, moisture-enhanced with four brining formulations, cooked to 60°C and 55°C, on day-0 (i. e., approximately 24 h after preparation) and day -30 after frozen (-20°C) storage and 48-72 h thawing.

Sample	Treatment	60°C		55°C	
		Day-0	Day-30	Day-0	Day-30
Uncooked	NaCl + STP	5.78 ± 0.11 ^{aX}	5.49 ± 0.37 ^{abY}	5.93 ± 0.15 ^{abX}	5.84 ± 0.16 ^{abX}
	NaCl + STP + CPC	5.88 ± 0.08 ^{bX}	5.95 ± 0.09 ^{bcX}	6.00 ± 0.11 ^{bX}	5.87 ± 0.11 ^{abX}
	NaCl + STP + LA	5.54 ± 0.14 ^{aX}	5.45 ± 0.09 ^{aX}	5.64 ± 0.27 ^{aX}	5.58 ± 0.33 ^{aX}
	NaCl + STP + SM	6.26 ± 0.10 ^{cX}	6.27 ± 0.12 ^{cdX}	6.47 ± 0.18 ^{cX}	6.37 ± 0.11 ^{bcX}
Cooked	NaCl + STP	6.02 ± 0.12 ^{bcX}	5.99 ± 0.05 ^{bcX}	6.15 ± 0.09 ^{bX}	6.15 ± 0.13 ^{bcX}
	NaCl + STP + CPC	6.08 ± 0.11 ^{bcX}	6.08 ± 0.04 ^{cX}	6.10 ± 0.08 ^{bX}	6.10 ± 0.11 ^{bX}
	NaCl + STP + LA	5.85 ± 0.15 ^{bX}	5.78 ± 0.18 ^{bX}	5.85 ± 0.22 ^{abX}	5.78 ± 0.19 ^{aX}
	NaCl + STP + SM	6.36 ± 0.17 ^{cX}	6.40 ± 0.07 ^{dX}	6.47 ± 0.10 ^{cX}	6.41 ± 0.14 ^{cX}

NaCl = Sodium Chloride, 0.5%; STP = Sodium Tripolyphosphate, 0.25%; CPC = Cetylpyridinium Chloride, 0.2%;
LA = Lactic Acid, 0.3%, SM = Sodium Metasilicate, 0.2%. Mean values with different lower case letter in the same column are significantly different ($P < 0.05$). Mean values with different upper case letter in the same row with in each cooking temperature are significantly different ($P < 0.05$).

Table 6.2. Water activity values (mean ± standard deviation) of uncooked and cooked roasts, moisture-enhanced with four brining formulations, cooked to 60°C and 55°C, on day-0 (i e., approximately 24 h after preparation) and on day-30 after frozen (-20°C) storage and 48-72 h thawing.

Sample	Treatment	60°C		55°C	
		Day-0	Day-30	Day-0	Day-30
Uncooked	NaCl + STP	0.989 ± 0.007 ^{aX}	0.988 ± 0.006 ^{aX}	0.988 ± 0.005 ^{aX}	0.983 ± 0.003 ^{aX}
	NaCl + STP + CPC	0.990 ± 0.004 ^{aX}	0.992 ± 0.004 ^{aX}	0.996 ± 0.004 ^{bY}	0.984 ± 0.002 ^{aX}
	NaCl + STP + LA	0.988 ± 0.004 ^{aX}	0.990 ± 0.002 ^{aX}	0.993 ± 0.003 ^{abX}	0.986 ± 0.003 ^{aX}
	NaCl + STP + SM	0.990 ± 0.007 ^{aX}	0.991± 0.004 ^{aX}	0.994 ± 0.006 ^{abX}	0.986 ± 0.002 ^{aX}
Cooked	NaCl + STP	0.989 ± 0.007 ^{aX}	0.991± 0.003 ^{aX}	0.988 ± 0.002 ^{aX}	0.988 ± 0.002 ^{aX}
	NaCl + STP + CPC	0.989 ± 0.006 ^{aX}	0.992 ± 0.004 ^{aX}	0.988 ± 0.001 ^{aX}	0.987 ± 0.002 ^{aX}
	NaCl + STP + LA	0.991 ± 0.005 ^{aX}	0.992 ± 0.004 ^{aX}	0.987 ± 0.002 ^{aX}	0.986 ± 0.002 ^{aX}
	NaCl + STP + SM	0.990 ± 0.002 ^{aX}	0.993 ± 0.005 ^{aX}	0.987 ± 0.001 ^{aX}	0.988 ± 0.003 ^{aX}

NaCl = Sodium Chloride, 0.5%; STP = Sodium Tripolyphosphate, 0.25%; CPC = Cetylpyridinium Chloride, 0.2%; LA = Lactic Acid, 0.3%, SM = Sodium Metasilicate, 0.2%. Mean values with different lower case letter in the same column are significantly different ($P < 0.05$). Mean values with different upper case letter in the same row with in each cooking temperature are significantly different ($P < 0.05$).

Table 6.3. Fat and moisture contents (mean ± standard deviation) of uncooked and cooked roasts samples, moisture-enhanced with four brining formulations, cooked to 60°C and 55°C on day-0 (i. e., approximately 24 h after preparation).

Sample	Treatment	60°C		55°C	
		Fat content (%)	Moisture content (%)	Fat content (%)	Moisture content (%)
Uncooked	NaCl + STP	7.2 ± 2.8 [b]	71.1 ± 5.7 [ab]	1.4 ± 1.3 [a]	74.8 ± 1.7 [b]
	NaCl + STP + CPC	1.7 ± 1.8 [ab]	74.6 ± 1.0 [b]	2.2 ± 3.1 [a]	73.7 ± 1.2 [b]
	NaCl + STP + LA	2.0 ± 2.1 [ab]	73.6 ± 3.3 [b]	3.2 ± 3.0 [ab]	71.5 ± 5.2 [ab]
	NaCl + STP + SM	0.9 ± 0.3 [a]	74.1 ± 2.2 [b]	2.3 ± 3.1 [a]	74.2 ± 3.1 [b]
Cooked	NaCl + STP	5.8 ± 2.7 [ab]	63.3 ± 4.3 [a]	4.4 ± 2.2 [ab]	65.6 ± 3.8 [ab]
	NaCl + STP + CPC	4.5 ± 4.2 [ab]	63.9 ± 1.7 [a]	8.4 ± 3.3 [b]	66.2 ± 1.9 [ab]
	NaCl + STP + LA	2.5 ± 1.7 [ab]	66.9 ± 5.5 [ab]	3.7 ± 1.9 [ab]	64.4 ± 7.5 [ab]
	NaCl + STP + SM	5.0 ± 3.0 [ab]	69.9 ± 2.9 [ab]	2.8 ± 2.6 [ab]	70.1 ± 2.1 [a]

NaCl = Sodium Chloride, 0.5%; STP = Sodium Tripolyphosphate, 0.25%; CPC = Cetylpyridinium Chloride, 0.2%; LA = Lactic Acid, 0.3%, SM = Sodium Metasilicate, 0.2%. Mean values with different lower case letter in the same column are significantly different ($P < 0.05$).

Table 6.4. *E. coli* O157:H7 counts (log CFU/g; mean ± standard deviation) from uncooked beef roasts samples prior to cooking to 60°C and 55°C, moisture-enhanced with four brining formulations, on day-0 (i. e., approximately 24 h after preparation) and day-30 after frozen (-20°C) storage and 48-72 h thawing.

Treatment	60°C		55°C	
	Day-0	Day-30	Day-0	Day-30
NaCl + STP	6.9 ± 0.2^{bX}	6.7 ± 0.2^{bX}	5.9 ± 0.1^{bX}	5.9 ± 0.3^{bX}
NaCl + STP + CPC	6.1 ± 0.5^{aY}	5.6 ± 0.4^{aX}	4.4 ± 0.8^{aX}	3.8 ± 0.8^{aX}
NaCl + STP + LA	6.7 ± 0.4^{bX}	6.5 ± 0.3^{bX}	5.7 ± 0.4^{bX}	5.9 ± 0.4^{bX}
NaCl + STP + SM	6.9 ± 0.2^{bX}	6.4 ± 0.2^{bX}	5.6 ± 0.3^{bX}	5.4 ± 0.2^{bX}

NaCl = Sodium Chloride, 0.5%; STP = Sodium Tripolyphosphate, 0.25%; CPC = Cetylpyridinium Chloride, 0.2%; LA = Lactic Acid, 0.3%, SM = Sodium Metasilicate, 0.2%. Mean values with different lower case letter in the same column are significantly different ($P < 0.05$). Mean values with different upper case letter in the same row with in each cooking temperature are significantly different ($P < 0.05$).

Table 6.5. Total bacterial population (log CFU/g; mean ± standard deviation) from uncooked beef roasts samples prior to cooking to 60°C and 55°C, moisture-enhanced with four brining formulations, on day-0 (i.e., approximately 24 h after preparation) and day-30 after frozen (-20°C) storage and 48-72 h thawing.

Treatment	60°C		55°C	
	Day-0	Day-30	Day-0	Day-30
NaCl + STP	6.9 ± 0.2 ^{aX}	7.4 ± 0.8 ^{bX}	6.6±0.8 ^{bX}	6.1±0.1 ^{bX}
NaCl + STP + CPC	6.0 ± 0.4 ^{aX}	5.3 ± 0.9 ^{aX}	4.3±0.8 ^{aX}	4.2±1.0 ^{aX}
NaCl + STP + LA	6.7 ± 0.3 ^{aX}	7.2 ± 0.9 ^{bX}	6.0±0.6 ^{bX}	6.0±0.6 ^{bX}
NaCl + STP + SM	6.8 ± 0.2 ^{aX}	7.4 ± 1.1 ^{bX}	6.1±0.6 ^{bX}	5.9±0.9 ^{bX}

NaCl = Sodium Chloride, 0.5%; STP = Sodium Tripolyphosphate, 0.25%; CPC = Cetylpyridinium Chloride, 0.2%; LA = Lactic Acid, 0.3%, SM = Sodium Metasilicate, 0.2%. Mean values with different lower case letter in the same column are significantly different ($P < 0.05$). Mean values with different upper case letter in the same row with in cooking temperature are significantly different ($P < 0.05$).

Table 6.6. Cooking times (min) and final temperatures (°C) for roasts, moisture-enhanced with four brining formulations, cooked to 60°C and 55°C, on day -0 (i.e., approximately 24 h after preparation) and day-30 after frozen (-20°C) storage and 48-72 h thawing .

Treatment	Sample	Cooking Time				Final Temperature			
		60°C		55°C		60°C		55°C	
		Day-0	Day-30	Day-0	Day-30	Day-0	Day-30	Day-0	Day-30
NaCl + STP	1	156	135	129	139	65	64	55	59
	2	155	163	140	123	65	59	62	64
	3	124	130	115	122	69	67	63	63
	4	136	124	112	98	66	67	64	63
NaCl + STP + CPC	1	141	142	139	148	64	64	62	60
	2	124	126	141	124	68	64	62	61
	3	121	134	103	111	60	67	59	61
	4	128	106	110	121	66	63	60	63
NaCl + STP + LA	1	115	127	126	88	65	69	NA	46
	2	152	121	106	119	62	64	54	61
	3	112	114	110	112	58	69	55	66
	4	115	130	112	96	69	67	66	61
NaCl + STP + SM	1	167	156	136	146	65	65	63	62
	2	112	146	138	152	46	61	60	61
	3	135	112	114	121	68	63	58	65
	4	113	121	112	133	53	63	66	64

NaCl = Sodium Chloride, 0.5%; STP = Sodium Tripolyphosphate, 0.25%; CPC = Cetylpyridinium Chloride, 0.2%; LA = Lactic Acid, 0.3%, SM = Sodium Metasilicate, 0.2%. NA- Not Available

Table 6.7. Endpoint temperatures (°C) at five locations of roasts, moisture-enhanced with four brining formulations, cooked to 60°C, on day-0 (i. e., approximately 24 h after preparation) and day-30 after frozen (-20°C) storage and 48-72 h thawing.

Treatment	Sample	Day-0					Day-30				
		Side-1	Side-2	Top	Center	Bottom	Side-1	Side-2	Top	Center	Bottom
NaCl + STP	1	NA	NA	NA	NA	NA	73	74	71	60	70
	2	74	66	79	60	75	66	71	68	60	74
	3	81	80	64	60	88	81	82	63	60	88
	4	75	82	79	60	72	121	83	76	60	90
NaCl + STP + CPC	1	82	74	74	60	76	76	75	67	60	73
	2	71	56	63	60	66	77	87	86	60	86
	3	79	80	61	60	82	85	90	81	60	90
	4	90	90	68	60	86	84	79	48	60	84
NaCl + STP + LA	1	NA	NA	NA	NA	NA	88	95	79	60	74
	2	62	67	61	60	74	77	120	59	60	68
	3	77	83	49	60	73	88	91	67	60	87
	4	85	91	68	60	81	81	82	63	60	88
NaCl + STP + SM	1	75	76	70	60	82	79	74	73	60	77
	2	61	67	64	60	96	78	72	69	60	77
	3	88	87	72	60	89	76	82	61	60	76
	4	NA	NA	NA	NA	NA	82	83	77	60	69

NaCl = Sodium Chloride, 0.5%; STP = Sodium Tripolyphosphate, 0.25%; CPC = Cetylpyridinium Chloride, 0.2%; LA = Lactic Acid, 0.3%, SM = Sodium Metasilicate, 0.2%. NA- Not Available

Table 6.8. Endpoint temperatures (°C) at five locations of cooked roasts, moisture-enhanced with four brining formulations, cooked to 55°C, on day-0 (i.e., approximately 24 h after preparation) and day-30 after frozen (-20°C) storage and 48-72 h thawing.

Treatment	Sample	Day-0					Day-30				
		Side-1	Side-2	Top	Center	Bottom	Side-1	Side-2	Top	Center	Bottom
NaCl + STP	1	63	64	68	55	74	85	75	80	55	83
	2	75	84	61	55	86	90	89	62	55	80
	3	92	90	63	55	80	92	85	67	55	74
	4	64	92	69	55	72	87	87	49	55	92
NaCl + STP + CPC	1	NA	85	72	55	NA	69	67	67	55	NA
	2	84	87	71	55	85	85	78	66	55	81
	3	70	70	50	55	65	84	85	57	55	87
	4	82	81	54	55	86	82	75	53	55	84
NaCl + STP + LA	1	NA	NA	NA	NA	NA	75	71	46	55	NA
	2	82	89	57	55	84	83	83	86	55	86
	3	72	76	59	55	58	82	89	73	55	84
	4	90	88	64	55	90	77	86	54	55	89
NaCl + STP + SM	1	83	78	62	55	77	71	77	68	55	NA
	2	81	72	62	55	70	76	77	70	55	NA
	3	68	63	59	55	74	82	81	63	55	79
	4	90	88	64	55	90	84	84	67	55	74

NaCl = Sodium Chloride, 0.5%; STP = Sodium Tripolyphosphate, 0.25%; CPC = Cetylpyridinium Chloride, 0.2%; LA = Lactic Acid, 0.3%, SM = Sodium Metasilicate, 0.2%. NA- Not Available

Table 6.9. Final temperatures (°C) at five locations of cooked roasts, moisture-enhanced with four brining formulations, cooked to 60°C, on day -0 (i e., approximately 24 h after preparation) and day-30 after frozen (-20°C) storage and 48-72 h thawing.

Treatment	Sample	Day-0					Day-30				
		Side-1	Side-2	Top	Center	Bottom	Side-1	Side-2	Top	Center	Bottom
NaCl + STP	1	NA	NA	NA	NA	NA	57	50	55	64	61
	2	62	65	59	65	66	56	39	54	59	63
	3	69	62	65	69	68	65	67	61	67	66
	4	68	64	59	66	71	61	64	60	67	68
NaCl + STP + CPC	1	44	57	60	64	65	49	40	48	64	61
	2	77	63	71	68	71	68	55	47	64	57
	3	56	55	53	60	55	71	58	52	67	63
	4	54	56	54	66	54	51	57	50	63	44
NaCl + STP + LA	1	NA	NA	NA	NA	NA	61	37	54	69	67
	2	62	60	56	62	60	63	NA	58	64	63
	3	56	52	55	58	65	67	60	59	69	62
	4	66	61	69	69	67	64	67	61	67	66
NaCl + STP + SM	1	57	67	64	65	66	57	61	55	65	61
	2	59	61	59	46	50	52	NA	53	61	64
	3	56	67	60	68	64	59	56	43	63	65
	4	NA	NA	NA	NA	NA	56	60	59	63	53

NaCl = Sodium Chloride, 0.5%; STP = Sodium Tripolyphosphate, 0.25%; CPC = Cetylpyridinium Chloride, 0.2%; LA = Lactic Acid, 0.3%, SM = Sodium Metasilicate, 0.2%. NA- Not Available

Table 6.10. Final temperatures (°C) at five locations of cooked roasts, moisture-enhanced with four brining formulations, cooked to 55°C, on day -0 (i.e., approximately 24 h after preparation) and day-30 after frozen (-20°C) and 48-72 h thawing.

Treatment	Sample	Day-0					Day-30				
		Side-1	Side-2	Top	Center	Bottom	Side-1	Side-2	Top	Center	Bottom
NaCl + STP	1	48	60	44	55	50	51	68	53	59	61
	2	64	53	58	62	55	55	57	NA	64	69
	3	46	55	54	63	72	53	58	48	63	70
	4	58	56	54	64	70	62	59	50	63	60
NaCl + STP + CPC	1	44	50	55	62	NA	57	51	51	60	NA
	2	57	52	58	62	56	39	56	53	61	65
	3	68	60	50	59	65	56	61	45	61	65
	4	28	56	47	60	64	64	63	50	63	65
NaCl + STP + LA	1	NA	NA	NA	NA	NA	41	46	41	46	NA
	2	59	44	52	54	53	55	59	46	61	59
	3	62	51	42	55	60	70	54	62	66	63
	4	67	59	56	66	65	60	64	50	61	64
NaCl + STP + SM	1	52	60	46	63	70	68	68	64	62	61
	2	NA	NA	NA	NA	NA	47	49	52	61	61
	3	63	59	45	58	59	70	74	56	65	70
	4	67	59	56	66	65	73	64	54	64	72

NaCl = Sodium Chloride, 0.5%; STP = Sodium Tripolyphosphate, 0.25%; CPC = Cetylpyridinium Chloride, 0.2%; LA = Lactic Acid, 0.3%, SM = Sodium Metasilicate, 0.2%. NA- Not Available

Table 6.11. Cooking losses (%) for roasts, moisture-enhanced with four brining formulations, cooked to 60°C and 55°C, on day-0 (i. e., approximately 24 h after preparation) and day-30 after frozen (-20°C) storage and 48-72 h thawing .

Treatment	Sample	60°C		55°C	
		Day-0	Day-30	Day-0	Day-30
NaCl + STP	1	26.2	23.6	14.8	31.3
	2	30.4	22.9	31.5	31.5
	3	35.0	32.3	33.7	31.9
	4	21.9	34.2	33.0	29.6
NaCl + STP + CPC	1	11.5	22.7	28.2	21.1
	2	17.4	32.9	30.1	18.6
	3	30.0	30.9	23.9	25.1
	4	27.9	25.3	24.9	31.3
NaCl + STP + LA	1	21.7	33.1	3.2	11.9
	2	19.1	29.5	26.1	29.6
	3	34.7	38.4	26.4	36.8
	4	30.8	32.9	36.5	33.2
NaCl + STP + SM	1	23.3	21.9	24.6	15.8
	2	9.5	16.7	14.1	24.5
	3	37.3	18.3	10.8	30.8
	4	10.5	17.9	26.8	28.2

NaCl = Sodium Chloride, 0.5%; STP = Sodium Tripolyphosphate, 0.25%; CPC = Cetylpyridinium Chloride, 0.2%; LA = Lactic Acid, 0.3%, SM = Sodium Metasilicate, 0.2%.

Table 6.12. *E. coli* O157:H7 counts (log CFU/g) from six locations of cooked roasts, moisture-enhanced with four brining formulations, when cooked to internal temperatures of 60°C, on day -0 (i. e., approximately 24 h after preparation).

Treatment	Sample	Center			Side		
		Top	Middle	Bottom	Top	Middle	Bottom
NaCl + STP	1	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5
	2	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5
	3	<0.5	<0.5	<0.5	<0.5	<0.5	0.5
	4	<0.5	<0.5	<0.5	0.5	<0.5	<0.5
NaCl + STP + CPC	1	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5
	2	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5
	3	<0.5	1.9	<0.5	<0.5	<0.5	0.5
	4	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5
NaCl + STP + LA	1	<0.5	0.5	1.7	0.5	1.0	<0.5
	2	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5
	3	4.8	2.8	2.9	2.5	1.8	<0.5
	4	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5
NaCl + STP + SM	1	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5
	2	5.4	4.3	4.0	5.3	3.7	3.6
	3	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5
	4	5.0	3.5	3.3	3.3	3.0	3.1

NaCl = Sodium Chloride, 0.5%; STP = Sodium Tripolyphosphate, 0.25%; CPC = Cetylpyridinium Chloride, 0.2%; LA = Lactic Acid, 0.3%, SM = Sodium Metasilicate, 0.2%.

Table 6.13. *E. coli* O157:H7 counts (log CFU/g) from six locations of cooked roasts, moisture-enhanced with four brining formulations, when cooked to internal temperatures of 60°C, on day -30 after frozen (-20°C) storage and 48-72 h thawing.

Treatment	Sample	Center			Side		
		Top	Middle	Bottom	Top	Middle	Bottom
NaCl + STP	1	1.2	<0.5	<0.5	1.3	<0.5	<0.5
	2	1.8	<0.5	<0.5	1.1	<0.5	<0.5
	3	<0.5	<0.5	<0.5	0.5	<0.5	0.5
	4	<0.5	<0.5	<0.5	0.5	<0.5	2.0
NaCl + STP + CPC	1	0.5	<0.5	<0.5	<0.5	<0.5	<0.5
	2	0.5	<0.5	<0.5	<0.5	<0.5	<0.5
	3	<0.5	<0.5	<0.5	<0.5	0.5	0.5
	4	4.9	3.7	1.2	0.9	<0.5	<0.5
NaCl + STP + LA	1	1.0	1.2	1.1	1.0	<0.5	<0.5
	2	1.0	<0.5	<0.5	<0.5	<0.5	<0.5
	3	<0.5	<0.5	<0.5	<0.5	1.8	<0.5
	4	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5
NaCl + STP + SM	1	<0.5	<0.5	<0.5	<0.5	<0.5	0.5
	2	<0.5	<0.5	<0.5	1.4	<0.5	<0.5
	3	5.4	5.3	3.4	2.8	1.3	3.3
	4	4.9	3.8	2.8	NA	0.9	1.6

NaCl = Sodium Chloride, 0.5%; STP = Sodium Tripolyphosphate, 0.25%; CPC = Cetylpyridinium Chloride, 0.2%; LA = Lactic Acid, 0.3%, SM = Sodium Metasilicate, 0.2%.

Table 6.14. *E. coli* O157:H7 counts (log CFU/g) from six locations of cooked roasts, moisture-enhanced with four brining formulations, when cooked to internal temperatures of 55°C, on day -0 (i. e., approximately 24 h after preparation).

Treatment	Sample	Center			Side		
		Top	Middle	Bottom	Top	Middle	Bottom
NaCl + STP	1	4.3	3.9	2.6	3.3	4.3	2.8
	2	1.1	<0.5	<0.5	<0.5	<0.5	<0.5
	3	<0.5	<0.5	0.8	<0.5	<0.5	<0.5
	4	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5
NaCl + STP + CPC	1	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5
	2	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5
	3	4.4	2.5	1.7	2.9	<0.5	1.5
	4	2.5	1.5	0.8	<0.5	<0.5	<0.5
NaCl + STP + LA	1	4.7	4.4	3.5	2.9	3.8	2.8
	2	3.5	3.5	3.2	2.9	2.1	2.5
	3	4.8	4.0	3.9	2.3	2.4	2.9
	4	<0.5	<0.5	<0.5	<0.5	0.5	<0.5
NaCl + STP + SM	1	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5
	2	<0.5	1.5	1.7	3.8	2.5	1.8
	3	4.5	3.1	2.5	0.8	1.1	2.5
	4	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5

NaCl = Sodium Chloride, 0.5%; STP = Sodium Tripolyphosphate, 0.25%; CPC = Cetylpyridinium Chloride, 0.2%; LA = Lactic Acid, 0.3%, SM = Sodium Metasilicate, 0.2%.

Table 6.15. *E. coli* O157:H7 counts (log CFU/g) from six locations of cooked roasts, moisture-enhanced with four brining formulations, when cooked to internal temperatures of 55°C, on day -30 after frozen (-20°C) storage and 48-72 h thawing.

Treatment	Sample	Center			Side		
		Top	Middle	Bottom	Top	Middle	Bottom
NaCl + STP	1	0.5	<0.5	<0.5	<0.5	<0.5	<0.5
	2	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5
	3	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5
	4	4.5	2.9	2.1	2.3	1.3	1.9
NaCl + STP + CPC	1	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5
	2	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5
	3	3.8	<0.5	1.7	<0.5	<0.5	<0.5
	4	3.0	<0.5	<0.5	<0.5	0.5	<0.5
NaCl + STP + LA	1	5.2	5.0	4.3	4.9	3.7	3.5
	2	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5
	3	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5
	4	4.7	4.4	2.3	<0.5	<0.5	1.1
NaCl + STP + SM	1	3.0	<0.5	<0.5	<0.5	<0.5	0.5
	2	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5
	3	2.0	<0.5	<0.5	<0.5	<0.5	1.1
	4	0.5	<0.5	<0.5	<0.5	<0.5	<0.5

NaCl = Sodium Chloride, 0.5%; STP = Sodium Tripolyphosphate, 0.25%; CPC = Cetylpyridinium Chloride, 0.2%; LA = Lactic Acid, 0.3%, SM = Sodium Metasilicate, 0.2%.

Table 6.16. Total bacterial population (log CFU/g) from six locations of cooked roasts, moisture-enhanced with four brining formulations, when cooked to internal temperatures of 60°C, on day-0 (i. e., approximately 24 h after preparation).

Treatment	Sample	Center			Side		
		Top	Middle	Bottom	Top	Middle	Bottom
NaCl + STP	1	<0.5	2.3	0.5	2.5	0.5	<0.5
	2	<0.5	1.8	0.5	1.3	2.4	<0.5
	3	0.5	<0.5	<0.5	<0.5	<0.5	<0.5
	4	0.5	<0.5	<0.5	0.5	<0.5	<0.5
NaCl + STP + CPC	1	<0.5	0.5	0.5	<0.5	<0.5	0.5
	2	0.8	<0.5	0.5	<0.5	<0.5	<0.5
	3	0.5	1.8	<0.5	<0.5	0.5	0.5
	4	<0.5	<0.5	<0.5	0.5	0.5	0.5
NaCl + STP + LA	1	1.0	0.5	1.9	0.5	1.0	<0.5
	2	0.8	<0.5	<0.5	0.5	<0.5	0.5
	3	4.9	2.8	2.9	2.4	0.9	<0.5
	4	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5
NaCl + STP + SM	1	1.1	<0.5	<0.5	<0.5	<0.5	<0.5
	2	5.5	4.2	3.7	5.3	4.1	3.4
	3	1.7	<0.5	1.3	0.9	<0.5	1.4
	4	5.0	3.5	3.3	3.3	3.1	3.3

NaCl = Sodium Chloride, 0.5%; STP = Sodium Tripolyphosphate, 0.25%; CPC = Cetylpyridinium Chloride, 0.2%; LA = Lactic Acid, 0.3%, SM = Sodium Metasilicate, 0.2%.

Table 6.17. Total bacterial population (log CFU/g) from six locations of cooked roasts, moisture-enhanced with four brining formulations, when cooked to internal temperatures of 60°C, on day -30 after frozen (-20°C) storage and 48-72 h thawing.

Treatment	Sample	Center			Side		
		Top	Middle	Bottom	Top	Middle	Bottom
NaCl + STP	1	2.5	<0.5	<0.5	<0.5	2.2	1.9
	2	3.6	<0.5	<0.5	3.2	0.5	1.4
	3	1.2	1.3	0.5	0.8	0.8	<0.5
	4	<0.5	<0.5	<0.5	3.4	5.2	<0.5
NaCl + STP + CPC	1	1.1	<0.5	<0.5	1.0	<0.5	0.8
	2	2.3	<0.5	<0.5	<0.5	<0.5	1.5
	3	0.5	4.2	1.6	<0.5	<0.5	<0.5
	4	4.9	3.7	0.9	1.4	3.6	<0.5
NaCl + STP + LA	1	3.2	0.8	2.8	<0.5	2.7	0.8
	2	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5
	3	<0.5	0.8	2.3	<0.5	2.7	2.5
	4	0.9	<0.5	<0.5	<0.5	<0.5	<0.5
NaCl + STP + SM	1	<0.5	<0.5	<0.5	3.4	2.2	2.9
	2	2.7	0.5	2.5	<0.5	1.6	1.0
	3	5.4	5.3	3.4	2.8	1.2	3.2
	4	4.9	3.8	2.8		0.9	1.6

NaCl = Sodium Chloride, 0.5%; STP = Sodium Tripolyphosphate, 0.25%; CPC = Cetylpyridinium Chloride, 0.2%; LA = Lactic Acid, 0.3%, SM = Sodium Metasilicate, 0.2%.

Table 6.18. Total bacterial population (log CFU/g) from six locations of cooked roasts, moisture-enhanced with four brining formulations, when cooked to internal temperatures of 55°C, on day -0 (i. e., approximately 24 h after preparation).

Treatment	Sample	Center			Side		
		Top	Middle	Bottom	Top	Middle	Bottom
NaCl + STP	1	5.2	4.0	3.4	4.0	4.6	3.6
	2	1.7	3.1	2.1	2.4	1.0	1.0
	3	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5
	4	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5
NaCl + STP + CPC	1	<0.5	<0.5	<0.5	<0.5	<0.5	0.5
	2	<0.5	1.7	0.5	<0.5	<0.5	<0.5
	3	4.5	3.8	1.7	2.9	<0.5	0.9
	4	4.3	1.5	0.8	0.5	0.5	<0.5
NaCl + STP + LA	1	5.0	4.6	3.4	3.0	4.0	2.8
	2	3.9	3.4	3.4	3.4	2.2	2.7
	3	4.8	3.7	3.9	2.2	2.9	2.9
	4	<0.5	<0.5	<0.5	<0.5	1.5	<0.5
NaCl + STP + SM	1	3.6	2.4	<0.5	<0.5	<0.5	<0.5
	2	2.2	2	2.4	5.1	4.8	3
	3	4.5	2.6	2.3	0.8	1.1	2.4
	4	<0.5	<0.5	<0.5	<0.5	1.1	0.8

NaCl = Sodium Chloride, 0.5%; STP = Sodium Tripolyphosphate, 0.25%; CPC = Cetylpyridinium Chloride, 0.2%; LA = Lactic Acid, 0.3%, SM = Sodium Metasilicate, 0.2%.

Table 6.19. Total bacterial population (log CFU/g) from six different locations of cooked roasts, moisture-enhanced with four brining formulations, when cooked to internal temperatures of 55°C, on day -30 after frozen (-20°C) storage and 48-72 h thawing.

Treatment	Sample	Center			Side		
		Top	Middle	Bottom	Top	Middle	Bottom
NaCl + STP	1	3.6	4.0	3.4	0.8	<0.5	<0.5
	2	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5
	3	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5
	4	4.4	2.8	2.1	2.3	1.3	1.9
NaCl + STP + CPC	1	<0.5	<0.5	<0.5	<0.5	<0.5	1.4
	2	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5
	3	3.8	2.5	0.5	<0.5	<0.5	<0.5
	4	3	<0.5	<0.5	<0.5	0.5	<0.5
NaCl + STP + LA	1	5.5	4.9	4.3	5.0	3.8	3.8
	2	1.0	1.8	0.5	1.2	2.0	1.6
	3	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5
	4	4.6	4.4	2.4	<0.5	<0.5	0.5
NaCl + STP + SM	1	3.3	3.2	1.0	0.8	0.8	1.1
	2	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5
	3	<0.5	<0.5	<0.5	<0.5	<0.5	1.1
	4	0.5	<0.5	<0.5	<0.5	<0.5	<0.5

NaCl = Sodium Chloride, 0.5%; STP = Sodium Tripolyphosphate, 0.25%; CPC = Cetylpyridinium Chloride, 0.2%; LA = Lactic Acid, 0.3%, SM = Sodium Metasilicate, 0.2%.

Table 6.20. Percent of subsamples with undetectable (<0.5 log CFU/g) levels of *E. coli* O157:H7 and total bacterial population from total sixteen subsamples from six locations of roasts cooked to 60°C and 55°C, on day -0 (i. e., approximately 24 h after preparation) and on day-30 after frozen (-20°C) storage and 48-72 h thawing.

Subsample Location	*E. coli* O157:H7				Total Bacterial Population			
	60°C		55°C		60°C		55°C	
	Day-0	Day-30	Day-0	Day-30	Day-0	Day-30	Day-0	Day-30
Center Top	81.3	43.7	50.0	43.7	31.3	25.0	37..5	43.8
Side Top	68.8	46.7	56.3	87.5	37.5	53.3	43.8	68.8
Center Middle	68.8	75.0	50.0	81.3	50.0	50.0	31.3	56.3
Side Middle	75.0	75.0	56.3	81.3	50.0	31.3	37.5	68.8
Center Bottom	75.0	75.0	43.8	75.0	43.8	50.0	37.5	56.3
Side Bottom	75.0	62.5	56.3	68.8	56.3	37.5	37.5	56.3

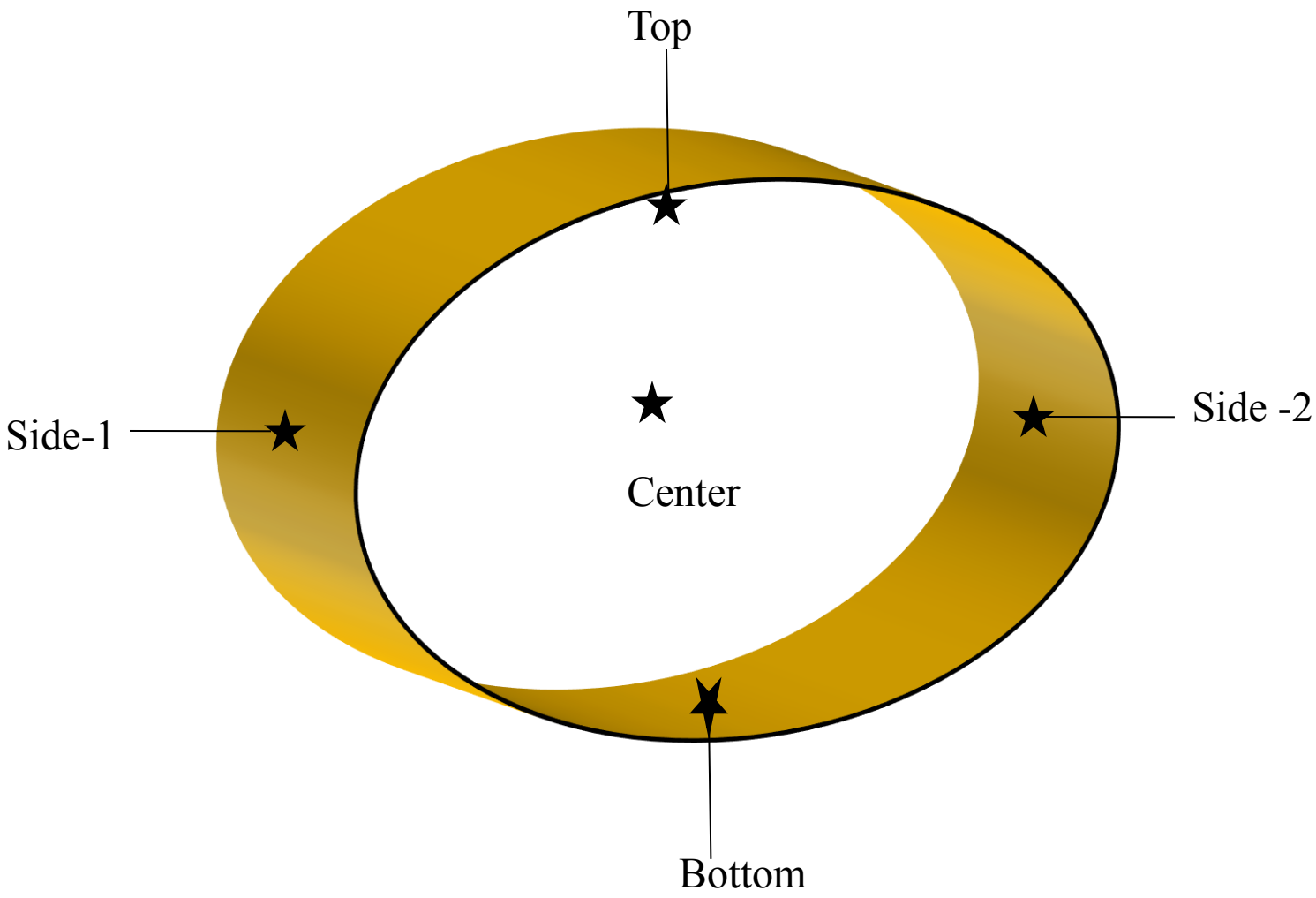

Figure 6.1. Location of five thermocouples in moisture-enhanced roast for temperature monitoring during cooking to internal temperatures of 60°C and 55°C in conventional kitchen oven and during 20-min resting period.

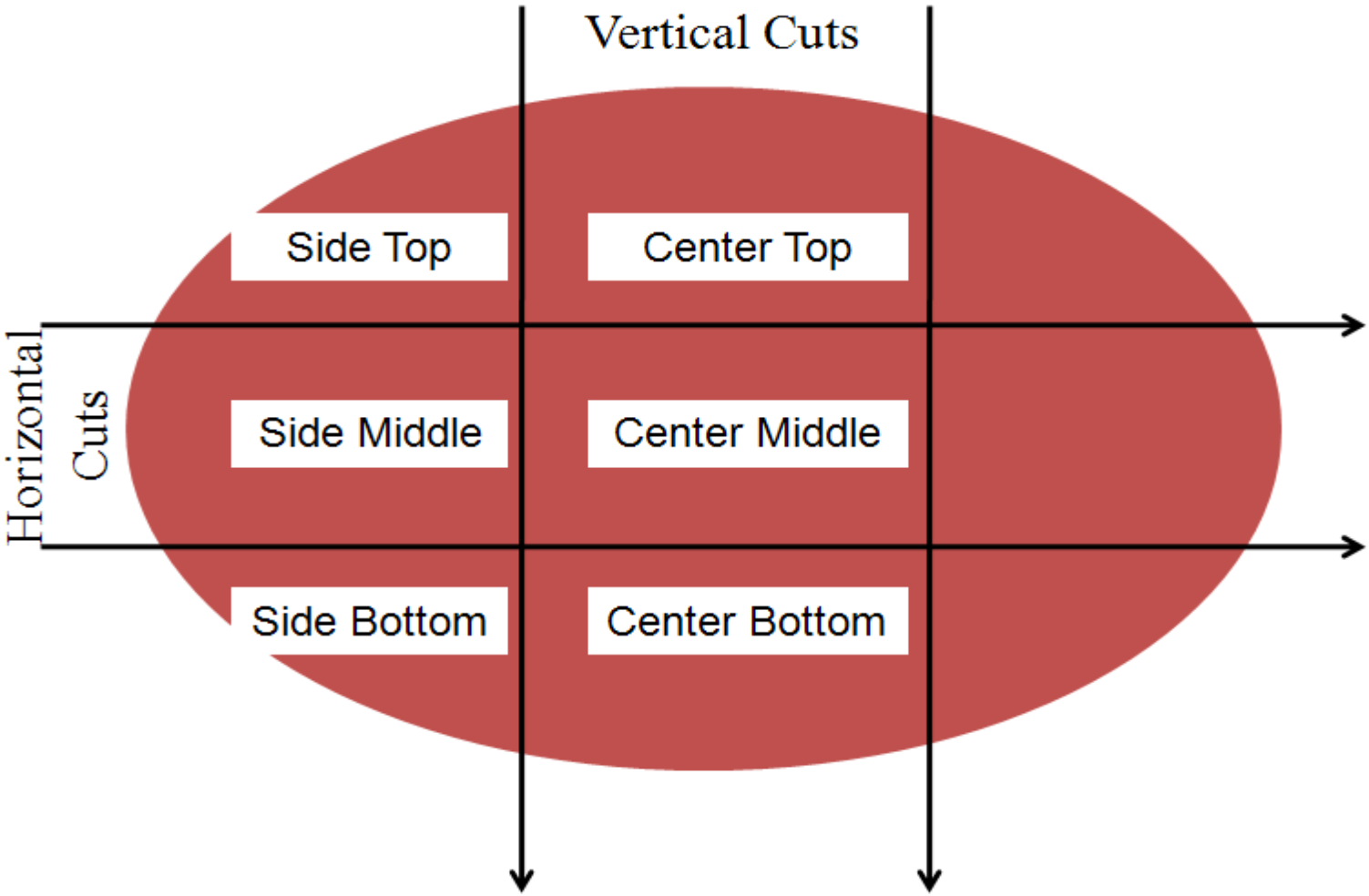

Figure 6.2. Six subsample locations extracted from cooked moisture-enhanced roast after cooking to internal temperatures of 60°C and 55°C.

CHAPTER 7

SURVIVAL AND REMOVAL OF *LISTERIA MONOCYTOGENES* CONTAMINATION FROM KITCHEN COUNTERTOP SURFACES

This study investigated the survival of *Listeria monocytogenes* without and with nutrient supplementation on kitchen countertop laminate surfaces, on laminate and corian surfaces, and evaluated the efficacy of four wiping materials to remove contamination. Laminate (2.5×4 cm) and corian (4.5×4.5 cm) surfaces were inoculated **(**5 log CFU/cm^2) with a 5-strain mixture of *L. monocytogenes* in 0.1 ml of ham homogenate, and incubated at 25±2°C at 50 and 90% RH for 96 h. Nutrient supplementation of coupon surfaces was accomplished by spreading 0.1 ml of uninoculated ham homogenate on inoculated areas every morning and evening simulating exposure of bacterial cells to nutrients during food preparation. At 0, 6, 24, 48, 72, and 96 h, laminate and corian surfaces were swabbed with kimwipes to recover surviving *L. monocytogenes* cells. Laminate surfaces were also first cleaned with handi-wipes®, heavy-wipes®, kitchen-cloth,® or paper-towel, and then swabbed with kimwipes to determine removed and remaining population. All kimwipes and wipes samples were analyzed for *L. monocytogenes* (PALCAM agar) and total bacterial (tryptic soy agar with 0.6% yeast extract) populations. *L. monocytogenes* cells with nutrient supplementation on laminate surfaces survived at higher levels compared to cells without nutrient supplementation, in the period of 48 to 96 h under both

environmental conditions. *L. monocytogenes* survival on laminate and corian surfaces did not differ significantly ($P \leq 0.05$), and decreased with time with total reductions of 5.4 log CFU/cm^2 after 96 h under both environmental conditions. Efficacy of the wiping materials to remove *L. monocytogenes* cells from laminate surfaces decreased with time at 50 and 90% RH. These data should encourage consumers to practice better hygienic practices when handling ready-to-eat meat and other food products on kitchen countertop surfaces.

7.1. INTRODUCTION

Listeria monocytogenes, a human foodborne pathogen, causes a deadly disease, especially in immunocompromised people, and is widely associated with class-1 recalls of potentially contaminated ready-to-eat (RTE) foods (Rodriguez et al., 2007a). Gombas et al. (2003) tested eight categories of RTE foods (luncheon meats, deli salads, fresh soft "Hispanic-style" cheeses, bagged salads, blue-veined and soft mold-ripened cheeses, smoked seafood, and seafood salads), collected over a period of time 14 to 23 months from retail markets at FoodNet sites in Maryland and northern California, for presence of *L. monocytogenes.* The overall prevalence of this pathogen in all food categories tested was 1.82%. However, contamination levels of *L. monocytogenes* were below 1 CFU/g in 99.6% of the positive samples (Gombas et al., 2003). Considering the prevalence of *L. monocytogenes* in RTE products at retail (Gombas et al., 2003), this pathogen can enter into the domestic kitchen along with contaminated food products. Food contact surfaces, like kitchen countertops, used for various food preparation tasks, can become contaminated with pathogens either by direct contact during food preparation or

indirectly through airborne particles (Kusumaningrum et al., 2003a). If these surfaces are not cleaned, pathogen cells can attach and survive on surfaces while residual food particles after food preparation can provide nutrients for already present cells to grow to dangerous levels, especially if surfaces are not cleaned for long periods of time. These contaminated surfaces could become sources of cross-contamination for clean foods during subsequent use. Nutrient availability (Allan et al., 2004; Parikh et al., 2009a; Yang et al., 2009a) and relative humidity levels (Kim et al., 2008) could influence attachment and survival of bacterial cells to different surfaces. Allan et al. (2004) found that preconditioning of surfaces simulating soiling in food processing environments can enhance survival of *L. monocytogenes* on stainless coupons. Studies (Kim et al., 2008; Palumbo and Williams, 1990; Stine et al., 2005) have reported variable effect of different relative humidities on survival of bacteria and viruses on food contact surfaces.

Tile, concrete, laminate, granite, silestone, ceramic, natural stone, engineered stone, quartz and soapstone are materials commonly used for furnishing kitchen countertop surfaces. Most published studies have compared differential survival of foodborne pathogens on materials like stainless steel, high density polyethylene (HDPE), polyethylene and polypropylene, glass etc, while very few studies have reported on survival of foodborne pathogens on materials used for kitchen countertops (Moore et al., 2007; Oliveria et al., 2006; Silva et al., 2008; Teixeira et al., 2007). Laminate and corian, two materials selected for the present study, are commonly used for furnishing kitchen countertop surfaces in domestic settings because they are versatile, inexpensive, durable, and available in multiple colors and designs.

Failure to effectively remove bacteria from food contact surfaces can have serious implications in the transmission of foodborne pathogens (DeVere and Purchase, 2007). Cleaning of food contact surfaces after food preparation could be used as a preventive measure against cross-contamination from these surfaces in the domestic kitchen. Commercially available wipes without and with antimicrobial components are commonly used for cleaning of contaminated food surfaces in food service and domestic settings. Wipes without antimicrobial components remove pathogen cells from surfaces without killing. Commercially available wipes without antibacterial components (e. g., Handi-wipes®, heavy-wipes®, kitchen-cloth®, paper-towel) differ in texture, and it should be interesting to know if these wipes also differ in their ability to remove pathogen cells from contaminated kitchen countertop surfaces.

Therefore, objectives of the present study were to: (i) investigate survival of *L. monocytogenes* artificially inoculated on laminate kitchen countertop surfaces without and with nutrient supplementation when exposed to dry (50% RH) or humid (90% RH) environmental conditions; (ii) investigate survival of *L. monocytogenes* on laminate and corian kitchen countertop materials when exposed to dry (50% RH) or humid (90% RH) environmental conditions with nutrient supplementation; and (iii) evaluate efficacy of four types of commercial wiping materials for removal of *L. monocytogenes* from artificially inoculated laminated kitchen countertop surfaces exposed to dry (50% RH) or humid (90% RH) environmental conditions for 96 h.

7.2. MATERIALS AND METHODS

7.2.1. Inoculum preparation

Five strains of *L. monocytogenes* (FSL J1-177 human sporadic; serotype 1/2b, FSL C1-056 human sporadic; serotype 1/2a, FSL N3-013 food epidemic, UK, Pâté; serotype 4b, FSL R2-499 human epidemic, sliced turkey 2000; serotype 1/2a, FSL N1-227 food epidemic, United States, ready to eat meat products, 1998-1999; serotype 4b), provided by Dr. Martin Wiedmann (Cornell University, Ithaca, NY, USA), and representing the genetic diversity of *L. monocytogenes* (Fugett et al., 2006), were used in the present study. Stock cultures were kept frozen (-70°C) in tryptic soy broth (TSB; Difco, Becton Dickinson, Sparks, MD) containing 0.6% yeast extract (YE; Acumedia, Baltimore, Md.). Strains were activated by transferring individually in TSBYE at 30°C for 22-24 h. After subculturing twice in TSBYE, overnight cultures were harvested by centrifuging individually (Eppendorf model 5810 R, Brinkmann Instruments Inc., Westbury, N.Y.) at 5000×*g* for 15 min at 4°C, washing with 10 ml of phosphate-buffered saline (PBS, pH 7.4; 0.2 g of KH_2PO_4, 1.5 g of $Na_2HPO_4 \cdot 7H_2O$, 8.0 g of NaCl, and 0.2 g of KCl in 1 liter of distilled water), centrifuging again as previously described, resuspending in 30 ml of ham homogenate (10% wt/vol), and habituating at 7°C for 2 days. Portions (10 ml) of ham homogenate with each habituated *L. monocytogenes* strain were combined on the day of the inoculation and this mixture was used for inoculation of coupon surfaces.

7.2.2. Ham homogenate preparation

Ham homogenate (10% wt/vol) was prepared from commercial ham without antimicrobials. A suspension was prepared by homogenizing (Masticator, IUL

Instruments, Barcelona, Spain) 10 g of ham with 90 ml of distilled water in whirl-Pak bags (12 oz, Nasco, Fort Atkinnson, WI) for 2 min. The suspension was subsequently passed through cheesecloth, autoclaved at 121°C for 15 min, and cooled to ambient temperature (25°C) before use (Yang et al., 2009a). Sterilized ham homogenate was used for habituation of *L. monocytogenes* strains and for nutrient supplementation of cells attached to coupon surfaces. Habituation of strains in ham homogenate simulated a real life scenario where contaminated RTE products are stored under refrigeration temperatures and pathogens cells entering into the domestic kitchen are acclimated to a low temperature food environment (Lianou et al., 2007; Yang et al., 2009).

7.2.3. Preparation and inoculation of coupon surfaces

Laminate coupons (5×4 cm), selected as one of the tested surfaces for *L. monocytogenes* survival, were kindly donated by Wilsonart International (Temple, TX). The coupons were sterilized before inoculation. All coupons, one the day before inoculation, were completely immersed for 30 min in ethanol (70%, v/v). The coupons were then drained and rinsed two times with two liters of sterile distilled water to remove ethanol residues. After rinsing, coupons were placed on a tray and dried for 5-6 h in a biosafety cabinet. The entire laminate coupon surface area (5×4 cm) was divided into two equal parts (2.5×4 cm) for inoculation. Corian coupons (4.5×4.5 cm), selected as another tested surface, were kindly donated by Dupont™Corian® (Greely, CO). Corian coupons were sterilized before inoculation by autoclaving at 121°C for 15 min.

On the day of inoculation, 0.1 ml of ham homogenate inoculated with the five-strain *L. monocytogenes* composite was spread evenly on the designated area on each individual laminate (2.5×4 cm) and corian (4.5×4.5 cm) coupon, to achieve target

inoculum levels of 5.7±0.5 log CFU/cm^2 and 5.3±0.5 log CFU/cm^2, respectively. After inoculation, coupons were placed on trays and stored in incubators maintained at two relative humidities (RH: 50±5 % and 90±5 %) at temperature of 25±2°C for 96 h. Relative humidity (RH) levels in incubators, set at 50±5 % and 90±5 %, were achieved by placing a saturated potassium sulfate solution (K_2SO_4) at the bottom of incubator 24 h before starting the experiment. The potassium sulfate solution was kept in the incubator with coupons for the entire length of the experiment and its volume was adjusted if RH fell below 90% (Yang et al., 2009). The relative humidities of the incubators were monitored daily with hygrometers (Time-Save Temperature & Humidity Data logger, Dickson, Addision, IL). The surviving bacterial cells were recovered from inoculated coupon surfaces on 0, 6, 24, 48, 72, and 96 h with kimwipes as described later.

7.2.4. Recovery of *L. monocytogenes* from coupon surfaces

The methodology of Vorst et al. (2004) for recovery of cells from coupon surfaces was adopted without any modification. Kimwipes® (Kimberly-Clark Corp., Roswell, Ga.) (11.4×21.5 cm) were cut into four pieces (5.6×12 cm), folded (3×3 cm) and autoclaved. The sterilized kimwipes were placed in a Whirl-Pak® bags (12 oz Nasco, Modesto, California) with 10 ml of maximum recovery diluent (MRD; 0.85% NaCl and 0.1% peptone), moistened, and squeezed with sterile forceps to remove excess solution. The coupon surface on the designated sampling point was swabbed with these moistened kimwipes ten times in horizontal and ten times in vertical direction. After swabbing, kimwipes were returned to the same Whirl-Pak®bag and an additional 10 ml of MRD was added into the bags. The kimwipes in the bag were homogenized for 2 min (Masticator,

IUL Instruments, Barcelona, Spain) and hand-massaged for 30 sec inside the same bag before microbiological analysis.

7.2.5. Procedure for survival and wiping studies

Laminate coupon surfaces were sterilized, inoculated, and incubated at two relative humidities (dry: 50±5% RH and humid: 90±5% RH) for 96 h at 25±2°C, as described earlier. A portion of 100µl of ham homogenate (10%: wt/vol) was deposited every morning and evening on one set of inoculated coupons for the entire length of the experiment while the other set of inoculated coupons did not receive any nutrient supplementation. Bacterial cells were supplemented with nutrients (ham homogenate) in a cycle starting from every morning at 8.00 am (0 h), and after 8 h (evening of the same day), and then after 16 h (next day morning). The nutrient supplementation cycle was repeated for 96 h (day 4). Bacterial cells were recovered from both sets of coupon surfaces with kimwipes on designated sampling times as described earlier.

In another survival study, laminate and corian coupons were sterilized, inoculated, and incubated at two relative humidities (dry: 50±5% RH and humid: 90±5% RH) for 96 h at 25±2°C with nutrient supplementation, and bacterial cells were recovered from these surfaces on designated sampling times, as described earlier.

Effect of wiping type on *L. monocytogenes* removal from laminate coupon surfaces was tested in another experiment. Each wipe type tested was cut and moistened with sterile distilled water (DW) before cleaning process. Amount of water used to moisten each wipe was enough for cleaning the coupon surface and not dripping from each wipe. Size and amount of distilled water used to moisten each wipe type are mentioned below in parentheses along with each wipe; (1) Handi-wipes® (HDW) (Size-

2×2cm^2, DW-100µl); (2) Heavy -wipes® (HW) (Size-2×2 cm^2, DW-300µl); (3) Kitchen-cloth® (KC) (Size-2×1cm^2, DW-200µl); (4) Paper-towel (PT) (Size-2×2 cm^2, DW-200µl). Laminate surfaces were sterilized, inoculated with the five strain *L. monocytogenes* mixture and incubated at two relative humidities (dry: 50±5% RH and humid: 90±5% RH) for 96 h at 25±2°C as described previously. Inoculated laminate coupon surface areas were cleaned with each wiping material type with one stroke in forward and backward direction on designated samplings. Each wipe type after the cleaning process, was placed in an 18-oz Whirl-Pak sterile bag with 6 ml MRD and pummeled for 2 min before microbiological analysis. Each laminate surface was swabbed with kimwipes after wiping process and analyzed microbiologically to recover left-over bacterial cells.

7.2.6. Microbiological analysis

All samples were plated on PALCAM (Difco, Becton Dickinson, Sparks, Md.) and Tryptic Soya Agar (TSA; Difco, Becton Dickinson, Sparks, Md) supplemented with 0.6% yeast extract (Acumedia, Lansing, MI; TSAYE) media for surviving *L. monocytogenes* and total bacterial populations, respectively. Colonies were enumerated after 48 h at 30°C (PALCAM agar), and 72h at 25°C (TSAYE).

7.2.7. Statistical analysis

Total bacterial and *L. monocytogenes* counts for all samples were converted into log CFU/cm^2 before statistical analysis. The survival experiments were conducted four times, and two individual samples within each replicate were analyzed for each sampling interval (n=8). Surviving bacterial counts (log CFU/cm^2) recovered from laminate surfaces without and with nutrient supplementation were analyzed for main effects of nutrient (N=2), sampling time (T=6), relative humidity (RH=2) and interactions (N×T,

T×RH, N×RH, and N×T×RH) of these factors. Surviving bacterial counts (log CFU/cm^2) recovered from laminate and corian surfaces with nutrient supplementation were analyzed for main effects of kitchen countertop material (KM=2), sampling time (T=6), relative humidity (RH=2) and interactions (KM×T, T×RH, KM×RH, and, KM×T×RH) of these factors. Efficacy experiments were conducted five times with two samples for each replicate. A total of ten samples (n=10) were analyzed for each sampling point. Bacterial cell counts (log CFU/cm^2) on each wiping material and recovered from laminate surface after the cleaning process were analyzed for main effects of wiping material (WM=4), sampling time (T=6) and relative humidity (RH=2) and the interactions (WM×T, T×RH, WM×RH, and WM×T×RH) of these factors.

Data were analyzed using the General Linear Model (GLM) of SAS® version 9.2 (SAS Institute Inc. Cary, NC). Means and standard deviations were calculated for the bacterial counts for samples. Multiple pairwise comparisons of the means were done using Tukey's honestly significance difference testes. Means were considered significantly different when P-values were less than 0.05.

7.3. RESULTS

7.3.1. Effect of nutrient supplementation on survival of *L. monocytogenes* on laminate surfaces

The average *L. monocytogenes* counts on the laminate surfaces were 5.7±0.5 log CFU/cm^2 immediately after inoculation (0 h) (Table 7.1). These counts were reduced by 1.6 and 2.8 log CFU/cm^2 at dry (50% RH) and humid (90% RH) environmental conditions, respectively, during the first 6 h of incubation when microorganisms did not

receive any nutrient supplementation (Table 7.1). Following this, nutrient supplementation significantly ($P \leq 0.05$) affected survival of *L. monocytogenes* cells on laminate surface under both environmental conditions. At 24 h (day 1) under humid (90 % RH) environmental conditions, *L. monocytogenes* levels when received nutrient supplementation on laminate surfaces were higher by 0.9 log CFU/cm^2 compared to levels without nutrient supplementation; a similar trend for *L. monocytogenes* population with and without nutrient supplementation was found in the period of 48 h (day 2) to 96 h (day 4) under both humidity conditions. *L. monocytogenes* cells with nutrient supplementation were 1.1 and 0.5 log CFU/cm^2 at 96 h under dry (50 % RH) and humid (90% RH) conditions, respectively. However, *L. monocytogenes* without any added nutrients were reduced to below detection (<0.3 log CFU/cm^2), with total reductions of 5.4 log CFU/cm^2, after 72 h (day 3) and 96 h (day 4), under dry (50 % RH) and humid (90% RH) environmental conditions, respectively (Table 7.1). Relative humidity significantly ($P \leq 0.05$) effected *L. monocytogenes* survival on laminate surfaces, and *L. monocytogenes* counts on laminate surface under humid (50% RH) environmental conditions were higher by 1.2 log CFU/cm^2 compared to levels under dry (90% RH) environmental conditions (Table 7.1).

7.3.2. Effect of material type on survival of *L. monocytogenes* on kitchen countertop surfaces

The initial *L. monocytogenes* levels on laminate and corian surfaces were 5.7±0.5 and 5.3±0.2 log CFU/cm^2, respectively (Table 7.2). Material type did not ($P \geq 0.05$) affect *L. monocytogenes* survival on these surfaces. Counts on both type of kitchen countertop surfaces decreased with time, reduced by 4.6-5.2 log CFU/cm^{2,} and 4.4-5.2 log

CFU/cm^2 after 4 days on laminate and corian surfaces, respectively under both environmental conditions. Relative humidity significantly ($P \leq 0.05$) effected survival of *L. monocytogenes* on these surfaces, and counts under humid environmental conditions were higher by 1.2 log CFU/cm^2 and 0.8 log CFU/cm^2 compared to levels under dry (50% RH) conditions on laminate and corian surfaces respectively, during first 6 h of contamination (Table 7.2).

7.3.3. Effect of wiping materials on *L. monocytogenes* removal from laminate coupon surfaces

Highest levels of *L. monocytogenes* were removed by each wiping material from laminate surfaces immediately after inoculation (0 h) and these levels ranged from 5.0-5.5 log CFU/cm^2 and 5.0-6.2 log CFU/cm^2 under dry (50% RH) and humid (90% RH) environmental conditions, respectively (Table 7.3). Efficacy of each wiping material to remove *L. monocytogenes* cells from laminate surfaces decreased with time under both environmental conditions. Wiping type significantly ($P \leq 0.05$) effected removal of *L. monocytogenes* from laminate surfaces. Even though no specific wiping material performed better for *L. monocytogenes* cells removal from laminate surfaces than others for entire sampling period; specifically paper-towel performed better compared to other three wipes (i. e., handy-wipes®, heavy-wipes® and kitchen-cloth®) for a period from 6-72 h under dry (50% RH), and handy-wipes® performed better compared to other three wipes for a period from 24-48 h under humid (90% RH) environmental conditions (Table 7.3). *L. monocytogenes* counts removed by four wipes from laminate surface differed by 1.6 and 0.5 log CFU/cm^2 at 6 h, 1.0 and 2.1 log CFU/cm^2 at 24 h (day1), 1.3 and 2.1 log CFU/cm^2 at 48 h (day 2), 1.7 and 1.0 log CFU/cm^2 at 72 h (day 3), and 0.3 and 0.7 log

CFU/cm^2 at 96 h (day 4), under dry and humid environmental conditions, respectively (Table 7.3). Similar to results of survival studies, relative humidity significantly ($P \leq 0.05$) effected efficacy of wiping materials for *L. monocytogenes* removal from laminate surfaces, and *L. monocytogenes* cells removed by handi-wipes®, heavy-wipes®, kitchen-cloth® and paper-towel from laminate surfaces under humid (90% RH) conditions were higher by 3.0, 2.6, 1.4 and 1.3 log CFU/cm^2 compared to levels removed by these wiping materials under dry (50% RH) environmental conditions (Table 7.3).

Leftover *L. monocytogenes* cells recovered with kimwipes from laminate surfaces after cleaning with each wiping material ranged from 2.7 to 3.7 and 3.2 to 3.8 log CFU/cm^2 under dry and humid environmental conditions, respectively, immediately after inoculation (0 h) (Table 7.4). Leftover *L. monocytogenes* levels recovered from laminate surfaces after cleaning deceased with time were in the ranges: 0.5-2.2 and 2.0-3.0 log CFU/cm^2 at 6 h, 0.6-1.3 and 1.3-2.0 log CFU/cm^2 at 24 h (day 1), 0.4-1.0 and 0.5-1.5 log CFU/cm^2 at 48 h (day 2), 0.4-1.1 and 0.4-1.2 log CFU/cm^2 at 72 h (day 3), and, <0.3-0.4 and <0.3-0.4 log CFU/cm^2 at 96 h (day 4) under dry (50% RH) and humid (90% RH) environmental conditions, respectively (Table 7.4).Total bacterial populations recovered on TSAYE plates from surfaces showed trends similar to *L. monocytogenes* counts under both environmental conditions (Tables 7.5-7.8).

7.4. DISCUSSION

Inoculation of laminate and corian surfaces with *L. monocytogenes* in ham homogenate simulated kitchen countertop surface contamination with purge from meat products during food preparation. Survival of *L. monocytogenes* was evaluated at relative

humidities represented maximum and minimum levels observed in humid and dry environments.

L. monocytogenes levels survived at higher levels on laminate kitchen countertop surfaces under humid compared to dry environmental conditions during the first 6 h of contamination (Tables 7.1 and 7.2). Similarly, Palumbo and Williams (1990) observed higher survival of *L. monocytogenes* levels suspended in distilled water under high relative humidity levels (75%) compared to very low relative humidity levels (1%) during 40 days of storage. Similarly, Vogel et al. (2010) observed higher survival of *L. monocytogenes* on stainless steel coupons at 43 and 75% RH compared to 2% RH during 20 days of storage. Kim et al. (2008) also observed higher survival for *Cronobacter sakazakii* at 100% RH compared to 48% when were dried over stainless steel coupons at 25°C for 42 days. The lower *L. monocytogenes* survival at dry (50 % RH) compared to humid (90 % RH) environmental conditions on surfaces could be due to low air moisture content and drying effect under lower relative humidity levels. Studies (Haysom and Sharp, 2005; De Cesare et al., 2003; Kusumaningrum et al., 2003a; Moore et al., 2003) have reported loss of viability of bacterial cells on surfaces within the first few hours of incubation as suspending media dried out (drying effect), and the drying rate of inoculum on surfaces could be influenced by variables like relative humidity, temperature and air circulation (De Ceasre et al., 2003). As could be expected, coupon surfaces appeared dry without visible moisture after 6 h under dry (50 % RH) compared to surfaces under humid (90 % RH) environmental conditions in the present study.

L. monocytogenes cells supplemented with nutrients on the laminate surfaces simulated reuse of contaminated kitchen countertop surfaces without cleaning after food

preparation. Cells that did not receive any nutrients simulated where contaminated kitchen countertop surfaces left unclean for extended periods of times after food preparation. Supplementation of *L. monocytogenes* cells with nutrients prolonged survival of these cells on laminate kitchen countertop surfaces especially under dry environmental conditions (Table 7.2) It has been previously reported that presence of macromolecular in the form residual food debris, milk, chicken and fat could provide nutrients, protect cells against dehydration, and eventually could prolong survival of cells on food contact surfaces (Ak et al., 1994; De Cesare et al., 2003; Kusumaningrum et al., 2003a; Moore et al., 2007; Yang et al., 2009a).

Laminate commonly known as "Formica" has a smooth surface with shallow scratches and depressions (Nyeleti et al., 2004). Corian® is an acrylic-based premium solid material that has nonporous and seamless surfaces with no micro cavities to harbor pathogen cells and low levels of contaminants could be easily cleaned from these surfaces (Anonymous, 2009). *L. monocytogenes* survived on laminate and corian surfaces for 4 days and populations of these pathogens were reduced by 4 to 5 logs under humid and dry environmental conditions (Table 7.3). Moore et al. (2007) also reported survival of *Salmonella* Typimurium on laminate surface for 6 h and populations were reduced by 3.3 log CFU/25cm^2 during this time period. Humphrey et al. (1994) reported that *Salmonella spp.* could persist on Formica for at least 24 h when these surfaces get contaminated following egg preparation.

Survival of *L. monocytogenes* cells on kitchen countertop surfaces could be interpreted as potential of cells for cross-contamination (Moore et al., 2007). Higher levels of cross- contamination could be expected immediately after contamination and

this potential decreased as contamination became older. Additionally, higher cross-contamination levels could be expected under humid compared to dry environmental conditions first few hours of contamination.

According to manufacturers of four wiping materials (i. e., handi wipes®, heavy wipes®, Kitchen cloth® and paper towel) tested in this study did not have antibacterial component. This claim was supported by the results since *L. monocytogenes* levels (5.0-6.2 log CFU/cm^2) removed from laminate surfaces by each wiping material immediately after inoculation (0 h) were not statistically ($P > 0.05$) different from levels (5.7 log CFU/cm^2) recovered for these surfaces by kimwipes (Tables 7.1-7.3) (Lee et al., 2007).

Wiping materials were most efficient for *L. monocytogenes* removal from laminate kitchen countertop surfaces immediately (0 h) after contamination (Table 7.3). As explained by Bloomfield and Scott (1997) food particles and loosely attached bacterial cells could be removed after normal cleaning. However, strongly attached bacterial cells, usually invisible, could have not been removed from surfaces. Contaminated laminate kitchen countertop surfaces looked clean after wiping with each material tested with one stroke forward and backward direction. However, strongly attached listerial cells were recovered as leftover cells when laminate surfaces were swabbed with kimwipes after the cleaning material wiping process (Tables 7.3 and 7.4). Efficacy of wiping materials to remove *L. monocytogenes* cells and counts of leftover recovered with kimwipes after cleaning process, decreased as contamination became older under both environmental conditions (Tables 7.3 and 7.4). Lower viability of *L. monocytogenes* cells on laminate surfaces with time, as discussed earlier, could be the reason for lower removal of *L. monocytogenes* cells with wiping materials as

contamination became older (Moore et al., 2007). Similarly, lower survival of *L. monocytogenes* cells on laminate surface under dry environmental conditions could be the reason for lower efficacy of all wiping materials under dry (50 % RH) compare to humid (90 % RH) environmental conditions during the first 6 h of contamination.

7.5. CONCLUSIONS

The results of this study indicated that kitchen countertop surfaces can become contaminated with high levels of *L. monocytogenes* when used for preparation of contaminated ready-to-eat meat products. Pathogen cells could survive on these surfaces extended times periods if these surfaces not cleaned before subsequent use and these contaminated kitchen countertop surfaces could act as reservoirs for bacteria and sources of cross-contamination of clean products during meal preparation. Higher survival and cross-contamination levels of *L. monocytogenes* could be expected from these contaminated kitchen countertop surfaces in regions with high compared to lower humidity levels. Cleaning of contaminated kitchen countertop surfaces with commercially available wipes with one stroke in the forward and backward direction could remove the majority of pathogen cells especially if surfaces were cleaned immediately after contamination. Further studies are needed to test if cleaning of surfaces with commercially wipes using multiple strokes and/or combined with antibacterial sprays could be effective for removal of leftover pathogen cells to achieve total sanitation on these surfaces. These data could be used for development of cleaning recommendations for kitchen countertops under different environmental conditions.

Table 7.1 (Appendix Figure 18). *L. monocytogenes* (log CFU/cm^2; mean ± standard deviation) recovered from laminate kitchen countertop surfaces, without and with nutrient supplementation, incubated at 50±5% and 90±5% relative humidity for 96 h at 25±2°C

Sampling Time (h)	Not added Nutrients		Added Nutrients	
	50%	90%	50%	90%
0	5.7 ± 0.5 ^{aX}	5.7 ± 0.5 ^{aX}	5.7 ± 0.5 ^{aX}	5.7 ± 0.5 ^{aX}
6	2.9 ± 0.9 ^{bX}	4.1 ± 1.2 ^{bX}	2.9 ± 0.9 ^{bX}	4.1 ± 1.2 ^{bX}
24	2.3 ± 0.6 ^{bcX}	2.4 ± 1.7 ^{cX}	2.3 ± 0.8 ^{bcX}	3.3 ± 1.0 ^{bX}
48	<1.2 ± 0.8 ^{cX}	<0.7 ± 0.8 ^{dX}	<1.7 ± 0.9 ^{cX}	<1.6 ± 1.1 ^{cX}
72	<0.5 ± 0.6 ^{cX}	<0.3 ^{dY}	<1.4 ± 0.7 ^{cX}	<1.2 ± 1.1 ^{cX}
96	<0.3^{cX}	<0.3 ^{dY}	<1.1 ± 0.1 ^{cX}	<0.5 ± 0.6 ^{cX}

Mean values with different lower case letter in the same column are significantly different (P<0.05). Mean values with different upper case letter in the same row are significantly different (P<0.05).

Table 7.2 (Appendix Figure 19). *L. monocytogenes* (log CFU/cm^2; mean ± standard deviation) recovered from laminate and corian kitchen countertop surfaces, incubated at 50±5% and 90±5% relative humidity levels for 96 h at 25±2°C with nutrient supplementation.

Sampling Time (h)	Laminate		Corian	
	50%	90%	50%	90%
0	5.7 ± 0.5 ^{aX}	5.7 ± 0.5 ^{aX}	5.3 ± 0.3 ^{aX}	5.3 ± 0.3 ^{aX}
6	2.9 ± 0.9 ^{bX}	4.1 ± 1.2 ^{abX}	3.0 ± 1.0 ^{bX}	3.8 ± 1.2 ^{abX}
24	2.3 ± 0.8 ^{bcX}	3.3 ± 1.0 ^{bX}	2.4 ± 0.4 ^{bcX}	<2.5 ± 1.8 ^{bcX}
48	<1.7 ± 0.9 ^{bcX}	<1.6 ± 1.1 ^{bcX}	<0.8 ± 1.1 ^{cdX}	<1.7 ± 1.7 ^{bcX}
72	<1.4 ± 0.7 ^{cX}	<1.2 ± 1.1 ^{cX}	<0.7 ± 0.7 ^{cdX}	<1.5 ± 1.9 ^{cX}
96	<1.1 ± 0.1 ^{cX}	<0.5 ± 0.6 ^{cX}	<0.1± 0.2 ^{dX}	<0.9 ± 1.7 ^{cX}

Mean values with different lower case letter in the same column are significantly different (P<0.05). Mean values with different upper case letter in the same row are significantly different (P<0.05).

Table 7.3 (Appendix Figures 20 and 21). *L. monocytogenes* (log CFU/cm^2; mean ± standard deviation) removed by each wiping material after cleaning laminate coupon surfaces incubated at 50±5% and 90±5% relative humidity levels for 96 h at 25±2°C with nutrient supplementation

Sampling Hour (h)	50%				90%			
	Handi Wipes®	Heavy Wipes®	Kitchen Cloth®	Paper Towel	Handi Wipes®	Heavy Wipes®	Kitchen Cloth®	Paper Towel
0	5.3 ± 1.1 ^{aX}	5.5 ± 0.5 ^{aX}	5.2 ± 1.1 ^{aX}	5.0 ± 0.9 ^{aX}	5.8 ± 0.2 ^{aX}	5.5 ± 0.5 ^{aX}	6.2 ± 0.1 ^{aX}	5.0 ± 0.9 ^{aX}
6	1.7 ± 1.2 ^{bX}	2.6 ± 1.1 ^{bXY}	3.3 ± 1.1 ^{bXY}	3.3 ± 0.6 ^{abXY}	4.7 ± 0.5 ^{abY}	4.2 ± 0.8 ^{aXY}	4.7 ± 0.6 ^{aY}	4.6 ± 0.3 ^{aY}
24	<1.9 ± 1.9 ^{bXY}	<1.6 ± 1.9 ^{bX}	<1.9 ± 1.9 ^{bXY}	2.6 ± 1.0 ^{bXY}	4.0 ± 0.9 ^{abY}	<1.9 ± 0.6 ^{bXY}	3.7 ± 0.8 ^{bXY}	3.3 ± 1.2 ^{abXY}
48	<1.4 ± 1.4 ^{bXY}	<0.7 ± 1.2 ^{bX}	<1.6 ± 1.5 ^{bXY}	2.1 ± 1.1 ^{bXY}	3.0 ± 1.1 ^{bY}	<0.9 ± 1.3 ^{bX}	2.5 ± 1.3 ^{bXY}	2.3 ± 1.8 ^{bXY}
72	<2.1 ± 0.5 ^{bX}	<0.6 ± 1.0 ^{bX}	1.8 ± 0.8 ^{bX}	2.3 ± 0.7 ^{bX}	1.8 ± 1.2 ^{bcX}	<1.0 ± 1.6 ^{bX}	<1.2 ± 1.7 ^{cX}	<2.0 ± 1.6 ^{bX}
96	<1.2±1.2 ^{bX}	1.2 ± 0.3 ^{bX}	<0.9 ± 0.7 ^{bX}	0.9 ± 1.2 ^{bX}	<0.5 ± 0.6 ^{cX}	<0.0 ± 0.7 ^{bX}	<-0.2 ^{cX}	<-0.2 ^{bX}

Mean values with different lower case letter in the same column are significantly different (P<0.05). Mean values with different upper case letter in the same row are significantly different (P<0.05).

Table 7.4 (Appendix Figures 22 and 23). *L. monocytogenes* (log CFU/cm^2; mean ± standard deviation) recovered from laminate coupon surfaces after cleaning surfaces with each wiping material, incubated at 50±5% and 90±5% relative humidity levels for 96 h at 25±2°C with nutrient supplementation.

Sampling Hour (h)	50%				90%			
	Handi Wipes®	Heavy Wipes®	Kitchen Cloth®	Paper Towel	Handi Wipes®	Heavy Wipes®	Kitchen Cloth®	Paper Towel
0	2.7±1.1 ^{aX}	3.4 ± 1.1 ^{aX}	2.8±1.4 ^{aX}	3.7 ± 1.0 ^{aX}	3.2±0.8 ^{aX}	3.8±1.0 ^{aX}	3.7±0.7 ^{aX}	3.7 ± 1.0 ^{aX}
6	0.8±0.6 ^{bXY}	<0.5 ± 0.4 ^{bX}	<0.8±0.5 ^{bXY}	2.2 ± 0.8 ^{bX}	2.0±0.7 ^{abXY}	2.3±0.9 ^{abX}	2.1±0.9 ^{bY}	3.0 ± 1.3 ^{abX}
24	<1.1±0.9 ^{bX}	<0.8 ± 0.9 ^{bX}	<0.6±0.5 ^{bX}	<1.3 ± 0.8 ^{bX}	1.9±0.8 ^{abX}	<1.3±1.3 ^{bX}	<1.9±0.7 ^{bcX}	<2.0 ± 1.3 ^{bX}
48	<0.8±0.7 ^{bX}	<0.5 ± 0.4 ^{bX}	<0.4±0.4 ^{bX}	<1.0 ± 0.9 ^{bX}	<1.2±0.7 ^{bX}	<0.5±0.4 ^{bX}	<0.8±1.0 ^{bcX}	<1.5 ± 1.3 ^{bX}
72	<0.5±0.3 ^{bX}	<0.4 ± 0.2 ^{bX}	<0.5±0.5 ^{bX}	<1.1 ± 0.9 ^{bX}	<0.6±0.5 ^{bX}	<0.4±0.3 ^{bX}	<0.9±0.9 ^{bcX}	<1.2 ± 1.4 ^{bX}
96	<0.3 ^{cY}	<0.3 ^{cY}	<0.4±0.3 ^{bX}	<0.4 ± 0.2 ^{bX}	<0.3 ^{cY}	<0.3 ^{cY}	<0.4±0.3 ^{cX}	<0.3 ^{cY}

Mean values with different lower case letter in the same column are significantly different (P<0.05). Mean values with different upper case letter in the same row are significantly different (P<0.05).

Table 7.5 (Appendix Figure 24). Total bacterial population (log CFU/cm^2; mean ± standard deviation) recovered from laminate kitchen countertop surfaces, without and with nutrient supplementation, incubated at 50±5% and 90±5% relative humidity for 96 h at 25±2°C

Sampling Time (h)	Not added Nutrients		Added Nutrients	
	50%	90%	50%	90%
0	5.7 ± 0.5 ^{aX}	5.7 ± 0.5 ^{aX}	5.7 ± 0.5 ^{aX}	5.7 ± 0.5 ^{aX}
6	3.1 ± 0.9 ^{bX}	4.3 ± 1.0 ^{aX}	3.1 ± 0.9 ^{bX}	4.2 ± 1.2 ^{abX}
24	2.5 ± 0.5 ^{bX}	2.5 ± 1.7 ^{bX}	2.4 ± 0.9 ^{bcX}	3.5 ± 1.0 ^{bcX}
48	1.8 ± 0.6 ^{bcX}	<1.4 ± 0.9 ^{bcX}	<1.7 ± 1.1 ^{bcX}	2.0 ± 0.8 ^{cX}
72	<1.0 ± 0.7 ^{cX}	<0.9 ± 0.6 ^{cX}	<1.4 ± 0.8 ^{cX}	<1.3 ± 1.1 ^{cX}
96	<0.7 ± 0.6 ^{cX}	<0.8 ± 0.9 ^{cX}	<1.1 ± 0.6 ^{cX}	<1.0 ± 0.8 ^{cX}

Mean values with different lower case letter in the same column are significantly different ($P<0.05$). Mean values with different upper case letter in the same row are significantly different ($P<0.05$).

Table 7.6 (Appendix Figure 25). Total bacterial population (log CFU/cm^2; mean ± standard deviation) recovered from laminate and corian kitchen countertop surfaces, incubated at 50±5% and 90±5% relative humidity levels for 96 h at 25±2°C with nutrient supplementation.

Sampling Hour (h)	Laminate		Corian	
	50%	90%	50%	90%
0	5.7 ± 0.5 ^{aX}	5.7 ± 0.5 ^{aX}	5.2 ± 0.4 ^{aX}	5.4 ± 0.3 ^{aX}
6	3.1 ± 0.9 ^{bX}	4.2 ± 1.2 ^{abX}	3.2 ± 0.9 ^{bX}	3.8 ± 1.2 ^{abX}
24	2.4 ± 0.9 ^{bcX}	3.5 ± 1.0 ^{bX}	2.4 ± 0.5 ^{bcX}	2.8 ± 1.3 ^{bX}
48	<1.7 ± 1.1 ^{bcX}	2.0 ± 0.8 ^{bcX}	<1.2 ± 1.0 ^{cdX}	<2.1 ± 1.6 ^{bcX}
72	<1.4 ± 0.8 ^{bcX}	<1.3 ± 1.1 ^{cX}	<0.9 ± 1.0 ^{cdX}	<1.6 ± 1.9 ^{bcX}
96	<1.1 ± 0.6 ^{cX}	<1.0 ± 0.8 ^{cX}	<0.3 ± 0.6 ^{dX}	<0.5 ± 1.3 ^{cX}

Mean values with different lower case letter in the same column are significantly different ($P<0.05$). Mean values with different upper case letter in the same row are significantly different ($P<0.05$).

Table 7.7 (Appendix Figures 26 and 27). Total bacterial population (log CFU/cm^2; mean ± standard deviation) removed by each wiping material after cleaning laminate coupon surfaces incubated at 50±5% and 90±5% relative humidity levels for 96 h at 25±2°C with nutrient supplementation.

Sampling Hour (h)	50%				90%			
	Handi Wipes®	Heavy Wipes®	Kitchen Cloth®	Paper Towel	Handi Wipes®	Heavy Wipes®	Kitchen Cloth®	Paper Towel
0	5.8 ± 0.3 ^{aX}	5.6 ± 0.5 ^{aX}	6.0 ± 0.2 ^{aX}	5.1 ± 0.8 ^{aX}	5.8 ± 0.2 ^{aX}	5.6 ± 0.5 ^{aX}	6.1 ±0.2 ^{aX}	5.1 ± 0.8 ^{aX}
6	2.4 ± 1.3 ^{bX}	2.8 ± 1.5 ^{bX}	3.4 ± 0.8 ^{bXY}	3.5 ± 0.6 ^{abXY}	4.5 ± 0.8 ^{abX}	4.2 ± 0.7 ^{abXY}	5.1 ± 0.5 ^{abX}	4.7 ± 0.5 ^{aY}
24	3.0 ± 0.8 ^{bXY}	2.0 ± 0.8 ^{bcX}	2.8 ±1.5 ^{bXY}	3.1 ± 0.9 ^{bXY}	4.6 ± 0.6 ^{abY}	<2.5 ± 1.8 ^{bX}	4.3 ± 0.5 ^{bY}	4.2 ± 0.7 ^{aY}
48	<1.6 ± 1.6 ^{bXY}	<1.1 ± 1.0 ^{bcX}	<1.8 ± 1.8 ^{bXY}	2.6 ± 0.7 ^{bXY}	3.5 ± 0.8 ^{bY}	<1.4 ± 1.4 ^{bX}	3.2 ± 0.9 ^{bcY}	3.7 ± 0.7 ^{aY}
72	2.3 ± 0.6 ^{bXY}	<0.7 ± 1.2 ^{cX}	2.5 ± 0.7 ^{bXY}	2.7 ± 0.7 ^{bXY}	2.3 ± 1.4 ^{bcXY}	<0.7 ± 1.3 ^{bX}	2.4 ± 1.5 ^{cXY}	3.6 ± 0.9 ^{aY}
96	1.9 ± 1.1 ^{bX}	1.3 ± 1.5 ^{bcX}	<1.2 ± 1.5 ^{bX}	1.6 ± 1.2 ^{bX}	<1.1 ± 1.5 ^{cX}	<0.7 ± 1.4 ^{bX}	<0.5 ± 0.9 ^{cX}	0.6 ± 0.9 ^{bX}

Mean values with different lower case letter in the same column are significantly different (P<0.05). Mean values with different upper case letter in the same row are significantly different (P<0.05).

Table 7.8 (Appendix Figures 28 and 29). Total bacterial population (log CFU/cm^2; mean ± standard deviation) recovered from laminate coupon surfaces after cleaning surfaces with each wiping material, incubated at 50±5% and 90±5% relative humidity levels for 96 h at 25±2°C with nutrient supplementation.

Sampling Hour (h)	50%				90%			
	Handi Wipes®	Heavy Wipes®	Kitchen Cloth®	Paper Towel	Handi Wipes®	Heavy Wipes®	Kitchen Cloth®	Paper Towel
0	3.3 ± 0.8 ^{aX}	3.5 ± 0.9 ^{aX}	3.8 ± 0.8 ^{aX}	3.5 ± 1.0 ^{aX}	3.5 ± 0.7 ^{aX}	3.5 ± 0.9 ^{aX}	3.9 ± 0.6 ^{aX}	3.8 ± 1.0 ^{aX}
6	<1.1 ± 1.1 ^{bXY}	<0.8 ± 0.6 ^{bX}	1.0 ± 0.7 ^{bXY}	2.5 ± 0.7 ^{abY}	2.2 ± 0.7 ^{abXY}	2.3 ± 0.8 ^{abXY}	2.3 ± 0.9 ^{abXY}	3.3 ± 1.3 ^{aY}
24	<1.3 ± 1.2 ^{bXY}	1.4 ± 0.8 ^{bXY}	<0.9 ± 0.6 ^{bX}	<1.6 ± 0.9 ^{bXY}	2.5 ± 0.7 ^{abY}	<1.4 ± 1.2 ^{bcXY}	1.9 ± 0.6 ^{bcXY}	2.8 ± 1.0 ^{aY}
48	<0.9 ± 0.9 ^{bXY}	<0.7 ± 0.6 ^{bX}	<0.7 ± 0.6 ^{bX}	1.5 ± 0.9 ^{bXY}	1.7 ± 0.8 ^{bXY}	<0.8 ± 0.7 ^{bcXY}	<1.2 ± 1.2 ^{bcXY}	2.4 ± 1.0 ^{abY}
72	<0.6 ± 0.2 ^{bX}	<0.5 ± 0.4 ^{bX}	<0.8 ± 0.6 ^{bX}	1.3 ± 1.1 ^{bX}	<1.1 ± 0.9 ^{bX}	<0.5 ± 0.4 ^{cX}	<1.1 ± 1.2 ^{bcX}	<2.1 ± 1.3 ^{abX}
96	<0.3 ^{cX}	<0.3 ^{cX}	<0.5 ± 0.4 ^{bX}	<0.5 ± 0.3 ^{bX}	<0.6 ± 0.6 ^{bX}	<0.3 ^{dX}	<0.5 ± 0.4 ^{cX}	<0.5 ± 0.4 ^{bX}

Mean values with different lower case letter in the same column are significantly different (P<0.05). Mean values with different upper case letter in the same row are significantly different (P<0.05).

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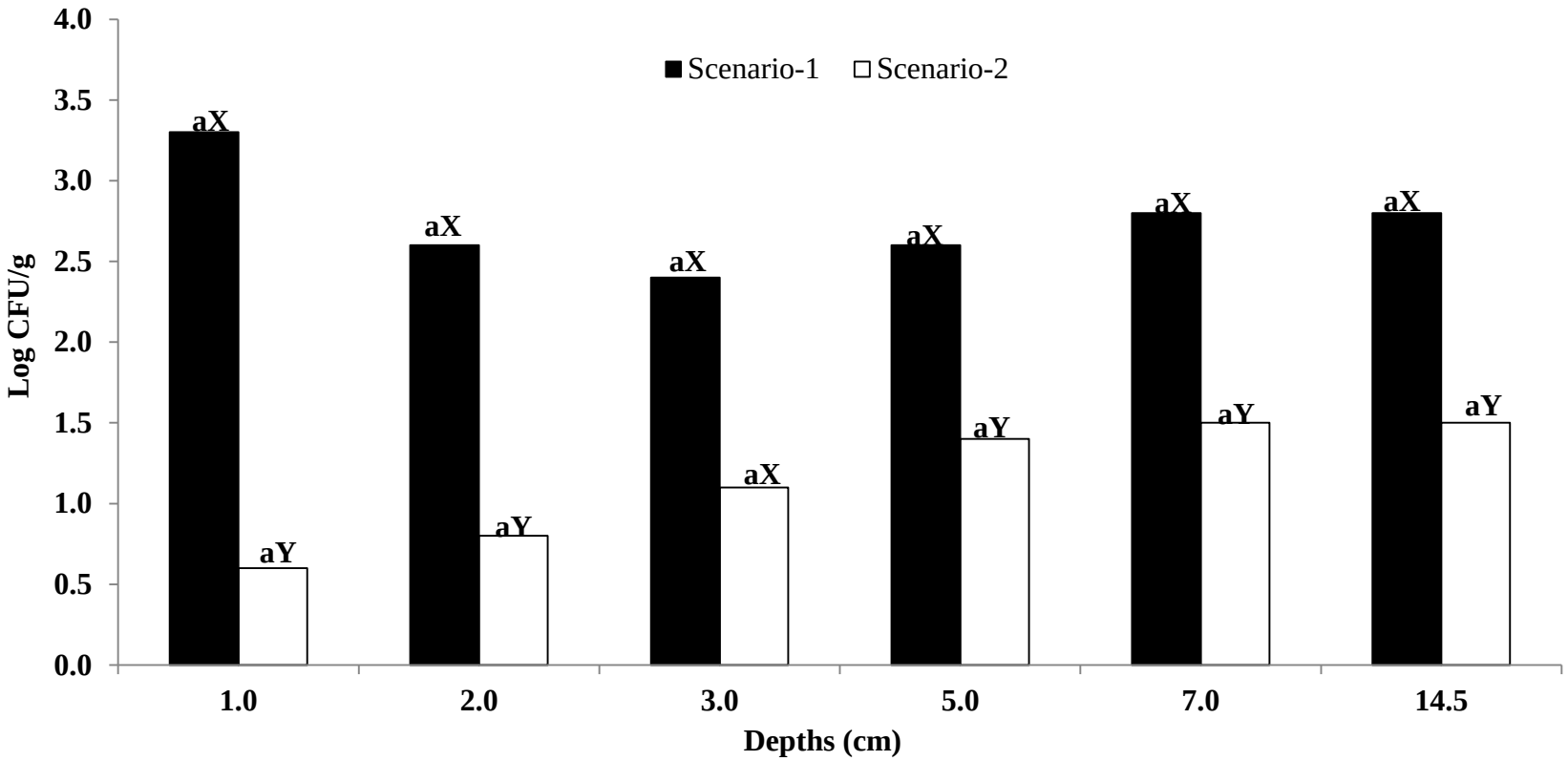
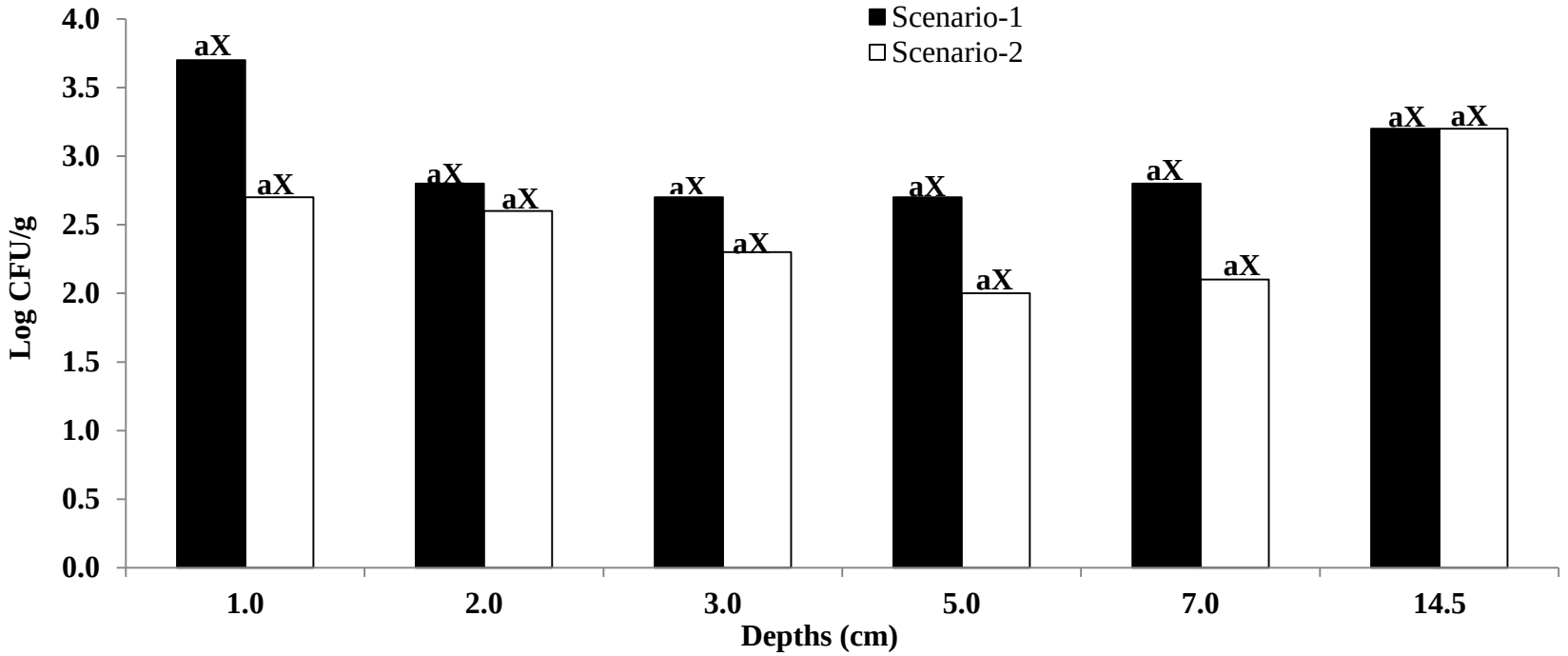
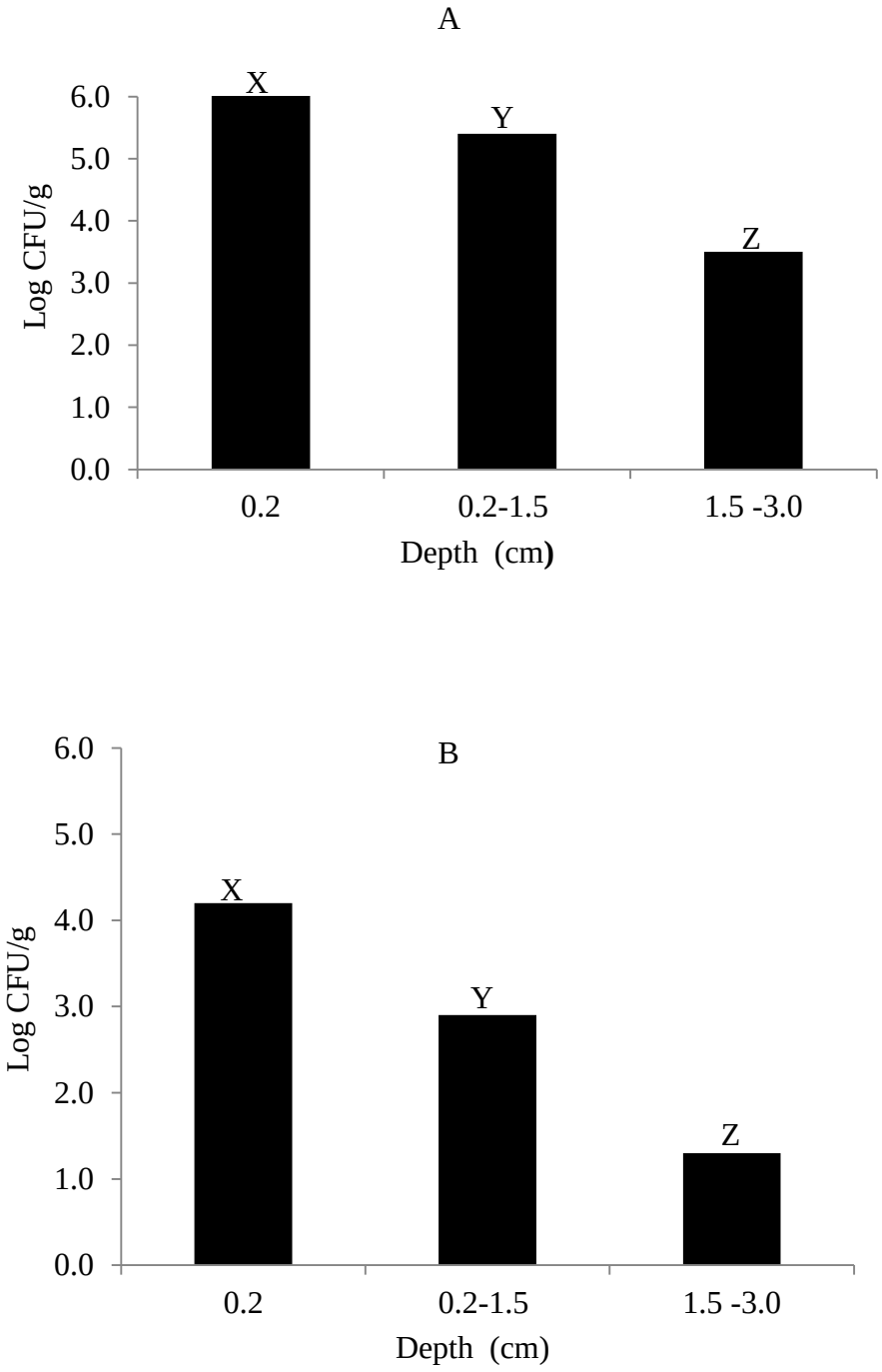
APPENDIX



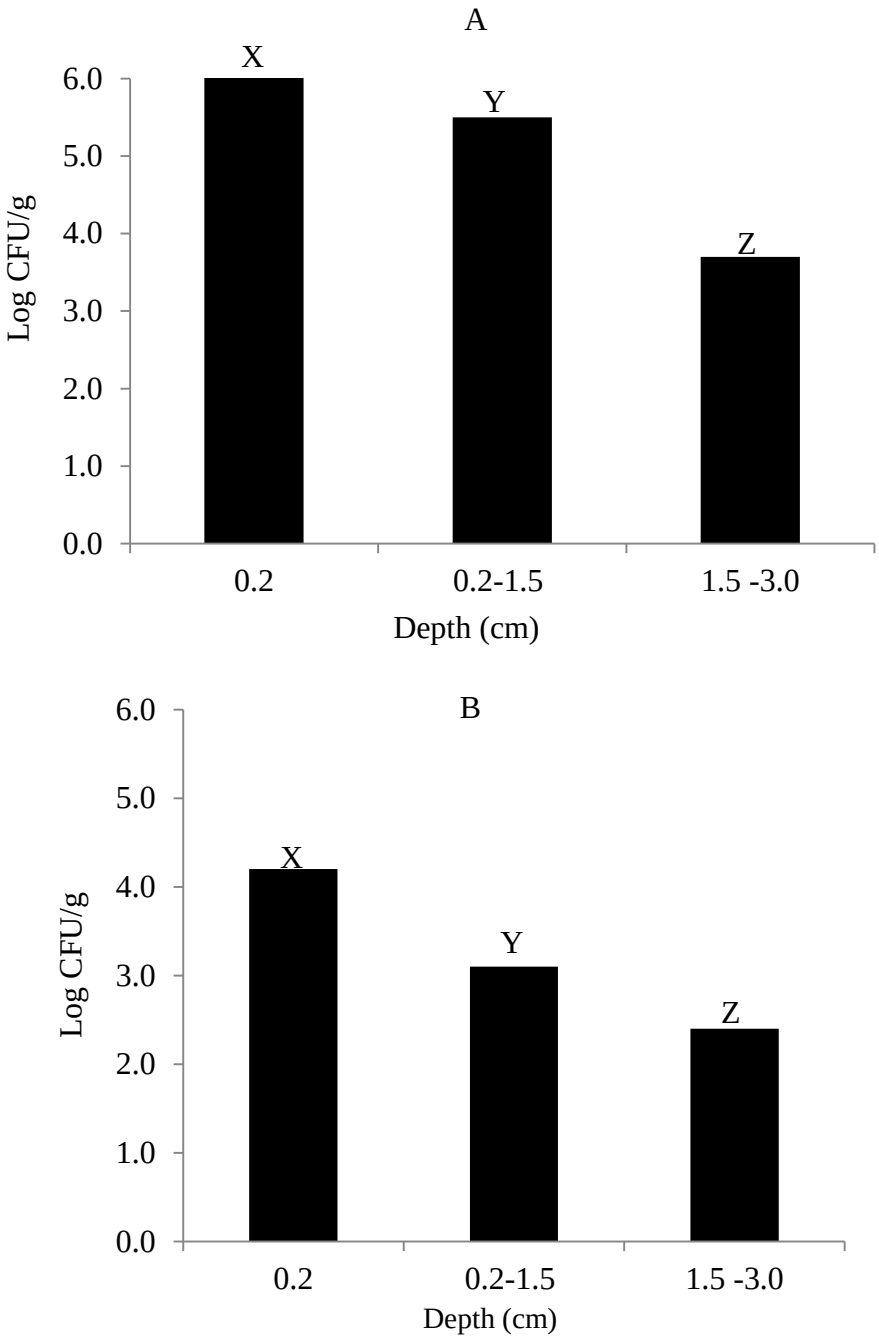
Appendix Figure 1 (Table 3.2). *E. coli* O157:H7 counts (mean±standard deviation; log CFU/g) and percent of inoculated pathogen cells transferred to different depths of knuckles after injection, when pathogen cells inoculated on the knuckle surface (contamination scenario-1) and in solutions, i. e. water or brine solution (Contamination scenario-2). Surface inoculation level: 4.7±0.3 log CFU/g. Water and brine solution inoculation levels: 3.7±0.1 and 3.4±0.2 log CFU/ml, respectively. Mean values for different depths with different lower case letters with in each contamination scenario in figure are different ($P \leq 0.05$). Mean values for both contamination scenarios with different upper case letters within each depth in figure are different ($P \leq 0.05$).



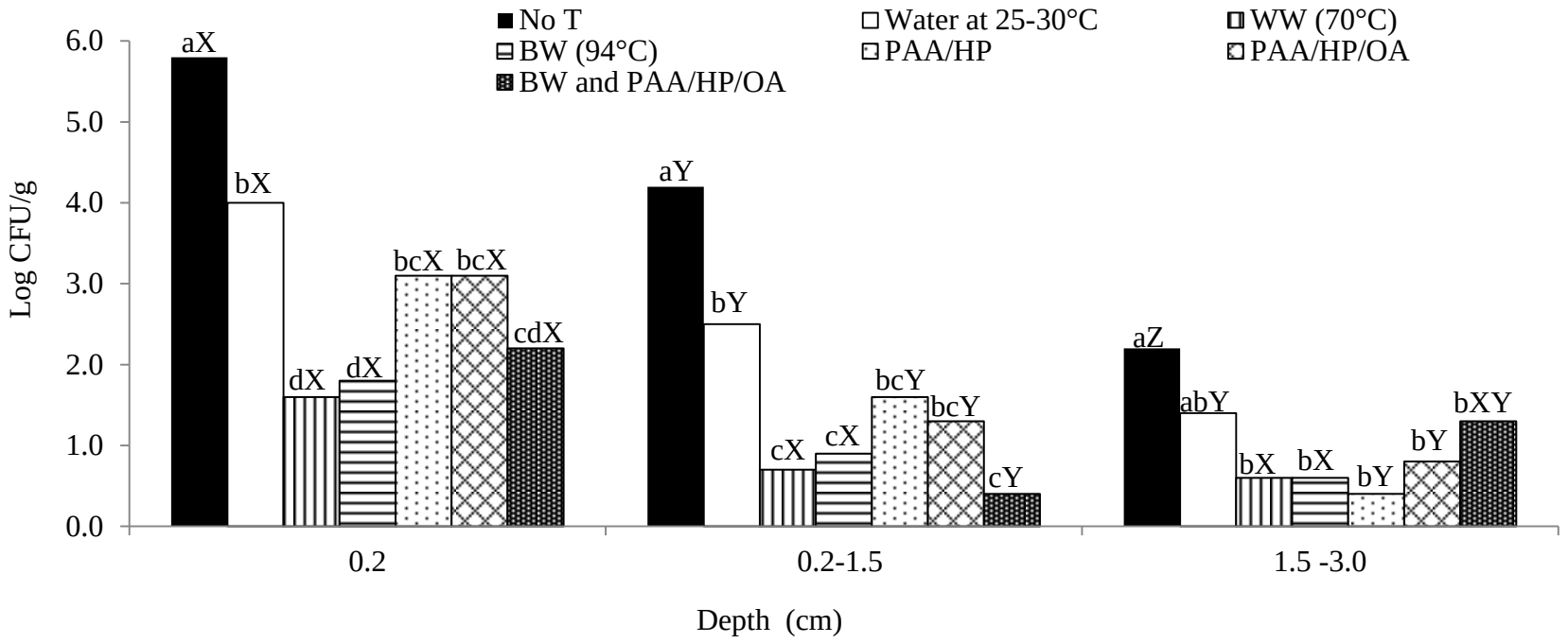
Appendix Figure 2 (Table 3.3). Total bacterial populations (mean±standard deviation; log CFU/g) and percent of inoculated pathogen cells transferred to different depths of knuckles after injection when pathogen cells, inoculated on the knuckle surface (contamination scenario-1) and in enhancement solutions, i. e. water or brine solution (Contamination scenario-2). Surface inoculation level: 4.7±0.3 log CFU/g. Water and brine solution inoculation levels: 3.7±0.1 and 3.4±0.2 log CFU/ml, respectively. Mean values for different depths with different lower case letters with in each contamination scenario in figure are different ($P \leq 0.05$). Mean values for both contamination scenarios with different upper case letters within each depth in figure are different ($P \leq 0.05$).



Appendix Figure 3 (Table 4.1 and 4.2). *E. coli* O157:H7 population vertically translocated to three depths of 3.0 cm thick beef steaks surface inoculated at high (A: 7.0 log CFU/g) and low (B: 4.2 log CFU/g) levels after processing with blade tenderizer. Mean values with different letters within a figure are different ($P<0.05$).

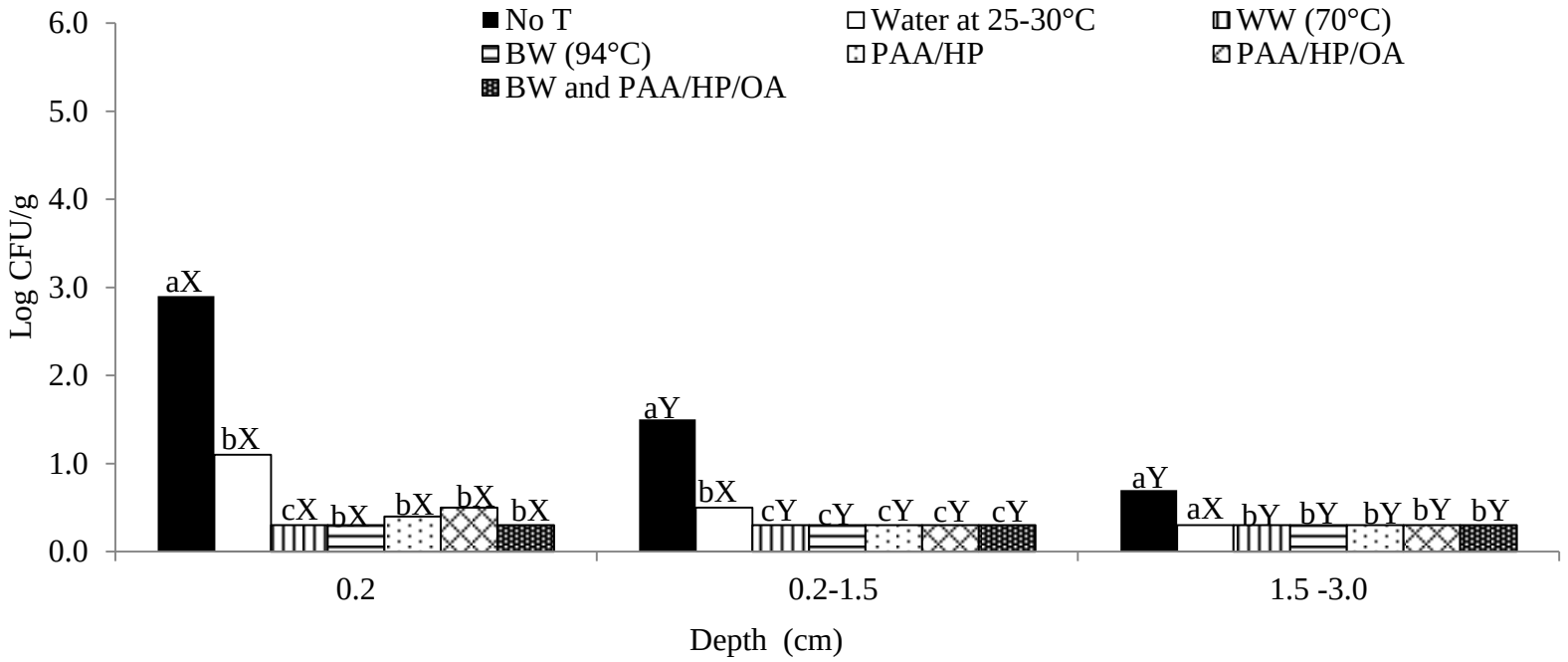


Appendix Figure 4 (Table 4.3 and 4.4). Total bacterial population vertically translocated to three depths of 3.0 cm thick beef steaks surface inoculated at high (A: 7.0 log CFU/g) and low (B: 4.2 log CFU/g) levels after processing with blade tenderizer. Mean values with different letters within a figure are different ($P<0.05$).

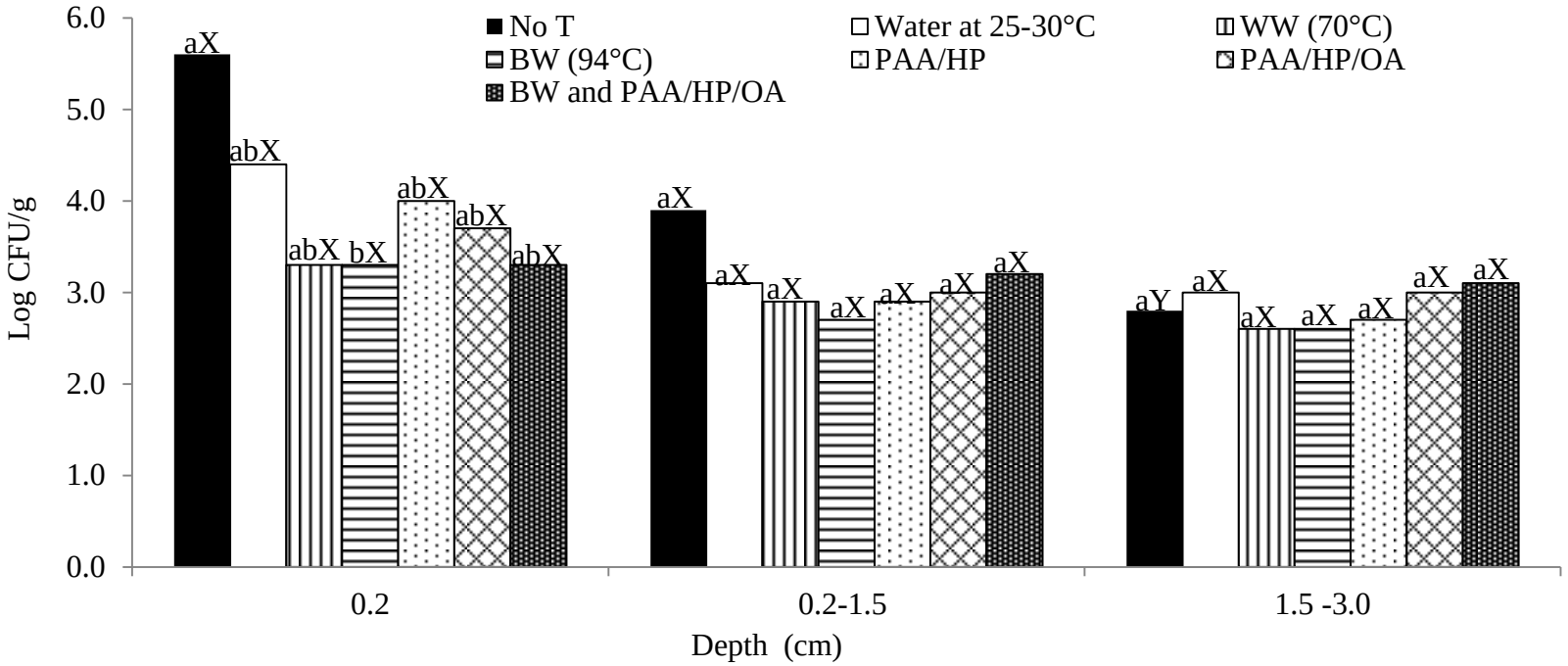


Appendix Figure 5 (Table 4.1). *E. coli* O157:H7 population (high contamination level: 7.0 log CFU/g) horizontally transferred to three depths of an additional 3.0 cm thick beef steaks after processing with blade tenderizer that was previously sanitized or unsanitized (control). Exposure time of blades tenderizer for each sanitation treatment was 30 sec. Mean values within specific sanitation treatment for different beef steak depths not followed by same upper case letter within a figure are significantly different ($P < 0.05$) from each other. Mean values within each specific steak depth for different sanitation treatments not followed by same lower case letter within a figure are significantly different ($P < 0.05$) from each other.

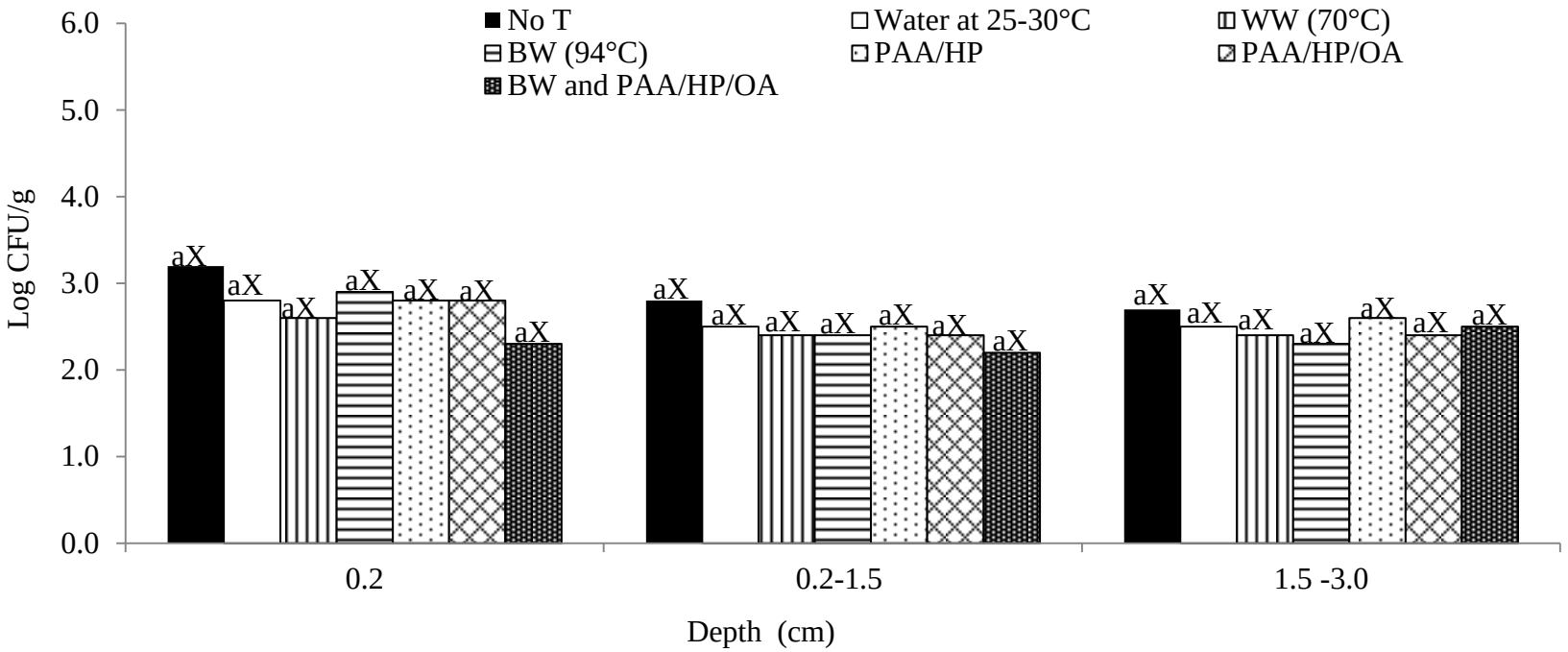
No T = no treatment (unsanitized blades); WW = warm water, water at 70°C; BW = boiling water, water at 94°C; PAA = peroxyacetic acid; HP = hydrogen peroxide; OC = octanic acid; PAA/HP (Oxonia Active®) and PAA/HP/OA (Vortexx™) = 2500 ppm. Detection limit to detect *E. coli* O157:H7 at different depths of tenderized beef steak was below 0.3 log CFU/g.



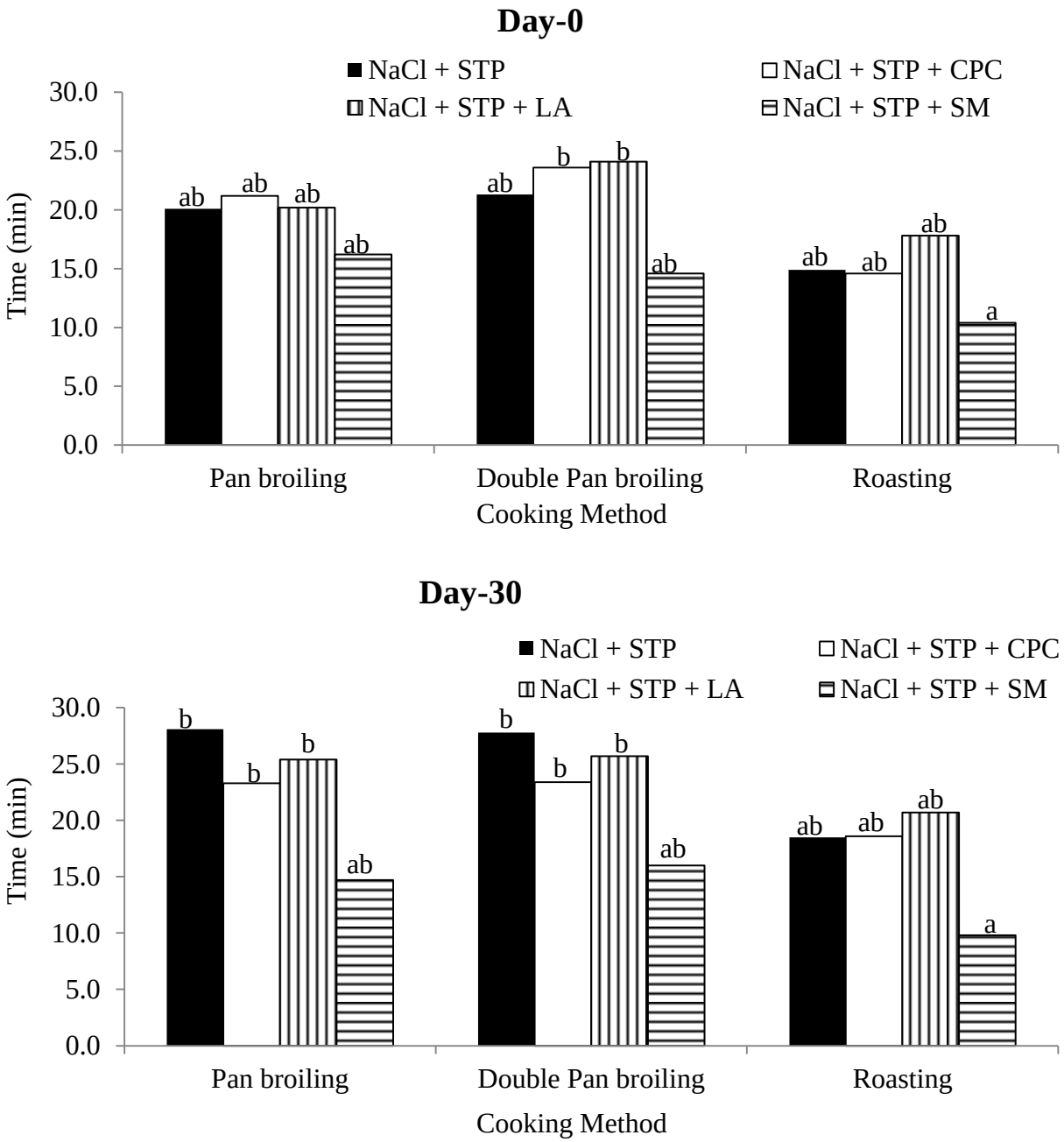
Appendix Figure 6 (Table 4.2). *E. coli* O157:H7 population (low contamination level: 4.2 log CFU/g) horizontally transferred to three depths of an additional 3.0 cm thick beef steaks after processing with blade tenderizer that was previously sanitized or unsanitized (control). Exposure time of blades tenderizer for each sanitation treatment was 30 sec. Mean values within specific sanitation treatment for different beef steak depths, not followed by same upper case letter within a figure are significantly different ($P < 0.05$) from each other. Mean values within each specific steak depth for different sanitation treatments, not followed by same lower case letter within a figure are significantly different ($P < 0.05$) from each other.
No T = no treatment (unsanitized blades); WW = warm water, water at 70°C; BW = boiling water, water at 94°C; PAA = peroxyacetic acid; HP = hydrogen peroxide; OC = octanic acid; PAA/HP (Oxonia Active®) and PAA/HP/OA (Vortexx™) = 2500 ppm. Detection limit to detect *E. coli* O157:H7 at different depths of tenderized beef steak was below 0.3 log CFU/g.



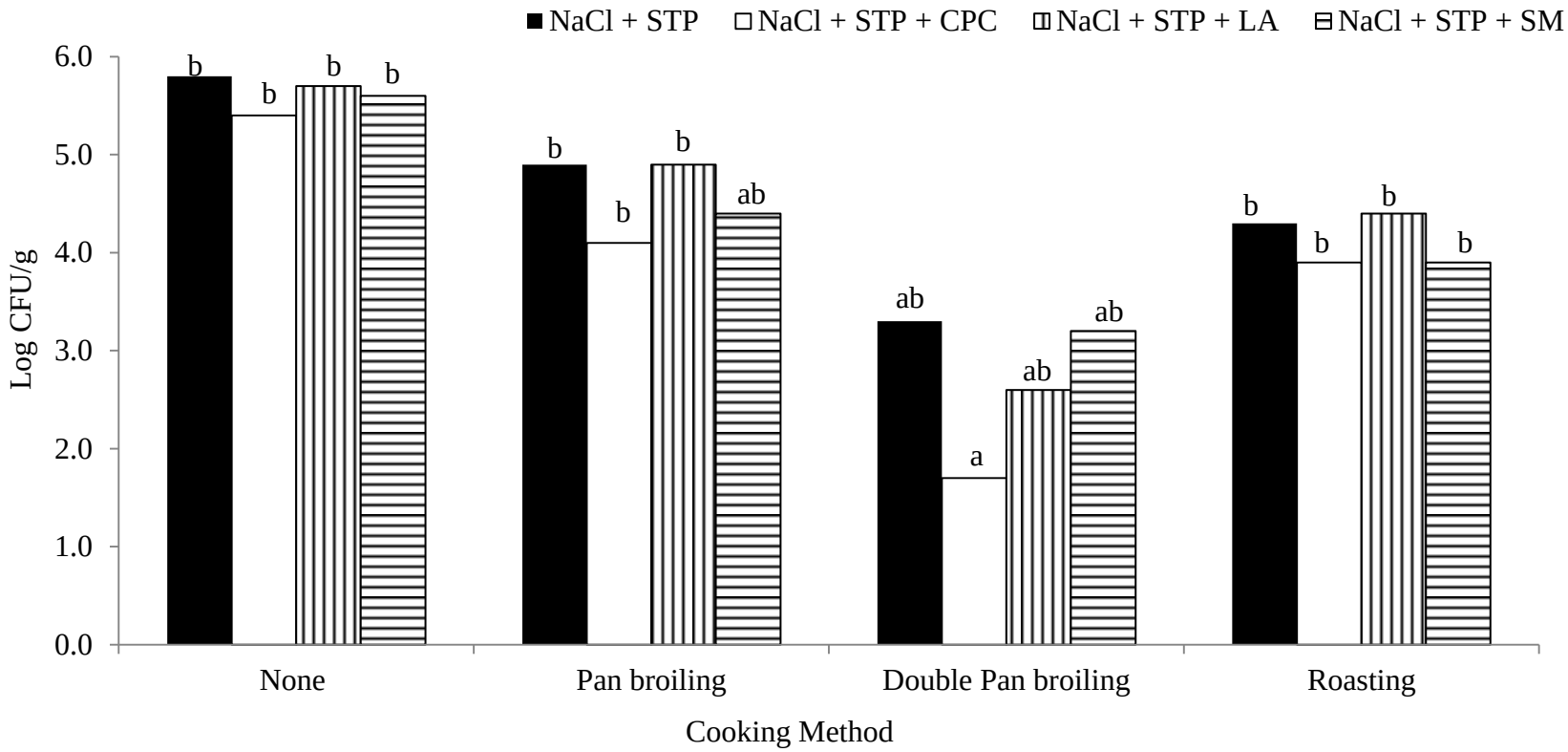
Appendix Figure 7 (Table 4.3). Total bacterial population (high contamination level: 7.0 log CFU/g) horizontally transferred to three depths of an additional 3.0 cm thick beef steaks after processing with blade tenderizer that was previously sanitized or unsanitized (control). Exposure time of blades tenderizer for each sanitation treatment was 30 sec. Mean values within specific sanitation treatment for different beef steak depths, not followed by same upper case letter within a figure are significantly different ($P < 0.05$) from each other. Mean values within each specific steak depth for different sanitation treatments, not followed by same lower case letter within a figure are significantly different ($P < 0.05$) from each other. No T = no treatment (unsanitized blades); WW = warm water, water at 70°C; BW = boiling water, water at 94°C; PAA = peroxyacetic acid; HP = hydrogen peroxide; OC = octanic acid; PAA/HP (Oxonia Active®) and PAA/HP/OA (Vortexx™) = 2500 ppm. Detection limit to detect total bacterial population at different depths of tenderized beef steak was below 0.3 log CFU/g.



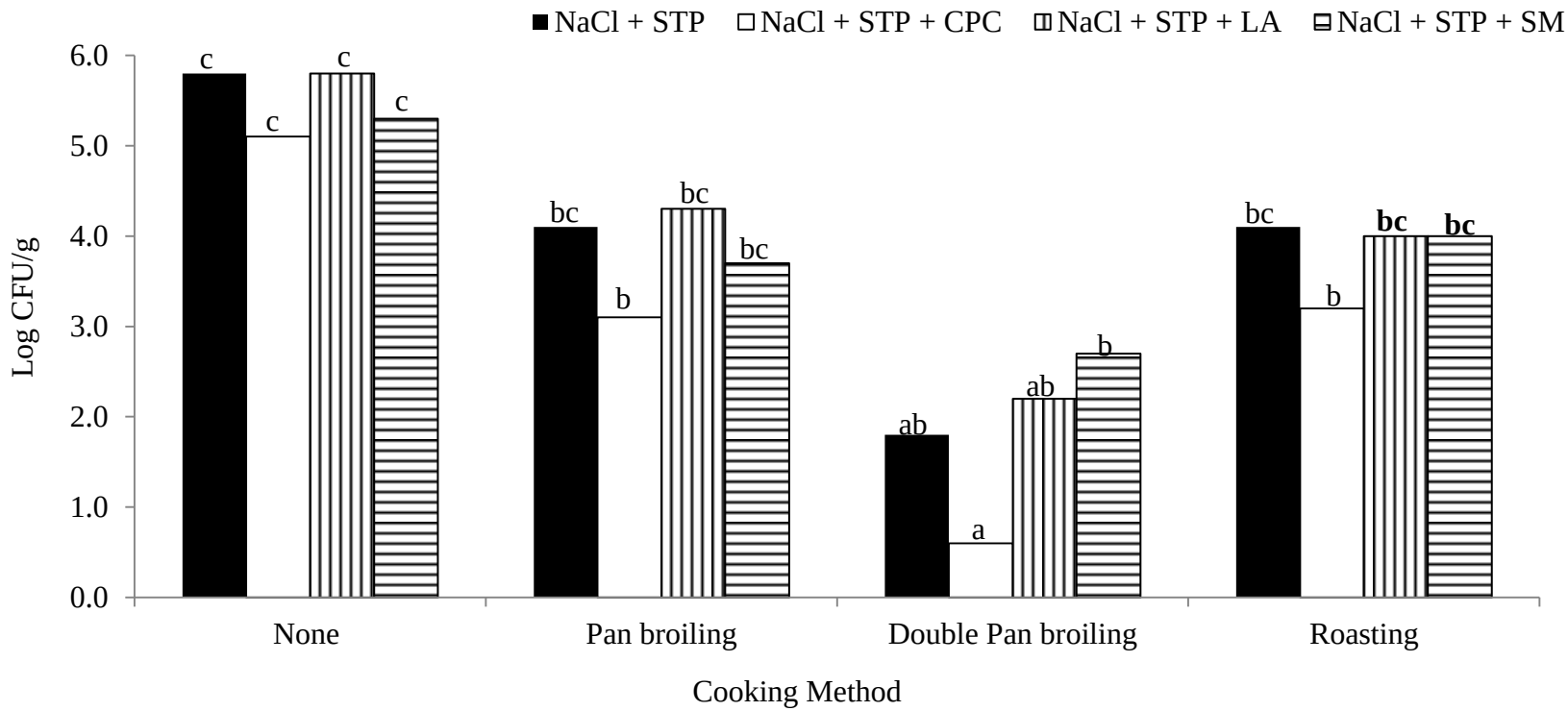
Appendix Figure 8 (Table 4.4). Total bacterial population (low contamination level: 4.2 log CFU/g) horizontally transferred to three depths of an additional 3.0 cm thick beef steaks after processing with blade tenderizer that was previously sanitized or unsanitized (control). Exposure time of blades tenderizer for each sanitation treatment was 30 sec. Mean values within specific sanitation treatment for different beef steak depths, not followed by same upper case letter within a figure are significantly different ($P < 0.05$) from each other. Mean values within each specific steak depth for different sanitation treatments, not followed by same lower case letter within a figure are significantly different ($P < 0.05$) from each other.No T = no treatment (unsanitized blades); WW = warm water, water at 70°C; BW = boiling water, water at 94°C; PAA = peroxyacetic acid; HP = hydrogen peroxide; OC = octanic acid; PAA/HP (Oxonia Active®) and PAA/HP/OA (Vortexx™) = 2500 ppm. Detection limit to detect total bacterial population at different depths of tenderized beef steak was below 0.3 log CFU/g.



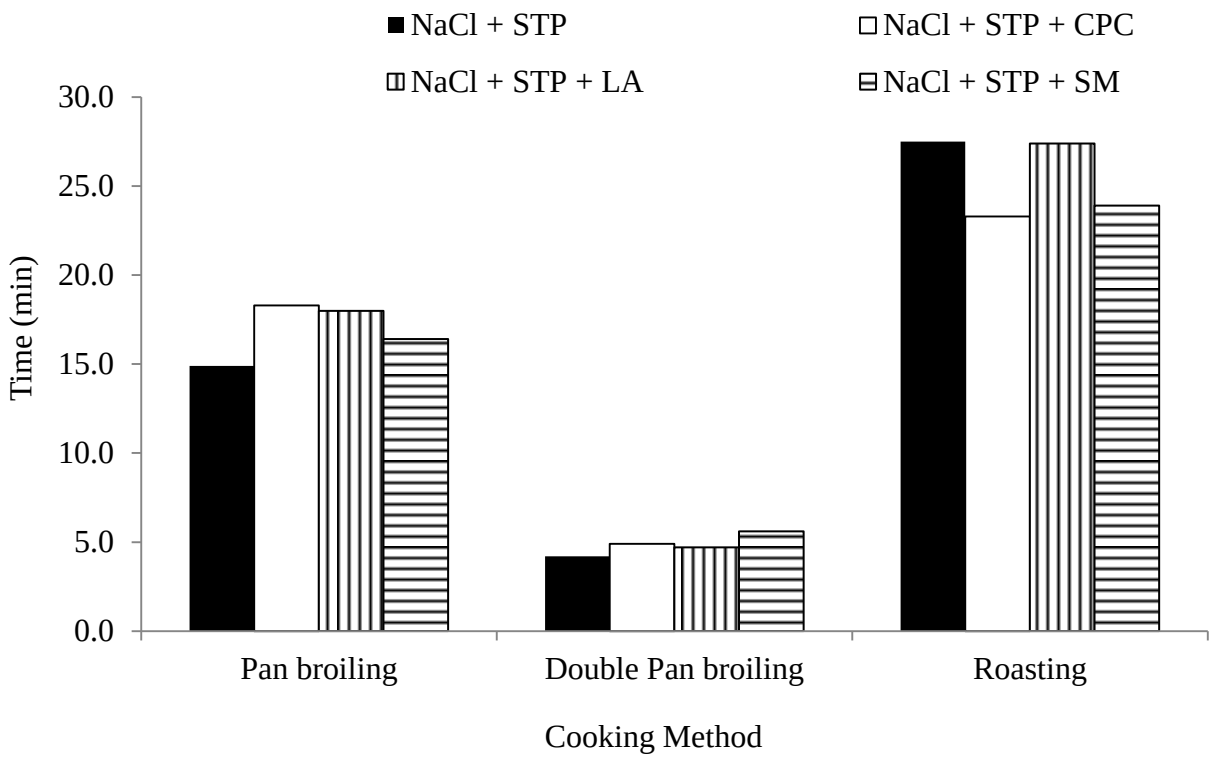
Appendix Figure 9 (Table 5.4). Cooking losses (mean ± standard deviation) of uncooked/cooked beef steaks (2.5-cm thickness), moisture-enhanced with four brining formulations, on day-0 and day-30 after frozen (-20°C) storage when cooked with three methods to 60°C. Mean values with different letters within a figure are different ($P<0.05$).
NaCl = Sodium Chloride, 0.5%; STP = Sodium Tripolyphosphate, 0.25%; CPC = Cetylpyridinium Chloride, 0.2%; LA = Lactic Acid, 0.3%, SM = Sodium Metasilicate, 0.2%.



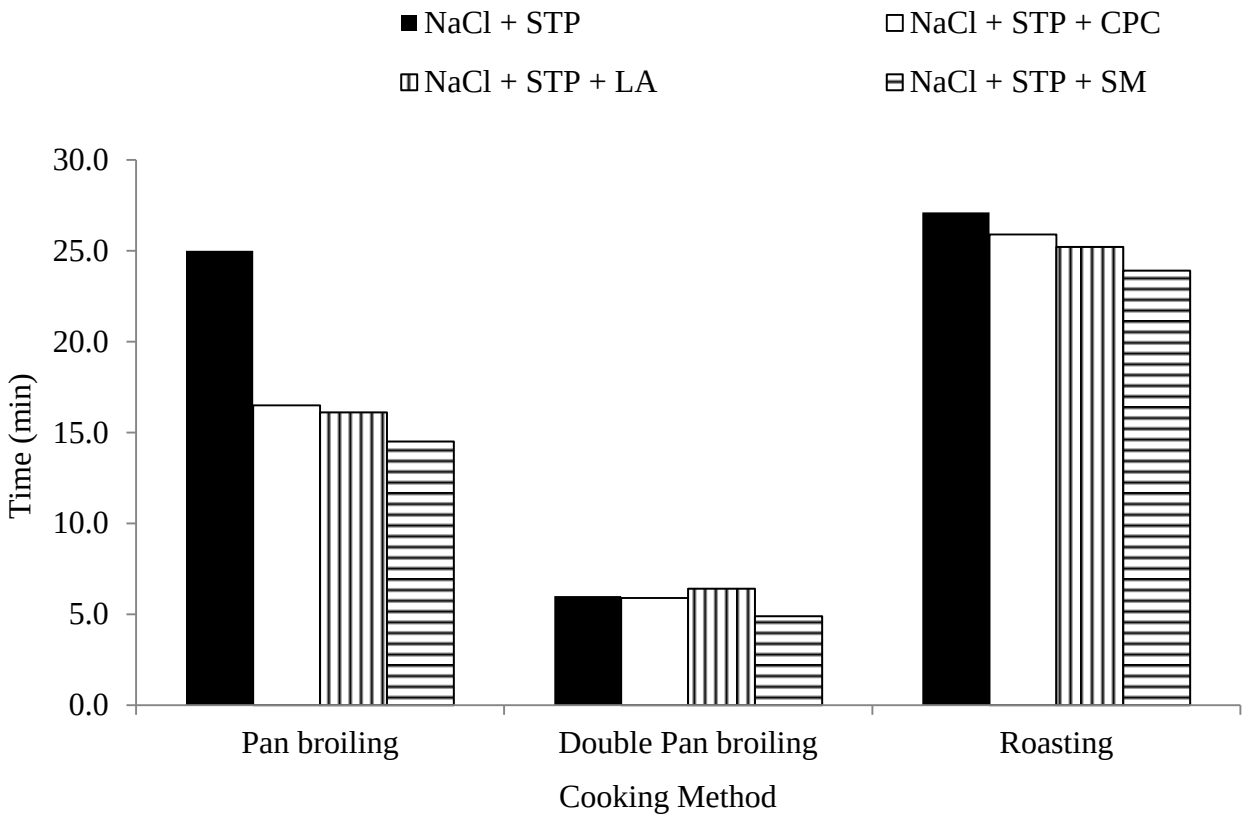
Appendix Figure 10 (Table 5.5). *E. coli* O157:H7 population (log CFU/g; mean ± standard deviation) in uncooked/cooked beef steaks (2.5-cm thickness), moisture-enhanced with four brining formulations on day-0 after cooking with three methods to 60°C. Mean values with different letters within a figure are different ($P<0.05$).
NaCl = Sodium Chloride, 0.5%; STP = Sodium Tripolyphosphate, 0.25%; CPC = Cetylpyridinium Chloride, 0.2%; LA = Lactic Acid, 0.3%, SM = Sodium Metasilicate, 0.2%.



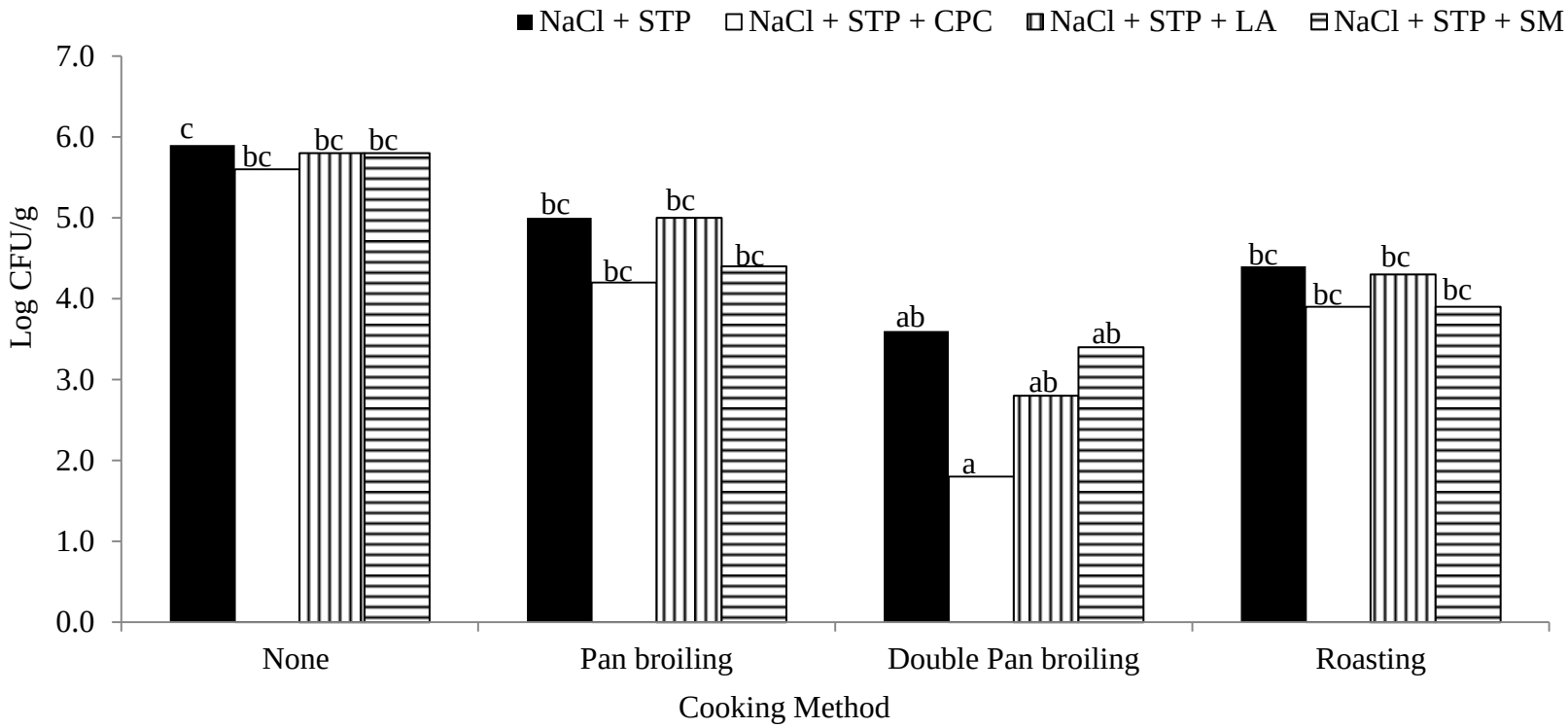
Appendix Figure 11 (Table 5.5). *E. coli* O157:H7 population (log CFU/g; mean ± standard deviation) in uncooked/cooked beef steaks (2.5-cm thickness), moisture-enhanced with four brining formulations, after frozen (-20°C) storage for 30 days and after cooking with three methods to 60°C. Mean values with different letters within a figure are different ($P<0.05$).
NaCl = Sodium Chloride, 0.5%; STP = Sodium Tripolyphosphate, 0.25%; CPC = Cetylpyridinium Chloride, 0.2%; LA = Lactic Acid, 0.3%, SM = Sodium Metasilicate, 0.2%.



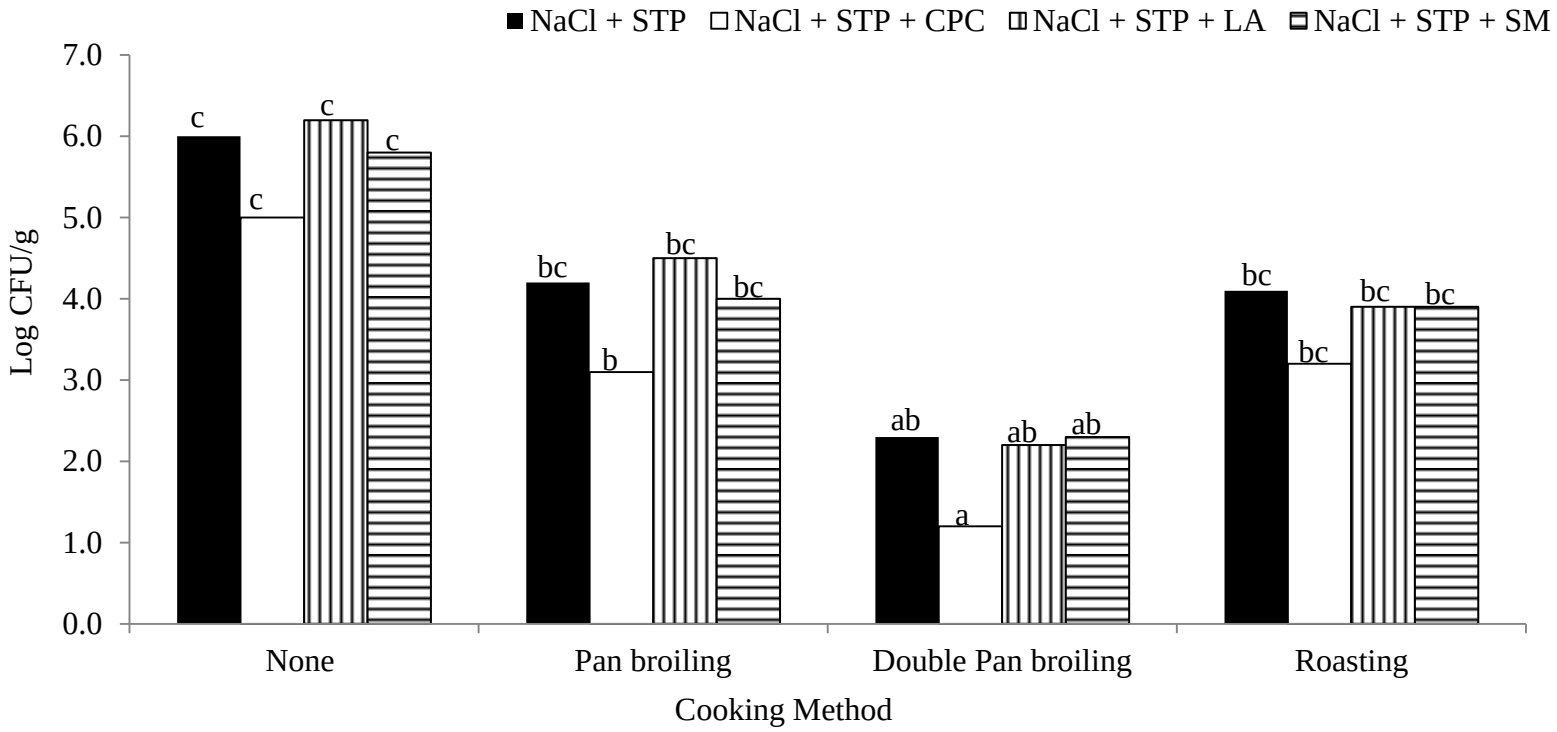
Appendix Figure 12 (Table 5.6). Cooking times (mean ± standard deviation) for uncooked/cooked beef steaks (2.5-cm thickness), moisture-enhanced with four brining formulations, on day-0 after cooking with three methods to 60°C.
NaCl = Sodium Chloride, 0.5%; STP = Sodium Tripolyphosphate, 0.25%; CPC = Cetylpyridinium Chloride, 0.2%; LA = Lactic Acid, 0.3%, SM = Sodium Metasilicate, 0.2%.



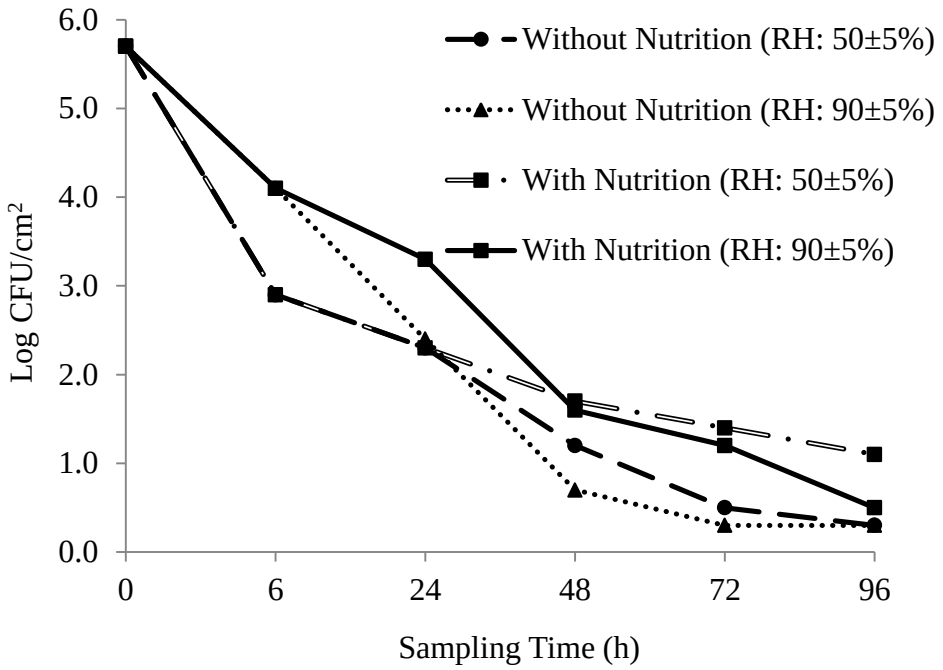
Appendix Figure 13 (Table 5.6). Cooking times (mean ± standard deviation) for uncooked/cooked beef steaks (2.5-cm thickness), moisture-enhanced with four brining formulations, on day-30 after cooking with three methods to 60°C.
NaCl = Sodium Chloride, 0.5%; STP = Sodium Tripolyphosphate, 0.25%; CPC = Cetylpyridinium Chloride, 0.2%; LA = Lactic Acid, 0.3%, SM = Sodium Metasilicate, 0.2%.



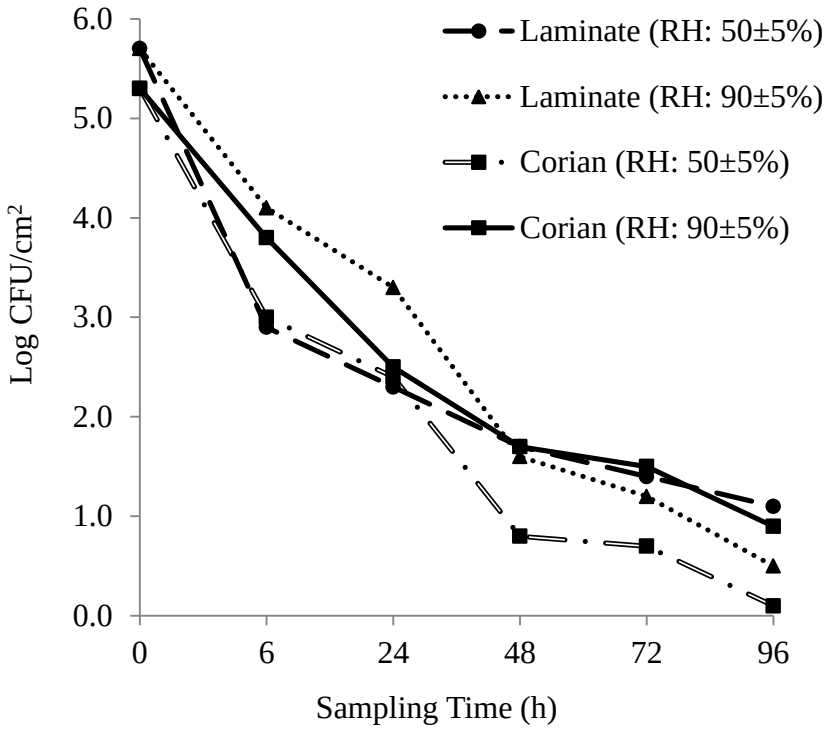
Appendix Figure 14 (Table 5.7). Total bacterial population (log CFU/g; mean ± standard deviation) of uncooked/cooked beef steaks (2.5-cm thickness), moisture-enhanced with four brining formulations, on day-0 after cooking with three methods to 60°C. Mean values with different letters within a figure are different ($P<0.05$).
NaCl = Sodium Chloride, 0.5%; STP = Sodium Tripolyphosphate, 0.25%; CPC = Cetylpyridinium Chloride, 0.2%; LA = Lactic Acid, 0.3%, SM = Sodium Metasilicate, 0.2%.



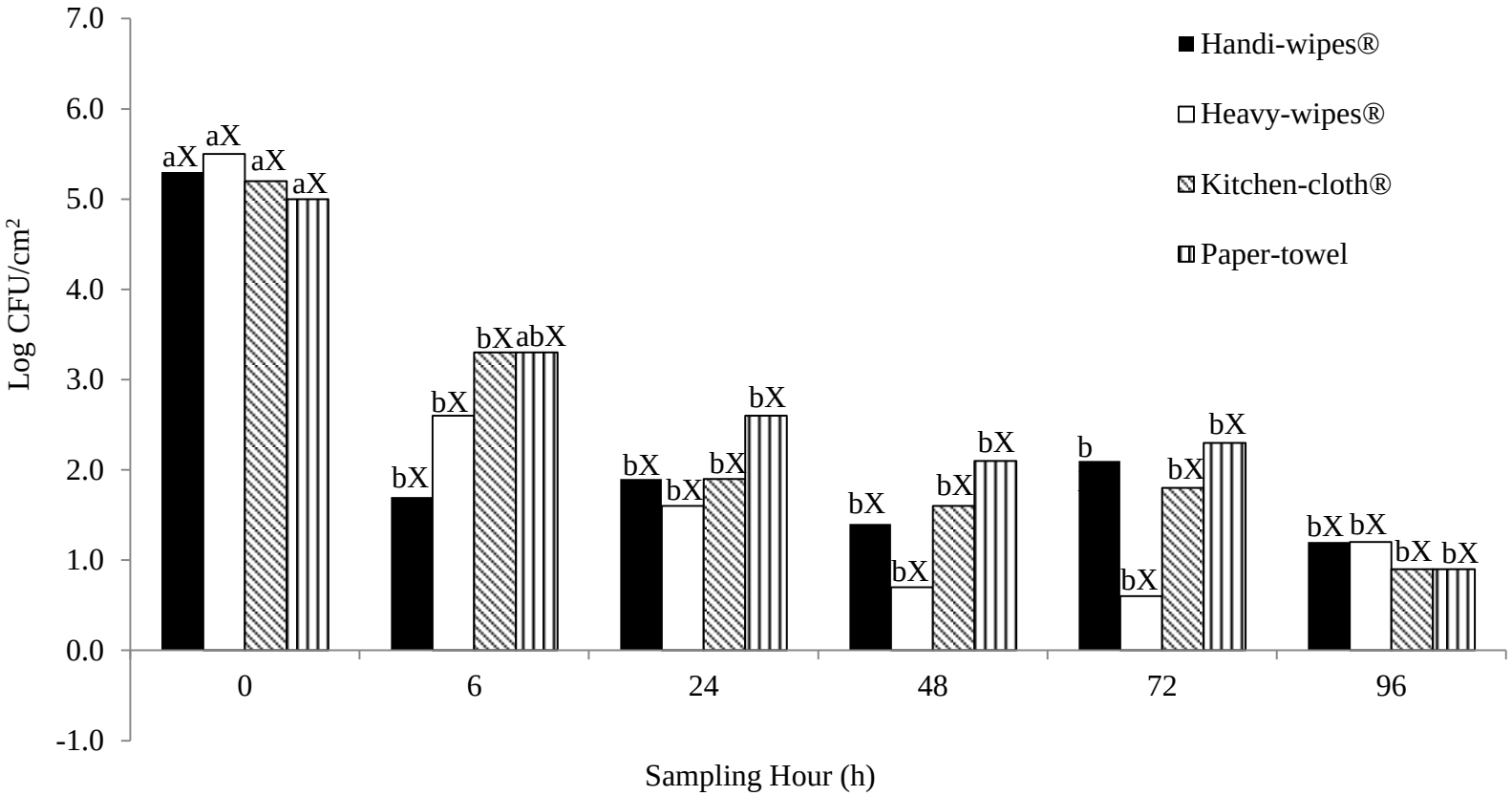
Appendix Figure 15 (Table 5.7). Total bacterial population (log CFU/g; mean ± standard deviation) of uncooked/cooked beef steaks (2.5-cm thickness), moisture-enhanced with four brining formulations, after frozen (-20°C) storage for 30 days and after cooking with three methods to 60°C. Mean values with different letters within a figure are different ($P<0.05$).
NaCl = Sodium Chloride, 0.5%; STP = Sodium Tripolyphosphate, 0.25%; CPC = Cetylpyridinium Chloride, 0.2%; LA = Lactic Acid, 0.3%, SM = Sodium Metasilicate, 0.2%.



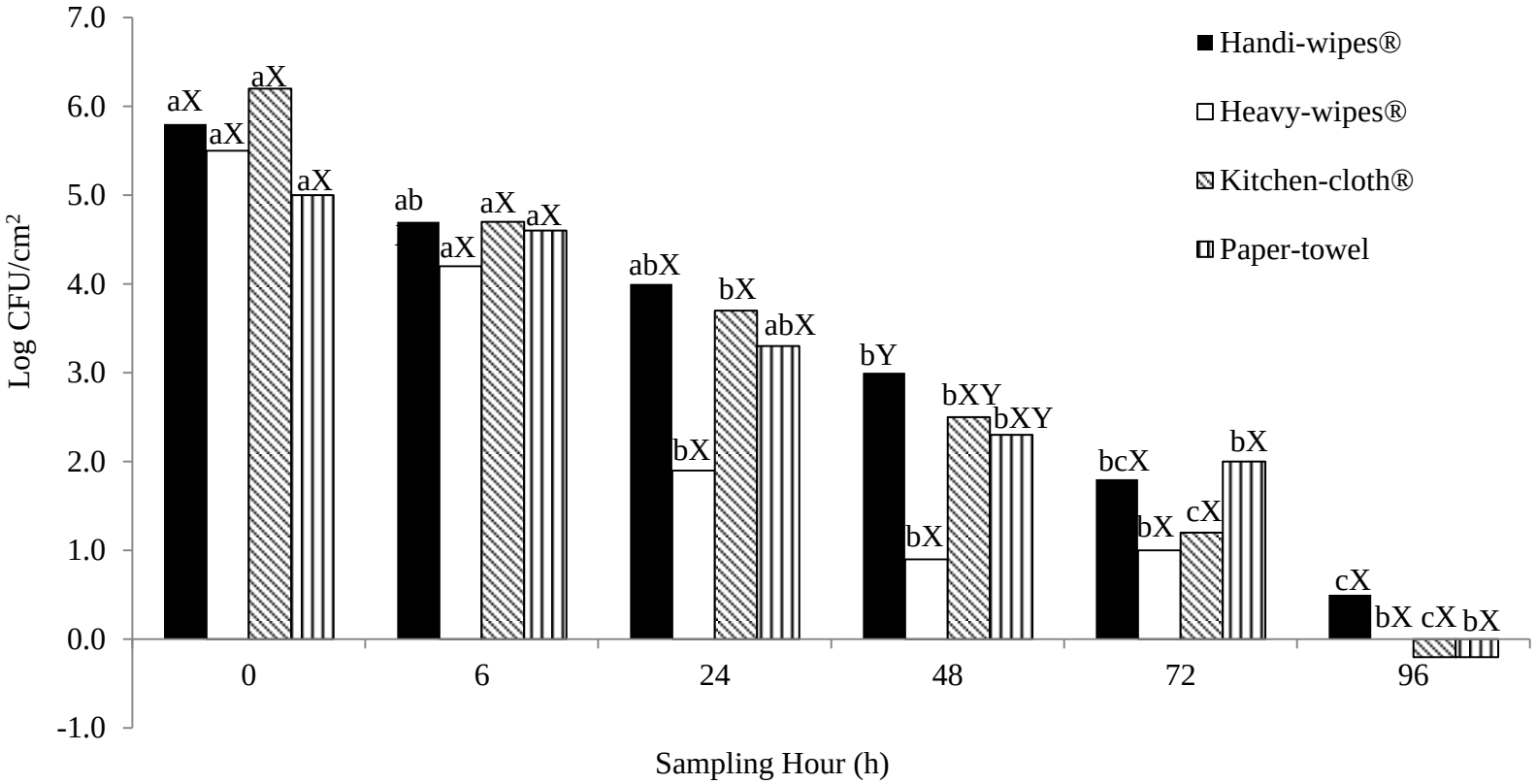
Appendix Figure 16 (Table 7.1). *L. monocytogenes* (log CFU/cm^2; mean ± standard deviation) recovered from laminate kitchen countertop surfaces, without and with nutrient supplementation, incubated at 50±5% and 90±5% relative humidity for 96 h at 25±2°C.



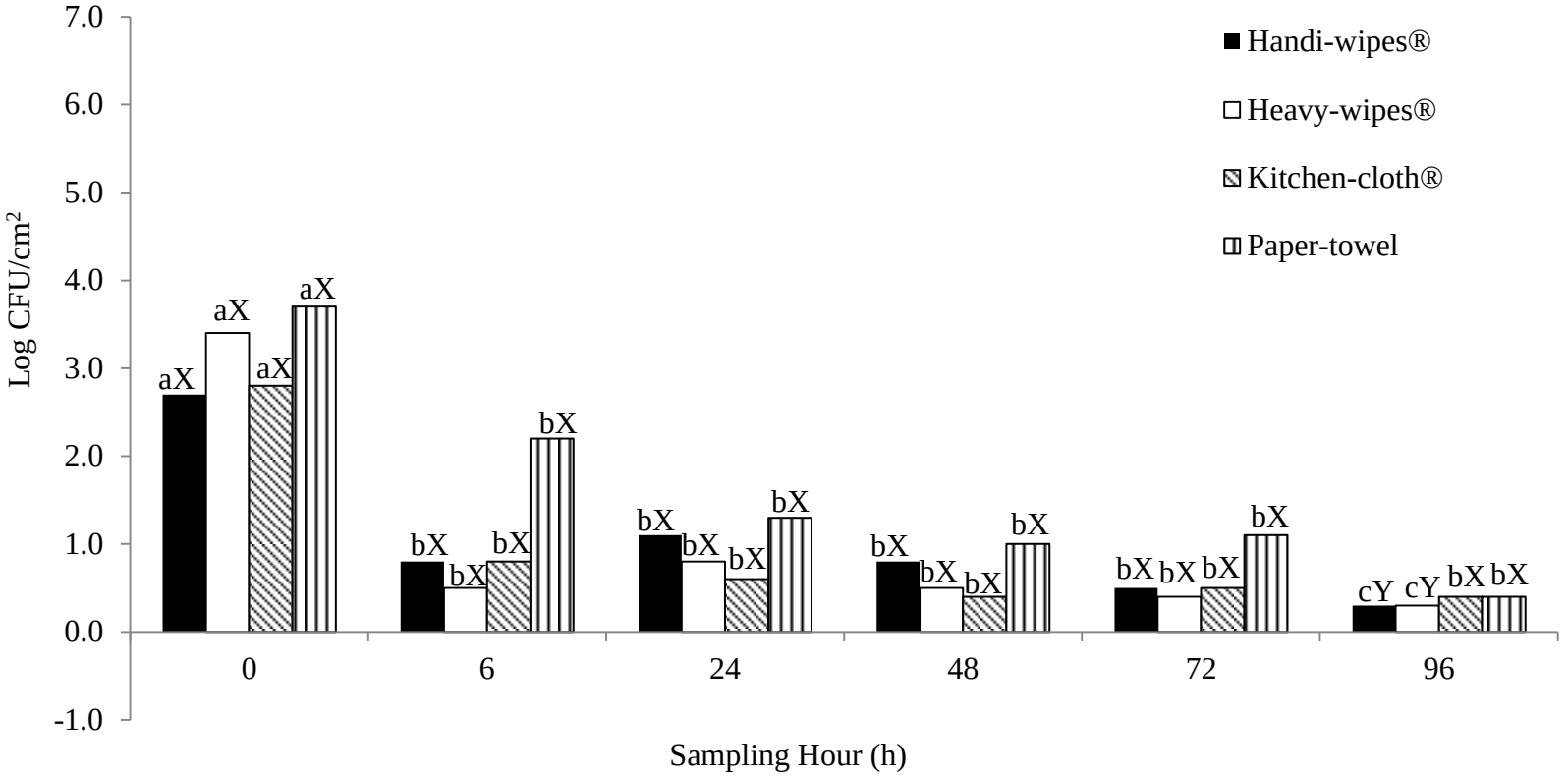
Appendix Figure 17 (Table 7.2). *L. monocytogenes* (log CFU/cm^2; mean ± standard deviation) recovered from laminate and corian kitchen countertop surfaces, incubated at 50±5% and 90±5% relative humidity levels for 96 h at 25±2°C with nutrient supplementation.



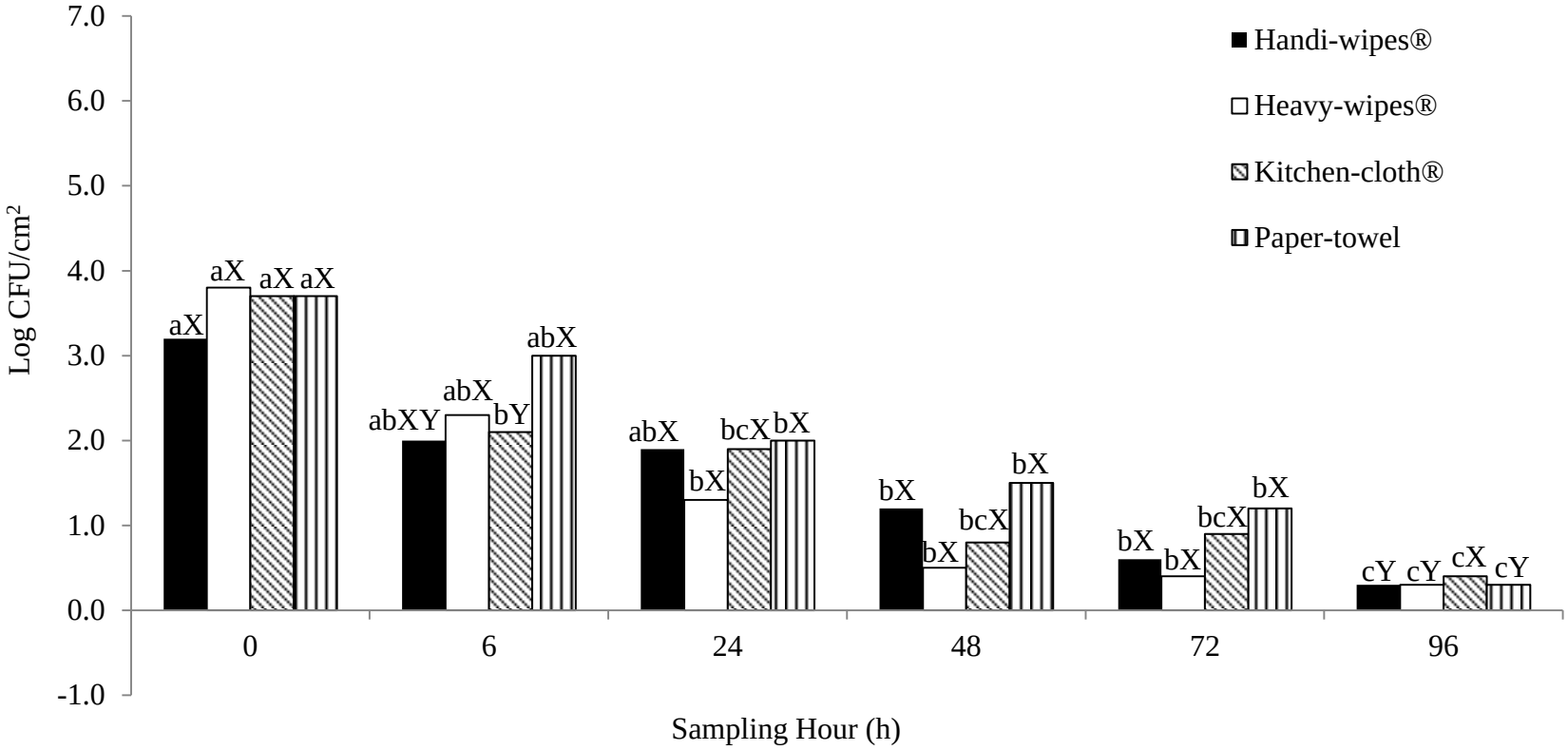
Appendix Figure 18 (Table 7.3). *L. monocytogenes* (log CFU/cm^2; mean ± standard deviation) removed by each wiping material after cleaning laminate coupon surfaces incubated at 50±5% relative humidity level for 96 h at 25±2°C with nutrient supplementation. Mean values not followed by same upper and lower case letter within a figure are significantly different ($P < 0.05$) from each other.



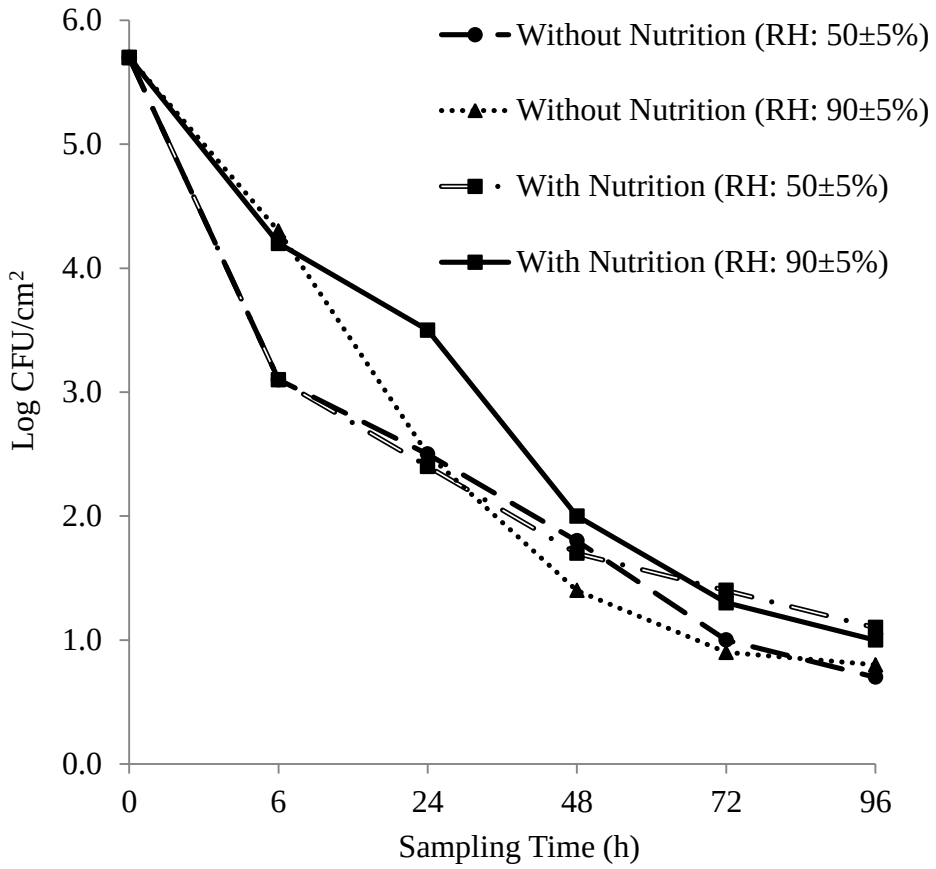
Appendix Figure 19 (Table 7.3). *L. monocytogenes* (log CFU/cm^2; mean ± standard deviation) removed by each wiping material after cleaning laminate coupon surfaces incubated at 90±5% relative humidity level for 96 h at 25±2°C with nutrient supplementation. Mean values not followed by same upper and lower case letter within a figure are significantly different ($P < 0.05$) from each other.



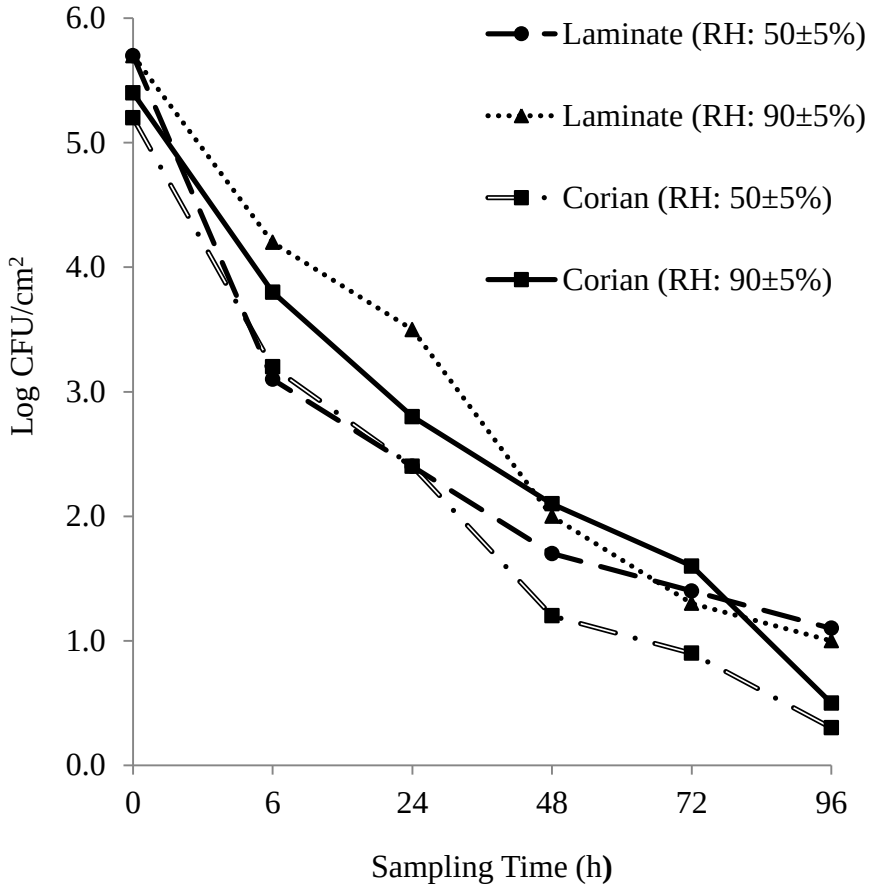
Appendix Figure 20 (Table 7.4). *L. monocytogenes* (log CFU/cm^2; mean ± standard deviation) recovered from laminate coupon surfaces after cleaning with each wiping material, incubated at 50±5% relative humidity level for 96 h at 25±2°C with nutrient supplementation. Mean values not followed by same upper and lower case letter within a figure are significantly different ($P < 0.05$) from each other.



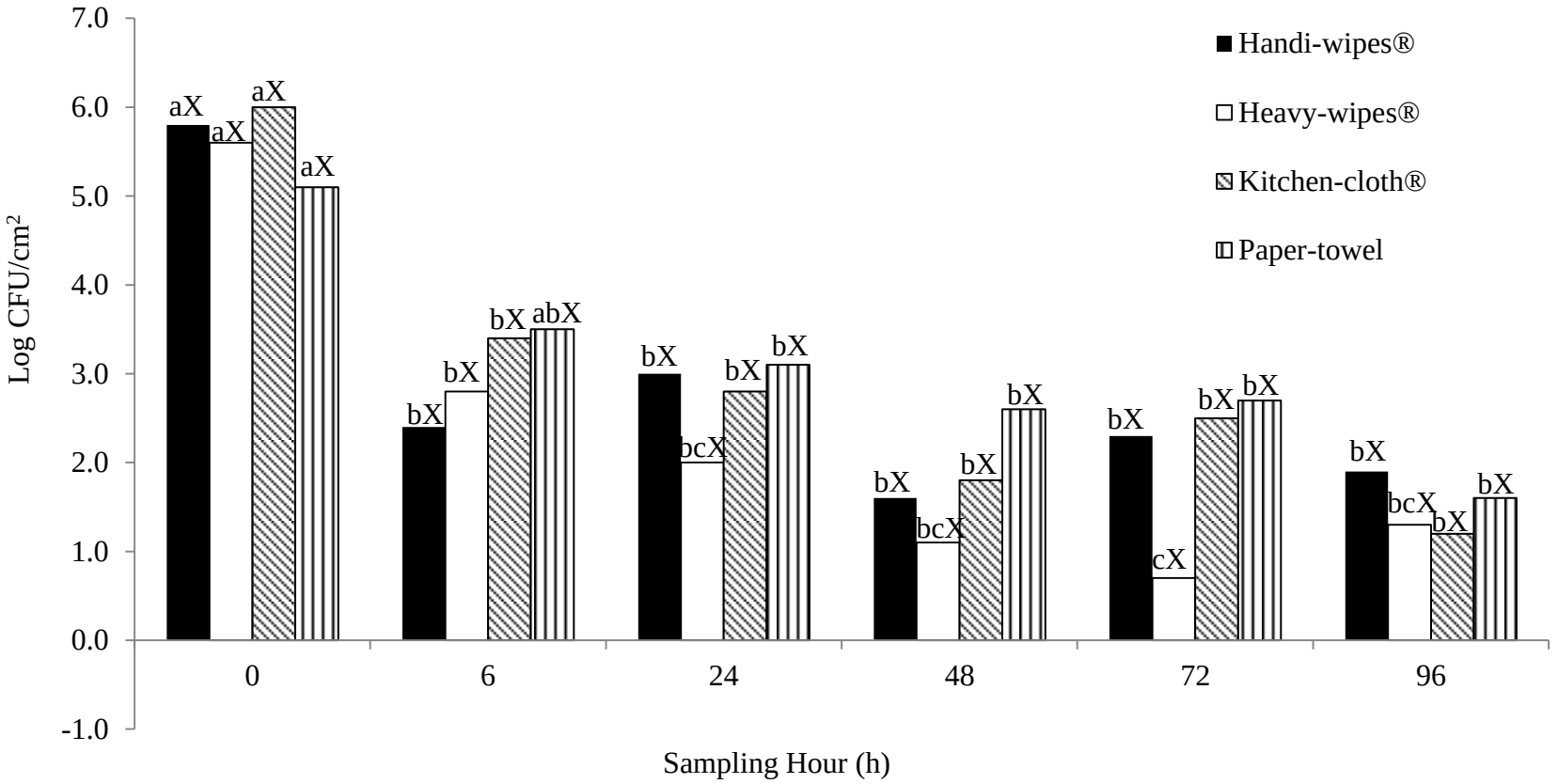
Appendix Figure 21 (Table 7.4). *L. monocytogenes* (log CFU/cm^2; mean ± standard deviation) recovered from laminate coupon surfaces after cleaning surfaces with each wiping material, incubated at 90±5% relative humidity level for 96 h at 25±2°C with nutrient supplementation. Mean values not followed by same upper and lower case letter within a figure are significantly different ($P < 0.05$) from each other.



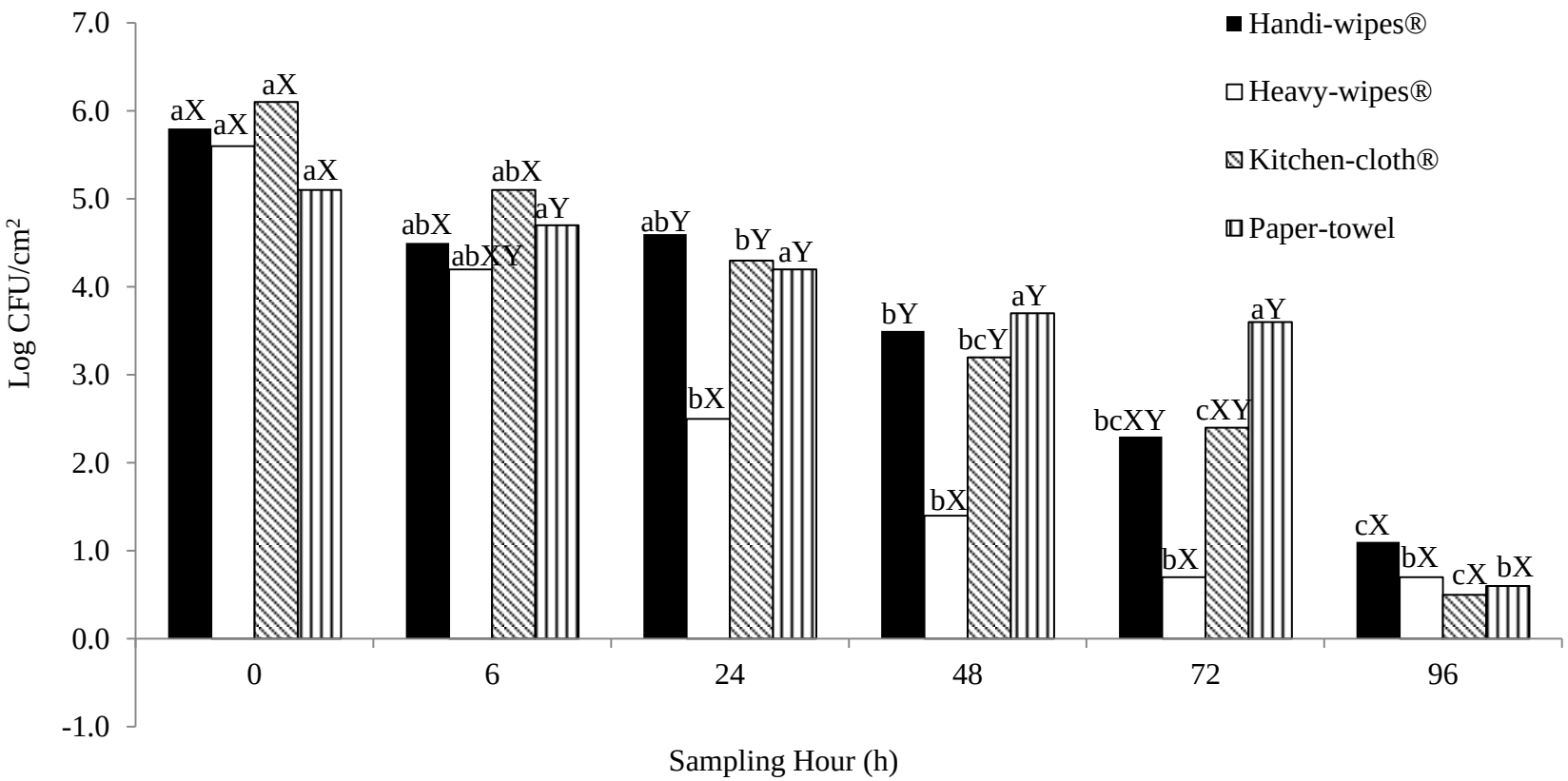
Appendix Figure 22 (Table 7.5). Total bacterial population (log CFU/cm^2; mean ± standard deviation) recovered from laminate kitchen countertop surfaces, without and with nutrient supplementation, incubated at 50±5% and 90±5% relative humidity for 96 h at 25±2°C.



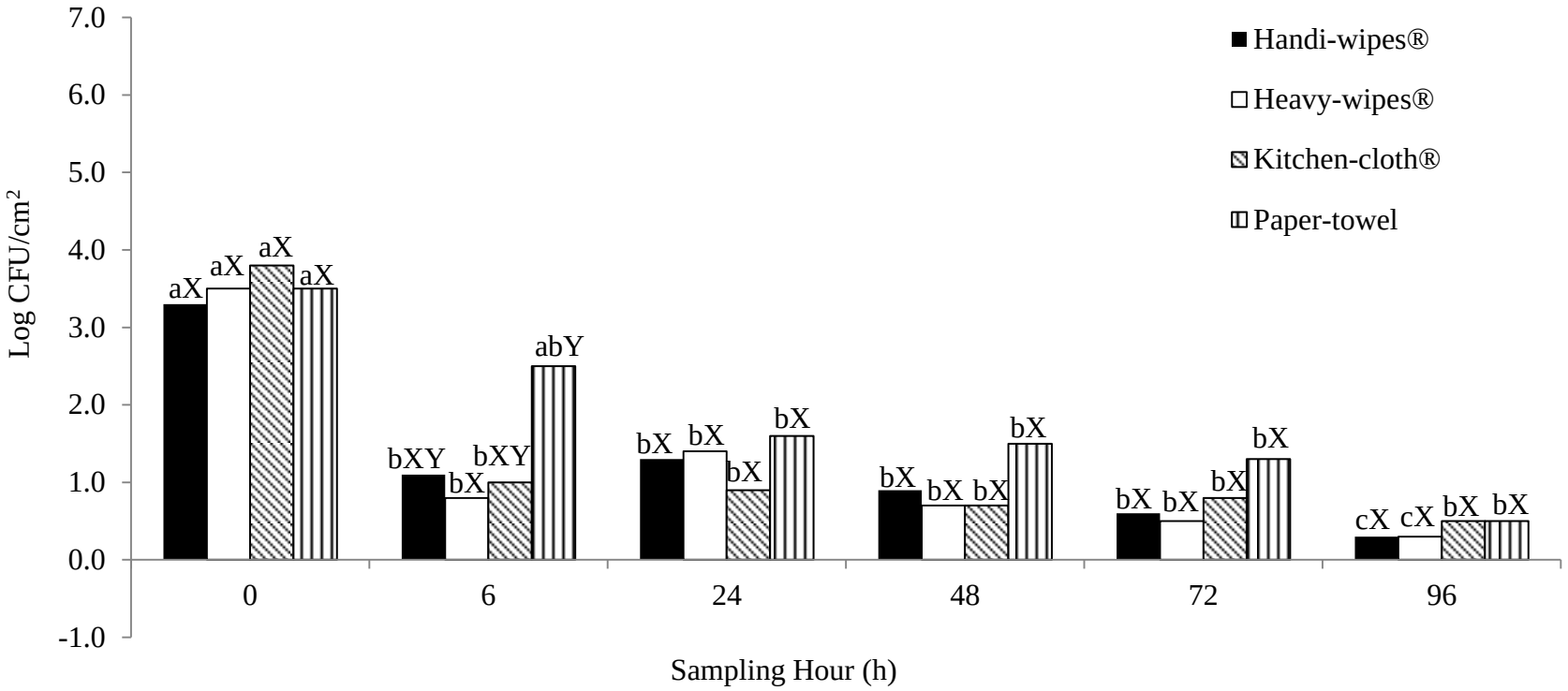
Appendix Figure 23 (Table 7.6). Total bacterial population (log CFU/cm^2; mean ± standard deviation) recovered from laminate and corian kitchen countertop surfaces, incubated at 50±5% and 90±5% relative humidity levels for 96 h at 25±2°C with nutrient supplementation.



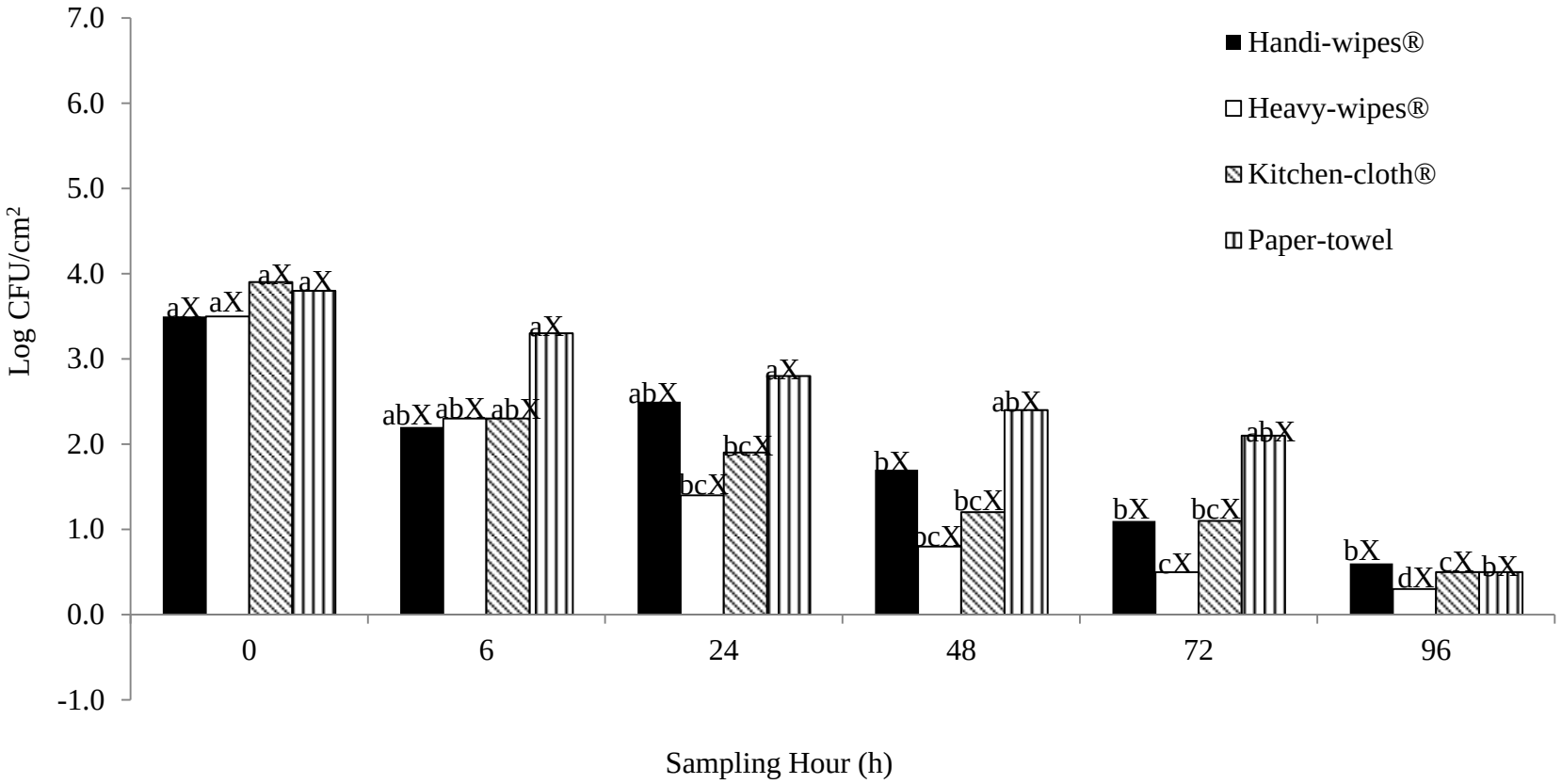
Appendix Figure 24 (Table 7.7). Total bacterial population (log CFU/cm^2; mean ± standard deviation) removed by each wiping material after cleaning laminate coupon surfaces incubated at 50±5% relative humidity level for 96 h at 25±2°C with nutrient supplementation. Mean values not followed by same upper and lower case letter within a figure are significantly different ($P < 0.05$) from each other.



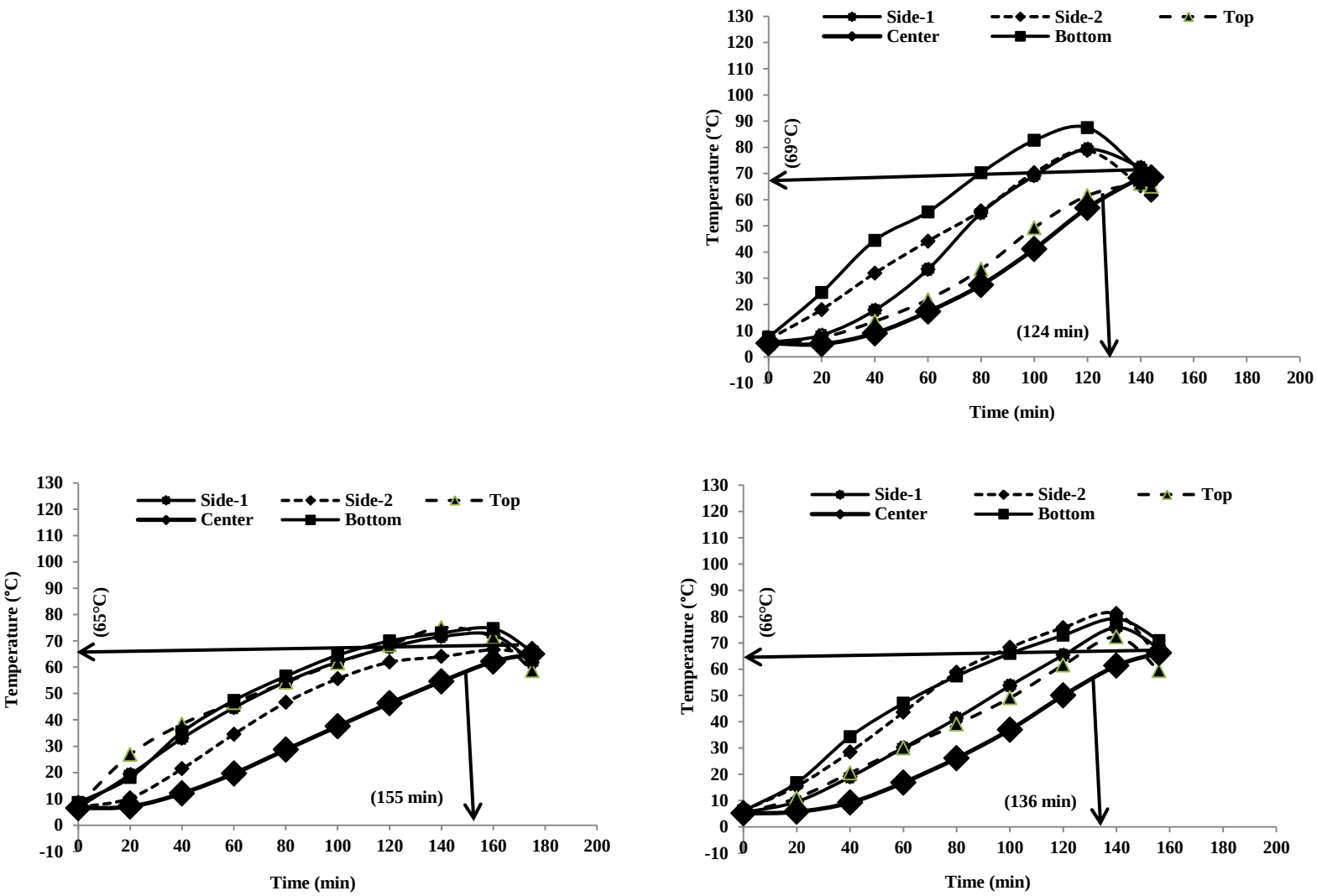
Appendix Figure 25 (Table 7.7). Total bacterial population (log CFU/cm^2; mean ± standard deviation) removed by each wiping material after cleaning laminate coupon surfaces incubated at 90±5% relative humidity level for 96 h at 25±2°C with nutrient supplementation. Mean values not followed by same upper and lower case letter within a figure are significantly different ($P < 0.05$) from each other.



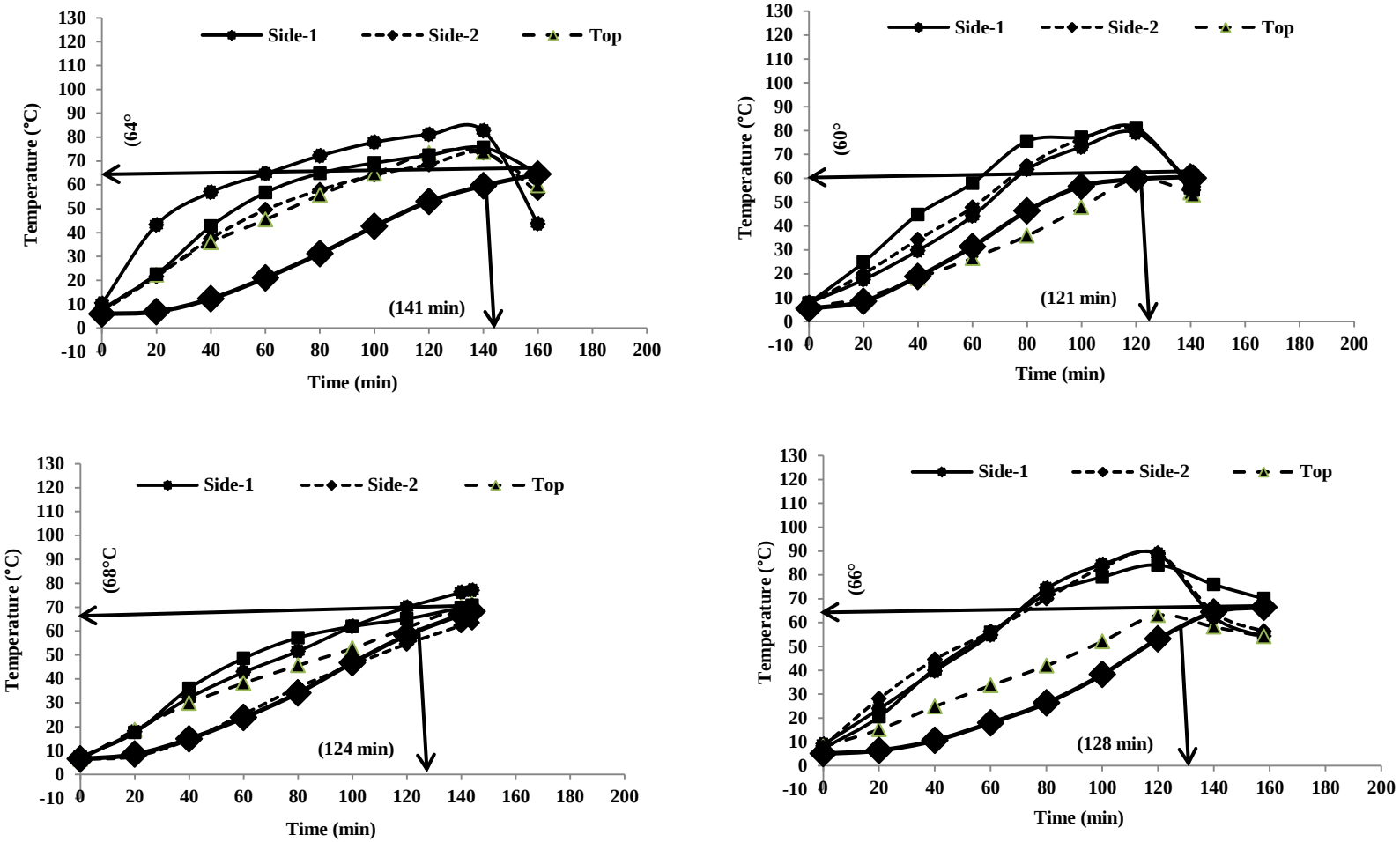
Appendix Figure 26 (Table 7.8). Total bacterial population (log CFU/cm^2; mean ± standard deviation) recovered from laminate coupon surfaces after cleaning with each wiping material, incubated at 50±5% relative humidity level for 96 h at 25±2°C with nutrient supplementation. Mean values not followed by same upper and lower case letter within a figure are significantly different ($P < 0.05$) from each other.



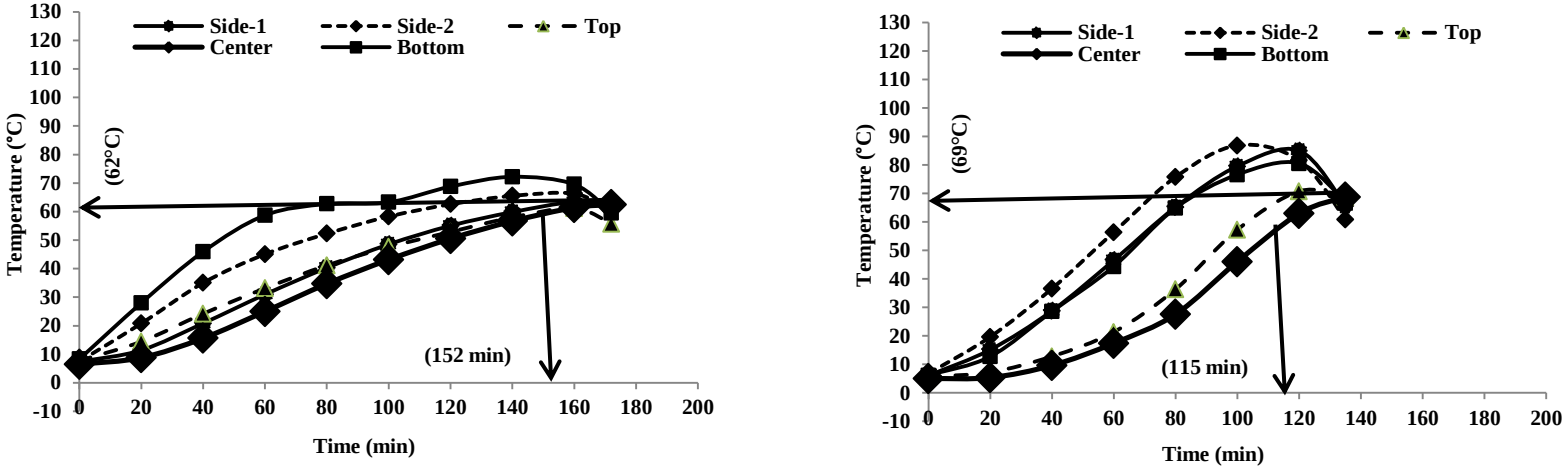
Appendix Figure 27 (Table 7.8). Total bacterial population (log CFU/cm^2; mean ± standard deviation) recovered from laminate coupon surfaces after cleaning surfaces with each wiping material, incubated at 90±5% relative humidity level for 96 h at 25±2°C with nutrient supplementation. Mean values not followed by same upper and lower case letter within a figure are significantly different ($P < 0.05$) from each other.



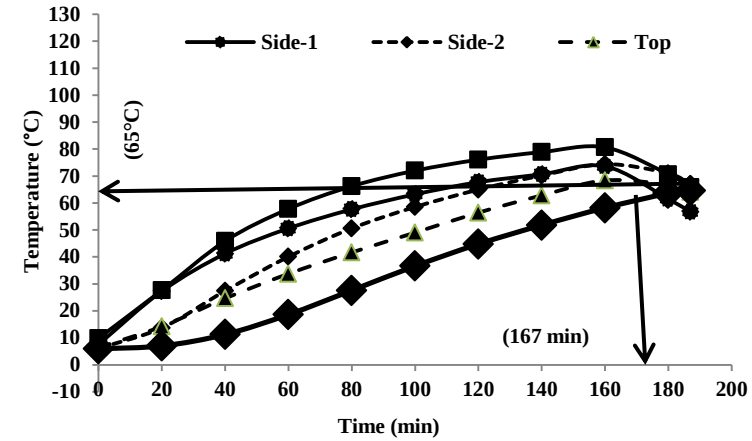
Appendix Figure 30. Cooking time and temperature profile of four roasts moisture-enhanced with NaCl+STP cooked to 60°C on day-0 (i. e., approximately 24 h after preparation).

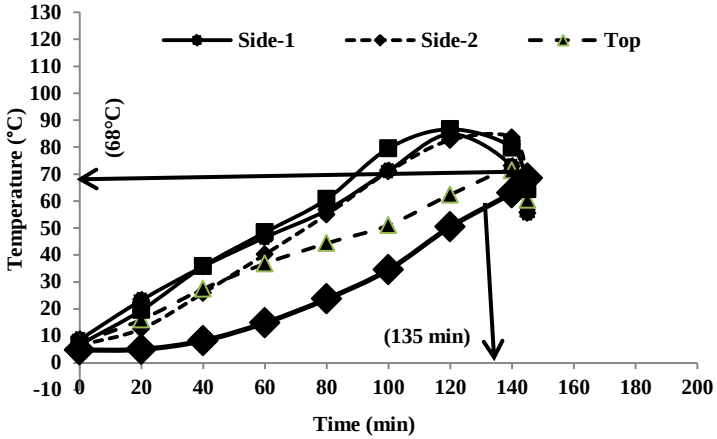


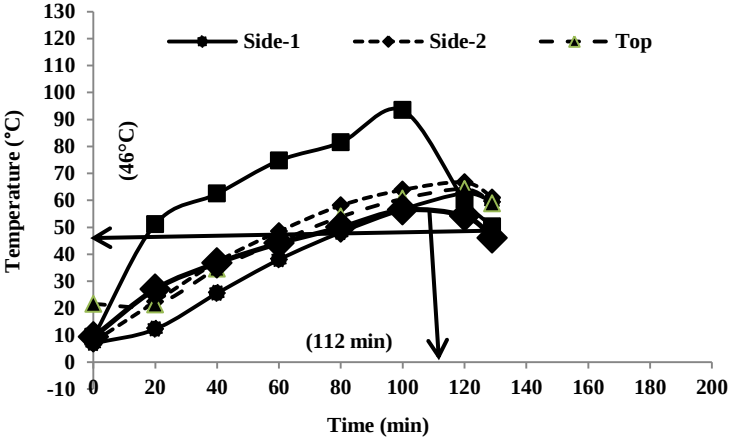
Appendix Figure 31. Cooking time and temperature profile of four roasts moisture-enhanced with NaCl+STP+CPC cooked to 60°C on day-0 (i. e., approximately 24 h after preparation).



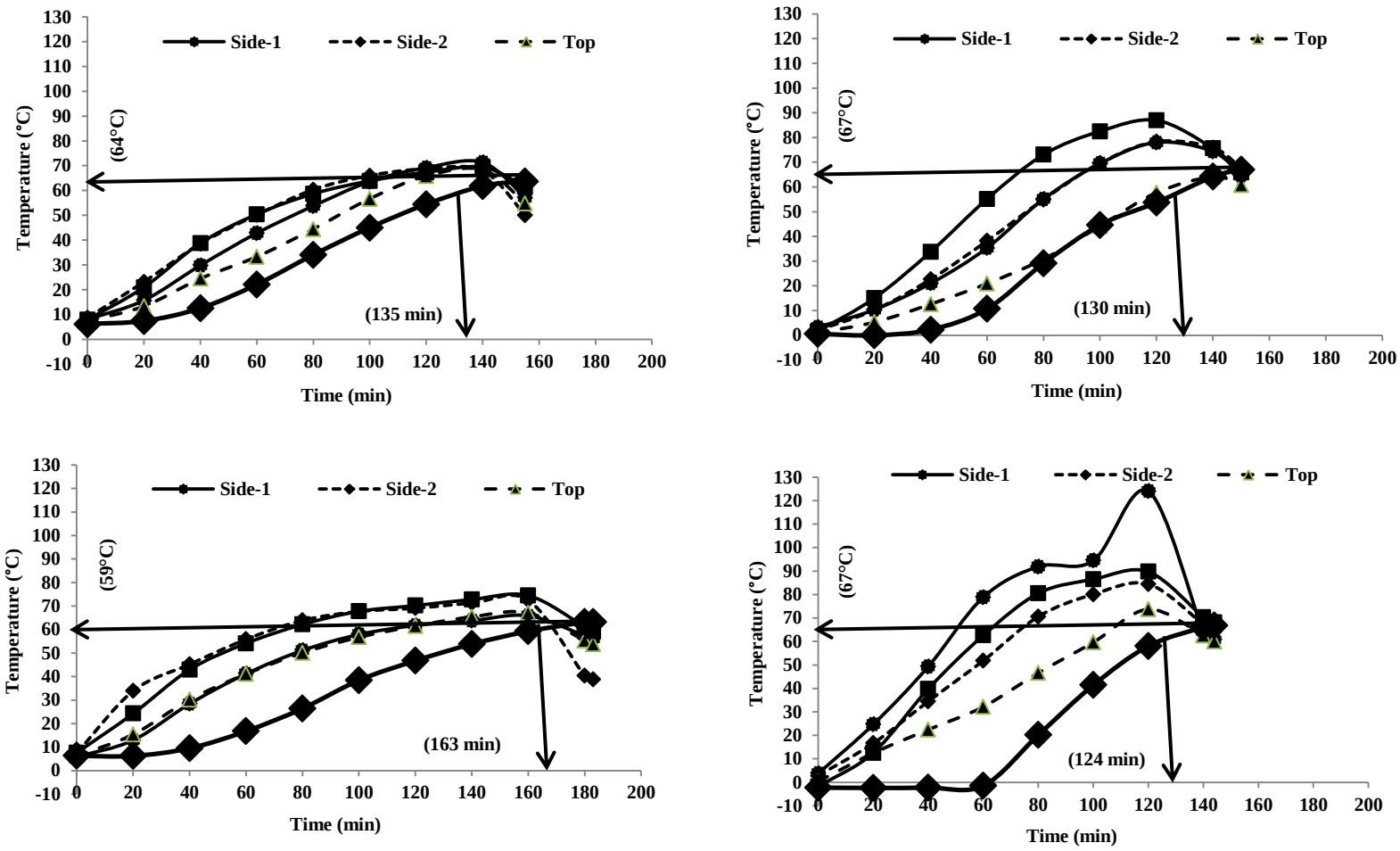
Appendix Figure 32. Cooking time and temperature profile of four roasts moisture-enhanced with NaCl+STP+LA cooked to 60°C on day-0 (i. e., approximately 24 h after preparation).



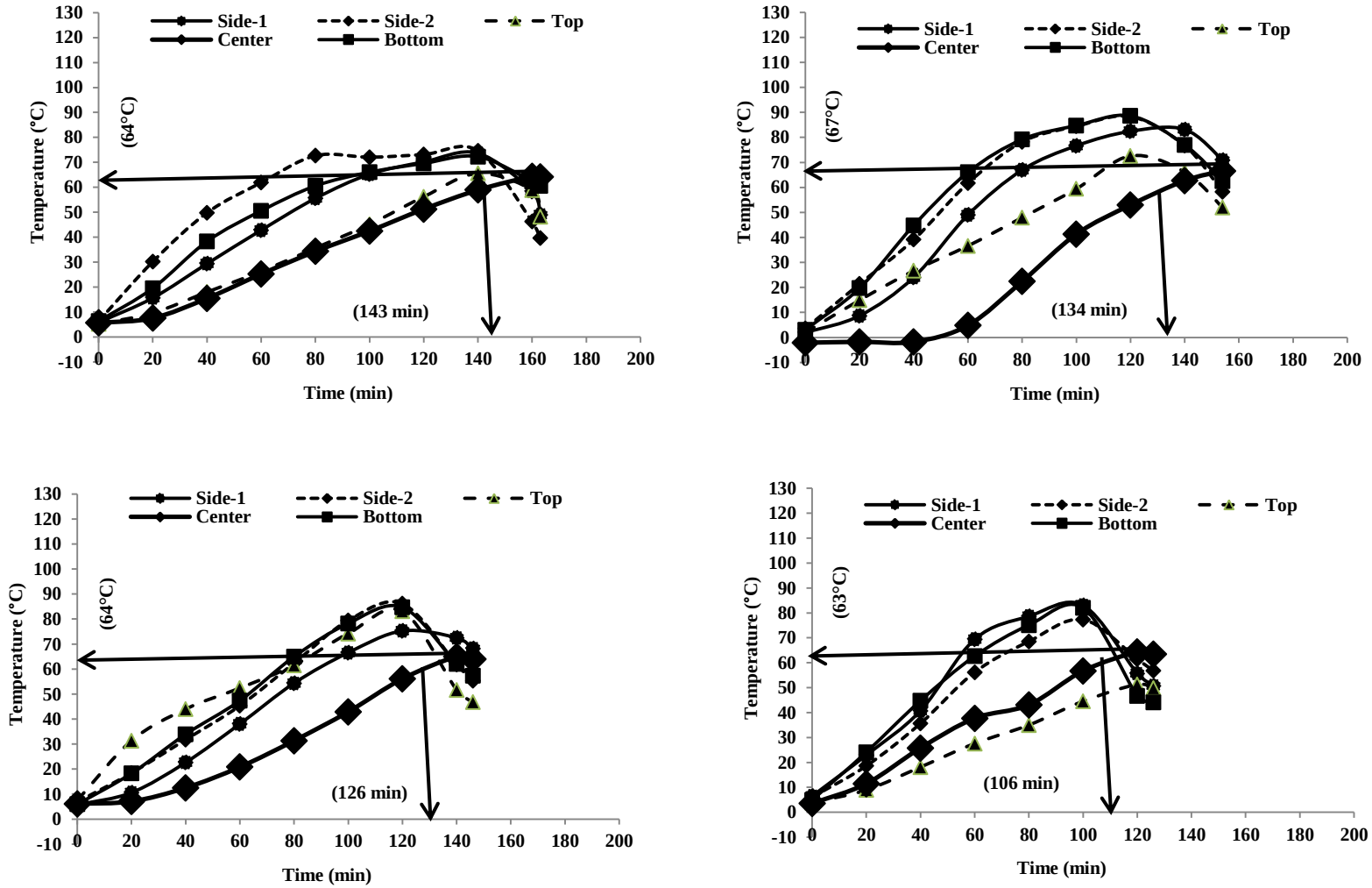




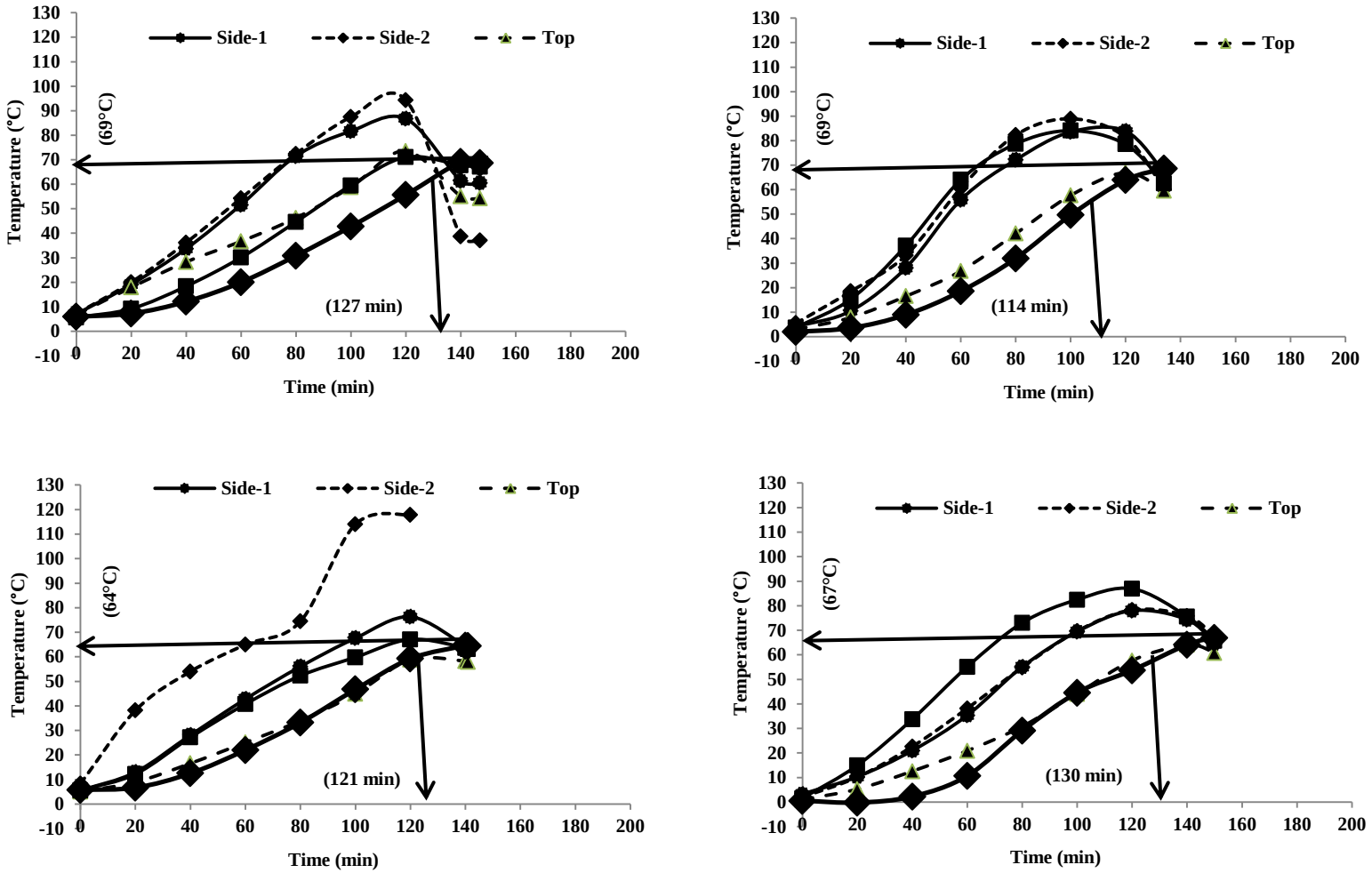
Appendix Figure 33. Cooking time and temperature profile of four roasts moisture-enhanced with NaCl+STP+SM cooked to 60°C on day-0 (i. e., approximately 24 h after preparation).



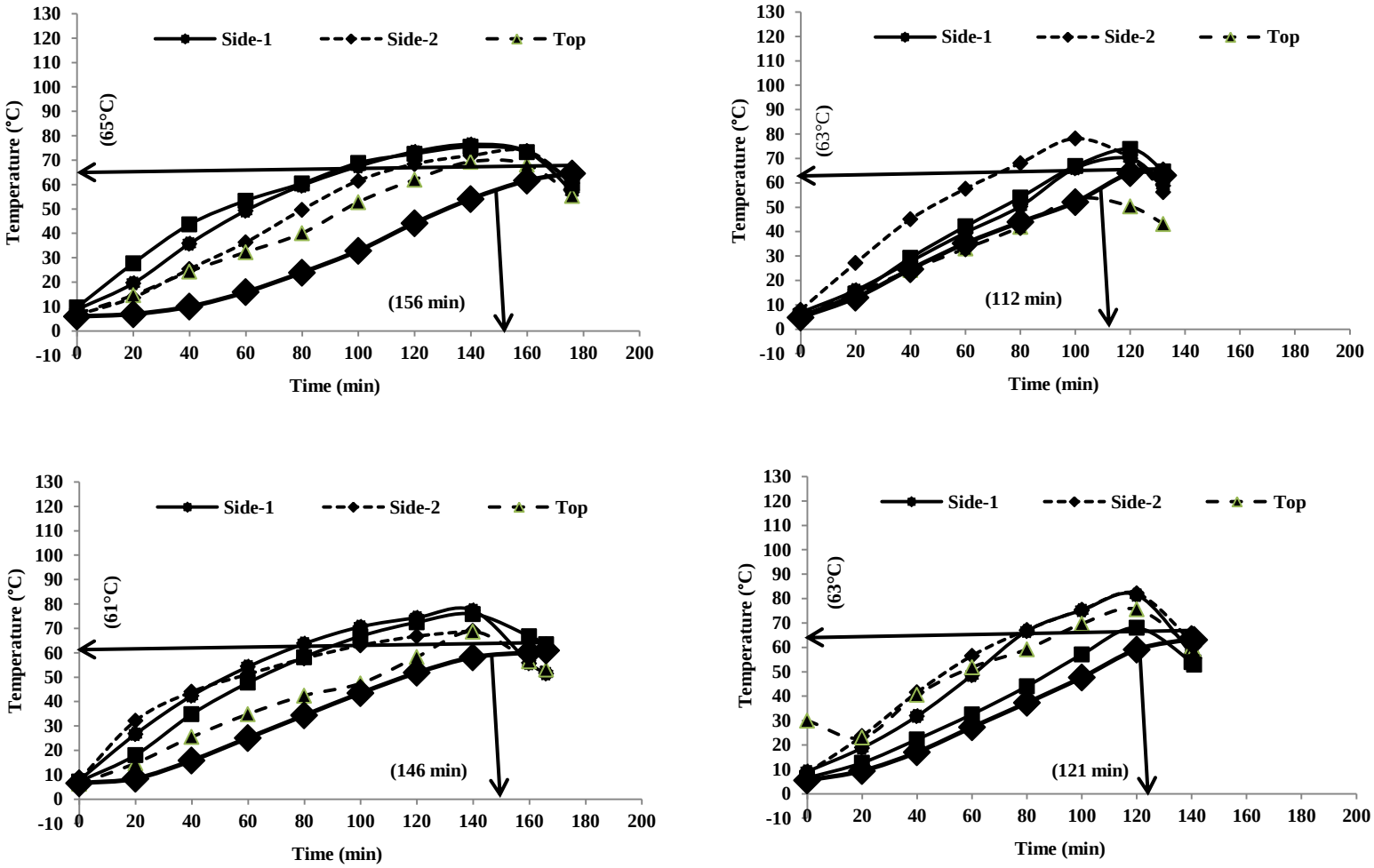
Appendix Figure 34. Cooking time and temperature profile of four roasts moisture-enhanced with NaCl+STP cooked of 60°C on day-30 after frozen (-20°C) storage and 48-72 h thawing.



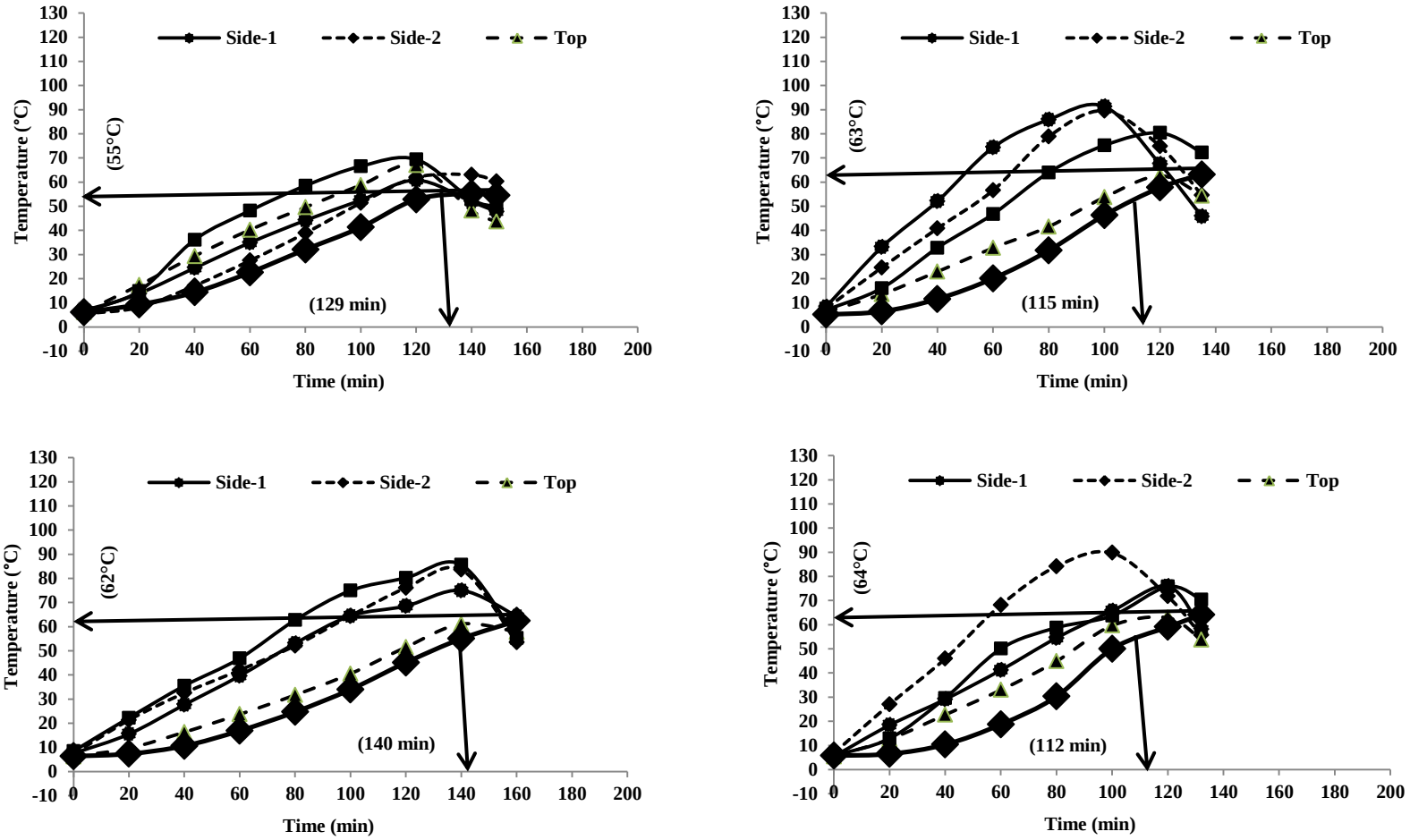
Appendix Figure 35. Cooking time and temperature profile of four roasts moisture-enhanced with NaCl+STP+CPC cooked to 60°C on day-30 after frozen (-20°C) storage and 48-72 h thawing.



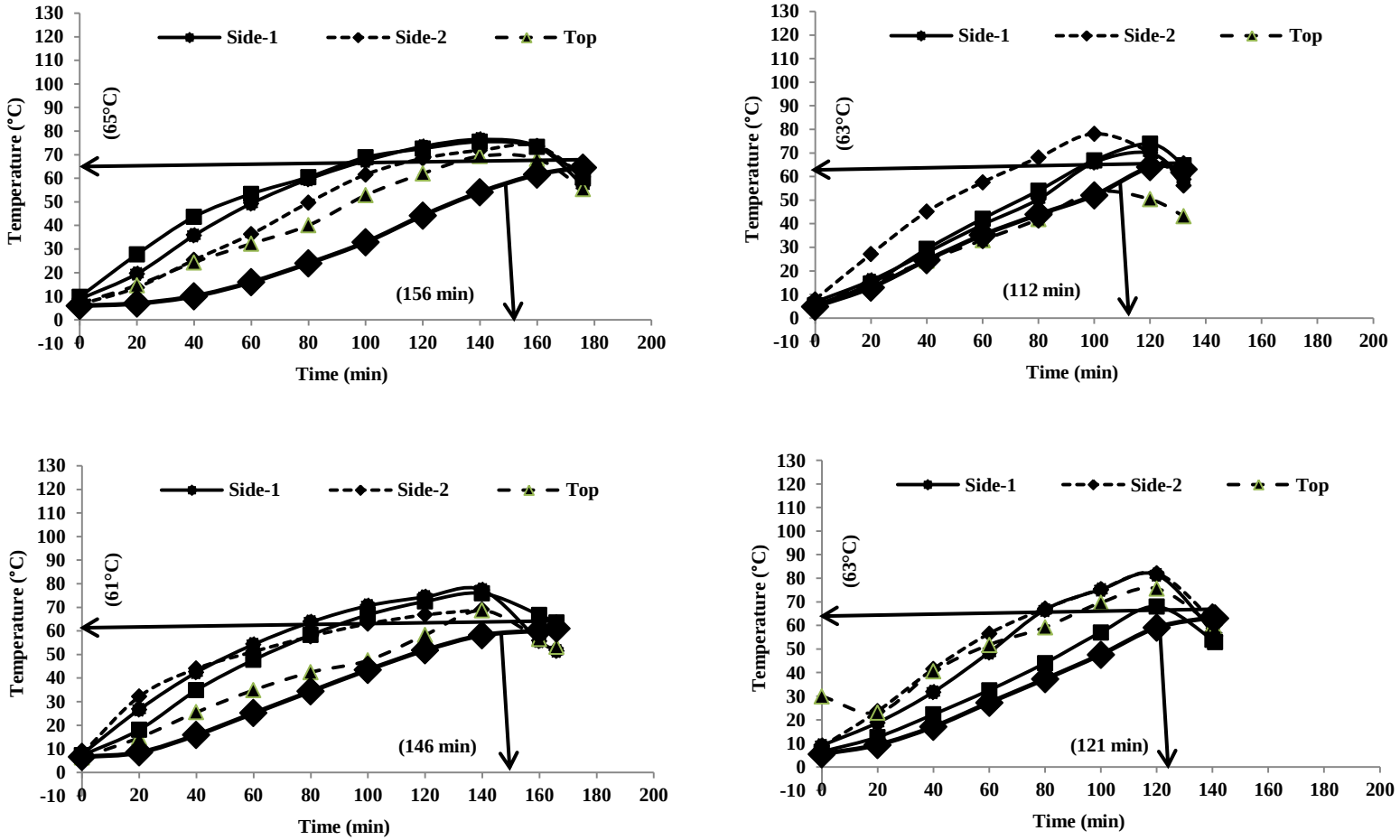
Appendix Figure 36. Cooking time and temperature profile of four roasts moisture-enhanced with NaCl+STP+LA cooked to 60°C on day-30 after frozen (-20°C) storage and after 48-72 h thawing.



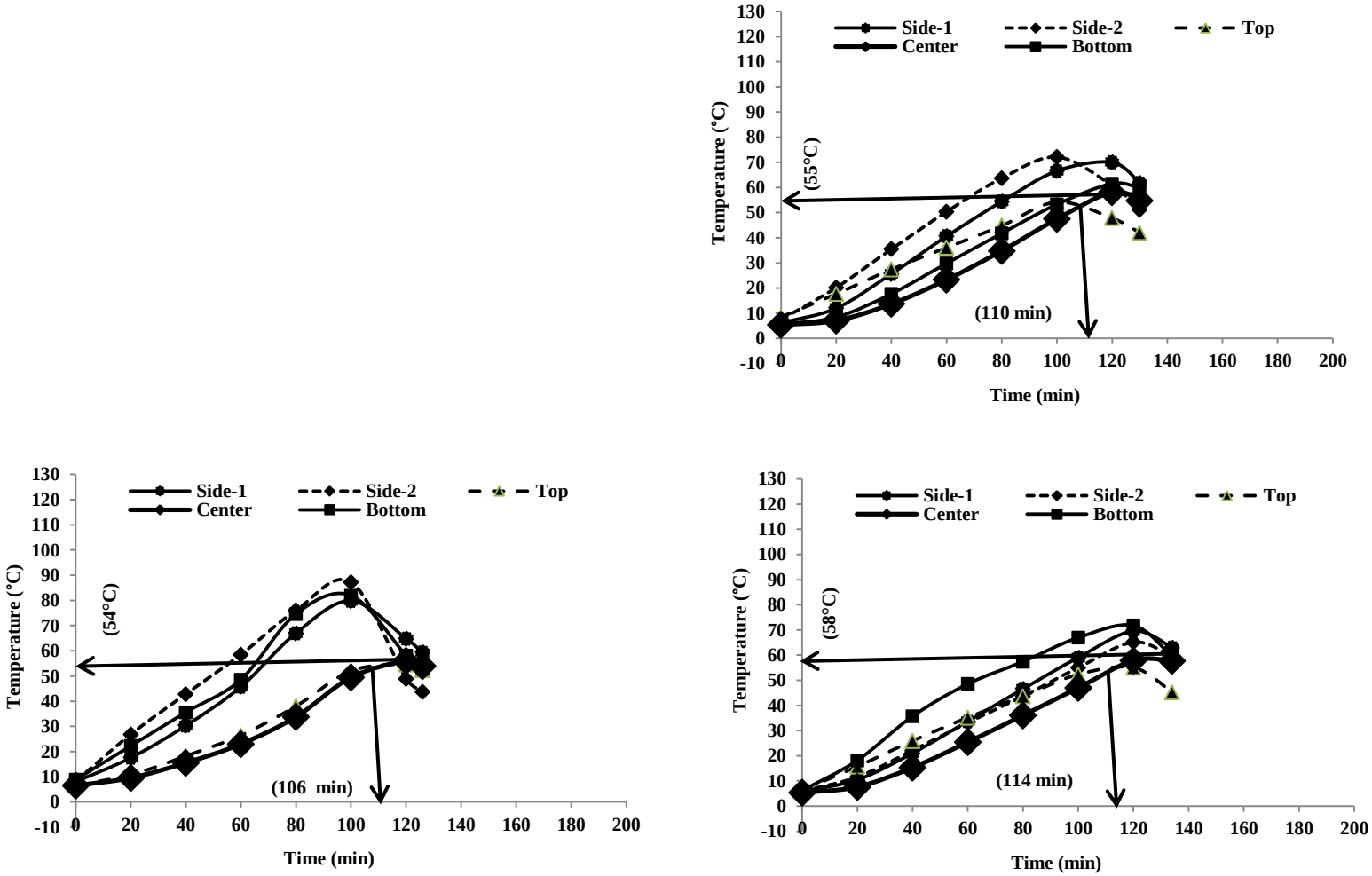
Appendix Figure 37. Cooking time and temperature profile of four roasts moisture-enhanced with NaCl+STP+SM cooked to 60°C on day-30 after frozen (-20°C) storage and 48-72 h thawing.



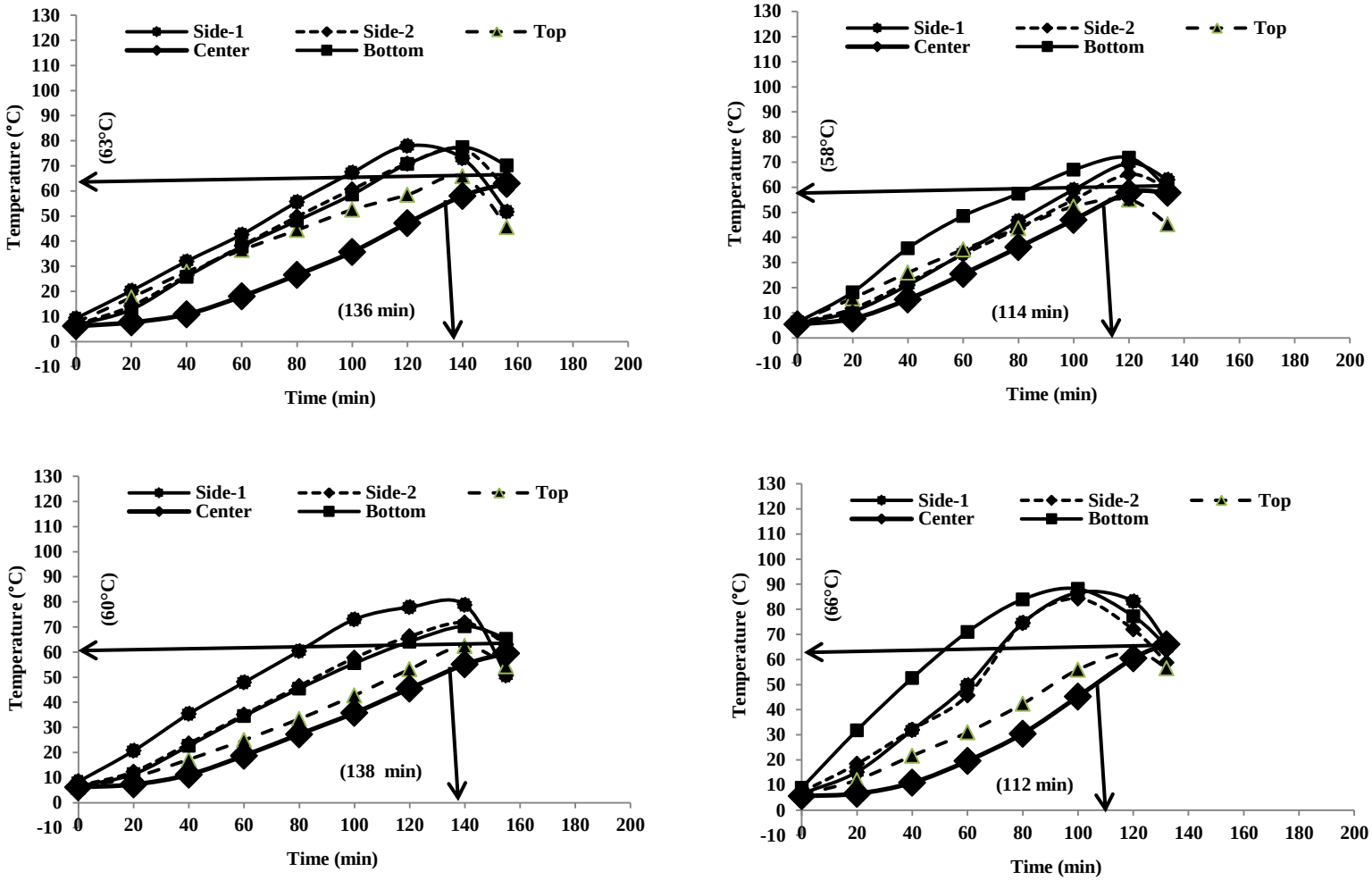
Appendix Figure 38. Cooking time and temperature profile of four roasts moisture-enhanced with NaCl+STP cooked to 55°C on day-0 (i. e., approximately 24 h after preparation).



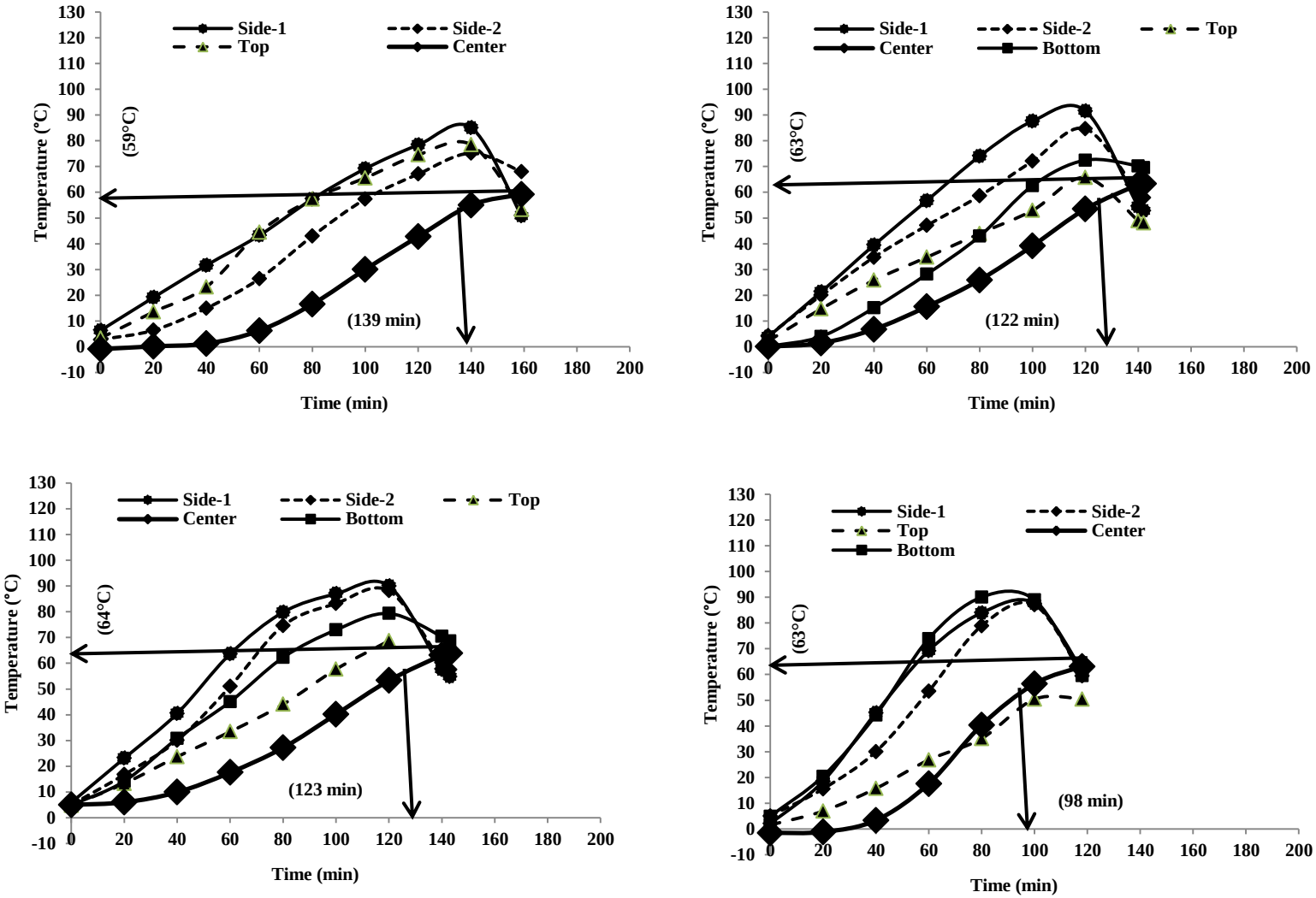
Appendix Figure 39. Cooking time and temperature profile of four roasts moisture-enhanced with NaCl+STP+CPC cooked to 55°C on day-0 (i. e., approximately 24 h after preparation).



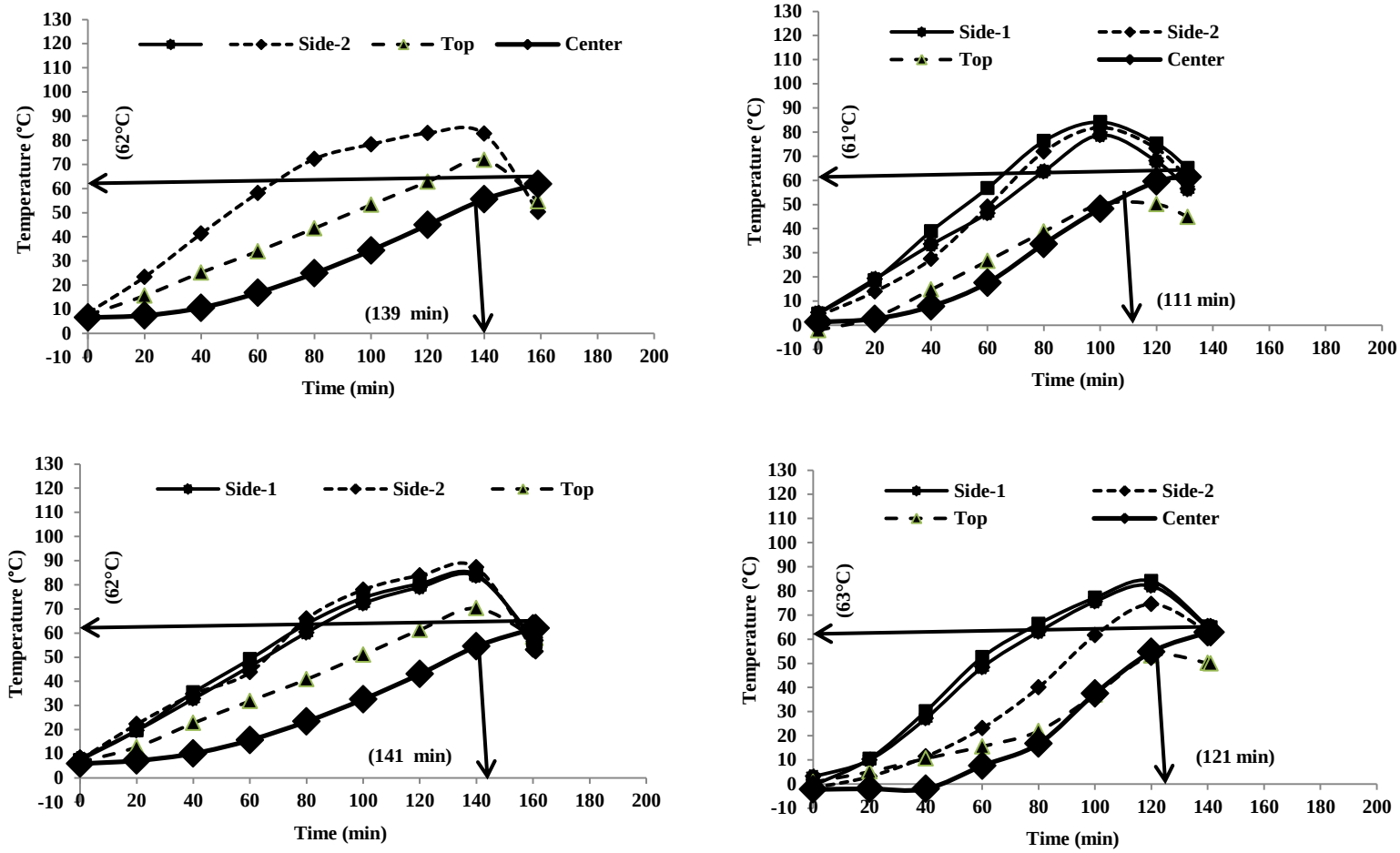
Appendix Figure 40. Cooking time and temperature profile of four roasts moisture-enhanced with NaCl+STP+LA cooked to to 55°C on day-0 (i. e., approximately 24 h after preparation).



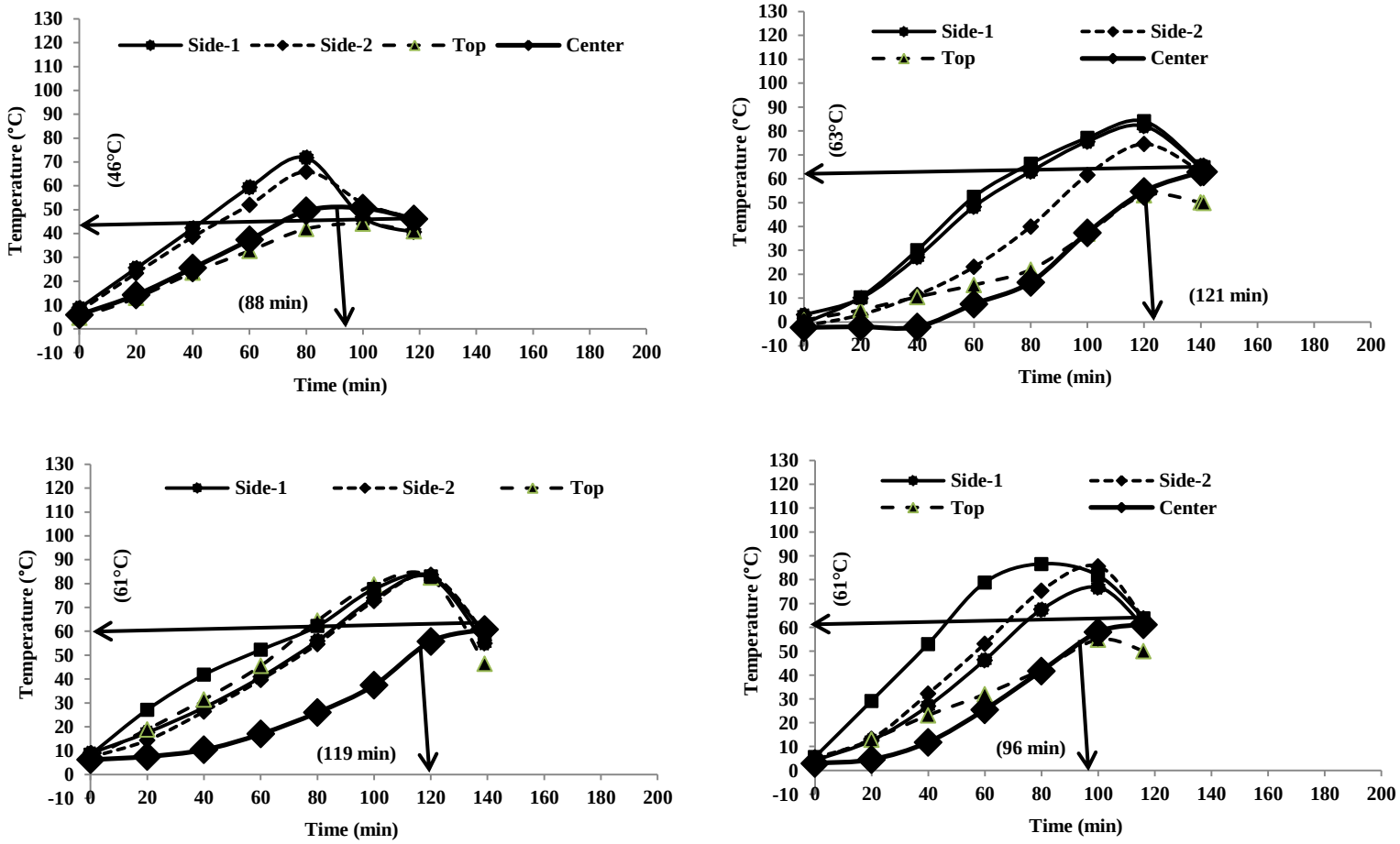
Appendix Figure 41. Cooking time and temperature profile of four roasts moisture-enhanced with NaCl+STP+SM cooked to 55°C on day-0 (i. e., approximately 24 h after preparation).



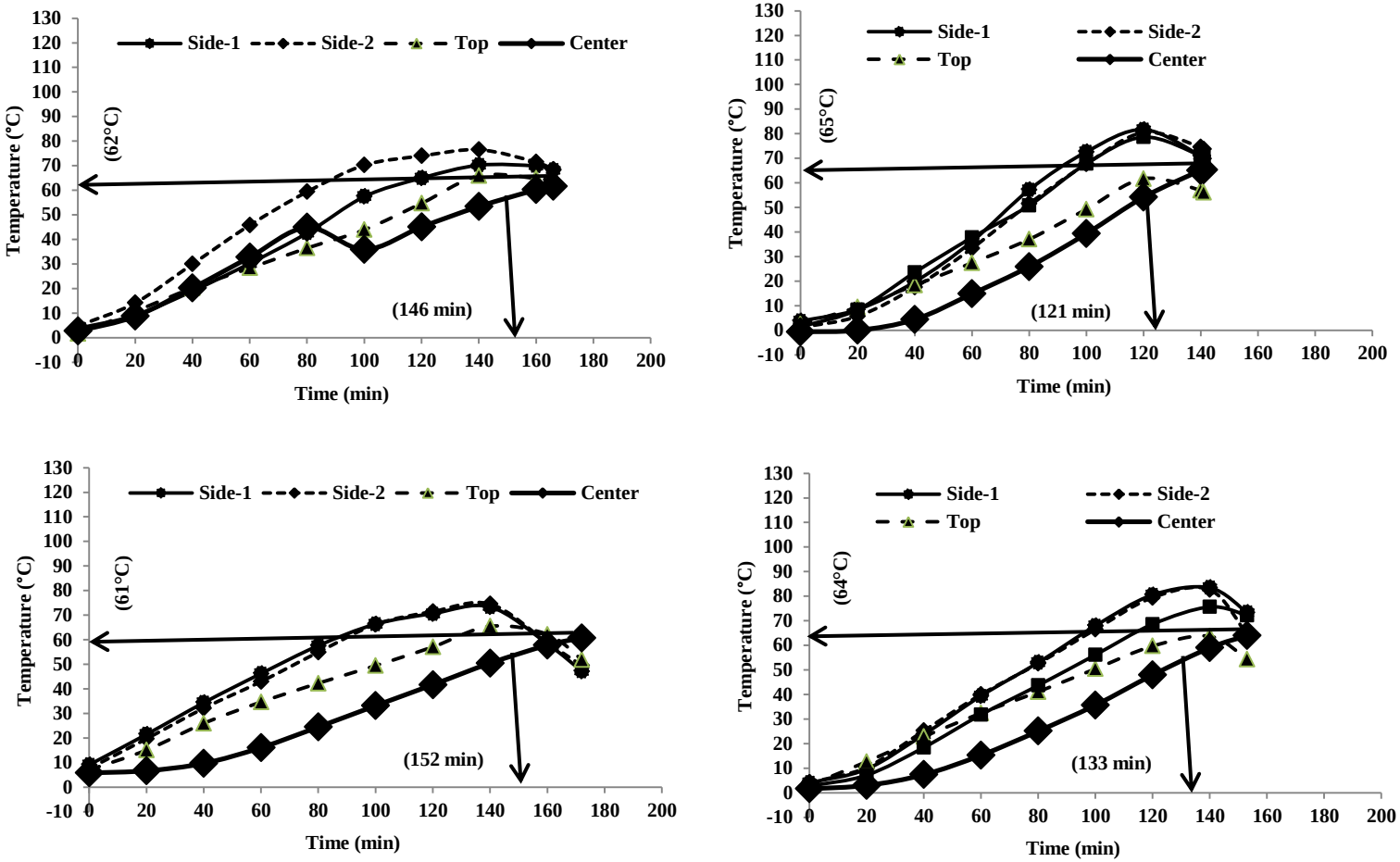
Appendix Figure 42. Cooking time and temperature profile of four roasts moisture-enhanced with NaCl+STP cooked to 55°C on day-30 after frozen (-20°C) storage and 48-72 h thawing.



Appendix Figure 43. Cooking time and temperature profile of four roasts moisture-enhanced with NaCl+STP+CPC cooked to 55°C on day-30 after frozen (-20°C) storage and after 48-72 h thawing.



Appendix Figure 44. Cooking time and temperature profile of four roasts moisture-enhanced with NaCl+STP+LA cooked to of 55°C on day-30 after frozen (-20°C) storage and after 48-72 h thawing.



Appendix Figure 45. Cooking time and temperature profile of four roasts moisture-enhanced with NaCl+STP+SM cooked to 55°C on day -30 after frozen (-20°C) storage and 48-72 h thawing.