DISSERTATION

EFFECTS OF BRINING INGREDIENTS AND ANTIMICROBIALS ON THERMAL INACTIVATION OF *ESCHERICHIA COLI* O157:H7 IN A MEAT MODEL SYSTEM AND CONTROL OF *LISTERIA MONOCYTOGENES* IN FRANKFURTERS

Submitted by

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In partial fulfillment of the requirements

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WE HERBY RECOMMEND THAT THE DISSERTATION PREPARED UNDER OUR SUPERVISION BY OLEKSANDR ANATOLIEVICH BYELASHOV ENTITLED EFFECTS OF BRINING INGREDIENTS AND ANTIMICROBIALS ON THERMAL INACTIVATION OF *ESCHERICHIA COLI* O157:H7 IN A MEAT MODEL SYSTEM AND CONTROL OF *LISTERIA MONOCYTOGENES* IN FRANKFURTERS BE ACCEPTED AS FULFILLING IN PART REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY.

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ABSTRACT OF THE DISSERTATION

EFFECTS OF BRINING INGREDIENTS AND ANTIMICROBIALS ON THERMAL INACTIVATION OF *ESCHERICHIA COLI* O157:H7 IN A MEAT MODEL SYSTEM AND CONTROL OF *LISTERIA MONOCYTOGENES* IN FRANKFURTERS

Microbial food safety has been one of the most important challenges for the meat industry and regulatory agencies during the last two decades owing to outbreaks by pathogens such as *Escherichia coli* O157:H7 and *Listeria monocytogenes* traced to contaminated products, and associated with costly product recalls from the market. Among others, *E. coli* O157:H7 infections have been associated with undercooked contaminated brine-injected meats. *L. monocytogenes* is of particular concern in ready-to-eat (RTE) meat and poultry products.

One part of this dissertation evaluated the effect of brining ingredients, as well as existing and novel antimicrobials, on the fate of *E. coli* O157:H7 during storage and on its thermal (65°C) inactivation in an inoculated (7 log CFU/g) brine-injected meat (two fat levels) model system. The following compounds, alone or in combinations, were mixed with inoculated ground meat: sodium chloride, sodium tripolyphosphate, sodium pyrophosphate, potassium lactate, sodium diacetate, lactic acid, acetic acid, citric acid, nisin, pediocin, sodium metasilicate, cetylpyridinium chloride (CPC), and hops beta acids. Overall, findings showed that common brining ingredients, sodium chloride and sodium

phosphates, did not affect ($P \geq 0.05$) the pathogen during storage and neither protected nor sensitized it to heat. Among tested antimicrobials, CPC was the only antimicrobial that reduced (by approximately 1 log-cycle) *E. coli* O157:H7 during storage. The effect of fat content on the fate of *E. coli* O157:H7 was negligible. Thermal treatment reduced pathogen numbers by 1.5 to 2.5 log-units. CPC-, nisin- and pediocin- treated samples showed an enhanced ($P < 0.05$) thermal destruction of the bacterium, compared to the sodium chloride plus sodium tripolyphosphate control treatments, while other compounds did not influence thermal inactivation.

Another study examined the effect of lactic acid (LA) dipping solutions on *L. monocytogenes* numbers on surface-inoculated (4.4 log CFU/cm^2) frankfurters, and determined parameters (temperature: 4 to 55°C; LA concentration: 1 to 3%; and exposure time: 15 to 120 sec) achieving 1 and 2 log-unit immediate reductions. These reductions may allow processors to meet regulatory requirements, as it is required that post-lethality treatments must reduce the pathogen by at least 1 log-cycle, while processing plants employing treatments that reduce the pathogen by at least 2 log-cycles should be subject to less frequent microbial sampling and testing. Distilled water, at all temperatures, and LA applied at 4°C reduced pathogen counts by approximately 1 log-cycle. Overall, the magnitude of the antimicrobial effect of LA against *L. monocytogenes* increased with solution concentration, temperature, and to a lesser extent, by dipping time. A 2-log reduction was obtained by 1% LA applied at 55°C for 60 s or by 3% LA applied at 25°C for 120 s. A developed prediction equation for *L. monocytogenes* reduction included significant ($P < 0.05$) effects of the linear terms of concentration, time, temperature, and interaction of concentration and temperature; other tested parameters (other interactions,

quadratic and cubic terms) did not affect ($P \geq 0.05$) the reduction within the range of the tested experimental conditions. This equation may help processors to vary parameters (temperature, LA concentration and time) of post-lethality treatments to achieve a 1 or 2 log-unit reduction of *L. monocytogenes* and to meet regulatory requirements.

Another study evaluated the effect (immediate and during 90-d storage) of LA (5% vol/vol) and sodium lauryl sulfate (SLS; 0.5% wt/vol), sprayed individually or as a mixture (LA/SLS), against *L. monocytogenes* on surface-inoculated (4.8 log CFU/cm^2) frankfurters. The LA/SLS was applied before or after inoculation. Spraying with distilled water, LA or SLS after inoculation reduced numbers of *L. monocytogenes* by 1.3 ± 0.2, 1.8 ± 0.5 and 2.0 ± 0.4 log CFU/cm^2, respectively. Reduction by LA/SLS mixture applied after inoculation (2.8 ± 0.2 log CFU/cm^2) was higher ($P < 0.05$) than that achieved by the mixture applied before inoculation (1.8 ± 0.4 log CFU/cm^2). Further, treatments that contained LA delayed growth and decreased growth rate of the pathogen.

A last study evaluated the fate of *L. monocytogenes* on surface-inoculated (1.8 log CFU/cm^2) frankfurters formulated with or without 1.5% potassium lactate and 0.1% sodium diacetate (PL/SD) and stored under fluctuating conditions. These conditions imitated pre-shipment storage (24 h, 4°C), temperature mishandling during distribution (7 h, 7°C followed by 7 h, 12°C), and storage before purchase (60 d, 4°C; SBP). At 0, 20, 40, and 60 d of SBP, samples were exposed to conditions that followed those encountered during transportation from retail to consumers (3 h, 23°C). Then, vacuum-packages were opened or kept intact at 4 or 7°C for 14 d (SHF). *L. monocytogenes* numbers were relatively stable on products with PL/SD regardless of storage conditions; but, they increased on samples without PL/SD. In vacuum-packages, during SHF at 4°C, the

pathogen grew faster ($P < 0.05$) on aged frankfurters (20 d of SBP) compared to frankfurters that were fresh (0 d of SBP); a similar trend was seen in opened packages. At 7°C in opened packages growth rates (0.35 ± 0.02 log CFU/cm^2/d) were the highest on fresh product. By day-40 of SBP *L. monocytogenes* reached high levels and grew slowly or stayed constant during SHF.

Overall, the results of the studies reported in this dissertation may be useful in developing storage recommendations and interventions to control *L. monocytogenes* on frankfurters, as well as in developing and improving brining recipes to control *E. coli* O157:H7 in moisture-enhanced meat products. Further, these data may be useful in pathogen risk assessments for RTE and moisture-enhanced meat products.

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DEDICATION

This dissertation is dedicated to the memory of Dr. David F. Cox. His initial academic background came from animal science and quantitative genetics. Dr. Cox taught statistics for biological sciences at Iowa Sate University for 30 years and consulted graduate students and their advisors about experimental design and data analysis. He became my close friend and mentor during my graduate study at ISU and remained so throughout most of my work at Colorado Sate University.

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CHAPTER 1

INTRODUCTION

In recent decades *Escherichia coli* O157:H7 has been involved in human illnesses and deaths traced to undercooked contaminated non-intact meat products, while *Listeria monocytogenes* infections are mostly associated with ready-to-eat (RTE) meat and poultry products. Outbreaks and deaths are usually followed by market recalls of potentially contaminated products. Thus, in addition to loss of life, processors also suffer direct financial losses due to product destruction, and indirect losses associated with consumers' avoidance of recalled brands and accompanying declines in product sales *(194).* Brands not associated with contaminated foods may also experience temporary declines in sales, as consumer confidence in microbial safety of affected products is compromised *(194).*

The interior of wholesome muscles is generally sterile *(184).* However, pathogens such as *E. coli* O157:H7 may be present on the surface of meat, on injection needles, or in meat enhancement solutions, and may become internalized during injection of meat with brines *(121, 184).* Thus, the pathogen has been involved in several outbreaks of infection linked to non-intact steaks or roasts *(41, 111, 209).* Currently, the U.S. Department of Agriculture Food Safety and Inspection Service (USDA-FSIS) considers *E. coli* O157:H7 as an adulterant in all non-intact beef products, or beef cuts intended to be processed into non-intact products *(202).* Thus, processors are required to

re-evaluate their Hazard Analysis and Critical Control Point (HACCP) plans and revise control measures for the pathogen *(209)*. These measures may include the use of antimicrobials in enhancement solutions *(184)*.

Unlike raw non-intact meat products which may become contaminated with *E. coli* O157:H7, RTE meats generally do not contain the bacterium, as it is destroyed during adequate processing. However, *L. monocytogenes*, which is widely distributed in the environment, may contaminate RTE meats after the lethality processing step, and then multiply during refrigerated storage. Therefore, USDA-FSIS enforces a "zero-tolerance" *(200, 201)* policy for *L. monocytogenes* in RTE meat and poultry products. It also requires food manufacturers to control *L. monocytogenes* in products that allow for pathogen proliferation, and may be recontaminated through exposure to the processing environment after the lethality step *(207)*. Specifically, the industry is required to use one out of three alternatives: a post-lethality inactivation treatment combined with a growth inhibitor of *L. monocytogenes*, a post-lethality inactivation treatment or a growth inhibitor, or sanitation measures combined with frequent environmental testing *(207)*. The first alternative provides a higher stringency of control than the other two, and it involves less frequent USDA-FSIS testing *(210)*.

In the quantitative assessment of relative risk to public health from foodborne *L. monocytogenes* among 23 categories of RTE foods, frankfurters consumed without reheating were identified as a high relative risk product that can cause listeriosis *(59)*. This designation was based on the fact that frankfurters are frequently contaminated with *L. monocytogenes*, support rapid growth of the pathogen even under refrigeration, may be stored for a long time, and are consumed frequently and in relatively large quantities *(59)*.

Regulatory agencies also provide recommendations for safe food practices at retail and at the consumer level. The Food and Drug Administration's (FDA) Food Code provides food handling recommendations to food service establishments, restaurants, nursing homes, and other institutions *(60, 61)*. It is suggested that packages of commercially manufactured RTE foods that are opened in food establishments should be stored for ≤7 days at or below 5°C *(60, 61)*. The USDA-FSIS also recommends general procedures of safe food handling for consumers, suggesting that frankfurters should be stored at 40 °F (4.4 °C) in opened packages for ≤ 7 d or in vacuum-packages for ≤ 14 d *(211)*. These documents are updated and re-issued as new scientific information becomes available.

Development of new federal regulations, standards and recommendations for processors has induced an intensified search for novel antimicrobials by both industry and academia. Some of these compounds include organic acids *(11, 73, 131, 133, 134, 144, 169)*, organic acids salts *(11, 12, 73, 74, 80, 113, 124, 131, 133, 169, 174, 222, 223)*, hops beta acids *(176, 177)*, nisin *(26, 148, 160, 218, 226)*, pediocin *(26, 42, 170)*, sodium metasilicate *(151, 155, 220)*, cetylpyridinium chloride *(21, 38, 53, 93, 94, 102, 117, 143, 152, 178)*, sodium lauryl sulfate *(88, 161, 188, 189, 230)* and many others *(44)*. Some of these are approved by the regulatory agencies for various purposes and/or have shown a promising effect against pathogens in foods under certain laboratory conditions. However, there is a need for evaluation of new compounds as well as re-evaluation of existing ones, alone or in combinations, against *E. coli* O157:H7 in non-intact meat products, and against *L. monocytogenes* in RTE meats. These studies may help processors to meet regulatory requirements and make their products safer for consumers.

The overall goal of studies included in this dissertation was to evaluate the effect of brining ingredients and antimicrobials against *E. coli* O157:H7 in a brine-injected meat model system, to develop post-lethality interventions to control *L. monocytogenes* on frankfurters, and to determine the fate of *L. monocytogenes* on frankfurters during storage under variable conditions. To achieve this goal, four major objectives were addressed:

- Screen effects of brining ingredients, as well as existing and novel antimicrobials, against *E. coli* O157:H7 during storage and cooking in a meat model system. Results of this study may be useful in developing new brining formulations that enhance thermal inactivation of *E. coli* O157:H7.
- Evaluate the effect of lactic acid (LA) solutions on *L. monocytogenes* numbers on surface-inoculated frankfurters and identify parameters (temperature, concentration and time) achieving 1 and 2 log-unit immediate reductions. A developed equation may help manufacturers to adjust parameters of surface treatment with LA to achieve a 1 or 2 log-unit reduction of *L. monocytogenes* and meet USDA-FSIS requirements.
- Examine immediate and residual effects of LA, alone or in combination, with sodium lauryl sulfate (SLS) against *L. monocytogenes* on surface-inoculated frankfurters. Considering that processing contamination of frankfurters may take place before or after spraying with antimicrobials, the effects of spraying with LA/SLS mixture applied before or after inoculation were assessed. These results may help processors to better control *L. monocytogenes* in frankfurters and to comply with federal regulations.
- Evaluate the fate of *L. monocytogenes* on inoculated frankfurters stored under variable conditions to which the product may be exposed during the time between manufacturing and consumption. Data obtained in this study may be useful in developing

and updating storage recommendations for frankfurters and in efforts to establish safety-based consume-by date labels.

CHAPTER 2

REVIEW OF LITERATURE

Control of *Escherichia coli* O157:H7 in Brine-injected Beef Products

E. coli O157:H7

Escherichia coli are gram-negative, aerobic or facultative anaerobic, motile or nonmotile rods that belong to the family of Enterobacteriaceae *(17, 87, 109)*. Most of the strains are non-pathogenic and may be present as normal microflora in the digestive tract of warm-blooded animals and humans serving various beneficial functions to the host *(17, 87, 109)*. However, some strains may cause enteric or extraintestinal infections to humans *(17, 87, 109)*. There have been hundreds of serotypes identified based on lipopolysaccharide side chains on the cell wall (O-antigen) and by the flagellar H antigen *(87, 166)*.

Based on the pathogenicity, *E. coli* can be divided into six classes: enteropathogenic (EPEC), enterotoxigenic (ETEC), enterohemorrhagic (EHEC), enteroinvasive (EIEC), enteroaggregative (EAEC), and diffusely adherent (DAEC) *(109)*. Shiga toxin-producing *E. coli* (STEC) is a subset of *E. coli* with the ability to produce the potent cytotoxins, Shiga-toxins I and/or II *(109)*.

Enterohemorrhagic *E. coli* are STEC that are associated with gastrointestinal disease in humans, resulting in life-threatening complications such as hemolytic uremic-

syndrome (HUS) *(96)*. Genetically, STEC can be identified by the presence of either (or both) of the Shiga-toxin genes, *stxI* and *stxII*. In addition to containing *stxI* and/or *stxII*, EHEC are identified genetically by the presence of *eae* or *saa* genes *(81, 148)*. The *eae* gene encodes for intimin which is an outer membrane protein used in bacterial attachment to intestinal epithelial cells *(81)*; *saa* encodes for an outer membrane autoagglutinating adhesion protein that is associated with pathogens that cause HUS and lack the *eae* gene *(148)*. The most commonly known EHEC is *E. coli* O157:H7, which has been associated with many foodborne outbreaks *(38)*. *E. coli* O157:H7 is genetically identified by the presence of *stxI* and/or *stxII*, *rfbE*, and *flicC* genes *(81, 87)*. The *rfbE* gene encodes for the unique enzyme used in the production of the O157 extracellular antigen; and *fliC* encodes for a structural protein unique to the H7 flagellar antigen *(89)*.

The infective dose of *E. coli* O157:H7 is known to be very low (<10 cells) *(87)*. Ingestion of the pathogen may result in colonization of the colon where the pathogen produces Shiga-toxins *(87)*. Shiga-toxins bind to the cellular Gb3 receptor that is located in the vascular epithelium, colon, and kidneys, enter into the endosomes, and become transported to the trans-Golgi network and the endoplasmic reticulum, where one of the toxin subunits enters the cytoplasmic matrix *(81)*. This subunit inhibits protein production by enzymatic depurination of ribosomal ribonucleic acid (rRNA), which leads to cellular death *(81)*. As a consequence, humans may develop a HUS, which can be characterized by hemolytic anemia, acute renal failure and a low platelet count *(87)*. The HUS is fatal in 5% of cases, and approximately 8% of the survived patients have serious aftereffects of the disease including neurological disorders, blindness, paralysis, and renal compromise *(87)*.

Non-intact beef products

Approximately 74% of beef carcass meat weight is represented by low-value cuts, as most valued cuts come from the loin and rib area *(129)*. Based on a 2003 survey conducted on behalf of the National Cattlemen's Beef Association (NCBA), it was estimated that approximately 94% of beef processing plants utilize mechanical tenderization of lower value cuts, and approximately 24% of the plants use enhancement solutions *(136)*. It also was estimated that approximately 18% of retail beef products are either mechanically tenderized or moisture-enhanced *(136)*.

The USDA-FSIS defines intact beef cuts as a cut of whole muscle that has not been injected, mechanically tenderized or reconstructed *(203)*. The Food and Drug Administration (FDA) has provided a similar definition for such products: "whole muscle beef that is not injected, mechanically tenderized, reconstructed, or scored and marinated, from which beef steaks may be cut" *(60)*. These cuts may include products such as steaks, roasts, briskets, and beef for stir-frying or cubes for stew *(203)*. Non-intact beef products include intact cuts that have been ground, injected with solutions, marinated, restructured, or tenderized by needling, cubing, frenching or pounding devices *(203)*. These processing techniques inevitably involve a potential for pathogen internationalization if it is present on the exterior of the product *(110, 185, 203)*. The internalization may occur by direct translocation of bacteria from the exterior to the interior of the cuts via contaminated blades, needles, recycled injection fluid, or by combining meat pieces into restructured product *(120, 184, 185)*.

The interior of intact beef products is usually protected from microbial contamination, even if bacteria are present on the surface *(203)*. The prevalence of *E.*

coli O157:H7 on the surface of intact subprimal beef cuts prior to further processing may be less then 0.083% *(100)*. Also, only 3 to 4% of the bacteria may be transferred from the meat surface into the interior of the meat *(185)*. Most of the cells are transferred into the topmost 1 cm; however, some cells become translocated into the deeper tissues of the meat *(120)*. Furthermore, it was reported that cooking steaks to at least medium rare (63°C) may ensure microbial safety *(76, 77)*.

In 2002, the USDA-FSIS conducted a comparative risk assessment for intact (non-tenderized) and non-intact (tenderized) beef *(204)*. It was estimated that *E. coli* O157:H7 prevalence was 0.000026 and 0.000037% for intact and tenderized steaks, respectively *(204)*. It also was concluded that risk of *E. coli* O157:H7 infection from contaminated tenderized products is very low and similar to that from intact beef, as the pathogen does not typically survive thermal treatment applied during cooking *(204)*. Similarly, the National Advisory Committee on Microbiological Criteria for Foods (NACMCF) found that risk of *E. coli* O157:H7 infection from contaminated non-intact beef is low (1 illness per 14.2 million steaks) and similar to that from intact steaks (1 illness per 15.9 millions of steaks) *(134)*. However, these reports were based on limited data. Still, outbreaks have been linked to non-intact beef products. There is a need for more research on pathogen prevalence in non-intact beef and its survival during thermal treatment, cooking practices and heat transfer in products, modeling the industry tenderization procedures, heat resistance of *E. coli* O157:H7 strains, and dose-response relationship in humans *(134, 204)*.

There is no federal regulation which requires labling of mechanically tenderized or brine-injected beef products *(120)*. Therefore, if these products are not specifically

labeled as non-intact, consumers may perceive tenderized/injected beef products as intact beef cuts *(184)*. If such products are intentionally or unintentionally undercooked, pathogens may survive in the interior of the meat and therefore represent a health risk to the consumers *(184)*.

E. coli O157:H7 infection outbreaks traced to contaminated meat

In 1999, it was estimated that *E. coli* causes more than 73,000 illnesses in the U.S. resulting in more than 2000 hospitalizations and 60 deaths *(126)*. The United States Centers for Disease Control and Prevention (CDC) Foodborne Diseases Active Surveillance Network (FoodNet) data showed that the relative incidence of STEC *E. coli* O157 infection in the U.S. in 2008 was 1.12 cases per hundred thousand individuals *(39)*. *E. coli* O157:H7 has become the most frequent STEC serotype in North America *(87)* since it was first recognized as a foodborne pathogen after two outbreaks of gastrointestinal illness in the U.S. traced to undercooked beef in 1982 *(163)*. Up until 2002, there had been at least 350 outbreaks of *E. coli* O157:H7 infection in 49 states with 41% of outbreaks traced to contaminated ground beef *(91, 98, 159)*. In 1993, a large multistate outbreak traced to undercooked hamburgers from a fast-food restaurant chain sickened 501 individuals resulting in 151 hospitalizations and three deaths *(15)*. In September 2007, approximately 9.8 million kg of ground beef products were recalled nationwide because of possible contamination with STEC serovariant O157:H7 *(212)*. The contaminated product led to at least 40 confirmed cases of foodborne illness *(38)*.

The presence of STEC in beef has been of serious concern for the beef industry for a number of years. For the last two decades, contaminated ground beef was a primary source of foodborne *E. coli* O157:H7 infection; but it was not the only beef product that

has been implicated in the disease. Contrary to above mentioned risk assessments, which concluded that risk of *E. coli* O157:H7 infection from tenderized products is very low, mechanically tenderized and injected beef products (intact beef cuts that were injected with solutions, needled, cubed, or pounded) *(204, 209)* also have been implicated in outbreaks of the disease *(209)*. The first documented outbreak traced to these products occurred in Michigan in 2000 *(209)*.

Another multistate outbreak of the disease traced to blade tenderized and injected with marinade steaks sold by door-to-door vendors occurred in 2003 *(110)*. During this outbreak, contaminated steaks sickened a total of twelve people (ten culture-confirmed cases) in five states and resulted in a nationwide recall of 335,506 kg of potentially contaminated beef products *(110)*. In the processing plant investigation, it was revealed that the steaks were injected with water and flavorings at 12%, and then went through a blade tenderizing apparatus multiple times *(110)*. The injection/tenderization equipment was cleaned and sanitized on a daily basis, but the complete disassembly was performed only once a week, suggesting possible harborage of the pathogen in the equipment *(110)*. The outbreak investigation also revealed that the steaks were cooked directly from the frozen state without prior thawing, which could have resulted in undercooking of meat, and therefore, in the survival of the pathogen *(110)*.

In 2004, another outbreak was traced to non-intact beef steaks served at four different locations of a national restaurant chain in Colorado (Denver metropolitan area) *(209)*. As a result of the outbreak the manufacturer recalled approximately 184,158 kg of potentially contaminated product *(208)*. Most recently, in 2007, there were three separate

outbreaks in Pennsylvania, California, and Michigan linked to non-intact beef products *(200)*.

Brine-injection of meat

Typically beef products are injected with brines at 7 to 15% (i.e., to 107 to 115% of original weight of meat) *(128)*. Brines usually contain sodium chloride and phosphates *(128)*. Sodium chloride is usually added at the level of 0.5% (wt/wt) of product. It increases the water holding capacity (WHC) of the meat by lowering the isoelectric point of meat proteins *(128)*, and by extraction and solubilization of myofibrillar proteins *(182)*. Phosphates (usually used at the level of 0.25% wt/wt) increase the meat pH, and therefore move it away from the isoelectric point of meat *(128, 182)*. This increases the amount of negative charges on meat proteins allowing more water to be bound to proteins and increasing the WHC. The primary phosphate salt which is used in brines is sodium or potassium tripolyphosphate, but other phosphates, including sodium pyrophosphates, tetrasodium pyrophosphates, sodium hexametophosphates, alone or in combination can be added with tripolyphosphates. Other ingredients including organic acids *(130, 132)*, sodium citrate/acetate *(104, 186)*, sodium/potassium lactate *(49, 102, 104)*, calcium chloride *(11, 18)*, calcium lactate *(111)*, soy protein isolates *(227)*, beef broth *(11, 111)*, rosemary extract *(104)*, glucose *(18, 102)*, kappa carrageenan *(111)*, bromelain *(105)*, and others. Some of these are added to the brines to improve functionality or microbial quality of meat.

Antimicrobial compounds that were tested in foods other than non-intact meat, but may potentially be added to brining solutions, include hops beta acids *(177, 178)*, nisin *(26, 149, 161, 218, 226)*, pediocin *(26, 42, 171)*, sodium metasilicate *(152, 156,*

220), and cetylpyridinium chloride *(21, 38, 53, 92, 93, 101, 116, 144, 153, 179)*. All these ingredients may be used alone or in combination after careful consideration as they may display additive, synergetic or antagonistic effects. In addition, the efficacy of antimicrobials can be affected by the properties of meats, including their fat and moisture contents, pH, and water activity. For example, calcium chloride is a potent tenderizer, but it should not be used as a mixture with phosphates, as calcium-phosphate complexes can be formed, reducing the effectiveness of each individual compound *(174)*. In addition, some ingredients may affect the susceptibility of pathogens (if present in the interior of meat) to heat during cooking. Mukherjee et al. *(130, 131)* demonstrated that calcium lactate (0.63%) and potassium lactate (1.8%) protected *E. coli* O157:H7 during cooking (after overnight refrigerated storage) of ground beef, whereas citric (0.2%) and acetic (0.3%) acids enhanced pathogen destruction.

Nisin is a bacteriocin produced by some lactic acid bacteria strains, which sensitizes pathogens to heat in liquids, such as milk *(149, 161, 218, 226)*. It is approved by the USDA-FSIS for use in ready-to eat (RTE) meat and poultry products, including soups, sausages, cured meats, salads, sauces, and dressings *(213)*. Pediocin is also an antimicrobial peptide, which is produced by *Pediococcus* spp. *(68, 194)*. Its use in foods is not currently approved in the U.S., but the antimicrobial had shown a promising effect against *L. monocytogenes* in RTE meats *(42)*. Both of these antimicrobials are more effective against Gram-positive bacteria compared to Gram-negatives, as Gram-negative bacteria have a more complex cell wall structure which prevents the penetration of bacteriocins inside the cell *(68, 194)*. However, the effectiveness of nisin and pediocin against Gram-negative bacteria, including *E. coli* O157:H7, may be improved when they

are combined with permeabilizers of bacterial outer membrane *(68, 194)*. For example, Ca^{2+} and Mg^{2+} ion chelators, such as ethylene diamine tetra acetate (EDTA) or phosphates, can facilitate access of these peptides inside of the bacteria *(48, 68, 187, 191, 194)*.

Sodium metasilicate is an alkaline antimicrobial approved by the USDA-FSIS as an ingredient of raw meat and poultry marinades, and as a surface treatment of beef carcasses, subprimals, and trimmings, and RTE meats *(213)*. CPC is a cationic quaternary ammonium compound approved for surface decontamination of raw poultry carcasses prior or after dipping into a chiller *(213)*. It is a potent antimicrobial, whith demonstrated effectiveness against a broad range of pathogens, including *Salmonella* Typhimurium, *E. coli* O157:H7, *L. monocytogenes*, and *Staphylococcus aureus* in inoculated beef products *(153, 180)*. Hops beta acids also are approved by the USDA-FSIS for use on RTE meats and their casings, and in salad dressings *(213)*. It has been reported that hops beta acids are effective against *L. monocytogenes* in broth medium *(178)* and in controlling the pathogen on frankfurters *(177)*.

Published reports regarding the effect of existing brining ingredients on the heat destruction of *E. coli* O157:H7 are scarce. Further, there is a need for the evaluation of novel antimicrobials as a part of brining formulation against *E. coli* O157:H7 during storage and cooking of moisture-enhanced meat products.

Control of *Listeria monocytogenes* in RTE Meats

L. monocytogenes and listeriosis

L. monocytogenes is a psychrotrophic, facultative anaerobic, Gram-positive, salt- and pH-tolerant bacterium which is commonly found in the environment *(167)*. It is a

short (0.5 μm x 1-2 μm) catalase-positive motile rod which does not form spores *(167)*. *L. monocytogenes* can be classified into at least thirteen serotypes (1/2a, 1/2b, 1/2c, 3a, 3b, 3c, 4a, 4ab, 4b, 4c, 4d, 4e, 7) based on the recognition of surface antigens by specific antisera *(167, 224)*.

L. monocytogenes is a facultative intracellular foodborne pathogen which causes listeriosis, a potentially deadly disease most commonly affecting immunocompromised people *(125, 167)*. The symptoms of listeriosis may include diarrhea, fever, headache, and myalgia, septicema, meningitis, enceplalitis, abortion, or stillbirth *(167)*. Listeriosis has the highest hospitalization rate (92.2%) and the second highest mortality rate (20%) among known foodborne illnesses *(126)*.

Most of the current epidemiological knowledge about listeriosis has come from outbreak investigations, while most of illnesses are sporadic *(59)*. In 2003, the FDA, USDA-FSIS, and CDC conducted a quantitative assessment of relative risk to public health from foodborne *L. monocytogenes*. Based on the hazard characterization step of risk assessment, it was concluded that RTE meat and dairy products are most frequently associated with foodborne listeriosis in the U.S. and globally *(59)*. It also was reported that serotype 4b was implicated in approximately 70% of documented outbreaks *(59)*. The FoodNet data showed that the relative incidence of listeriosis in the U.S. in 2008 was 0.29 cases per hundred thousand individuals *(39)*. The actual incidence of the disease was estimated to be at least twice as high due to underreporting *(217)*.

L. monocytogenes prevalence in RTE meats

Listeriosis outbreaks traced to frankfurters have resulted in nonregulatory surveys to determine the incidence of *L. monocytogenes* in this product. Wang and Muriana

(219) examined 20 brands of retail frankfurters and found that, on average, 7.5% of samples were contaminated with *L. monocytogenes*. The pathogen was found in liquid exudates, but not inside of frankfurters, suggesting that contamination occurred after the thermal treatment involved in frankfurter processing *(219)*. A Canadian survey *(196)* showed that 17% of retail frankfurters were contaminated with *L. monocytogenes*. Between 1990 and 2000, the USDA-FSIS conducted microbiological testing in 1,800 federally inspected production facilities *(114)*. It was reported that the cumulative 10-year prevalence of *L. monocytogenes* in small-diameter cooked sausages and deli meats was 3.56 and 5.16%, respectively *(114)*. It also was reported that the prevalence of *L. monocytogenes* was higher in RTE products that were subjected to post-cooking handling, including peeling, slicing, dicing and packaging *(114)*. Further, results indicated that prevalence of the pathogen tended to decrease between 1990 and 1999, suggesting improvements in plant sanitation and use of post-lethality interventions *(114)*. In 2003, the Agricultural Research Service of the USDA published results of a two-year study which evaluated prevalence of *L. monocytogenes* in frankfurters that came from 12 separate producers (9 large and 3 small plants) *(216)*. In that study, 532 of 32,800 (1.6%) packages tested positive for the pathogen *(216)*. In 2007, the USDA-FSIS analyzed 8,687 RTE meat and poultry products at high risk for causing listeriosis (sampling project 001 RTE) and found 46 samples positive for the pathogen (0.53%) *(214)*.

Despite the downward trend in the contamination rates over the years, current prevalence of *L. monocytogenes* in RTE meat and poultry products indicates that there is a need for further improvement in production practices and antimicrobial interventions.

Dry fermented sausages also may become contaminated with *L .monocytogenes.* Thirty-four percent of raw meat samples used in processing of dry fermented sausages were found to be contaminated with *L. monocytogenes (193).* Further, up to 72% of ground and stuffed sausages prior to aging, fermentation and drying may become contaminated with the pathogen *(193).* Traditional processing of fermented sausages includes aging of meat (with sodium chloride), fermentation (with added spices, sugar and sodium nitrate) with starter culture bacteria, and drying of the product *(146).* These processing steps may reduce numbers of *L. monocytogenes,* but do not always eliminate it from the final product, as it can tolerate salt, low water activity and low pH *(78).* Therefore, microbial survivors may acquire enhanced salt and acid tolerance or cross-protection to a variety of stresses *(119, 169).* In addition to potential survivors, sliced fermented sausages may become contaminated with *L. monocytogenes* from the processing environment or food-contact surfaces during peeling, slicing or packaging *(197).*

It was reported that in the U.S., the cumulative prevalence of *L. monocytogenes* in dry and semi-dry fermented sausages, including pepperoni, cervelat, several varieties of salami, summer sausage, sopresatte, and others was 3.25% *(114).* European studies showed a higher prevalence of *L. monocytogenes* in these types of products *(50, 193).* For example, it was reported that 15.2% of Italian fermented sausages were contaminated with *L. monocytogenes*, including the contamination with lineage I isolates *(50)*, which are most frequently associated with human illness *(225).* Thevenot et al. *(193)* reported that prevalence of the pathogen in dried sausages from 13 French processing plants was 10%. It was demonstrated that bacterial populations may be reduced during storage of

these products by hurdles such as low pH and low water activity *(90)*. However, the efficacy of these hurdles may depend on the physiological state of the pathogen and may be influenced by product storage conditions *(107)*.

Frankfurters as "a high risk product"

L. monocytogenes does not survive thermal treatment during manufacturing of frankfurters *(229)*. However, contamination may occur through direct contact of the cooked product with contaminated surfaces or from the air of the processing environment during peeling and packaging *(197)*. Frankfurters can support growth of *L. monocytogenes* even when stored in vacuum packages at refrigeration temperature *(59)*. Therefore, if contaminated, these products may pose a life-threatening hazard to the consumers, especially considering that some individuals may consume these products without adequate reheating *(59, 198)*. Further, contaminated frankfurter exudate may cross-contaminate food-contact surfaces, kitchen utensils and appliances, or other products *(219)*. Therefore, in the quantitative assessment of relative risk to public health from foodborne listeriosis among selected categories of RTE foods, non-reheated frankfurters were identified as a high risk product that can cause listeriosis *(59)*.

Listeriosis traced to RTE meats

Contaminated RTE meats, including frankfurters, have been implicated in outbreaks and sporadic cases of listeriosis in the U.S. In 1986 to 1987, the CDC conducted a population-based survey of listeriosis in the U.S. and found that sick individuals consumed nonreheated hot dogs significantly more frequently than other foods *(173)*. In 1988, CDC isolated *L. monocytogenes* from turkey frankfurters stored in a refrigerator of an individual who died of foodborne listeriosis *(32)*. The isolates had the

same Pulsed Field Gel Electrophoresis (PFGE) profile as those isolated from the patient's specimens; this led to a voluntary recall of the contaminated product *(32)*. In 1998 to 1999, a multistate outbreak of listeriosis traced to contaminated frankfurters made by a single processing plant caused 50 illnesses, three deaths and one miscarriage/abortion *(33, 34)*. In 2000, another outbreak of the disease linked to turkey delicatessen meat, caused 29 illnesses, four deaths and three miscarriages in ten states *(35)*. In 2002, a multistate outbreak of listeriosis linked to turkey delicatessen meats sickened 46 individuals, caused seven deaths and three miscarriages in the Northeastern U.S. *(37, 83)*. The most recent outbreak of listeriosis emerged in 2008 in Canada. According to the Public Health Agency of Canada (PHAC), there were 57 confirmed cases of the disease including 22 deaths, traced to RTE meat products manufactured by a Canadian processing plant *(27)*.

Regulatory policy on *L. monocytogenes* in the U.S.

The USDA-FSIS enforces a "zero-tolerance" policy for *L. monocytogenes* in RTE meat and poultry products *(201, 202)*. In 2008, the FDA also proposed to continue using the "zero-tolerance" policy for RTE foods that support growth of *L. monocytogenes*, but to set an allowable level (100 CFU/g) for non-meat products that do not support pathogen growth *(62)*. In addition to proper sanitation, USDA-FSIS requires the industry to apply control measures for *L. monocytogenes* in these products if they are exposed to the processing environment after the lethality processing step, and support growth of the pathogen *(206)*. Alternative 1 requires the use of a post-lethality processing intervention and antimicrobial agent or process that suppresses or limits the growth of *L. monocytogenes* on the product after it has been exposed to a processing environment. Under Alternative 2, processors must use either a post-lethality processing intervention or

an antimicrobial agent to control the pathogen. Processors that chose Alternative 3 are required to control the pathogen in RTE foods or the processing environment by implementing a sanitation program and are subjected to frequent FSIS testing. The USDA-FSIS will still test plants under all Alternatives, but the frequency and number of samples increases from Alternative 1 to Alternative 3. The chosen alternative must be included in the Hazard Analysis and Critical Control Point (HACCP) plan or prerequisite programs, and its effectiveness should be validated and the data shared with USDA-FSIS *(206)*. The USDA-FSIS developed a compliance guideline to assist processors in meeting regulatory requirements of the final rule *(210)*. The guideline suggests that the post-lethality treatment must reduce *L. monocytogenes* by at least 1 log-cycle *(210)*. Processing plants using interventions that reduce the bacterium by at least 2 log-cycles should be subject to less frequent microbial sampling and testing by USDA-FSIS *(210)*.

To achieve uniform and efficient food safety standards, FDA, CDC, USDA-FSIS and the Conference for Food Protection (CFP) issued the 2005 edition of the Food Code *(60, 61)*. This document helps state, local, and tribal food safety agencies in regulating institutions (nursing homes, day care centers, hospitals), and retail, vending, and food service establishments including grocery stores and restaurants *(60)*. As of 2005, the Food Code was adopted by 48 states as a model guideline for food safety. The document provides specific food handling guidelines including suggestions for time and temperature control of RTE foods *(60)*. Specifically, the document recommends that commercially manufactured vacuum-packaged RTE foods that were opened in a food establishment should to be clearly labeled with a date and time if they are intended to be

stored for more than 24 h before consumption or sale. The opened RTE foods should be stored for no longer than 7 days at or below 5°C *(60, 61)*.

The USDA-FSIS also provides suggestions for safe food handling at consumers' homes. The agency elaborated a Cold Storage Chart that contains time and temperature limits for safe storage of food products, including RTE meats. The chart suggests that frankfurters should be always stored at or below 40°F (4.4°C) *(211)*. The opened product packages should be stored for no longer than 7 days, and vacuum-packages should be stored for no longer than 14 days *(211)*. Although, according to a web-based survey, the majority of consumers store frankfurters within recommended time limits *(31)*, most of their refrigerators operate at temperatures higher than those recommended by the agencies *(99)*.

L. monocytogenes is generally considered as a target bacterium for control in refrigerated RTE meat and poultry products because of its virulence, ability to tolerate salt, and grow at refrigeration temperatures in vacuum-packaged or aerobically stored foods *(40, 60, 160)*. However, the infectious dose of the pathogen remains unknown. Therefore, there is no general agreement on performance standards or a specified allowable maximum increase in numbers of *L. monocytogenes* during storage of foods *(217)*. Despite this, FDA and USDA-FSIS safe time and temperature storage recommendations were designed to allow for no more than a 1-log growth of *L. monocytogenes (60)*. However, this performance standard does not mean that *L. monocytogenes* is always present or allowed to be present in foods, and does not set an acceptable level for the pathogen *(60)*. As a foundation for these recommendations, the agencies used different data sources including peer-reviewed journal articles, growth

modeling software and science-based reports such as the CDC/FDA/FSIS *L. monocytogenes* risk assessment *(40, 59, 60).* However, the available data contain a number of limitations. Specifically, most of the pathogen growth models were based on data collected with liquid food systems, and did not account for presence of spoilage bacteria and additives that may be present in processed foods and affect the metabolism of *L. monocytogenes (40).* Further, the majority of published studies on fate of the pathogen in foods were performed under constant storage conditions, and without considering temperature fluctuations to which foods may be exposed between processing and consumption.

In the quantitative assessment of relative risk to public health form foodborne listeriosis, the CDC/FDA/FSIS emphasized the importance of time/temperature control during storage of RTE foods *(59).* Because of this, the NACMCF provided federal food safety agencies with information for potential establishment of safety-based consume-by date labels (SBDL) for refrigerated RTE foods *(160).* The Committee concluded that application of SBDL at multiple points from food manufacturing to consumption may substantially reduce the incidence of human listeriosis. However, microbial challenge studies need to be performed to validate a SBDL *(160).*

Use of lactic acid to control *L. monocytogenes*

Lactic acid (LA) can be naturally present in many foods, or be produced by fermentation of carbohydrates by lactic acid bacteria, and may be added to perishable foods to extend their shelf life and to control pathogens *(192).* It is a generally recognized as safe (GRAS) food additive which can be used as an antimicrobial, for flavoring/flavor enhancing, or for pH control in non-meat products, at levels not to

exceed good manufacturing practices *(63)*. LA also is included in the USDA-FSIS list of safe and suitable ingredients for use in food production *(213)*. Specifically, it is approved for surface treatment of various products, including beef carcasses, beef and pork subprimal trimmings, beef jerky, frankfurters and other RTE meat and poultry products *(213)*. LA does not have to be declared on a product label when it does not have a lasting effect in a treated food *(213)*.

Similar to other organic acids, LA can damage bacterial cytoplasmic membranes *(23)*, increase cell permeability to other antimicrobials *(2)*, and inhibit metabolic reactions *(165)*. In addition, undissociated molecules of acids may disrupt the proton-motive force (PMF) of cells, which leads to metabolic exhaustion because most of the adenosine triphosphate (ATP) is used up in pumping protons out of the cell *(23, 221)*. It was demonstrated that the uptake of undissociated lactate ions into *E. coli* O157:H7 and *E. coli* O162 incubated in Luria Bertoni broth supplemented with 100 mmol/l D,L-lactate (pH 3.8) at 5, 20 and 37°C increased with the temperature, resulting in greater pathogen destruction *(101)*. Similarly, Venkitanarayanan et al. *(199)* reported that *E. coli* O157:H7 in 1.0 or 1.5% LA plus 0.1% H_2O_2 and 0.1% peptone water at 40°C was reduced from 6.5 log CFU/ml to undetectable levels (<10 CFU/ml) within 10 min, while it took twice as long to achieve the same reduction at 22°C. At 8°C, the combination of these chemicals reduced bacterial counts by only about 2.5 log-units *(199)*.

The bactericidal and bacteriostatic effects of LA as a single intervention or as a follow-up to other antimicrobial strategies on meat and poultry surfaces were demonstrated in numerous studies *(7, 47, 51, 75, 106, 181)*. Reported reductions in bacterial populations caused by organic acids vary considerably as a function of spraying

time, pressure, and temperature. Anderson and Marshall *(6)* showed that inactivation rates of *Enterobacteriaceae*, *E. coli* C5 and *S.* Typhimurium (ATCC9148) on inoculated beef dipped in acetic acid (1, 2, and 3%; 15 s) at 25, 40, 55, and 70°C increased with solution temperature and concentration. Similarly, it was reported that effectiveness of the acetic acid against generic *E. coli* and *Enterobacteriaceae*, and aerobic plate counts (APC) on beef carcasses increased with solution temperature *(5)*. It was reported that a remarkable reduction of *E. coli* and APC can be achieved with 4% LA applied at 55°C to beef carcasses under commercial plant conditions *(29, 30)*. Hardin et al. *(85)* reported that spraying artificially contaminated carcasses with water followed by LA (2%; 11 s) at 55°C was more effective in reduction of *E. coli* O157:H7 and *S.* Typhimurium compared to spraying with water alone.

Lactic acid also can be used as a surface treatment for control of *L. monocytogenes* on RTE meats *(11, 71, 73, 74, 133, 140, 145, 170, 183)*. For example, Palumbo et al. *(145)* reported that dipping frankfurters in LA (1.5 to 3%; 60 to 120 s) at ambient temperature reduced numbers of *L. monocytogenes* on inoculated frankfurters by 1.0 to 1.6 logs. Barmpalia et al. *(11)* reported that dipping inoculated frankfurters in LA (2.5%; 120 s) at 23°C reduced numbers of *L. monocytogenes* by 2.1 log CFU/cm^2 *(11)*. Similarly, Geornaras et al. *(74)* showed that pathogen numbers were reduced (1.8 log CFU/cm^2) by dipping frankfurters in LA (2.5%; 120 s) at 25°C. Despite reported interactions of LA concentration and temperature in reducing *E.* coli O157:H7 and *S.* Typhimurium on raw meat and poultry surfaces, in all published reports on control of *L. monocytogenes* on RTE meats, the chemical was exclusively used at ambient temperature.

Sodium lauryl sulfate

Sodium lauryl sulfate (SLS) is an anionic surfactant with a structural balance between a hydrophilic and hydrophobic group. Although not approved for use in meats, sodium lauryl sulfate (SLS) is a Generally Recognized as Safe (GRAS) food additive (0.001 to 0.5%) that is used as a wetting or whipping agent or as an emulsifier for vegetable oils, fruit juices, marshmallows and egg whites and other non-meat products *(63)*. SLS also is thought to damage the cell membranes and denature proteins of microorganisms when its activity is enhanced below pH 4.0 *(44)*. The bactericidal effect of SLS in combination with organic acids was documented against *S.* Typhimurium *(88, 190, 231)*, *Campylobacter jejuni (230),* and *E. coli* O157:H7 *(231)* on broiler skin. The chemical also is an effective ingredient of patented sanitizers for fresh produce *(189)* and surfaces in contact with foods *(162)*.

The primary mode of SLS antimicrobial action involves lysis of bacterial cells, general denaturation of proteins and enzymes, damage of cell membranes and changes in cell permeability *(2, 45, 86)*. The antibacterial effect of SLS increases at pH values below 4.0, with an optimum range between 1.5 and 3.0 *(44)*. As a surfactant, SLS may increase detachment of bacterial cells from surfaces by disruption of hydrophobic bonds and changing the conformation of the cell surface *(124, 137)*. Since microbial attachment varies among organisms and depends on magnitude of cell surface negative charge, surface hydrophobicity, extracellular polysaccharides and flagella *(52)*, the efficiency of the surfactant may vary as a function of these and other factors such as type of surface to which cells attach and concentration of the surfactant *(124)*.

Summary

Presence of *E. coli* O157:H7 and *L. monocytogenes* in meat and poultry products is of concern for regulatory agencies, public and processors. A need for the improvements in food safety has led to the intensified research. Still, there is a need for evaluation of the effect of brining ingredients and antimicrobials against *E. coli* O157:H7 in a brine-injected meat model system, development post-lethality interventions to control *L. monocytogenes* on frankfurters, and determination of the fate of *L. monocytogenes* on frankfurters during storage under variable conditions, to which the product may be exposed during the time between manufacturing and consumption.

CHAPTER 3

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

Abstract

Brine injection is used to increase the palatability of low-value meat cuts. *Escherichia coli* O157:H7 may become internalized during this process and cause human illness if meat is undercooked before consumption. The aim of this study was to evaluate the potential effect of brining ingredients on *E. coli* O157:H7 in a meat model system after simulated brining, storage, and cooking. Fresh beef knuckle or beef shoulder (2 replications, 3 samples/treatment/replication) were ground individually. Each type of meat (700 g batches) was mixed in a stand mixer with an 8-strain composite of rifampicin-resistant *E. coli* O157:H7 (7 ml) and brining solutions (63 ml; prepared in sterile distilled water, DW) to simulate product moisture-enhancement to 110% of initial weight. Samples (30 g) included no brining, DW, sodium chloride (NaCl, 0.5%; wt/wt), sodium tripolyphosphate (STP, 0.25%; wt/wt), sodium pyrophosphate (SPP, 0.25%; wt/wt), NaCl + STP, NaCl + SPP, NaCl + STP + potassium lactate (PL, 2%; wt/wt), NaCl + STP + sodium diacetate (SD, 0.15%; wt/wt), NaCl + STP + PL + SD, NaCl + STP + lactic acid (LA, 0.3%; vol/ wt), NaCl + STP + acetic acid (AA, 0.3%; vol/ wt),

NaCl + STP + citric acid (CA, 0.3%; wt/ wt), NaCl + STP + EDTA (20 mM; wt/wt) + nisin (0.0015%) or pediocin (1000 AU/g), NaCl + STP + sodium metasilicate (SM; 0.2%, wt/wt), NaCl + STP + cetylpyridinium chloride (CPC; 0.5%, vol/wt), and NaCl + STP + hops beta acids (HBA; 0.00055%, vol/wt). Samples were analyzed for pH, rifampicin-resistant *E. coli* O157:H7 (inoculum) on tryptic soy agar with 0.1% sodium pyruvate (TSAP) and 100 μg/ml rifampicin, and total microbial populations on TSAP immediately after mixing, storage (24 h at 4°C), and cooking to 65°C. Fat and moisture contents and water activity were measured after storage and cooking only. Cooking losses also were determined.

Fat contents of uninoculated knuckle and shoulder samples were 5.3 ± 2.4% and 15.3 ± 2.2%, respectively. Moisture contents were 72.3 ± 2.3% and 67.5 ± 4.0%, respectively. Overall, cooking tended to increase fat and decrease moisture content of ground knuckle samples, while the inverse effect was observed in ground beef shoulder samples. Cooking losses for NaCl + STP/SPP treatments were 13.1 ± 2.1 to 16.1 ± 3.0% and, in general, increased (20.7 ± 0.9 to 23.6 ± 1.1%) by organic acids, but decreased (0.7 ± 0.3 to 2.9 ± 1.2%) by PL or SM. The pH of uninoculated beef knuckle and beef shoulder were 5.75 ± 0.03 and 5.98 ± 0.04 units, respectively. Brining with acids, nisin or pediocin decreased ($P < 0.05$), while SM increased the pH of meat compared to NaCl + STP treated samples. In general, the pH did not change during storage, but increased ($P < 0.05$) after cooking. Water activity of uninoculated beef knuckle and beef shoulder samples was 0.985 ± 0.002 and 0.986 ± 0.003, respectively, and were decreased ($P < 0.05$) by PL. Total microbial counts were 3.3 ± 0.3 and 3.4 ± 0.2 log CFU/g for uninoculated beef knuckle and shoulder, respectively. The effect of beef type on

microbial counts, pH, and water activity was negligible. Overall, regardless of treatment and meat type, pathogen counts were similar to the total microbial counts. No reductions were obtained by brining solutions immediately or after storage, except for CPC, which reduced ($P < 0.05$) pathogen counts after storage by approximately 1 log-cycle. Cooking reduced 1.5 to 2.5 logs of the pathogen, while CPC-treated samples had the lowest ($P < 0.05$) counts compared to any other treatment. Other tested compounds did not influence thermal inactivation of the pathogen. These data may be useful in developing/improvement of brining recipes for control of *E. coli* O157:H7 in moisture-enhanced beef products.

Introduction

Most of a beef carcass is comprised of low-value muscle cuts *(129)*. Injection of such cuts with brines, or moisture enhancement, increases their tenderness and other palatability traits *(128)*. Therefore, consumers have a greater willingness to purchase injected beef compared to non-injected products *(151)*. Currently, approximately one out of four beef processors use enhancement solutions to improve the quality of low-value beef cuts *(136)*.

The interior of intact beef muscles is usually sterile; however, *Escherichia coli* O157:H7, if present on the surface of meat/injection needles or in brines, may become internalized during injection *(120, 184, 185, 203, 209)*. In 2002, the U.S. Department of Agriculture Food Safety and Inspection Service (USDA-FSIS) conducted a comparative risk assessment for intact (non-tenderized) and non-intact (tenderized) beef and concluded that risk of *E. coli* O157:H7 illness from both of these products is similar and

very low, as the pathogen does not typically survive thermal treatment applied during cooking *(204)*. However, injected beef products may not be labeled as "non-intact" *(120)*, and consumers may intentionally or unintentionally undercook them before consumption *(184)*. Because of this, *E. coli* O157:H7 has been involved in several outbreaks of infection in the United States traced to non-intact roasts or steaks *(41, 110, 209)*, which initiated costly recalls of potentially contaminated products *(206, 208, 212)*.

The USDA-FSIS has declared *E. coli* O157:H7 as an adulterant in all non-intact beef products, including those injected with brines, or beef cuts destined to be processed into non-intact products *(203)*. In 2005, the agency required that all non-intact beef processors re-assess their Hazard Analysis and Critical Control Point (HACCP) plans and revise their interventions for pathogen control *(209)*. These interventions may include use of antimicrobials in injection brines *(184)*.

Beef products are typically injected with solutions (7 to 15% pump rate; i.e., 107 to 115% of the initial weight), to contain 0.5% (wt/wt) of sodium chloride and 0.25% (wt/wt) of phosphates *(128)*. Food grade phosphates may inhibit growth of *E. coli* O157:H7 in liquid media *(141)*. However, at levels used in moisture-enhanced beef products, salt/phosphate combinations are generally ineffective as antimicrobials *(132, 222, 223)*. Other compounds, including organic acids and their salts, may be added to the solutions in order to improve microbial safety of non-intact beef *(130-132)*. Eilers et al. *(55)* reported that beef top round injected (10% pump rate) with lactic acid (3 M, pH 3) had lower aerobic plate counts compared to those injected with deionized water. Further, Mukherjee at al. *(130, 132)* showed that lactic (0.27%, wt/wt), citric (0.2% wt/wt) and acetic (0.3% vol/wt) acids enhanced heat destruction of *E. coli* O157:H7 in a model

system that simulated moisture-enhanced/restructured beef cooked to 60 or 65°C. Organic acid salts generally suppress growth of *E. coli* O157:H7 in beef *(222, 223)*; however, they may protect cells from thermal inactivation *(130)*.

Other ingredients, alone or in combination, that may potentially enhance pathogen inactivation during cooking of injected beef products include nisin, pediocin, sodium metasilicate, and cetylpyridinium chloride *(184)*. Nisin is an antimicrobial peptide that decreases the heat resistance of microorganisms in milk *(149, 161, 218, 226)* and it is approved by the USDA-FSIS for the in various ready-to eat (RTE) foods *(213)*. Pediocin is a bacteriocin that is currently not approved for food applications in the U.S., but it may potentially be used in combination with heat to effectively control pathogens in RTE meats *(42)*. Both of these bacteriocins are effective against Gram-positive bacteria, but the cell wall structure of Gram-negative bacteria is more complex, and does not allow the penetration of these compounds inside the cell *(68, 194)*. However, the antimicrobial spectrum of bacteriocins can be extended to Gram-negative pathogens, including *E. coli* O157:H7, when these antimicrobials are combined with compounds that permeate the outer membrane of bacteria. For example, chelating agents, such as food grade ethylene diamine tetra acetate (EDTA) or phosphates can bind Ca^{2+} and Mg^{2+} cations that stabilize lipopolysaccharide structures allowing penetration of bacteriocins inside the cell *(48, 68, 187, 191, 194)*.

Sodium metasilicate is approved by the USDA-FSIS as the component of marinades used for raw meat and poultry products, and as a surface treatment of beef carcasses, subprimals, and trimmings, and RTE meats *(213)*. Cetylpyridinium chloride (CPC) is approved as surface treatment of raw poultry carcasses either prior to or after

immersion in a chiller *(213)*. It was demonstrated that ground beef patties formulated from beef trimmings tumbled with sodium metasilicate or CPC had lower *E. coli* ATCC 11775 counts and better sensory properties compared to an untreated control *(152-154)*. Hops beta acids also are approved by the USDA-FSIS for use in casings and on RTE meats and in salad dressings used in refrigerated meat and poultry deli salads *(213)*. This compound demonstrated a promising antimicrobial effect against *Listeria monocytogenes* in broth medium *(178)* and in controlling the pathogen in RTE meats *(177)*.

Published reports regarding the effect of existing brining ingredients on the heat destruction of *E. coli* O157:H7 in brine-injected beef and its model systems are scarce. Further, there is a need for evaluation of antimicrobials as a part of brining formulation against *E. coli* O157:H7 during storage and cooking of moisture-enhanced beef products. Therefore, the objective of this study was to evaluate brining ingredients and antimicrobials for their potential effect on heat inactivation of *E. coli* O157:H7 in a moisture-enhanced beef model system. This system was comprised of two fat levels after simulated brine-injection, storage, and cooking to a medium-rare (65°C) degree of doneness. Data collected form this model system may be used for further studies, aiming to evaluate effects of chemicals on *E. coli* O157:H7 during cooking of injected meat cuts, using different cooking methods, cooking times, initial and endpoint temperatures, and product thicknesses.

Materials and Methods

Ground beef preparation

Fresh (approximately 72 h post mortem) beef knuckle (approximately 5% fat) or beef shoulder [approximately 15% fat; shoulder clod, IMPS/NAMP 114 *(135)*] were obtained from a meat packing plant in Northern Colorado. Each type of meat was individually ground through 0.95-cm and then 0.16-cm diameter plates (Hobart Mfg. Co., Troy, OH) in the Meat Science Laboratory, Department of Animal Sciences at Colorado State University. The meat was then transferred to the Pathogen Reduction Laboratory for inoculation, treatment and analyses.

Inoculum preparation

Rifampicin-resistant *E. coli* O157:H7 strains were generated *(97)* to enable tracking of the inoculum in meat samples and to detect cells injured by heat or brine ingredients and antimicrobials. Initial strains included ATCC 43888 (human clinical isolate), ATCC 43895 (hamburger isolate), ATCC 43895/ISEHGFP [hamburger isolate *(139)*; green fluorescent protein-positive strain)] and five strains previously isolated from cattle feces *(28)* including the following: C1-057, C1-072, C1-109, C1-154, and C1-158. The thermotolerance of rifampicin-resistant strains in tryptic soy broth (TSB; Difco, Becton Dickinson, Sparks, MD) at 60°C was similar to that of the initial strains (data not shown). Rifampicin-resistant strains were activated and subcultured individually (22 h at 35°C) in TSB supplemented with 100 μg/ml rifampicin (Sigma-Aldrich Inc., St. Louis, MO). Each culture (30 ml) was centrifuged, washed with phosphate buffered saline (PBS; pH 7.4; 0.2 g 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) and cell pellets were resuspended in 30 ml PBS. The eight strains were combined and used to inoculate the ground beef.

Sample preparation, inoculation and treatment

Ground meat (700 g) was mixed in a bowl-lift stand mixer (KitchenAid®, Professional 600, St. Joseph, MI) for 2 min at "speed 2" (55 revolutions per minute) with 7 ml of the inoculum (approximately 9 log CFU/ml) to achieve the inoculation level of 7 log CFU/g. Brining solutions (63 ml) were prepared fresh on the day of the experiment in sterile distilled water (DW) and then were added to the inoculated meat to simulate product moisture-enhancement to 110% of initial weight (10% pump rate). After addition, brining treatments were mixed with the meat for an additional 2 min at the same speed. The following ingredient combinations and their concentrations (on a finished product basis; wt/wt) were selected based on concentrations utilized by the industry, maximum allowable levels, and/or on published research:

1. Control (uninoculated)
2. Control (inoculated)
3. DW
4. Sodium chloride (NaCl, 0.5%; wt/wt; Fisher Scientific, Fair Lawn, NJ)
5. Sodium tripolyphosphate (STP, 0.25%; wt/wt; BK Giulini Corporation, Semi Valley, CA)
6. Sodium pyrophosphate (SPP, 0.25%; wt/wt; BK Giulini Corporation)
7. NaCl (0.5%) + STP (0.25%)
8. NaCl (0.5%) + SPP (0.25%)

9. NaCl (0.5%) + STP (0.25%) + potassium lactate (PL, 2%; wt/wt; PURAC America Inc., Lincolnshire, IL)

10. NaCl (0.5%) + STP (0.25%) + sodium diacetate (SD, 0.15%; wt/wt; Niacet Corporation, Niagara Falls, NY)

11. NaCl (0.5%) + STP (0.25%) + PL (2%) + SD (0.15%)

12. NaCl (0.5%) + STP (0.25%) + lactic acid (LA, 0.3%; vol/ wt; PURAC America Inc.)

13. NaCl (0.5%) + STP (0.25%) + acetic acid (AA, 0.3%; vol/ wt; EMD Chemicals Inc., Gibbstown, NJ)

14. NaCl (0.5%) + STP (0.25%) + citric acid (CA, 0.3%; wt/ wt; Fisher Scientific)

15. NaCl (0.5%) + STP (0.25%) + nisin (0.0015; added as Nisaplin®, kindly provided by Danisco USA Inc., New Century, KS) + EDTA (ethylene diamine tetra acetate, 20 mM; wt/wt; Fisher Scientific)

16. NaCl (0.5%) + STP (0.25%) + pediocin (1000 AU/g; added as ALTA™ 2341, kindly provided by Kerry Bio Science, Rochester, NY) + EDTA (20 mM)

17. NaCl (0.5%) + STP (0.25%) + sodium metasilicate (SM, 0.2%, wt/wt, added as AvGard® XP, kindly provided by Danisco USA Inc.)

18. NaCl (0.5%) + STP (0.25%) + cetylpyridinium chloride (CPC; 0.5%, vol/wt; added as Cecure™, kindly provided by Safe Foods Corporation, North Little Rock, AR)

19. NaCl (0.5%) + STP (0.25%) + hops beta acids (HBA; 0.00055%, vol/wt; kindly provided by S.S. Steiner Inc., New York, NY)

Inoculated and treated samples (30 g) were extruded into sterile plastic test tubes (2.5 × 10 cm) with a caulking gun (Facilities Maintenance, Colorado State University) and air pockets (when present) were removed, to ensure even cooking, by pressing the

meat with a spatula. Samples were either analyzed immediately after treatment or covered with sterile aluminum foil and stored (4°C, 24 h) to simulate brining. After storage, samples were cooked in a circulating water bath maintained at 75°C [cooking took approximately 12 min; injected steaks would likely be grilled/pan broiled, and it takes about 14 minutes for internal temperature of a 1.5 cm steak cooked on a grill to reach 65°C *(72)*] to an internal temperature of 65°C, simulating medium-rare degree of doneness of beef *(3)*. The temperature of the water in the water bath, and the internal temperature of samples intended for proximate analysis (Figure 3.1) was continuously monitored with thermocouples (Pico Technology Ltd., Cambridge, UK) and recorded with real-time data recording software (PicoScope 6, Pico Technology Ltd.). The water level in the water bath was maintained at about 2 cm above the level of ground beef in the tubes.

Microbial analyses

Samples were analyzed immediately after inoculation, after the 24-h storage period at 4°C, and after cooking. Samples were transferred into sterile bags (Whirl-Pack, Nasco, Modesto, CA) containing 70 ml of maximum recovery diluent (MRD; 0.85% of NaCl and 0.1% buffered peptone) and pummeled at speed of 8 strokes/s (Masticator, IUL Instruments, Barcelona, Spain) for 2 min. Serial dilutions of the homogenate were prepared in 0.1% buffered peptone water (Difco) and surface-plated on tryptic soy agar (Accumedia, Lansing, Mich.) with 0.1% sodium pyruvate (TSAP) and TSAP with 100 μg/ml rifampicin (Sigma Aldrich; TSAP+rif.) for enumeration of total microbial and rifampicin-resistant *E. coli* O157:H7 (inoculum) populations, respectively. Plates of both media were incubated at 35°C for 48 h.

Physico-chemical analyses

The pH (Denver Instruments, Arvada, CO) of plated samples was measured immediately after microbial analysis. Fat and moisture contents (AOAC International official methods [960.39 and 950.46.B, respectively]; *(8)*) and water activities (AquaLab model series 3, Decagon Devices, WA) were determined for samples before storage at 4°C (i.e., after inoculation and treatment) and after cooking. Cooking losses were determined by weighing separated fluid before the microbial analysis of the entire sample.

Statistical analysis

The study was repeated two times for each type of meat (i.e., two fat levels), with three individual samples analyzed at each sampling point. Bacterial counts were converted into log CFU/g before statistical analysis. Data were analyzed as a randomized complete block design testing for main effects of antimicrobials/ingredients, meat type, sampling time/cooking, and for interactions (antimicrobials/ingredients × meat type, antimicrobials/ingredients × sampling time/cooking, and meat type × sampling time/cooking) using PROC GLM procedure of SAS version 9.2. *(172)*. Tukey's Honestly Significant Differences test was used for multiple pairwise comparisons of the means *(172)*. Means were considered significantly different when P-values were less than 0.05.

Results and Discussion

Fat and moisture contents

Cooking times were similar for both types of meat. The average time (for both types of meat) for samples with initial internal temperature of 8 ± 2°C to reach 65°C (in a 75°C water bath) was 12.5 ± 0.4 min. Similarly, it was reported that it takes

approximately 14 min for the center of 1.5 cm beef steak to reach 65°C during cooking on a Sanyo® grill *(72)*. The temperature cooking profile for each type of meat averaged across treatments is shown on Figure 3.2.

Fat and moisture contents of uninoculated ground beef knuckle samples were 5.3 ± 2.4% and 72.3 ± 2.3%, respectively (Table 3.1). Similar values for ground beef knuckle were reported by Mukherjee et al. *(130, 132)*, and for beef top sirloin butt by Shao et al. *(176)*. Fat and moisture contents of uninoculated ground beef shoulder samples were 15.3 ± 2.2% and 67.5 ± 4.0%, respectively (Table 3.2). In the present study, none of the treatments affected ($P \geq 0.05$) fat or moisture contents of the samples. Similarly, Mukherjee et al. *(130)* reported that moisture contents of ground beef samples treated with organic acids and their salts varied within a narrow range, 71.7 ± 1.5 to 69.5 ± 0.4%. However, depending on experimental conditions, some organic acid salts may decrease the moisture content of meat. For example, Maca et al. *(122)* reported that cooked beef top round roasts injected (20% pump rate) with water containing 3% sodium lactate had 3.6% less moisture compared to water-treated control samples.

In general, fat content of beef knuckle samples tended to increase after cooking, but the increase was significant ($P < 0.05$) only for the NaCl + STP treatment. Cooking tended to decrease the moisture content, but the decrease was significant ($P < 0.05$) only for the NaCl + nisin + EDTA treatment. Other studies showed similar increases in fat content and decreases in moisture content during cooking of low-fat ground beef *(130, 199)*. In contrast with beef knuckle, beef shoulder fat content tended to decrease after cooking, while moisture content tended to increase. The fat content reduction, however, was significant ($P < 0.05$) only for the NaCl + STP treatment. Anderson and Berry *(4)*

also reported a decrease of fat content and increase in moisture content during cooking of high-fat ground beef.

Cooking losses

Cooking losses for DW-control beef knuckle and beef shoulder samples were 22.5 ± 0.8 and 19.9 ± 3.0%, respectively (Tables 3.1 and 3.2). The NaCl + STP/SPP treatments (without antimicrobials) decreased ($P < 0.05$) the cooking losses of beef knuckle samples; however, in beef shoulder samples, this decrease appeared insignificant ($P \geq 0.05$). Similarly, Shao et al. *(176)* reported that 1.5% NaCl plus 0.5% STP increased the cooking yield of restructured lean beef steaks which were pan-fried to a final temperature of 66°C. This was expected, as sodium chloride lowers the isoelectric point of meat proteins (about 5.2 to 5.3) and increases their solubilization, whereas sodium tripolyphosphate increases the pH, moving it further away form the isoelectric point *(128)*. In addition, sodium chloride improves the solubilization of meat proteins, which further increases the water holding capacity *(182)*.

When PL or sodium metasilicate (SM) were added, cooking losses decreased ($P < 0.05$) even further to 0.7 ± 0.3 to 2.9 ± 1.2%. Lactic, acetic and citric acids increased ($P < 0.05$) the moisture losses in beef knuckle samples; a similar trend ($P \geq 0.05$), was observed in ground beef shoulder samples. Similarly, Brewer and Novakofski *(22)* reported that cooking losses of a lean ground beef model system heated to 65°C were the highest when the pH of samples was close to the isoelectric point of meat proteins.

The pH of samples

The initial pH values of uninoculated ground beef knuckle and shoulder samples were 5.75 ± 0.03 and 5.98 ± 0.04, respectively (Tables 3.3 and 3.4). These pH values were within the normal range for ground beef.

Published reports on moisture enhancement of meat indicated that alkaline phosphates increased the pH of injected beef products *(13, 151)*. However, compared with published data, the numerical increases in pH in this study were insignificant. Brining treatments that contained lactic, acetic or citric acid, nisin + EDTA or pediocin + EDTA had lower ($P < 0.05$) initial pH values (5.23 ± 0.02 to 5.74 ± 0.39) than samples comprised of NaCl + STP only (5.94 ± 0.04 and 6.19 ± 0.08 for beef knuckle and beef shoulder, respectively). This was expected, as both acids and EDTA make aqueous solutions acidic *(27)*. Similarly, Mukherjee at al. *(130, 132)* reported that citric, lactic and acetic acids (0.2 to 0.3%) reduced the pH of ground beef. This also can explain the elevated cooking losses in samples treated with acids. Samples containing SM had the highest ($P < 0.05$) pH values (6.49 ± 0.14 and 6.83 ± 0.14, for beef knuckle and shoulder, respectively). Similarly, Quilo et al. *(155)* reported that ground beef patties made from beef trimmings that were tumbled (3 min 60 rpm) with 4% sodium metasilicate had higher pH values compared to patties prepared from untreated trimmings.

In general, the pH of samples did not change during storage at 4°C (24 h), but increased after cooking. This observation also was in agreement with previous reports, which demonstrated no changes in pH during storage (18 h at 4°C) of ground beef treated with organic acids and their salts, while there were increases in pH after cooking of

samples to 65°C *(130, 132)*. Other studies reported similar increases in pH during cooking of ground beef patties *(16, 199)*.

Water activity

The water activities of uninoculated ground beef knuckle and shoulder samples were 0.985 ± 0.002 and 0.986 ± 0.003, respectively (Tables 3.5 and 3.6), which were similar to what was previous reports *(121, 130)*. Johnston et al. *(94)* reported that water activities of ground beef chuck patties formulated with NaCl (1% wt/wt) + STP (0.25% wt/wt) were similar to those formulated with NaCl only, indicating that it was not affected by STP. However, it was demonstrated that NaCl (2.5% wt/wt) reduced the water activity of ground beef from 0.987 ± 0.001 (in control samples) to 0.957 ± 0.007 *(130)*. In our study, NaCl was used at a much lower concentration, 0.5%, and samples treated with NaCl + STP had only slightly lowered water activity compared to DW-control; in most cases, these reductions were not significant. However, samples containing PL had lower ($P < 0.05$) water activities (0.970 ± 0.000 to 0.973 ± 0.001) compared to any other treatment. This was expected, as numerous studies have demonstrated reductions in water activity of meat by lactate salts at levels similar or higher than those used in this study *(43, 121, 130, 147)*.

Overall, water activities of samples before storage were similar ($P \geq 0.05$) to those after cooking, except for the NaCl + STP + SM treatment (ground beef knuckle) which showed a lowered water activity, and NaCl +STP + pediocin + EDTA (ground beef shoulder) which showed an increased water activity following storage and cooking. These small changes may not have a practical significance, and their statistical significance may be explained by type I error, a phenomenon attributed to a multiple

comparisons test. Overall, results were somewhat similar to those reported by Mukherjee et al. *(130)*, which showed that the water activity of ground beef samples made with PL (1.8%, vol/wt) or NaCl (2.5%, wt/wt) decreased after storage, and then increased following heating to 65°C.

Microbial survival and inactivation

Total bacterial counts of uninoculated ground beef knuckle and shoulder samples were 3.3 ± 0.3 and 3.4 ± 0.2 log CFU/g, respectively (Tables 3.7 and 3.8). Rifampicin-resistant bacteria (representing *E. coli* O157:H7) were not detected (<0.52 log CFU/g) in uninoculated samples. In general, total bacterial and *E. coli* O157:H7 counts in inoculated samples were similar ($P < 0.05$), regardless of type of meat, sampling time, or antimicrobial/heat treatment. In contrary to this, Mukherjee et al. *(130, 132)* reported that *E. coli* O157:H7 reductions in ground beef following cooking in a water bath (to 60 or 65°C) were greater on selective media (modified eosin methylene blue agar plus novobiocin plus sorbitol *and* sorbitol MacConkey agar plus novobiocin plus potassium tellurite) compared to those on TSA. It was suggested *(130, 132)* that the higher reductions on selective media were due to microbial injuries by heat or antimicrobials. This phenomenon was not observed in our study, perhaps, because the selective medium used in this study (TSAP + rif) was not as harsh to the bacteria as selective media used in the studies of Mukherjee et al. *(130, 132)*.

Faith et al. *(57)* reported greater destruction of *E. coli* O157:H7 during production of full-fat (32% fat) pepperoni compared to the reduced-fat (15%) type. Contrary to these findings, Line et al. *(118)* demonstrated that, during heating (to 52 or 57°C) in a water bath, inoculated high-fat ground beef (30.5% fat) had higher *D*-values for *E. coli*

O157:H7 compared to those observed in low-fat (2% fat) samples. In our study, fat level (type of beef) had no effect ($P \geq 0.05$) on bacterial (total and *E. coli* O157:H7) counts, with few exceptions where this effect was negligible and had no practical significance. For example, NaCl + STP + PL - treated and then cooked beef knuckle had higher ($P < 0.05$; minimum significant difference 0.02 log CFU/g) pathogen counts (5.6 ± 0.0 log CFU/g) compared to beef shoulder samples treated with the same brine (5.5 ± 0.1 log CFU/g). Overall, findings agree with those of Stoltenberg et al. *(188)* who reported that fat content (10% or 25%) did not affect *E. coli* O157:H7 counts during the production of beef snack sticks formulated with or without encapsulated citric or lactic acid.

It is well established that antimicrobials work better in liquids, where they are more evenly distributed and have a better contact with bacterial cells, compared to solid heterogeneous foods *(68, 194)*. In ground beef, bacterial cells can be entrapped by meat particles and therefore become protected from antimicrobials. Additionally, meat has a strong buffering capacity, the ability of meat to resist the change in pH *(108)*, diminishing antimicrobial properties of acidic and alkaline antimicrobials. In this study, no immediate (i.e., after inoculation and treatment) reductions in bacterial populations were obtained by any treatment. Likewise, Mukherjee et al. *(130, 132)* reported no immediate or after storage (4°C, 18 h) reduction of *E. coli* O157:H7 in ground beef treated with organic acids and their salts.

Studies have demonstrated antimicrobial effects of CPC against a wide range of bacteria, including *Salmonella* Typhimurium, *E. coli* O157:H7, and *L. monocytogenes* in inoculated beef products *(153, 180)*. Pohlman et al. *(153)* showed that ground beef patties manufactured from *E. coli* ATCC 11775 - inoculated beef trimmings, tumbled (3

min; 16 rpm) with CPC (0.5%), had 0.6 log CFU/g lower counts, compared to the untreated control. In this study, pathogen counts remained relatively unchanged after 24-h storage in all treatments, except for CPC, which reduced ($P < 0.05$) pathogen and total bacterial counts by approximately 1 log-cycle.

Cooking of samples to 65°C resulted in *E. coli* O157:H7 reductions of 1.5 to 2.5 log CFU/g, which is lower than previously reported reductions (4 to 5 log CFU/g) in ground beef heated to 65°C *(130, 132)*. In those reports, cooking time was approximately 19 min *(130, 132)*, while in our study, the internal temperature of meat reached 65°C faster, in 12.5 ± 0.4 min, as the temperature in the water bath in our study was 5°C higher than that used by Mukherjee et al. *(130, 132)*. Therefore, the shorter exposure time of the bacteria to heat may explain the lower inactivation rates.

The NaCl, STP, SPP, NaCl + STP/SPP, and NaCl + STP combined with PL, PL/SD, LA, AA, CA, SM, or HBA did not enhance or protect ($P \geq 0.05$) thermal destruction of *E. coli* O157:H7. As previously stated, each of these ingredients may show antimicrobial properties, depending on concentration and application method. However, they were not effective under the conditions of this study.

As mentioned, EDTA facilitates penetration of bacteriocins, such as nisin or pediocin into Gram-negative bacteria *(48, 68, 187, 191, 194)*. Previous studies demonstrated antimicrobial properties of nisin in combination with EDTA against *E. coli* O157:H7 *(187, 191)*. Other reports showed only minor effects of this combination against this pathogen in beef *(48)*. Nevertheless, several studies indicated that nisin increases heat sensitivity of bacteria in milk *(149, 161, 218, 226)*. In our work, nisin + EDTA - treated samples had lower ($P < 0.05$) counts compared to those for the NaCl +

STP treatment (without antimicrobials). Similar reduction (0.6 to 0.7 logs) was achieved in samples treated with pediocin + EDTA. Chen et al. *(42)* reported that spraying frankfurters with pediocin (3,000 or 6,000 AU) before inoculation with *L. monocytogenes* (3.4 or 5.2 log CFU/g) reduced pathogen levels by 1.5 to 2.1 logs, while subsequent in-bag heating in a water bath (71 to 96°C, 30 to 120 s) further reduced bacteria by up to 5.2 logs. It was suggested that a combination of pediocin and thermal treatment provided enhanced microbial reduction *(42)*, which was confirmed by our findings.

Cooked samples treated with CPC had the lowest ($P < 0.05$) microbial counts (3.8 ± 0.3 and 3.7 ± 0.3 log CFU/g for knuckle and shoulder, respectively) compared to any other sample. CPC is a quaternary ammonium compound, which kills or injures cells by disruption of cytoplasmic membrane, resulting in leakage of cell constituents *(127)*. It also forms ionized compounds that interfere with bacterial metabolism *(44)*. Perhaps, *E. coli* O157:H7 exposure to CPC during storage of samples destroyed some bacterial cells and sensitized others to the subsequent heat exposure. As indicated earlier, this compound is approved for spraying/dipping poultry carcasses, but it is not approved for beef *(213)*. However, our results showed that use of CPC in moisture-enhanced beef products may substantially increase their microbial safety.

Conclusions

Overall, under the conditions of this study, the effect of the fat content (5 to 15%) on heat inactivation of *E. coli* O157:H7 at 65°C was negligible. No immediate reduction in bacterial numbers was achieved by any treatment. Furthermore, after 24 h of storage at 4°C, bacterial counts were not affected by any treatment, except for CPC which reduced pathogen levels by approximately 1 log-cycle. Surviving pathogen numbers in

cooked samples were the lowest in samples treated with CPC, while nisin and pediocin also increased heat inactivation. Other tested compounds, including NaCl, phosphates, hops beta acids, organic acids and their salts, or SM did not influence thermal inactivation of the pathogen.

These data should be useful in development and/or optimization of brining formulations to control of *E. coli* O157:H7 in moisture-enhanced beef products. This data should also be useful in development or updating risk assessments of *E. coli* O157:H7 infections from contaminated moisture-enhanced meat.

Table 3.1. Fat and moisture contents and cooking losses (mean ± standard deviation) of inoculated (*E. coli* O157:H7; 7 log CFU/g) ground beef knuckle (approximately 5% fat) that was treated with brining solutions, and then analyzed immediately, and after cooking to 65°C.

Treatment	Fat content (%)		Moisture content (%)		Cooking loss (%)
	Before storage	After cooking	Before storage	After cooking	After cooking
Control (uninoculated)	5.3±2.4 a	ND	72.3±2.3 a	ND	ND
Control (inoculated)	4.0±0.9 aA	4.9±1.4 aA	73.9±0.7 aA	74.0±2.6 aA	16.3±1.4 def
Distilled water	4.2±2.0 aA	3.9±1.2 aA	76.1±0.6 aA	74.9±1.4 aA	22.5±0.8 a
NaCl	3.9±2.1 aA	4.6±1.6 aA	75.6±1.3 aA	73.8±1.4 aA	17.4±1.6 bcde
STP	4.0±1.6 aA	4.3±1.9 aA	75.2±1.3 aA	73.7±1.9 aA	18.1±1.3 bcd
SPP	4.4±0.7 aA	3.8±1.3 aA	74.4±1.0 aA	74.6±2.1 aA	20.3±2.2 abc
NaCl + STP	3.7±1.4 aA	4.6±1.6 aB	74.8±1.9 aA	73.2±2.6 aA	13.1±2.1 def
NaCl + SPP	3.5±1.4 aA	3.9±1.4 aA	76.9±5.7 aA	73.7±2.3 aA	14.5±1.7 ef
NaCl + STP + PL	3.6±2.2 aA	4.1±1.6 aA	73.0±1.3 aA	73.0±0.7 aA	2.7±2.3 g
NaCl + STP + SD	3.6±1.5 aA	4.2±1.1 aA	74.6±1.8 aA	73.8±1.9 aA	14.5±2.0 def
NaCl + STP + PL + SD	3.4±2.3 aA	4.4±2.5 aA	73.4±1.3 aA	71.6±2.1 aA	2.9±1.2 g
NaCl + STP + LA	4.0±1.8 aA	4.7±1.8 aA	74.8±2.0 aA	73.2±2.3 aA	23.4±2.5 a
NaCl + STP + AA	3.7±1.5 aA	4.2±2.5 aA	75.5±0.9 aA	74.2±2.2 aA	23.6±1.1 a
NaCl + STP + CA	3.9±1.5 aA	4.9±2.0 aA	74.0±1.1 aA	72.6±2.9 aA	20.7±0.9 ab
NaCl + STP + nisin + EDTA	3.4±2.7 aA	5.1±1.6 aA	74.3±1.4 aA	72.2±0.5 aB	16.7±1.4 cdef
NaCl + STP + pediocin + EDTA	3.4±1.3 aA	3.9±1.7 aA	75.2±2.7 aA	73.7±1.6 aA	16.2±1.6 def
NaCl + STP + SM	4.0±2.3 aA	3.8±2.1 aA	73.8±1.9 aA	74.4±1.7 aA	1.4±0.8 g
NaCl + STP + CPC	4.0±3.9 aA	5.3±2.2 aA	76.6±6.9 aA	72.0±1.4 aA	14.4±1.1 def
NaCl + STP + HBA	4.4±2.9 aA	4.4±1.0 aA	74.0±2.8 aA	74.1±0.9 aA	13.6±1.3 ef

ND: Not done; NaCl: sodium chloride, 0.5%; STP: sodium tripolyphosphate, 0.25%; SPP: sodium pyrophosphate, 0.25%; PL: potassium lactate, 2%; SD: sodium diacetate, 0.15%; LA: lactic acid, 0.3%; AA: acetic acid, 0.3%; CA: citric acid, 0.3%; nisin: 0.0015%; EDTA: 20 mM; pediocin 1000 AU/g; SM: sodium metasilicate, 0.2%; CPC: cetylpyridinium chloride, 0.5%; HBA: hops beta acids, 0.00055%.

Mean values with different lowercase letters in the same column are significantly different ($P < 0.05$).

Mean values with different uppercase letters in the same row (within the same type of response variable) are significantly different ($P < 0.05$)

Table 3.2. Fat and moisture contents and cooking losses (mean ± standard deviation) of inoculated (*E. coli* O157:H7; 7 log CFU/g) ground beef shoulder (approximately 15% fat) that was treated with brining solutions, and then analyzed immediately, and after cooking to 65°C.

Treatment	Fat content (%)		Moisture content (%)		Cooking loss (%)
	Before storage	After cooking	Before storage	After cooking	After cooking
Control (uninoculated)	15.3±2.2 a	ND	67.5±4.0 a	ND	ND
Control (inoculated)	14.6±2.4 aA	13.9±1.3 aA	68.4±2.9 aA	68.6±1.0 aA	19.9±2.8 abc
Distilled water	13.9±1.6 aA	13.0±1.3 aA	69.8±2.4 aA	68.9±1.0 aA	19.9±3.0 abc
NaCl	14.0±1.7 aA	14.5±2.8 aA	69.8±2.2 aA	67.5±2.6 aA	16.2±3.0 abc
STP	14.6±2.6 aA	10.7±2.4 aA	70.1±3.0 aA	70.6±1.8 aA	16.2±1.3 abc
SPP	13.1±2.7 aA	11.3±1.2 aA	69.6±0.9 aA	69.6±1.2 aA	20.0±4.1 ab
NaCl + STP	16.0±1.8 aA	12.3±1.3 aB	68.4±5.4 aA	69.2±1.0 aA	14.3±4.1 abc
NaCl + SPP	11.9±1.7 aA	10.4±1.8 aA	71.6±1.3 aA	70.9±0.7 aA	16.1±3.0 abc
NaCl + STP + PL	14.1±3.0 aA	11.4±1.8 aA	69.1±3.4 aA	70.5±1.4 aA	1.2±1.0 d
NaCl + STP + SD	15.1±1.8 aA	13.0±1.5 aA	69.6±2.0 aA	69.2±1.0 aA	16.7±1.8 abc
NaCl + STP + PL + SD	16.0±1.8 aA	11.8±2.0 aA	67.6±2.7 aA	69.0±1.1 aA	1.3±1.0 d
NaCl + STP + LA	12.8±1.2 aA	12.6±3.1 aA	70.5±2.1 aA	67.9±2.3 aA	23.1±4.5 a
NaCl + STP + AA	13.4±1.3 aA	11.8±1.9 aA	69.7±3.0 aA	68.7±1.3 aA	22.3±1.3 a
NaCl + STP + CA	14.4±2.9 aA	12.6±0.7 aA	69.4±3.4 aA	68.7±1.0 aA	22.4±2.4 a
NaCl + STP + nisin + EDTA	16.0±2.2 aA	12.7±1.8 aA	68.4±2.7 aA	68.4±0.9 aA	19.4±2.7 abc
NaCl + STP + pediocin + EDTA	12.2±2.7 aA	11.5±1.6 aA	69.8±2.3 aA	69.6±1.2 aA	19.1±2.2 abc
NaCl + STP + SM	13.2±1.8 aA	12.9±2.3 aA	69.6±2.5 aA	70.0±2.1 aA	0.7±0.3 d
NaCl + STP + CPC	15.3±1.9 aA	12.7±1.7 aA	68.6±3.0 aA	69.1±2.0 aA	12.1±1.9 bc
NaCl + STP + HBA	14.8±1.8 aA	12.8±1.6 aA	69.5±2.3 aA	69.5±0.9 aA	10.7±3.3 c

ND: Not done; NaCl: sodium chloride, 0.5%; STP: sodium tripolyphosphate, 0.25%; SPP: sodium pyrophosphate, 0.25%; PL: potassium lactate, 2%; SD: sodium diacetate, 0.15%; LA: lactic acid, 0.3%; AA: acetic acid, 0.3%; CA: citric acid, 0.3%; nisin: 0.0015%; EDTA: 20 mM; pediocin 1000 AU/g; SM: sodium metasilicate, 0.2%; CPC: cetylpyridinium chloride, 0.5%; HBA: hops beta acids, 0.00055%.

Mean values with different lowercase letters in the same column are significantly different ($P < 0.05$).

Mean values with different uppercase letters in the same row (within the same type of response variable) are significantly different ($P < 0.05$).

Table 3.3. The pH values (mean ± standard deviation) of inoculated (*E. coli* O157:H7; 7 log CFU/g) ground beef knuckle (approximately 5% fat) that was treated with brining solutions, and then analyzed immediately, after storage at 4°C for 24 h, and after cooking to 65°C.

Treatment	Before storage	After storage	After cooking
Control (uninoculated)	5.75±0.03 bcde	ND	ND
Control (inoculated)	5.75±0.02 bcdeB	5.69±0.06 dB	5.98±0.03 fgA
Distilled water	5.74±0.03 cdeB	5.71±0.06 dB	5.98±0.02 gA
NaCl	5.71±0.01 cdeB	5.70±0.03 dB	6.01±0.03 efgA
STP	5.91±0.02 bcB	5.87±0.02 bcC	6.10±0.02 cdA
SPP	5.84±0.02 bcdeB	5.77±0.02 cdC	6.02±0.02 defgA
NaCl + STP	5.94±0.04 bcB	5.91±0.04 bcB	6.15±0.04 bcA
NaCl + SPP	5.90±0.04 bcdB	5.80±0.04 cdC	6.07±0.03 cdefA
NaCl + STP + PL	5.97±0.06 bB	5.97±0.05 bB	6.20±0.03 bA
NaCl + STP + SD	5.82±0.03 bcdeB	5.79±0.03 cdB	6.03±0.04 defgA
NaCl + STP + PL + SD	5.85±0.02 bcdeB	5.87±0.03 bcB	6.07±0.04 cdeA
NaCl + STP + LA	5.27±0.09 fB	5.34±0.09 eB	5.76±0.38 iA
NaCl + STP + AA	5.23±0.02 fB	5.25±0.03 eB	5.43±0.03 kA
NaCl + STP + CA	5.30±0.02 fB	5.33±0.04 eB	5.53±0.03 jA
NaCl + STP + nisin + EDTA	5.66±0.04 eB	5.67±0.04 dB	5.88±0.04 hA
NaCl + STP + pediocin + EDTA	5.67±0.06 deB	5.66±0.04 dB	5.87±0.04 hA
NaCl + STP + SM	6.49±0.14 aA	6.38±0.07 aA	6.47±0.06 aA
NaCl + STP + CPC	5.88±0.03 bcdeB	5.89±0.03 bcB	6.10±0.02 cdA
NaCl + STP + HBA	5.93±0.02 bcB	5.92±0.03 bcB	6.15±0.06 bcA

ND: Not done; NaCl: sodium chloride, 0.5%; STP: sodium tripolyphosphate, 0.25%; SPP: sodium pyrophosphate, 0.25%; PL: potassium lactate, 2%; SD: sodium diacetate, 0.15%; LA: lactic acid, 0.3%; AA: acetic acid, 0.3%; CA: citric acid, 0.3%; nisin: 0.0015%; EDTA: 20 mM; pediocin 1000 AU/g; SM: sodium metasilicate, 0.2%; CPC: cetylpyridinium chloride, 0.5%; HBA: hops beta acids, 0.00055%.
Mean values with different lowercase letters in the same column are significantly different ($P < 0.05$).
Mean values with different uppercase letters in the same row are significantly different ($P < 0.05$).

Table 3.4. The pH values (mean ± standard deviation) of inoculated (*E. coli* O157:H7; 7 log CFU/g) ground beef shoulder (approximately 15% fat) that was treated with brining solutions, and then analyzed immediately, after storage at 4°C for 24 h, and after cooking to 65°C.

Treatment	Before storage	After storage	After cooking
Control (uninoculated)	5.98±0.04 bcd	ND	ND
Control (inoculated)	5.89±0.04 bcdB	5.97±0.09 bcdeB	6.24±0.09 bcdA
Distilled water	5.91±0.04 bcdB	6.07±0.23 bcdAB	6.27±0.14 bcdA
NaCl	5.92±0.02 bcdB	5.98±0.08 bcdeB	6.29±0.13 bcdA
STP	6.08±0.06 bcdB	6.12±0.03 bcdB	6.37±0.10 bcA
SPP	6.07±0.05 bcdB	6.06±0.05 bcdB	6.31±0.06 bcdA
NaCl + STP	6.19±0.08 bB	6.21±0.07 bcB	6.39±0.10 bcA
NaCl + SPP	6.07±0.06 bcdB	6.06±0.05 bcdB	6.32±0.07 bcdA
NaCl + STP + PL	6.20±0.07 bB	6.27±0.07 bB	6.49±0.08 abA
NaCl + STP + SD	5.90±0.11 bcdB	5.89±0.20 bcdeB	6.18±0.03 bcdA
NaCl + STP + PL + SD	5.95±0.10 bcdC	6.05±0.03 bcdB	6.24±0.02 bcdA
NaCl + STP + LA	5.74±0.39 cdeA	5.61±0.16 deA	5.89±0.22 deA
NaCl + STP + AA	5.43±0.04 eB	5.46±0.05 eB	5.70±0.03 eA
NaCl + STP + CA	5.41±0.05 eB	5.45±0.12 eB	5.71±0.17 eA
NaCl + STP + nisin + EDTA	5.69±0.27 deA	5.71±0.19 cdeA	5.90±0.17 deA
NaCl + STP + pediocin + EDTA	5.69±0.22 deB	5.74±0.14 bcdeAB	5.97±0.08 cdeA
NaCl + STP + SM	6.83±0.14 aA	6.83±0.04 aA	6.93±0.04 aA
NaCl + STP + CPC	6.15±0.06 bcB	6.13±0.05 bcdB	6.32±0.04 bcdA
NaCl + STP + HBA	6.19±0.04 bA	6.01±0.43 bcdA	6.38±0.06 bcA

ND: Not done; NaCl: sodium chloride, 0.5%; STP: sodium tripolyphosphate, 0.25%; SPP: sodium pyrophosphate, 0.25%; PL: potassium lactate, 2%; SD: sodium diacetate, 0.15%; LA: lactic acid, 0.3%; AA: acetic acid, 0.3%; CA: citric acid, 0.3%; nisin: 0.0015%; EDTA: 20 mM; pediocin 1000 AU/g; SM: sodium metasilicate, 0.2%; CPC: cetylpyridinium chloride, 0.5%; HBA: hops beta acids, 0.00055%.
Mean values with different lowercase letters in the same column are significantly different ($P < 0.05$).
Mean values with different uppercase letters in the same row are significantly different ($P < 0.05$).

Table 3.5. Water activity values (mean ± standard deviation) of inoculated (*E. coli* O157:H7; 7 log CFU/g) ground beef knuckle (approximately 5% fat) that was treated with brining solutions, and then analyzed immediately, and after cooking to 65°C.

Treatment	Before storage	After cooking
Control (uninoculated)	0.985±0.002 ab	ND
Control (inoculated)	0.986±0.002 abA	0.983±0.002 aA
Distilled water	0.986±0.002 aA	0.984±0.004 aA
NaCl	0.984±0.003 abA	0.981±0.005 aA
STP	0.986±0.002 aA	0.984±0.004 aA
SPP	0.984±0.001 abA	0.983±0.005 aA
NaCl + STP	0.982±0.002 abA	0.981±0.006 aA
NaCl + SPP	0.981±0.002 abA	0.981±0.005 aA
NaCl + STP + PL	0.973±0.001 cA	0.970±0.004 bA
NaCl + STP + SD	0.981±0.003 abA	0.977±0.002 abA
NaCl + STP + PL + SD	0.971±0.002 cA	0.970±0.004 bA
NaCl + STP + LA	0.982±0.002 abA	0.980±0.003 aA
NaCl + STP + AA	0.981±0.003 abA	0.978±0.001 abA
NaCl + STP + CA	0.982±0.003 abA	0.977±0.002 abA
NaCl + STP + nisin + EDTA	0.981±0.002 abA	0.978±0.001 abA
NaCl + STP + pediocin + EDTA	0.980±0.002 abA	0.978±0.002 abA
NaCl + STP + SM	0.982±0.001 abA	0.979±0.003 abB
NaCl + STP + CPC	0.980±0.001 bA	0.978±0.002 abA
NaCl + STP + HBA	0.980±0.005 abA	0.979±0.001 aA

ND: Not done; NaCl: sodium chloride, 0.5%; STP: sodium tripolyphosphate, 0.25%; SPP: sodium pyrophosphate, 0.25%; PL: potassium lactate, 2%; SD: sodium diacetate, 0.15%; LA: lactic acid, 0.3%; AA: acetic acid, 0.3%; CA: citric acid, 0.3%; nisin: 0.0015%; EDTA: 20 mM; pediocin 1000 AU/g; SM: sodium metasilicate, 0.2%; CPC: cetylpyridinium chloride, 0.5%; HBA: hops beta acids, 0.00055%.
Mean values with different lowercase letters in the same column are significantly different ($P < 0.05$).
Mean values with different uppercase letters in the same row are significantly different ($P < 0.05$).

Table 3.6. Water activity values (mean ± standard deviation) of inoculated (*E. coli* O157:H7; 7 log CFU/g) ground beef shoulder (approximately 15% fat) that was treated with brining solutions, and then analyzed immediately, and after cooking to 65°C.

Treatment	Before storage	After cooking
Control (uninoculated)	0.986±0.003 aA	ND
Control (inoculated)	0.985±0.001 abA	0.986±0.006 abcA
Distilled water	0.986±0.001 aA	0.986±0.002 abcA
NaCl	0.982±0.001 abcdA	0.984±0.005 abcdA
STP	0.985±0.001 abcA	0.987±0.004 abA
SPP	0.985±0.001 abcA	0.988±0.004 aA
NaCl + STP	0.981±0.002 bcdA	0.982±0.004 bcdA
NaCl + SPP	0.982±0.001 abcdA	0.982±0.002 bcdA
NaCl + STP + PL	0.972±0.003 eA	0.973±0.004 eA
NaCl + STP + SD	0.980±0.002 dA	0.980±0.004 dA
NaCl + STP + PL + SD	0.970±0.000 eA	0.972±0.006 eA
NaCl + STP + LA	0.981±0.002 cdA	0.982±0.003 cdA
NaCl + STP + AA	0.982±0.001 abcdA	0.983±0.004 bcdA
NaCl + STP + CA	0.982±0.002 abcdA	0.983±0.003 abcdA
NaCl + STP + nisin + EDTA	0.981±0.001 abcdA	0.982±0.003 cdA
NaCl + STP + pediocin + EDTA	0.980±0.002 cdB	0.983±0.004 abcdA
NaCl + STP + SM	0.981±0.001 bcdA	0.982±0.004 bcdA
NaCl + STP + CPC	0.981±0.001 cdA	0.980±0.005 dA
NaCl + STP + HBA	0.982±0.001 abcdA	0.984±0.005 abcdA

ND: Not done; NaCl: sodium chloride, 0.5%; STP: sodium tripolyphosphate, 0.25%; SPP: sodium pyrophosphate, 0.25%; PL: potassium lactate, 2%; SD: sodium diacetate, 0.15%; LA: lactic acid, 0.3%; AA: acetic acid, 0.3%; CA: citric acid, 0.3%; nisin: 0.0015%; EDTA: 20 mM; pediocin 1000 AU/g; SM: sodium metasilicate, 0.2%; CPC: cetylpyridinium chloride, 0.5%; HBA: hops beta acids, 0.00055%.

Mean values with different lowercase letters in the same column are significantly different ($P < 0.05$).

Mean values with different uppercase letters in the same row are significantly different ($P < 0.05$).

Table 3.7. Total bacterial and *E. coli* O157:H7 populations (log CFU/g, mean ± standard deviation), recovered with tryptic soy agar supplemented with 0.1% pyruvate (TSAP) and TSAP supplemented with 100 µg/ml rifampicin (TSAP+rif) respectively, of inoculated (*E. coli* O157:H7; 7 log CFU/g) ground beef knuckle (approximately 5% fat) that was treated with brining solutions, and then analyzed immediately, after storage at 4°C for 24 h, and after cooking to 65°C.

Treatment	Bacterial populations (log CFU/g; mean ± standard deviation)					
	Before storage		After storage		After cooking	
	TSAP	TSAP+rif	TSAP	TSAP+rif	TSAP	TSAP+rif
Control (uninoculated)	3.3±0.3 b	<0.52	ND	ND	ND	ND
Control (inoculated)	7.3±0.1 Aa	7.2±0.1 aA	7.2±0.2 aA	7.1±0.1 aA	5.4±0.2 abcB	5.4±0.1 abB
Distilled water	7.2±0.1 aA	7.2±0.0 aA	7.2±0.1 aA	7.1±0.1 aA	5.1±0.2 bcdeB	5.1±0.2 abcdB
NaCl	7.3±0.1 aA	7.3±0.1 aA	7.1±0.1 aA	7.1±0.1 aA	5.4±0.2 abcB	5.4±0.1 abcB
STP	7.2±0.1 aA	7.2±0.1 aA	7.1±0.1 aA	7.1±0.1 aA	5.4±0.1 abcB	5.4±0.1 abcB
SPP	7.3±0.1 aA	7.3±0.1 Aa	7.1±0.1 aA	7.0±0.1 aA	5.3±0.2 abcdC	5.3±0.2 abcC
NaCl + STP	7.2±0.1 aA	7.3±0.1 aA	7.1±0.0 aA	7.1±0.0 aB	5.4±0.1 abcB	5.4±0.1 abC
NaCl + SPP	7.1±0.1 aA	7.1±0.2 aA	7.2±0.1 aA	7.2±0.1 aA	5.4±0.1 abcB	5.4±0.1 abcB
NaCl + STP + PL	7.1±0.1 aA	7.1±0.2 aA	7.2±0.1 aA	7.2±0.1 aA	5.6±0.0 aB	5.6±0.0 aB
NaCl + STP + SD	7.1±0.2 aA	7.2±0.3 aA	7.1±0.2 aA	7.1±0.1 aA	5.6±0.1 abB	5.6±0.1 aB
NaCl + STP + PL + SD	7.1±0.2 aA	7.2±0.3 aA	7.1±0.2 aA	7.1±0.1 aA	5.6±0.1 aB	5.6±0.1 aB
NaCl + STP + LA	7.2±0.1 aA	7.2±0.1 aA	7.1±0.1 aA	7.1±0.2 aA	5.0±0.2 cdefB	5.0±0.2 bcdB
NaCl + STP + AA	7.2±0.1 aA	7.2±0.1 aA	7.2±0.1 aA	7.2±0.0 aA	4.9±0.3 defB	4.9±0.2 cdB
NaCl + STP + CA	7.2±0.1 aA	7.3±0.1 aA	7.1±0.1 aA	7.1±0.1 aA	5.2±0.2 abcdB	5.1±0.3 abcdB
NaCl + STP + nisin + EDTA	7.3±0.2 aA	7.2±0.1 aA	7.1±0.1 aA	7.1±0.1 aA	4.7±0.4 fB	4.8±0.4 dB
NaCl + STP + pediocin + EDTA	7.2±0.1 aA	7.2±0.1 aA	7.1±0.2 aA	7.1±0.1 aA	4.7±0.2 efB	4.7±0.2 dB
NaCl + STP + SM	7.2±0.1 aA	7.2±0.1 aA	7.1±0.1 aA	7.1±0.1 aA	5.5±0.1 abB	5.5±0.1 aB
NaCl + STP + CPC	7.1±0.1 aA	7.1±0.1 aA	6.3±0.2 bB	6.3±0.2 bB	3.9±0.3 gC	3.8±0.3 eC
NaCl + STP + HBA	7.2±0.1 aA	7.2±0.1 aA	7.1±0.0 aA	7.0±0.1 aA	5.4±0.1 abcB	5.4±0.1 abcB

ND: Not done; NaCl: sodium chloride, 0.5%; STP: sodium tripolyphosphate, 0.25%; SPP: sodium pyrophosphate, 0.25%; PL: potassium lactate, 2%; SD: sodium diacetate, 0.15%; LA: lactic acid, 0.3%; AA: acetic acid, 0.3%; CA: citric acid, 0.3%; nisin: 0.0015%; EDTA: 20 mM; pediocin 1000 AU/g; SM: sodium metasilicate, 0.2%; CPC: cetylpyridinium chloride, 0.5%; HBA: hops beta acids, 0.00055%.

Mean values with different lowercase letters in the same column are significantly different ($P < 0.05$).

Mean values with different uppercase letters in the same row (within each plating medium) are significantly different ($P < 0.05$).

Table 3.8. Total bacterial and *E. coli* O157:H7 populations (log CFU/g, mean ± standard deviation), recovered with tryptic soy agar supplemented with 0.1% pyruvate (TSAP) and TSAP supplemented with 100 µg/ml rifampicin (TSAP+rif) respectively, of inoculated (*E. coli* O157:H7; 7 log CFU/g) ground beef shoulder (approximately 15% fat) that was treated with brining solutions, and then analyzed immediately, after storage at 4°C for 24 h, and after cooking to 65°C.

Treatment	Bacterial populations (log CFU/g; mean ± standard deviation)					
	Before storage		After storage		After cooking	
	TSAP	TSAP+rif	TSAP	TSAP+rif	TSAP	TSAP+rif
Control (uninoculated)	3.4±0.2 bA	<0.52 bB	ND	ND	ND	ND
Control (inoculated)	7.2±0.1 aA	7.2±0.1 aA	7.3±0.2 aA	7.3±0.2 aA	5.5±0.1 abcdB	5.5±0.1 abB
Distilled water	7.2±0.1 aA	7.2±0.1 aA	7.2±0.1 aA	7.2±0.1 aA	5.4±0.0 abcdB	5.3±0.1 abcdB
NaCl	7.2±0.2 aA	7.2±0.2 aA	7.1±0.1 aA	7.1±0.1 aA	5.4±0.1 abcdB	5.5±0.1 abB
STP	7.2±0.1 aA	7.2±0.1 aA	7.3±0.2 aA	7.2±0.2 aA	5.5±0.1 abcB	5.6±0.1 abB
SPP	7.2±0.1 aA	7.2±0.1 aA	7.1±0.0 aA	7.1±0.1 aA	5.5±0.0 abcdB	5.5±0.0 abB
NaCl + STP	7.2±0.1 aA	7.2±0.2 aA	7.3±0.2 aA	7.3±0.2 aA	5.5±0.1 abcB	5.6±0.1 abB
NaCl + SPP	7.1±0.1 aA	7.2±0.1 aA	7.3±0.1 aA	7.3±0.1 aA	5.4±0.1 abcdB	5.4±0.1 abcB
NaCl + STP + PL	7.1±0.1 aA	7.2±0.1 aA	7.1±0.1 aA	7.2±0.1 aA	5.5±0.1 abcB	5.5±0.1 abB
NaCl + STP + SD	7.1±0.1 aA	7.1±0.1 aA	7.2±0.2 aA	7.2±0.2 aA	5.7±0.1 aB	5.7±0.1 aB
NaCl + STP + PL + SD	7.2±0.2 aA	7.2±0.1 aA	7.2±0.1 aA	7.1±0.1 aA	5.5±0.1 abcdB	5.6±0.1 abB
NaCl + STP + LA	7.2±0.1 aA	7.1±0.1 aA	7.0±0.2 aA	7.1±0.2 aA	5.4±0.2 abcdB	5.4±0.2 abcB
NaCl + STP + AA	7.1±0.1 aA	7.1±0.1 aA	7.2±0.1 aA	7.2±0.1 aA	5.2±0.1 bcdeB	5.2±0.1 bcdB
NaCl + STP + CA	7.2±0.1 aA	7.2±0.1 aA	7.2±0.2 aA	7.2±0.2 aA	5.2±0.1 cdeB	5.2±0.1 bcdB
NaCl + STP + nisin + EDTA	7.1±0.1 aA	7.1±0.1 aA	7.2±0.2 aA	7.3±0.2 aA	4.9±0.2 eB	4.9±0.1 dB
NaCl + STP + pediocin + EDTA	7.2±0.1 aA	7.2±0.1 aA	7.2±0.1 aA	7.2±0.1 aA	5.1±0.1 deB	5.0±0.2 cdB
NaCl + STP + SM	7.2±0.1 aA	7.2±0.1 aA	7.2±0.2 aA	7.2±0.2 aA	5.6±0.1 abcB	5.6±0.1 abB
NaCl + STP + CPC	6.9±0.3 aA	6.9±0.4 aA	6.1±0.1 bB	6.1±0.1 bB	3.7±0.4 fC	3.7±0.3 eC
NaCl + STP + HBA	7.2±0.1 aA	7.1±0.1 aA	7.2±0.2 aA	7.2±0.2 aA	5.6±0.1 abB	5.6±0.1 abB

ND: Not done; NaCl: sodium chloride, 0.5%; STP: sodium tripolyphosphate, 0.25%; SPP: sodium pyrophosphate, 0.25%; PL: potassium lactate, 2%; SD: sodium diacetate, 0.15%; LA: lactic acid, 0.3%; AA: acetic acid, 0.3%; CA: citric acid, 0.3%; nisin: 0.0015%; EDTA: 20 mM; pediocin 1000 AU/g; SM: sodium metasilicate, 0.2%; CPC: cetylpyridinium chloride, 0.5%; HBA: hops beta acids, 0.00055%.

Mean values with different lowercase letters in the same column are significantly different ($P < 0.05$).

Mean values with different uppercase letters in the same row (within each plating medium) are significantly different ($P < 0.05$).

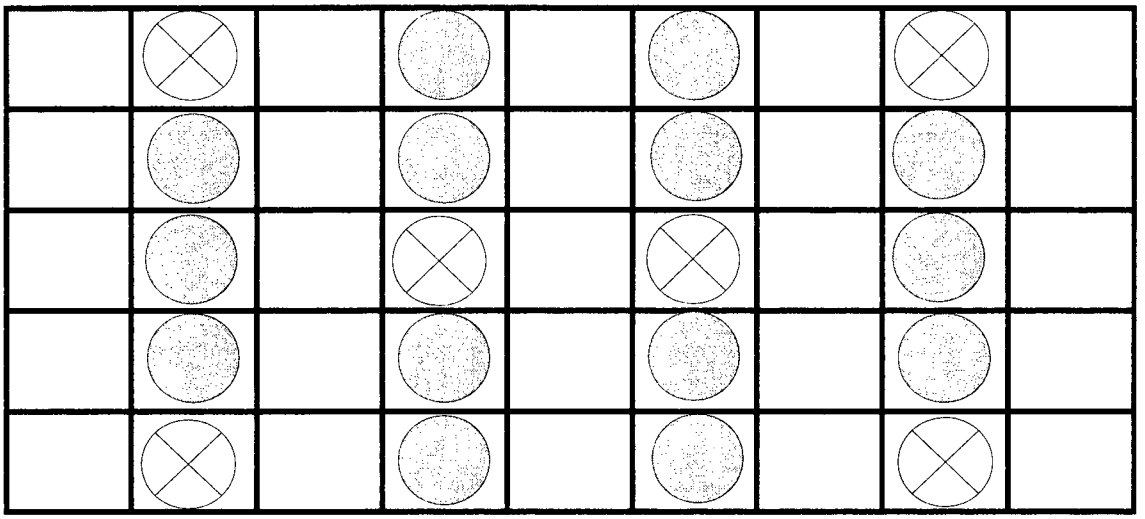


Figure 3.1. Schematic representation of the position of meat samples (indicated as circles) in the water bath during simulated cooking to an internal temperature of 65°C. Thermocouples were placed in meat samples intended for proximate analysis (circles with an ×).

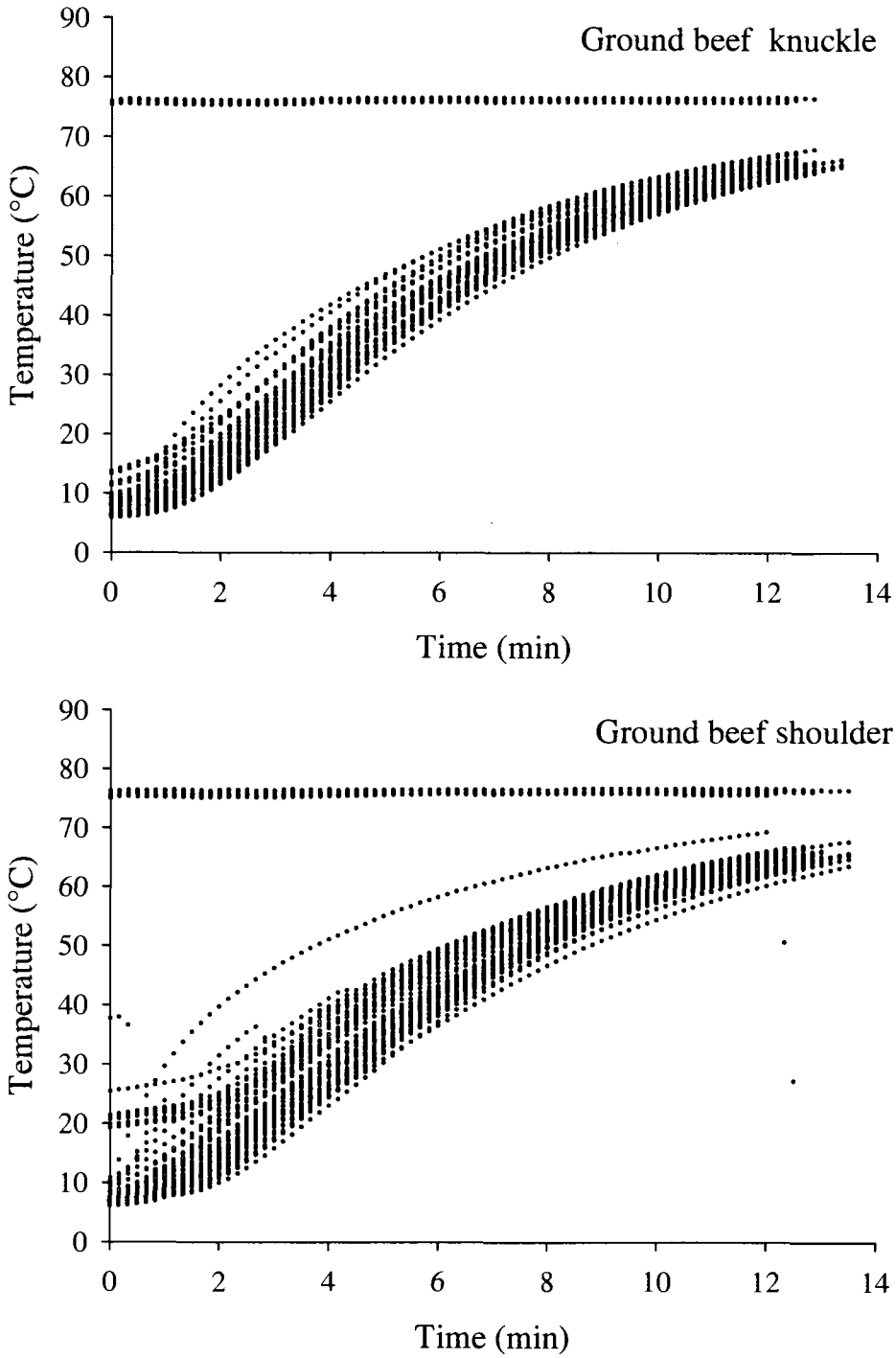


Figure 3.2. Cooking temperature profiles of ground beef shoulder and ground beef knuckle during heating in a water bath set at 75°C.

CHAPTER 4

Reduction of *Listeria monocytogenes* on Frankfurters by Lactic Acid Treatments Applied at Various Temperatures

Abstract

United States regulations require ready-to-eat meat and poultry processors to control *Listeria monocytogenes* using interventions that may include antimicrobials that reduce post-processing contamination by at least 1 log-cycle. If the treatment achieves ≥2 log reduction, the plant is subject to less frequent microbial testing. Lactic acid (LA) may be useful as a post-lethality intervention and its antimicrobial properties may increase with temperature of application. This study was conducted to evaluate the effect of LA solution temperature on *L. monocytogenes* counts on inoculated frankfurters and to identify parameters (temperature, concentration and time) that achieve 1 and 2 log-unit immediate reductions. Frankfurters (2 to 4 replications, 2-3 samples/treatment/replication) were surface-inoculated with a 10-strain mixture of *L. monocytogenes* (4.4 ± 0.1 log CFU/cm^2) and then dipped in distilled water (4, 25, 40, or 55°C) or LA (0 to 3%; 4, 25, 40, or 55°C) for 0 to 120 s. Samples were analyzed for pathogen (PALCAM agar) and total microbial counts (tryptic soy agar with 0.6% yeast extract). Data were analyzed by regression to evaluate the effects of tested conditions.

Distilled water, at any temperature, and LA applied at 4°C reduced pathogen numbers by approximately 1 log-cycle. Combinations delivering a 2-log reduction included 3% LA applied at 25°C for 120 s or 1% applied at 55°C for 60 s. The regression equation for *L. monocytogenes* reduction included ($P < 0.05$) effects of concentration, time, and temperature, and the interaction of concentration and temperature; other tested parameters (other interactions, quadratic and cubic terms) did not affect ($P \geq 0.05$) the reduction within the experimental range examined. Results indicated that effectiveness of LA against *L. monocytogenes* increased with solution temperature (in the range of 0.6 to 2.8 log CFU/cm^2). The developed equation may allow processors to vary conditions of treatment with LA to achieve a 1 or 2 log-unit reduction of the pathogen and comply with federal regulations.

Introduction

Among other ready-to-eat (RTE) foods, non-reheated frankfurters contaminated with *Listeria monocytogenes* have been implicated in outbreaks of listeriosis *(34, 35, 37)*. Generally, *L. monocytogenes* become inactivated by thermal treatment applied during processing of frankfurters *(229)*. However, the pathogen is widely distributed in the environment and may be present in food processing facilities and, therefore, contaminate frankfurters after thermal processing and during peeling or packaging *(197)*.

In processing facilities, 1.6% of packaged frankfurters are estimated to be contaminated with *L. monocytogenes (216)*. Frankfurters may support pathogen growth even in vacuum-packages and at refrigeration temperatures, and are frequently consumed without reheating *(59)*. Therefore, non-reheated frankfurters were classified as a "high

risk" product *(59)*. Currently, the Food Safety and Inspection Service of the U.S. Department of Agriculture (USDA-FSIS) enforces a "zero tolerance" rule for *L. monocytogenes* in RTE meat and poultry products *(201, 202)*. In 2003 the USDA-FSIS issued an interim final rule that requires food processors to control *L. monocytogenes* in RTE meat and poultry products, that support growth, using one out of three control alternatives *(207)*. Alternative 1 requires use of a post-lethality treatment *and* an antimicrobial agent or process capable of reducing numbers of *L. monocytogenes* or suppressing/limiting growth of the pathogen *(207)*. Those adopting Alternative 2 must use either a post-lethality treatment *or* an antimicrobial agent to control *L. monocytogenes (207)*. Processors that choose Alternative 3 are required to implement sanitation programs that control pathogen contamination and are subject to frequent USDA-FSIS environmental testing *(207)*. The stringency of the alternative approaches decreases from Alternative 1 to Alternative 3 *(207)*.

The USDA-FSIS also developed a compliance guideline to assist processors in meeting regulatory requirements of the final rule *(210)*. The guideline suggests that the post-lethality treatment must reduce the pathogen by at least 1 log-cycle, while processing plants employing treatments that reduce the pathogen by at least 2 log-cycles should be subject to less frequent microbial sampling and testing by USDA-FSIS *(210)*.

Lactic acid is a "Generally Recognized as Safe" (GRAS) cost-effective food additive which is commonly used for decontamination of beef carcasses, extension of shelf life, and pathogen control in perishable foods *(192)*. Lactic acid may also be used as a post-lethality surface treatment of RTE meat and poultry products to reduce numbers of *L. monocytogenes* and to prevent, delay or impede pathogen growth *(11, 73, 74, 140,*

145, 170, 183, 228). Reductions of bacterial populations on fresh meat treated with organic acids may increase with the duration of treatment and the temperature of solutions *(1, 85, 113, 215).* However, there are no published reports on the effect of lactic acid solution temperature on *L. monocytogenes* reduction on inoculated frankfurters.

The objective of this study was to evaluate the effect of lactic acid solution temperature on *L. monocytogenes* counts on inoculated frankfurters, and to identify parameters (temperature, concentration, and time) achieving 1 and 2 log-unit immediate (no storage) reductions.

Materials and Methods

Preparation of frankfurters

Frankfurters were prepared with fresh pork (70 to 72% lean) and beef (76 to 78% lean) shoulders at a 60:40 ratio of meat ingredients. Both types of meat were ground through a 0.79-cm grinder plate, and mixed *(168)* with ice, sodium chloride, dextrose, corn syrup solids, polyphosphate (Heller, Inc., Bedford Park, IL), sodium nitrite, sodium erythrobate, and spices (AC Legg Co., Birmingham, AL). The emulsion was prepared under vacuum (0.5 bar) in a bowl chopper (RMF, Kansas City, MO). The meat emulsion was then stuffed (Handmann, model VF 50, Biberach/Riss, Germany) into 22-mm peelable cellulose casings (Nojax® Viskase Co., Inc., Darien, IL) and linked at 7.3 ± 0.3 cm length. The linked product was weighed and then hung on racks and cooked in a humidity-controlled smokehouse (Alkar, DEC Intl. Inc., Lodi, WI). The cooking cycle consisted of the following steps: steam cooking for 10 min at 43°C and 68% relative humidity (RH) followed by 20 min at 63°C and 50% RH; hot smoking (liquid smoke;

Zesti Smoke, Monterrey, TN) for 30 min; cooking for 20 min at 63°C and 50% RH followed by 20 min at 71°C and 51% RH; cooking at 76°C and 100% RH until the internal temperature of the product reached 71.1°C, and showering with tap water (23 ± 2°C) for 20 min. Frankfurters were re-weighed to determine the cooking yield and then stored in a walk-in refrigerator at 4.0°C for 16 to 17 h, peeled, and kept frozen at -20°C for up to 3 months until used.

Inoculum preparation

The following *L. monocytogenes* strains were used for inoculation: N1-225 and N1-227 [serotype 4b, associated with 1998–1999 listeriosis outbreak traced to hot dogs *(34)*], 558 (serotype 1/2, pork meat isolate), NA-1 (serotype 3b, pork sausage isolate), N-7150 (serotype 3a, meat isolate), R2-500 and R2-501 [serotype 4b, associated with 2000 listeriosis outbreak linked to soft cheese *(36)*], and R2-763, R2-764 and R2-765 [serotype 4b, isolates associated with 2002 listeriosis outbreak linked to sliceable turkey deli meats *(37)*] *(67)*. Strains N1-225, N1-227, R2-500, R2-501, R2-763, R2-764, and R2-765 were kindly provided by Dr. M. Wiedmann (Cornell University, Ithaca, NY). Cultures were stored as frozen (-70°C) in tryptic soy broth (TSB; Difco, Becton Dickinson, Sparks, MD) supplemented with 0.6% yeast extract (YE; Acumedia, Lancing, MI) and 20% glycerol. Working cultures of each *L. monocytogenes* strain were prepared by transferring a loopful of stock culture into 10 ml of TSB (Difco) supplemented with 0.6% yeast extract (Difco; TSBYE) followed by incubation at 30°C for 22 h, and then subcultured twice under the same conditions. Then, 10 ml of each culture were transferred into individual sterile NALGENE® Oak Ridge tubes (Nalgene, Nalge Nunc, Rochester, NY), and centrifuged (Eppendorf model 5810 R, Brinkman Instruments Inc.,

Westbury, N.Y., 4,629 × *g*, 15 min, 4°C). Cell pellets formed by centrifugation were resuspended in 10 ml of 0.1% phosphate buffered saline (PBS; pH 7.4; 0.2 g 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) and washed by centrifugation as described above. After washing, each culture pellet was resuspended in sterile frankfurter homogenate (10% wt/wt in distilled water) and then habituated for 72 h at 4°C *(117)*. Habituated cells were combined and serially diluted to approximately 10^7 log CFU/ml in frankfurter homogenate before inoculation.

Inoculation of frankfurters

The ten-strain mixture of *L. monocytogenes* inoculum was spread (0.2 ml/frankfurter) with a sterile bent glass rod over the surface of each frankfurter under a biological safety cabinet (Nuaire, model NU-425-400, Plymouth, MN). After inoculation (4.4 log CFU/cm^2), samples were covered with alcohol-sanitized aluminum foil, and stored at 4°C for 10 min to allow attachment of bacterial cells before dipping samples in treatment solutions.

Treatment of frankfurters

The study was comprised of two sets of treatment combinations; data from the first set was used for the development of an *initial* equation, while data from the second set was used to validate the *initial* equation. Both sets of data were combined for development of the *final* equation. All lactic acid solutions (LA; Birko Co., Denver, CO) were prepared fresh in sterile distilled water (DW; 4°C) on the day of the experiment, and kept in the refrigerator (4°C) or in a water bath (25, 40, or 55°C). For development of the *initial* equation, we used the following treatments: DW or LA at 4°C (1, 2 or 3%; 30 or 120 s), 25°C (1, 2, or 3%; 15, 30, 60, 120 s plus 1.5% for 30 and 60 s), and 55°C (1, 2 or

3%; 15, 30, 60, 120 s plus 1.5% for 15 and 30 s). For validation of the *initial* equation, we used a second set of data collected from samples dipped (15 or 30 s) in the following treatments: DW or LA (1, 2, or 3%) solutions at 25, 40, and 55°C; these conditions likely to be used by the industry. For each treatment, approximately 20 frankfurters were immersed in 1.5 liters of solutions in sterile mixing bowls at the specified temperature and for specific times. The temperature of solutions in bowls during sample treatment was monitored with sanitized alcohol thermometers. The temperature of treatment solutions (25, 40, and 55°C) was reduced by 1 to 3°C within the first 15 s of dipping frankfurters (initial temperature of frankfurters was 4°C) and then remained relatively stable. The temperature of solutions applied at 4°C was not affected by immersion of frankfurters. After immersion, all samples were drained for 30 s before the analysis.

Microbiological analysis

Following draining, frankfurters were placed into sterile 24-oz bags (Whirl-Pak, Nasco, Modesto, CA) containing 50 ml of maximum recovery diluent (MRD; 0.85% of NaCl and 0.1% buffered peptone). Bags were manually shaken 30 times for approximately 30 s to detach the cells. Aliquots of appropriate dilutions were prepared in 0.1% buffered peptone water (Difco) and surface-plated onto PALCAM agar (Difco) or tryptic soy agar with 0.6% yeast extract (TSAYE) for enumeration of the pathogen and total microbial populations, respectively. Inoculated PALCAM agar plates were incubated at 30°C for 48 h and typical colonies of *L. monocytogenes* were counted after incubation. The TSAYE plates were incubated at 25 ± 2°C for 72 h and all bacterial colonies were counted. The counts were converted into log CFU/cm^2.

Physico-chemical analyses

The pH of frankfurters in MRD was measured after sample pummeling (2 min; Masticator, IUL Instruments, Barcelona, Spain) using a pH meter fitted with a glass electrode (Denver Instruments, Arvada, Colo.). Water activity (a_w) values (AquaLab model series 3, Decagon Devices, WA) were determined on day-0. The fat and moisture contents of frankfurters were determined according to the AOAC International official methods (960.39 and 950.46.B, respectively) *(8).*

Data analysis

As stated, the study consisted of two sets of treatment combinations; data from the first set (four replications) was used for the development of an *initial* equation, while data from the second set (two replications) was used to validate the *initial* equation. Both sets of data were combined for development of the *final* equation. Each replication was performed using a new batch of frankfurters and 2 or 3 samples per treatment. Data were analyzed using PROC MIXED procedures of SAS, version 9.2 *(172)* to evaluate the main effects of tested conditions (concentration, temperature, and time) and their interactions (time × temperature, concentration × time, and concentration × temperature). A regression equation for the prediction of *L. monocytogenes* reductions was developed using PROC REG procedure of SAS. Parameters, considered while developing the model included linear, quadratic, and cubic terms of concentration, temperature, and time; and two-way interactions: time × temperature, concentration × time, and concentration × temperature. Stepwise, forward and backward model selection methods, semi-automated processes of building a model by adding or removing variables based on the *t*-statistics of their estimated coefficients, were used to evaluate statistical contribution of individual terms in the model. Then, a goodness-of-fit test was used via the PROC

RSREG procedure to determine if the model fit the data *(172)*. Equation, selected by this process was called the *initial* equation.

The *initial* model developed using a first set of data was validated visually (scatter plot of predicted vs. observed values) using a second set of data. To generate a *final* prediction equation, validation data were combined with data used for the *initial* equation development and the model was refit. The predicted-residual-sum-of squares (PRESS) procedure was used to assess precision of the developed equation *(143)*.

Results and Discussion

Physico-chemical characteristics

The cooking yield of frankfurters was 91 ± 2% and the water activity was 0.974 ± 0.005. The fat and moisture contents were 15.2 ± 2.4 and 58.9 ± 2.0%, respectively. Similar physico-chemical properties of frankfurters were previously reported by Shen et al. *(177)*. The initial pH of frankfurters was 6.04 ± 0.11 and it was reduced ($P < 0.05$) by lactic acid (LA; averaged across all other variables) (Table 4.1). The interaction of exposure time and concentration were important ($P < 0.05$) in explaining variability, indicating that extent of pH reduction by LA increased with time of dipping or solution temperature. Overall pH means, averaged across dipping times, of frankfurters treated with 3% LA and at 55°C were 5.84 ± 0.11 and 5.91 ± 0.12 units, respectively.

Microbial reductions

Initial *L. monocytogenes* (PALCAM agar) and total microbial counts (TSAYE) on inoculated control frankfurters were 4.4 ± 0.1 and 4.3 ± 0.2 log CFU/cm^2, respectively (Figures 4.1 and 4.2). Overall, *L. monocytogenes* and total microbial counts in treated

samples were similar and therefore, *L. monocytogenes* counts were used for model development. Regardless of dipping time, distilled water (DW) applied at any temperature and lactic acid (LA) at 4°C reduced numbers of *L. monocytogenes* by approximately 1 log CFU/cm^2 (Figure 4.1). At 4°C, reductions caused by LA were similar ($P \geq 0.05$) to those caused by DW, which indicated that most of the observed decrease in pathogen numbers caused by LA at low temperature could be due to the physical removal of the cells. A 2-log reduction was obtained with 3% LA at 25°C applied for 120 s, or by 1% at 55°C applied for 60 s (Figure 4.1). Such reduction, after in-plant validation for a specific product, may be desirable for RTE meat and poultry processors. In addition to the enhancement of product microbial safety, it may help them to become less frequently tested by USDA-FSIS. In general, magnitude of the reduction increased with LA concentration, temperature, and, to a lesser extent by dipping time (Figure 4.1).

Organic acids are known to disrupt the cytoplasmic membranes of bacterial cells *(23)*. It also was demonstrated that the uptake of undissociated lactate ions into *E. coli* O157:H7 and *E. coli* O162 incubated in Luria Bertoni broth supplemented with 100 mmol/l D,L-lactate (pH 3.8) at 5, 20 and 37°C increased with the temperature, resulting in higher pathogen inactivation rates *(113)*. Similarly, Venkitanarayanan et al. *(215)* reported that *E. coli* O157:H7 (five strain mixture) in 1.0 or 1.5% LA plus 0.1% H_2O_2 and 0.1% peptone water at 40°C was reduced from 6.5 log CFU/ml to undetectable levels (<10 CFU/ml) within 10 min, while it took twice as long to achieve the same reduction at 22°C. At 8°C, the combination of these chemicals reduced pathogen counts by only 2.5 log-cycles *(215)*. The increased penetration of lactate ions at elevated temperatures and

accompanying enhanced microbial inactivation is thought to be due alterations in cell membranes which facilitates such penetration *(95)*.

Equation development

The data from the first set of treatments was used for the *initial* model development. Results of the three types of regression model selection techniques used, forward, backward, and stepwise, all were consistent and delivered the same model. The selected model included ($P < 0.05$) terms of concentration, time, temperature, and an interaction of concentration and temperature. Other tested parameters (other interactions, quadratic and cubic terms) did not ($P \geq 0.05$) improve the equation, and therefore were not included. The selected *initial* equation was:

Reduction (log CFU/cm^2) = 0.602604 − (0.03518 × concentration) + (0.00362 × time) + (0.00684 × temperature) + (0.00944 × concentration × temperature).

The lack-of-fit test measures the variation of the data around the fitted model, and is thought to be a stringent tool for determining adequacy of a prediction equation *(46, 232)*. Gao et al. *(69)* estimated the optimal process parameters (temperature, pressure and pressure holding time) for a 6-log-unit reduction of *L. monocytogenes*, and successfully used the lack-of-fit test in determining if the observed inactivation rates agreed with predicted values. Similarly, the lack-of-fit test was used in the hazard characterization part of a *L. monocytogenes* risk assessment in ready-to-eat foods as a selection tool for appropriate equations for the development of a dose-response curve *(58)*. No ($P \geq 0.05$) lack-of-fit was detected in the present study, indicating that the developed equation fit the data. Further, the best-fit equation explained 86% of variability in the data (the coefficient of determination adjusted for the number of independent variables in the

regression model [R^2_{adj}] was 0.86), suggesting good agreement between predicted and observed values.

Equation validation using collected data

The *initial* equation was validated using data from the second set of treatments. Each observation in the second set was predicted using the *initial* equation, and predicted *vs.* observed values were plotted for visual assessment of the fit. The *L. monocytogenes* data used for model validation (visual comparison between predicted and observed reductions; Figure 4.3) are shown in Table 4.2. In this set of data, the magnitude of pathogen reduction also increased with LA concentration, temperature, and dipping time. As expected, at 40°C, pathogen reductions were higher than reductions caused by LA at 25°C, but were lower than those at 55°C. Overall, total microbial counts (Appendix Table 3) were similar to counts of *L. monocytogenes.*

The R^2_{adj} for the equation derived from the validation data was 0.77. The scatter plot of observed versus predicted reductions (Figure 4.3) showed that predicted reductions compared well with those measured, indicating that the equation provided a good description for the data used for its development. Ölmez and Aran *(142)*, Fernandez et al. *(65)*, Eifert et al. *(54)*, and Buchanan and Golden *(24)* also used scatter plots of observed versus predicted microbiological (including *L. monocytogenes*) data to measure the reliability of developed prediction equations. Although there is room for improvement of the developed equation, model validation indicated good reliability. In the 1-log reduction region, most points appeared close (above or below) to the line of equivalence, indicating that predicted reductions were similar to those obtained for the validation (Figure 4.3). In the 2-log reduction region, most points appeared below the

line of equivalence, indicating that at this region the model predicts slightly lower reductions than those obtained for model validation (Figure 4.3). This small underestimation in reductions of *L. monocytogenes* may provide an extra safety margin in commercial operations.

After validation, parameters of the *initial* model were re-estimated using both sets of data and the resulted equation was termed the *final* equation. The *final* equation was: Reduction (log CFU/cm^2) = 0.58980 − (0.04407 × concentration) + (0.00341 × time) + (0.00752 × temperature) + (0.01002 × concentration × temperature).

This model showed no lack-of-fit ($P \geq 0.05$), included the same parameters as the *initial* equation, but the coefficients changed slightly, and the R^2_{adj} was reduced from 0.86 to 0.82. The predicted residual sum of squares (PRESS) was 13.4, and the standard deviation of the prediction error, i.e. the estimated difference between predicted and actual values ([PRESS/n]^{½}) *(143)*, was 0.3 log CFU/cm^2. This indicates that based on the "empirical rule" *(143)*, 68, 95, and 99.7% of observed reductions will lie within 0.3, 0.6 and 0.9 log CFU/cm^2 from the predicted values, respectively. The response surface plots (Figure 4.4) show that predicted reductions of *L. monocytogenes* on frankfurters dipped in LA at 4°C were only slightly affected by dipping time and were not affected by LA concentration, but at 25, 40, or 55°C they increased with the magnitude of these parameters. Therefore, processors using LA to control *L. monocytogenes* on frankfurters should consider its application at elevated temperatures, provided that subsequent cooling is rapid and efficient. Predicted solution temperatures needed to achieve a 2 log-unit reduction are presented in Table 4.3.

Comparisons of predicted reductions with published data

All published reports on control of *L. monocytogenes* with organic acids on RTE meats describe studies where LA solutions were applied at ambient temperature, and only some of them provided numerical values (23 or 25°C). This does not allow for validation of the developed equation with published data; the equation need to be validated when such data become available. Nevertheless, some examples of published data on reductions of *L. monocytogenes* on various RTE meats compared with predicted log reductions by the *final* equation are presented in Table 4.4. Magnitudes of *L. monocytogenes* inactivation on frankfurters or smoked sausage from the literature generally agree with those predicted by the equation. Specifically, predicted reductions (1.6 to 1.7 log-units) were slightly below reductions (1.8 to 2.1 logs) shown by Barmpalia et al. *(11)* and Geornaras et al. *(74)*, but were above reductions (0.7 logs) demonstrated by Sofos et al. *(183)*.

The equation slightly overestimated reductions demonstrated by Palumbo et al. *(145)* (Table 4.4). This overestimation (observed: 1.0 to 1.6 *vs.* predicted 1.4 to 1.8 logs) was attributed to specific experimental conditions and product characteristics. For example, Palumbo et al. *(145)* did not report the temperature of dipping solutions. However, to compare predicted reductions with those from studies with no temperature indication, it was assumed that samples were dipped in solutions at 25°C. If the actual temperature of dipping solutions in those studies was 12 to 20°C, the predicted reductions at these temperatures are similar (data not shown) to the actual published values.

Unlike reported *L. monocytogenes* reductions by LA at ambient temperature on frankfurters or smoked sausage, which generally agree with those predicted by the

developed *final* equation (Table 4.4), the actual reductions on sliced bologna, ham, and turkey breast were much lower (0.2 to 0.5 logs) than the predicted values (1.2 to 1.5 logs). The discrepancy between observed reductions in sliced products and predicted reductions derived from data collected from frankfurters may be explained by the differences in topography and texture of product surfaces. Frankfurters or smoked sausages usually have a smooth and relatively firm surface which formed during thermal processing of products in a smokehouse. In contrast with these products, the surface of sliced products is relatively rough and porous. This allows for the penetration of cells into the sliced product matrix during inoculation and their entrapment in crevices, which may provide protection of cells from physical removal and from contact with antimicrobials, while this phenomenon seemed to be less pronounced on products with smoother surfaces.

Conclusions

Results indicated that the effectiveness of LA against *L. monocytogenes* on frankfurters increased with lactic acid solution concentration (1 to 3%), temperature (4 to 55°C), and to a lesser extent, by exposure time (15 to 120 sec). One log-unit of the pathogen may be physically removed by dipping frankfurters for at least 15 s in DW at any temperature. The developed *final* equation may allow processors to vary conditions (lactic acid concentration and temperature, and exposure time) of treatment of frankfurters to achieve a 2 log-unit reduction of the pathogen and comply with federal regulations. Provided that results of this study are validated in-plant, processors using warm (55°C) lactic acid (3% for 15 s or 2% for 30 s) for dipping frankfurters should be subject to less frequent USDA-FSIS verification testing, since a 2-log reduction can be achieved.

Table 4.1. The pH (mean ± standard deviation) of *L. monocytogenes*-inoculated frankfurters that were left undipped or dipped in distilled water (DW) or lactic acid (1.0, 2.0, and 3.0%) at 25, 40, or 55°C for 15 or 30 s.

Dipping time (s)	Lactic acid concentration (%)	Temperature of dipping solution (°C)		
		25	40	55
0 (Undipped)	—	6.04±0.11 aA	6.04±0.11 aA	6.04±0.11 aA
15	0 (DW)	6.03±0.08 aA	6.01±0.10 aA	6.01±0.07 abcA
	1	6.04±0.07 aA	5.99±0.05 abA	6.02±0.06 abA
	2	5.99±0.09 abA	5.95±0.08 abcA	5.93±0.10 abcdA
	3	5.97±0.10 abA	5.94±0.10 abcA	5.94±0.11 abcdA
30	0 (DW)	5.92±0.11 abA	5.94±0.09 abcA	5.88±0.10 cdeA
	1	5.92±0.12 abA	5.91±0.11 abcA	5.89±0.09 bcdeA
	2	5.89±0.09 bA	5.86±0.08 bcA	5.83±0.12 deA
	3	5.85±0.16 bA	5.83±0.11 cA	5.77±0.13 eA

Within a column, means lacking a common lowercase letter are significantly different ($P < 0.05$). Within a row, means lacking a common uppercase letter are significantly different ($P < 0.05$).

Table 4.2. Survivors of *L. monocytogenes* (PALCAM agar; log CFU/cm^2; mean ± standard deviation) on inoculated frankfurters that were left undipped or dipped in distilled water (DW) or lactic acid (1.0, 2.0, and 3.0%) at 25, 40, or 55°C for 15 or 30.

Dipping time (s)	Lactic acid concentration (%)	Temperature of dipping solution (°C)		
		25	40	55
0 (Undipped)	—	4.5±0.1 aA	4.5±0.1 aA	4.5±0.1 aA
15	0 (DW)	3.5±0.2 bA	3.4±0.5 bA	3.6±0.2 bA
	1	3.6±0.2 bcA	3.1±0.4 bcAB	2.9±0.2 cdB
	2	3.5±0.1 bcA	2.5±0.3 cdeB	2.1±0.3 efB
	3	3.1±0.4 bcA	2.3±0.4 deB	1.7±0.2 fB
30	0 (DW)	3.5±0.3 bcA	3.4±0.4 bA	3.2±0.2 bcA
	1	3.5±0.2 bcA	2.9±0.4 bcdAB	2.4±0.2 deB
	2	3.2±0.3 bcA	2.6±0.3 cdeAB	1.9±0.4 efB
	3	2.9±0.8 bcA	2.1±0.2 eB	1.7±0.4 fB

Within a column, means lacking a common lowercase letter are significantly different ($P < 0.05$). Within a row, means lacking a common uppercase letter are significantly different ($P < 0.05$).

Table 4.3. Predicted solution temperatures (°C) needed to achieve a 2 log-unit reduction of *L. monocytogenes* on surface-inoculated frankfurters by dipping in lactic acid (1.0 to 3.0%) for 15 to 120 s*.

Exposure time (s)	Lactic acid concentration (%)										
	1.0	1.2	1.4	1.6	1.8	2.0	2.2	2.4	2.6	2.8	3.0
15	>55	>55	>55	>55	>55	55	51	48	46	43	41
20	>55	>55	>55	>55	>55	54	51	48	45	43	41
25	>55	>55	>55	>55	>55	54	50	47	45	42	40
30	>55	>55	>55	>55	>55	53	49	47	44	42	40
35	>55	>55	>55	>55	>55	52	49	46	43	41	39
40	>55	>55	>55	>55	55	51	48	45	43	41	39
45	>55	>55	>55	>55	54	51	48	45	42	40	38
50	>55	>55	>55	>55	54	50	47	44	42	40	38
55	>55	>55	>55	>55	53	49	46	43	41	39	37
60	>55	>55	>55	>55	52	49	46	43	41	38	37
65	>55	>55	>55	>55	51	48	45	42	40	38	36
70	>55	>55	>55	55	51	47	44	42	39	37	36
75	>55	>55	>55	54	50	47	44	41	39	37	35
80	>55	>55	>55	53	49	46	43	40	38	36	35
85	>55	>55	>55	52	48	45	42	40	38	36	34
90	>55	>55	>55	51	48	44	42	39	37	35	33
95	>55	>55	55	51	47	44	41	39	36	35	33
100	>55	>55	54	50	46	43	40	38	36	34	32
105	>55	>55	53	49	45	42	40	37	35	34	32
110	>55	>55	52	48	45	42	39	37	35	33	31
115	>55	>55	51	47	44	41	38	36	34	32	31
120	>55**	55	50	46	43	40	38	36	34	32	30

*Reduction (log CFU/cm^2) = 0.58980 − (0.04407 × concentration) + (0.00341 × time) + (0.00752 × temperature) + + (0.01002 × concentration × temperature).

**To achieve a 2 log-unit reduction, lactic acid solution temperatures should be above those evaluated in the present study.

Table 4.4. Observed (published) *L. monocytogenes* reductions in inoculated RTE meat and poultry products dipped in lactic acid compared to those predicted by the equation developed in this study.

Product	Lactic acid concentration (%)	Dipping time (s)	Solution temperature (°C)	Reductions (log CFU)		Reference
				Reported	Predicted	
Frankfurters	2.5	120	23	2.1	1.6	Barmpalia et al. *(11)*
Smoked sausage	2.5	120	25	1.4	1.7	Geornaras et al. *(73)*
Frankfurters	2.5	120	25	1.8	1.7	Geornaras et al. *(74)*
Ham (sliced)	2.5	120	25	0.3	1.7	Geornaras et al. *(71)*
Beef/pork bologna (sliced)	2.5	120	25	0.4	1.7	Geornaras et al. *(71)*
Pork bologna (sliced)	2.5	60	—*	0.5	1.5	Samelis et al. *(170)*
Frankfurters	2	30	—	0.7	1.3	Sofos et al. *(183)*
Uncured turkey breast (sliced)	1	60	—	0.2	1.2	Sofos et al. *(183)*
Uncured turkey breast (sliced)	2	60	—	0.2	1.4	Sofos et al. *(183)*
Cured turkey breast (sliced)	2	60	—	0.5	1.4	Sofos et al. *(183)*
Pork bologna (sliced)	2	30	—	0.4	1.3	Sofos et al. *(183)*
Frankfurters	2	60	—	1	1.4	Palumbo et al. *(145)*
Frankfurters	2	120	—	1.2	1.6	Palumbo et al. *(145)*
Frankfurters	1.5	120	—	1.1	1.5	Palumbo et al. *(145)*
Frankfurters	3	120	—	1.6	1.8	Palumbo et al. *(145)*

*Temperature of dipping solutions was not indicated, though for the comparisons of observed (published) with predicted reductions it was assumed that solutions were at 25°C.

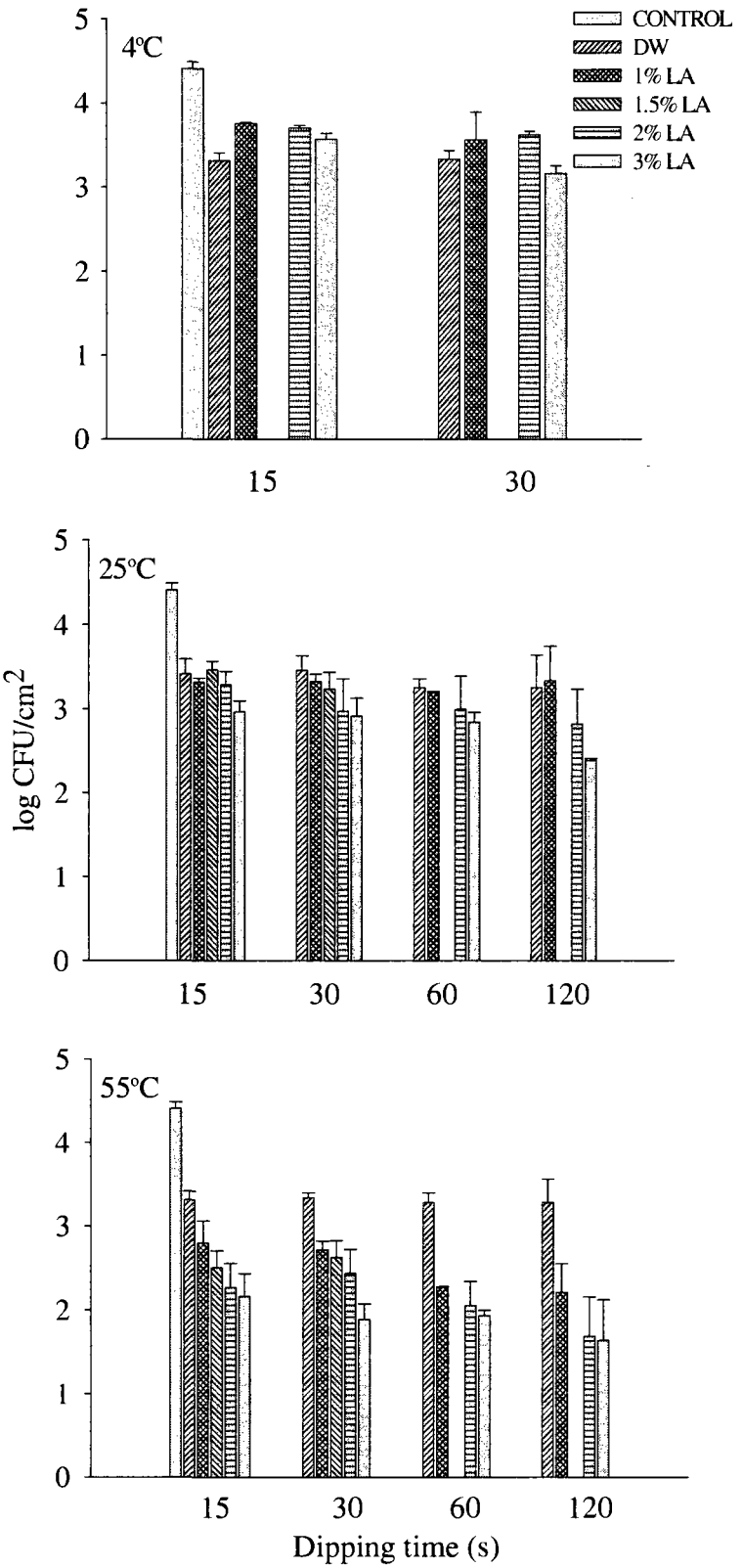


Figure 4.1 (Appendix Table 2). Survivors of *L. monocytogenes* (PALCAM agar) on inoculated frankfurters that were left undipped (control) or dipped in distilled water (DW), lactic acid (1.0, 1.5, 2.0, and 3.0%; LA) at 4, 25 or 55°C for 0 to 120 s. Bars show standard deviations.

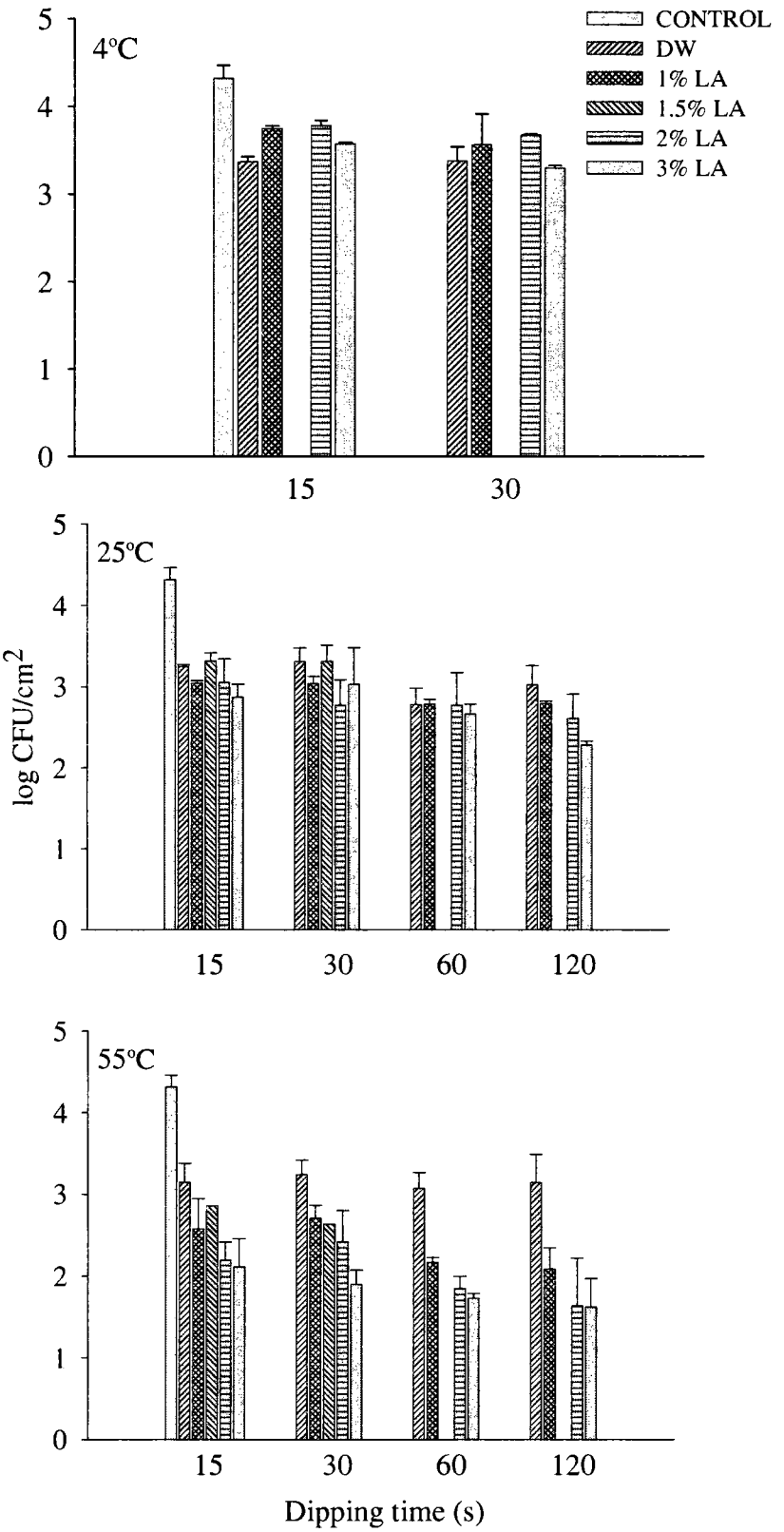


Figure 4.2 (Appendix Table 2). Survivors of total microbial populations (TSAYE) on inoculated frankfurters that were left undipped or dipped in distilled water (DW), lactic acid (1.0, 1.5, 2.0, and 3.0%; LA) at 4, 25 or 55°C for 0 to 120 s. Bars show standard deviations.

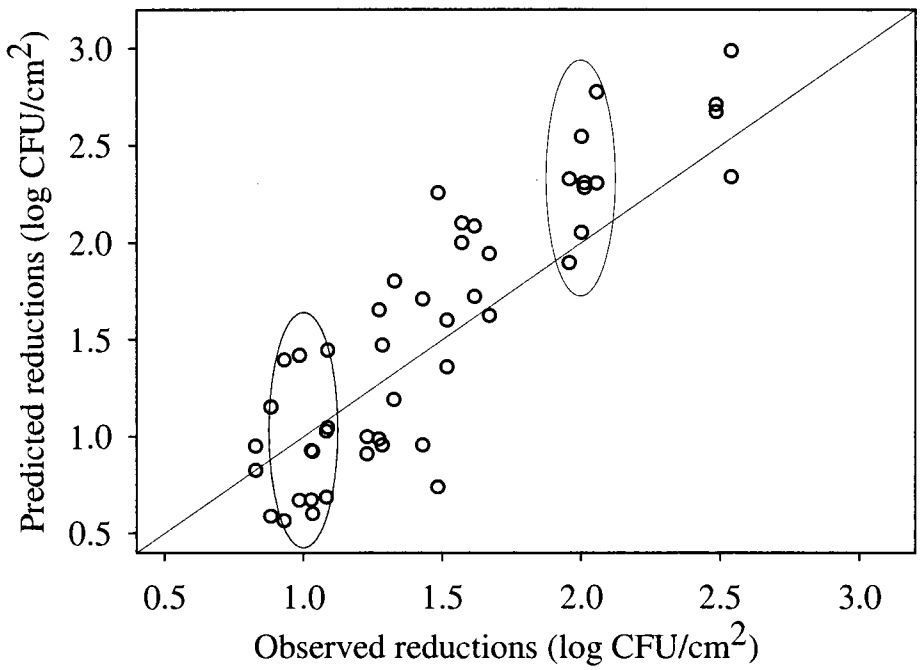


Figure 4.3. Comparisons of surviving populations of *L. monocytogenes* from observed data with predictions. The center line is the line of equivalence.

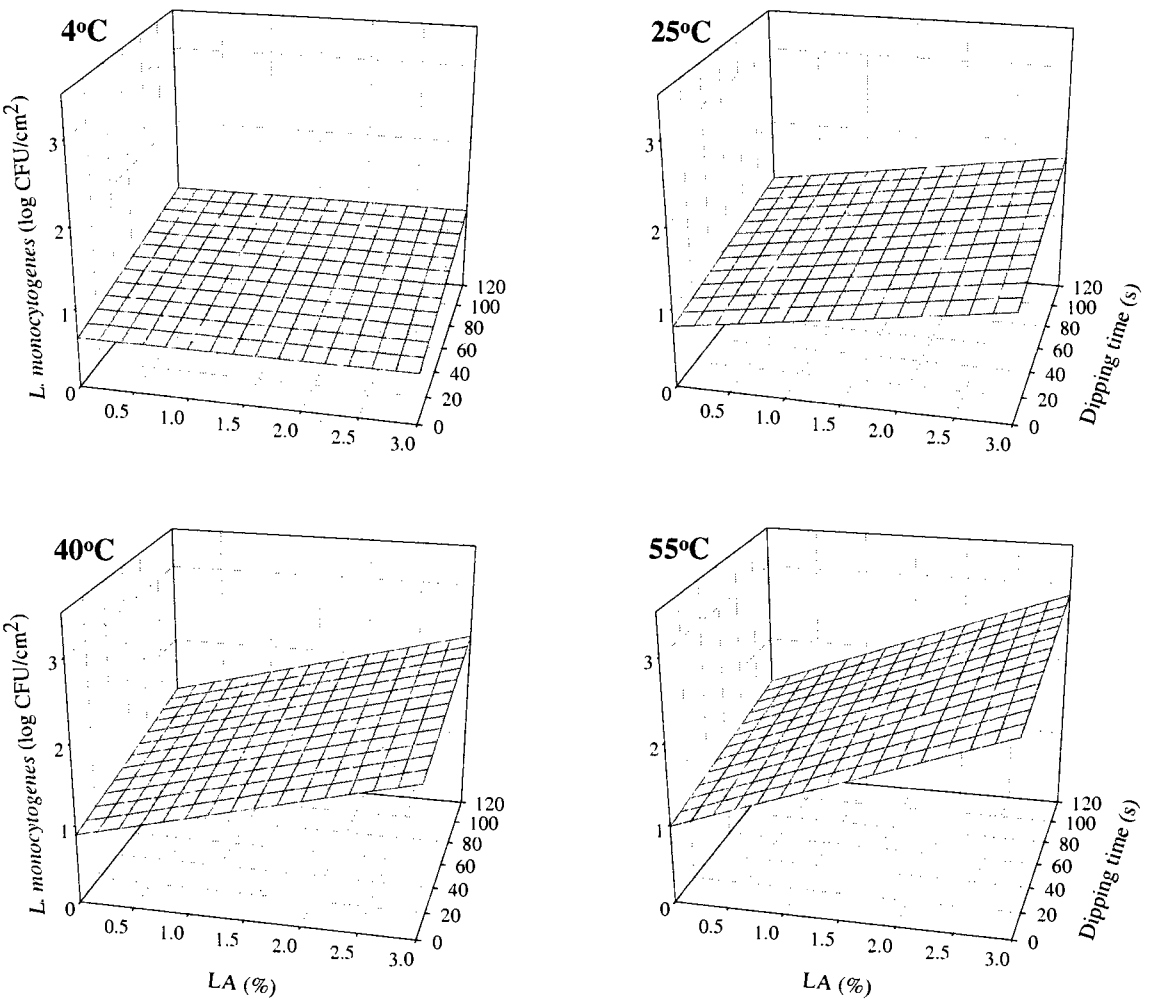


Figure 4.4. Response surface plots showing predicted reductions of *L. monocytogenes* on frankfurters under given conditions: lactic acid (LA) concentration 0 to 3%; temperature of dipping solutions 4, 25, 40, or 55°C; dipping time 15 to 120 s; and initial level of the pathogen 4.4 ± 0.1 log CFU/cm^2.

CHAPTER 5

Control of *Listeria monocytogenes* on Vacuum-Packaged Frankfurters Sprayed with Lactic acid Alone or in Combination with Sodium Lauryl Sulfate

Abstract

United States regulations require that processors apply lethal or inhibitory antimicrobials to ready-to-eat (RTE) meat and poultry products that support growth of *Listeria monocytogenes* and may be exposed to the processing environment after a lethality treatment. This study evaluated lactic acid (LA; 5% v/v) and sodium lauryl sulfate (SLS; 0.5% w/v), individually or as a mixture (LASLS), for control of *L. monocytogenes* on frankfurters. Frankfurters were inoculated with a 10-strain mixture of *L. monocytogenes*, sprayed (10 s, 20 bar, 23 ± 2°C) with antimicrobials or distilled water before (LASLS or DW) or after (LA, SLS, LASLS, or DW) inoculation (4.8 ± 0.1 log CFU/cm^2), vacuum-packaged, and stored at 4°C for 90 days. Samples were analyzed for the pathogen (PALCAM agar) and total microbial counts (TSAYE) during storage. Spraying with DW, LA or SLS after inoculation reduced numbers of *L. monocytogenes* by 1.3 ± 0.2, 1.8 ± 0.5 and 2.0 ± 0.4 log CFU/cm^2, respectively. The LASLS mixture applied before or after inoculation reduced pathogen populations by 1.8 ± 0.4 and 2.8 ± 0.2 log CFU/cm^2, respectively. No further reduction by any treatment was observed

during storage. Bacterial growth curves were fitted using the model of Baranyi and Roberts and indicated that the lag-phase duration of the bacterium on control samples (13.85 to 15.18 days) was extended by spraying with all solutions containing LA. For example, LA suppressed growth of *L. monocytogenes* for 39.14 to 41.01 days. Also, pathogen growth rates were lower on frankfurters sprayed after inoculation with LA or LASLS compared to those sprayed with DW. Therefore, spraying frankfurters with a mixture of lactic acid and sodium lauryl sulfate may be considered as an antilisterial alternative in RTE meat and poultry products.

Introduction

Ready-to-eat (RTE) meat and poultry products contaminated with *Listeria monocytogenes* have been implicated in several outbreaks of listeriosis in the U.S. *(34, 35, 37).* According to the quantitative assessment of relative risk to public health from foodborne listeriosis among selected categories of RTE foods, nonreheated frankfurters were classified as a high risk product that can cause listeriosis *(59). L. monocytogenes* does not survive thermal treatment involved in frankfurter processing *(229).* However, contamination may occur through direct contact of the cooked product with contaminated surfaces of the processing environment during peeling and packaging *(197).*

The U.S. Department of Agriculture Food Safety and Inspection Service (FSIS) enforces a "zero-tolerance" rule for *L. monocytogenes* in RTE meats *(201, 202).* In addition to proper sanitation, FSIS also requires the industry to apply control measures for *L. monocytogenes* in these products if they are exposed to the processing environment after the lethality processing step, and support growth of the pathogen *(207).* Specifically,

the industry is required to employ one of three alternatives: a post-lethality inactivation treatment combined with a *L. monocytogenes* growth inhibitor, a post-lethality inactivation treatment or a growth inhibitor, or sanitation measures and environmental testing. The chosen alternative must be included in the Hazard Analysis and Critical Control Point (HACCP) plan or prerequisite programs, and its effectiveness should be validated and the data shared with FSIS *(207)*. The FSIS developed a compliance guideline to assist processors in meeting regulatory requirements of the final rule *(210)*. The guideline suggests that the post-lethality treatment must reduce pathogens by at least 1 log-cycle, while processing plants using treatments that cause a reduction of the pathogen by at least 2 log-cycles should be subject to less frequent microbial sampling testing by FSIS *(210)*.

Lactic acid (LA) is a Generally Recognized as Safe (GRAS) compound that has a long history of being utilized as an acidulant in a wide variety of foods and is currently used by the meat industry for decontamination of beef carcasses *(192)*. Also, LA has been found to be effective against *L. monocytogenes* when applied as a surface treatment of RTE meats *(11, 71, 74, 133)*. Palumbo and Williams *(145)* first documented the bactericidal and bacteriostatic effect of LA as a surface treatment of frankfurters, inoculated with *L. monocytogenes*. In a recent study, Geornaras et al. *(74)* showed a similar effect with LA as a dipping solution of frankfurters inoculated with a 10-strain mixture of *L. monocytogenes* that were grown under conditions similar to those in processing plants; dipping in a 2.5% aqueous solution of LA (v/v) reduced the initial levels of *L. monocytogenes* on the surface of the frankfurters by 1.8 log CFU/cm^2,

subsequently increased the lag phase, and lowered the pathogen growth rate during storage in vacuum-packages at 10°C for 48 days.

Sodium lauryl sulfate (SLS) is an acid anionic surfactant which is approved by the U.S. Food and Drug Administration as a whipping or wetting agent or as an emulsifier in a wide variety of foods such as egg whites, fruit juices, vegetable oils and other food products *(63)*. However, the compound is not included in the current FSIS list of "Safe and Suitable Ingredients Used in the Production of Meat and Poultry Products" *(213)*. The antimicrobial properties of SLS, alone or combined with other antimicrobials, against pathogens in foods and surfaces in contact with food are well documented *(88, 162, 189, 190, 230)*. The primary mode of SLS action involves lysis of bacterial cells, general denaturation of proteins and enzymes, damage of cell membranes and changes in cell permeability *(2, 45, 86)*. The antibacterial effect of SLS increases at pH values below 4.0, with an optimum range between 1.5 and 3.0 *(44)*.

The objective of the present study was to determine the effect of spraying with LA or SLS individually or as a mixture against *L. monocytogenes* on frankfurters. Considering the possibility of product exposure to the processing environment and contamination with *L. monocytogenes* after spraying, an additional objective was to evaluate the effect of the LASLS mixture applied before and after simulated contamination.

Materials and Methods

Preparation of frankfurters

Frankfurters were prepared with 60% fresh pork (pork shoulder, 70 to 72% lean) and 40% fresh beef (beef shoulder, 76 to 78% lean). The meat was ground through a 0.79-cm grinder plate, and combined with ice, sodium chloride, dextrose, corn syrup solids, polyphosphate (Heller, Inc., Bedford Park, IL), sodium nitrite, sodium erythrobate, and spices (AC Legg Co., Birmingham, AL) before emulsification, according to the recipe used by Samelis et al. *(168).* The emulsion was prepared in a vacuum (0.5 bar) bowl chopper (RMF, Kansas City, MO). Then, the meat batter was stuffed (Handmann, model VF 50, Biberach/Riss, Germany) into 22-mm peelable cellulose casings (Nojax® Viskase Co., Inc., Darien, IL) and linked at 6.3 ± 0.3 cm length. The linked product was weighed, hung on racks and cooked in a humidity-controlled smokehouse (Alkar, DEC Intl. Inc., Lodi, WI). The cooking cycle was designed in preliminary trials and consisted of the following stages: cooking for 10 min at 43°C and 68% relative humidity (RH) followed by 20 min at 63°C and 50% RH; hot smoking for 30 min; cooking for 20 min at 63°C and 50% RH followed by 20 min at 71°C and 51% RH; and steam cooking at 76°C and 100% RH until the internal temperature of the product reached 71.1°C. Liquid smoke (Zesti Smoke, Monterrey, TN) was applied during the cooking cycle. At the end of the cycle, frankfurters were showered with tap water (23 ± 2°C) for 20 min and then stored in a walk-in refrigerator at 4.0°C for 16 to 17 h. The following day, frankfurters were reweighed to determine the cooking yield, peeled, and taken into the microbiology laboratory for treatment, inoculation, vacuum-packaging and storage.

Preparation of inoculum

The *L. monocytogenes* inoculum consisted of the following ten strains: N1-225 and N1-227 [serotype 4b, associated with 1998–1999 listeriosis outbreak traced to hot dogs *(34)*], 558 (serotype 1/2, pork meat isolate), NA-1 (serotype 3b, pork sausage isolate), N-7150 (serotype 3a, meat isolate), R2-500 and R2-501 [serotype 4b, associated with 2000 listeriosis outbreak traced to soft cheese *(36)*], and R2-763, R2-764 and R2-765 [serotype 4b, isolates associated with 2002 listeriosis outbreak traced sliceable turkey deli meats *(37)*] *(67)*. Strains N1-225, N1-227, R2-500, R2-501, R2-763, R2-764, and R2-765 were kindly provided by Dr. M. Wiedmann (Cornell University, Ithaca, NY). Cultures were maintained as frozen (-70°C) stocks in tryptic soy broth (TSB; Difco, Becton Dickinson, Sparks, Md.) supplemented with 0.6% yeast extract (YE; Acumedia, Lansing, MI) and 20% glycerol. Working cultures of each *L. monocytogenes* strain were prepared by transferring a loopfull of stock culture into 10 ml of TSB (Difco) supplemented with 0.6% yeast extract (Difco; TSBYE) followed by incubation at 30°C for 22 h. Two consecutive 22-h transfers of each *L. monocytogenes* strain were prepared in TSBYE at 30°C. Then, 5 ml of each culture were transferred into a sterile centrifuge tube. Cells were harvested individually by centrifugation (Eppendorf model 5810 R, Brinkman Instruments Inc., Westbury, NY, 4,629 × *g*, 15 min, 4°C) and the supernatant discarded. Culture pellets were resuspended in 10 ml of 0.1% phosphate buffered saline (PBS; pH 7.4; 0.2 g 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) and washed by centrifugation as described above. After washing, each culture pellet was resuspended in autoclave-sterilized frankfurter homogenate (10% wt/wt in distilled water) and then habituated for 72 h at 4°C *(117)*.

Habituated cells were combined and serially diluted to approximately 10^7 log CFU/ml in frankfurter homogenate before inoculation.

Inoculation of frankfurters

Before or after spraying, samples were surface-inoculated with 0.2 ml of a ten-strain composite of *L. monocytogenes* under a biological safety cabinet. The inoculum was spread with a sterile bent glass rod over the entire surface of each frankfurter to obtain a target inoculum level of approximately 4.8 log CFU/cm^2. After inoculation, frankfurters were covered with sanitized aluminum foil, and mantained at 4°C for 15 min to allow attachment of the cells before being surface-treated or vacuum-packaged.

Treatment of frankfurters

Freshly prepared spraying solutions (23 ± 2°C) of 5% (vol/vol) lactic acid (LA; Birko Co. Denver, CO) and 0.5% (wt/vol) sodium lauryl sulfate (SLS; Stepanol®, Stepan Co., Northfield, IL) were applied after inoculation only, whereas SLS mixed with LA (LASLS) or distilled water (DW) were applied before and after inoculation. Inoculated samples that were not treated served as control. After inoculation and cell attachment (for LA, SLS, DW, or LASLS treatments), or before inoculation (for DW or LASLS treatments), frankfurters were transferred into a class II biological safety cabinet (Nuaire, model NU-425-400, Plymouth, MN), placed on sterile grill wire netting and sprayed from the distance of 15 cm (23 ± 2°C; 20 bar, nozzle type: H1/8vv-95015, Spraying Systems Co., Wheaton, IL; custom-built spraying system, Chad Co., Olathe, KS) with antimicrobials or DW for 5 s, turned over, sprayed for another 5 s, and then drained for 1 min. Following treatment, inoculated samples were placed into vacuum bags (two frankfurters per bag; 15 by 22 cm, 3 mil std barrier, nylon/polyethylene vacuum pouch,

Koch, Kansas City, Mo.) and vacuum-packaged (Hollymatic Corp., Countryside, IL); frankfurters treated before inoculation were inoculated, mantained at 4°C for 15 min, and then vacuum-packaged.

Microbiological analysis

The packages were opened aseptically, two frankfurters from each package were transferred into sterile 24 oz bags (Whirl-Pak, Nasco, Modesto, CA) and 50 ml of maximum recovery diluent (MRD; 0.85% of NaCl and 0.1% buffered peptone) were added to each bag. Samples were shaken manually 30 times for approximately 30 s to detach the cells. Ten-fold serial dilutions of the liquid were prepared in 0.1% of buffered peptone water (Difco). Aliquots of appropriate dilutions were surface-plated onto PALCAM agar (Difco) or tryptic soy agar with 0.6% yeast extract (TSAYE) for enumeration of the pathogen and total microbial populations, respectively. Inoculated PALCAM agar plates were incubated at 30°C for 48 h and typical colonies of *L. monocytogenes* were counted after incubation. The TSAYE plates were incubated at 25 ± 2°C for 72 h and all bacterial colonies were counted. The counts were expressed as log CFU/cm^2.

Physical and chemical analyses

The pH of frankfurters in MRD was determined after sample pummeling (2 min; Masticator, IUL Instruments, Barcelona, Spain) using a pH meter fitted with a glass electrode (Denver Instruments, Arvada, Colo.). Water activity (a_w) values (AquaLab model series 3, Decagon Devices, Wash.) were determined on day-0. The fat and moisture contents of frankfurters were determined according to the AOAC International official methods (960.39 and 950.46.B., respectively) *(8)*.

Data analysis

The experiment was conducted two times, each with three samples per treatment at each sampling time. The treatments were: DW and LASLS (sprayed before or after inoculation); LA and SLS (sprayed after inoculation only), and an untreated control. The samples were analyzed eleven times during the storage (0, 7, 14, 21, 28, 35, 42, 52, 62, 77 and 90 days). Data were analyzed as a randomized complete block design with factorial arrangement of the treatments using the General Linear Model procedure of SAS *(172).* The analysis of variance model included the main effects of spraying treatment and storage time, and interactions of these factors. In addition, main effects of LA and SLS and their interactions were evaluated. Differences were considered statistically significant when the associated P-value was less than 0.05. We used DMFIT software (Institute of food Research, Reading, UK), kindly provided by Dr. J. Baranyi, to estimate the following: upper and lower asymptotes (Y_0 and Y_{end}), parameters which indicate initial and end points of sigmoid curve, respectively, (these values do not necessarily show precise numbers of the pathogen at day-0 and day-90); length of lag periods, time during which bacteria adjust to a new environment before initiation of growth; and growth rates of *L. monocytogenes (10).*

Results and Discussion

Product characteristics

The cooking yield of the frankfurters was 92 ± 1.0% and the estimated surface area of each frankfurter was 52.12 cm^2. The fat and moisture contents were 20 ± 0.5%

and 58.9 ± 0.5%, respectively. Water activity of the product after cooking and inoculation was 0.976 ± 0.002 and it was not affected by treatment.

Total microbial populations

Initial counts of *L. monocytogenes* (PALCAM agar) and total microbial populations (TSAYE) on inoculated control frankfurters were 4.79 ± 0.11 and 4.83 ± 0.09 log CFU/cm^2, respectively (Figures 5.1 and 5.2). The overall average (over storage temperature and treatments) of TSAYE counts did not differ ($P \geq 0.05$) from the overall average of the PALCAM agar counts. This indicated that most of the bacterial population was represented by *L. monocytogenes* and that most of the microbial reduction by surface treatments was due to physical removal or killing rather than sublethal injury. Previous reports showed similar results *(11, 168),* whereas others demonstrated significant differences in *L. monocytogenes* and total microbial counts on surface-treated vacuum-packaged frankfurters during storage at refrigeration temperature *(74).* Discrepancies in results may be due to antimicrobials included in formulations or used for surface-treatment, types and levels of background microflora, differences in chemical composition and physical properties of products tested, types of inoculum, and storage conditions of products.

Product pH

Initial pH of the inoculated frankfurters that were not sprayed with DW or antimicrobials was 6.04 ± 0.4 (Table 5.1). The pH values of LA and LASLS solutions were 1.77 and 1.81, respectively, and treatment with the solutions reduced ($P < 0.05$) product pH by 0.21 to 0.29 pH units. The pH of the SLS spraying solution was 8.34; however, spraying with SLS or DW did not affect ($P \geq 0.05$) the pH of the frankfurters.

The pH of frankfurters sprayed with solutions that contained LA was relatively stable throughout the entire storage period, whereas it decreased ($P < 0.05$) after 62 to 77 days in all other samples, presumably due to accumulation of microbial metabolites on the product surface during storage, since total microbial populations reached high numbers on day-62 (Figure 5.2).

Antimicrobial effects

All spraying solutions that were applied after inoculation of the frankfurters (DW, LA, SLS, and LASLS) caused immediate reduction in pathogen counts (Figures 5.1 and 5.2). Specifically, spraying with DW and LA reduced pathogen numbers by 1.3 ± 0.2 and 1.8 ± 0.5 log CFU/cm^2, respectively. The reduction caused by LA was not different ($P \geq 0.05$) from that caused by DW, which indicated that part of the observed decrease in pathogen levels caused by LA could be due to the physical removal of the cells. The extent of the effect of LA against *L. monocytogenes* was similar to that reported by Barmpalia et al. *(11)* who found that dipping inoculated frankfurters in a 2.5% solution of LA (120 s, 23 ± 2°C) reduced pathogen numbers by 2.0 log CFU/cm^2. Also, a 2-log reduction of *L. monocytogenes* on frankfurters following dipping (30 s, 20°C) in a 3.4% LA solution was reported by Nuñez de González et al. *(140).* The SLS treatment reduced pathogen numbers by 2.0 ± 0.4 log CFU/cm^2, a reduction numerically higher, but not statistically different ($P \geq 0.05$) from that caused by DW or LA. Spraying with LASLS after inoculation reduced initial levels of *L. monocytogenes* by 2.8 ± 0.2 log CFU/cm^2; the mixture was more effective than LA and it was the only treatment that was more effective ($P < 0.05$) than DW applied after inoculation. The increased effectiveness of SLS combined with LA was expected, since the antimicrobial properties of SLS increase

at low pH *(44)*. This observation also was in agreement with previous reports which demonstrated enhanced reduction of *Salmonella* Typhimurium on chicken broiler skin when SLS and LA were combined *(88, 190)*.

As expected, spraying with DW before inoculation did not affect initial numbers of the pathogen; however, LASLS, applied before inoculation reduced numbers of subsequently inoculated cells by 1.8 ± 0.4 log CFU/cm^2 (Table 5.2 and Figure 5.1). The decrease in pathogen levels caused by the LASLS mixture sprayed before inoculation was similar ($P \geq 0.05$) to reductions caused by any treatment (except for the same mixture) applied after inoculation. However, the reduction in cell numbers caused by the LASLS solution applied before contamination were probably due to killing, rather than physical removal, which may have been involved in at least part of the reduction caused by spraying with DW, LA, or SLS after inoculation. The effect associated with the pre-inoculation treatment may be of particular importance for control of *L. monocytogenes* in processing environments where contamination may take place after application of the post-lethality treatment.

None of the treatments reduced pathogen levels during storage. The lag phase duration of the untreated control samples was 13.85 to 15.18 days and it was similar to that of samples treated with DW before and SLS after inoculation (Figure 5.1 and Table 5.2). Pathogen growth initiation was delayed, compared to the above treatments, on samples treated with solutions containing LA. Similar to our findings, Samelis et al. *(170)* reported a growth inhibitory effect of 5% LA, when sliced pork bologna was dipped in the solution for 1 min before inoculation and storage at 4°C for 120 days.

L. monocytogenes grew at a slower rate on untreated samples (0.049 to 0.056 log CFU/cm^2/day) than on samples surface-treated with DW or antimicrobials (Table 5.2). There may be two possible explanations to this phenomenon. First, spraying may have resulted in residual moisture on the product surface, even though the internal a_w of frankfurters was not affected. This may result in increased growth rates of the pathogen on the frankfurter/packaging material interface *(170)*. Second, the initial pathogen counts on untreated samples were closer to the stationary phase than those on samples where initial numbers were reduced by spraying. Therefore, initial points of sigmoid curves for treated samples (Y_0 = 1.53 to 3.52) were lower than those of control samples (Y_0 = 4.58 to 4.70). Differences in initial points of the curves resulted in different lengths of the mid-phase part of the curves which could potentially affect estimated growth rates and contribute to observed increases in growth rates on treated samples. Nevertheless, treatments in which LA was applied after inoculation (LA and LASLS) reduced the growth rate of *L. monocytogenes* compared to DW applied after inoculation; this was consistent with reports by Geornaras et al. *(71, 74)*.

L. monocytogenes maximum cell counts on control samples and on samples treated with DW (before or after inoculation) or SLS reached approximately 7 log-cycles by day-62 (Figure 5.1). Numbers of pathogen cells also reached similar levels on samples treated with all solutions that contained LA, but later during storage (day-90).

Bacteriostatic properties of LA as a dipping solution against *L. monocytogenes* in vacuum-packaged RTE meats stored at refrigerated temperatures are well documented *(11, 71, 74, 170)*. The inhibitory effects of LA may be explained by the acidification of the cell membrane and the diffusion of protons inside the cell. In response to the increase

of intracellular pH, bacterial cells attempt to maintain the internal pH close to neutrality by actively pumping the protons out, using the process that requires the hydrolysis of adenosine triphosphate (ATP) *(19, 23)*. Therefore, maintaining the pH homeostasis is an energy-demanding process that makes it difficult for microorganisms to multiply.

As a surfactant, SLS may increase detachment of bacterial cells from surfaces by disruption of hydrophobic bonds and changing the conformation of the cell surface *(124, 137)*. Since microbial attachment varies among organisms and depends on magnitude of cell surface negative charge, surface hydrophobicity, extracellular polysaccharides and flagella *(52)*, the efficiency of the surfactant may vary as a function of these and other factors such as type of surface to which cells attach and concentration of the surfactant *(124)*. Raiden et al. *(158)* reported that 0.1% SLS aqueous solution (22 or 40°C) did not enhance removal of *Salmonella* and *Shigella* spp. from strawberries, tomatoes, and green-leaf lettuce. In the present study, pathogen reduction caused by spraying with SLS was similar ($P \geq 0.05$) to that caused by DW which indicated that SLS did not affect removal of *L. monocytogenes* from frankfurters.

As an antimicrobial, SLS may exhibit bactericidal properties in liquid media by damaging cell membranes, denaturing proteins and enzymes *(44)* or lysing bacterial cells *(2, 45, 86)* when its activity is enhanced at pH values below 4.0 *(44)*. Because of all these reasons, SLS is included in patented sanitizers for fresh produce *(189)* and surfaces in contact with foods *(162)*. However, Raiden et al. *(157)* demonstrated the ability of *Salmonella* and *Shigella* spp. to survive in 0.1% SLS at 22 or 40°C for up to 32 h. In that study, survival of the pathogens could have been attributed to binding of SLS and inactivation by amino acids *(84)* of TSA broth which was used for cell cultivation before

unwashed cultures (2.5 ml) were added to 0.1% SLS aqueous solution (250 ml). Under the conditions of this study, spraying frankfurters with SLS alone did not result in significant pathogen reduction compared to DW. The low inhibitory effect of the chemical may be explained by the high pH (8.34) of the solution, complexity of SLS interactions with organic compounds *(84, 164)* and, perhaps, because cells attached to frankfurter surface were not fully exposed to the antimicrobial as opposed to experiments which demonstrated bactericidal/bacteriolytic properties of the compound in liquids *(2, 45, 86)*.

Results suggest that both antimicrobials (LA and SLS) may contribute to the enhanced effectiveness of the mixture, perhaps by mutual reinforcement. First, as mentioned before, SLS decreases surface tension of solutions and, therefore, improves "wettability" *(138, 164)* of the frankfurter surface allowing more uniform distribution of LA molecules and, possibly, bringing them closer to the bacterial cell surface. Second, low pH affects electrostatic charges of surfactant molecules and substrates (cell surfaces) may cause increased adsorption of SLS *(164)* into cell walls, thereby leading to accelerated damage of the cytoplasmic membrane. Third, LA increases cell permeability and sensitizes bacterial cells to SLS *(2)*; hence, combination of the chemicals may inflict simultaneous injuries.

The results of this study demonstrated that, under the conditions evaluated, spraying inoculated frankfurters with lactic acid (5%), sodium lauryl sulfate (0.5%) or distilled water reduced numbers of *L. monocytogenes* by 1.3 to 2.0 log CFU/cm^2. The mixture of lactic acid and sodium lauryl sulfate applied after inoculation provided greater pathogen reduction (2.8 ± 0.2 log CFU/cm^2) than that caused by lactic acid used

individually. The combination of the antimicrobials applied before simulated contamination reduced pathogen numbers by 1.8 ± 0.4 log-cycles, which may be of particular importance in situations where contamination may occur after spraying. The mixture was more effective when applied after inoculation, compared to that applied before inoculation, possibly because some cells were removed from the surface of the frankfurters. Sodium lauryl sulfate, used alone, did not show a bacteriostatic effect, as it was applied at pH of 8.34; however, all treatments that contained lactic acid, even when applied before simulated contamination, delayed growth of the pathogen.

Conclusions

Based on these results, spraying frankfurters with a mixture of lactic acid and sodium lauryl sulfate can be considered for use as a post-lethality treatment which reduces *L. monocytogenes* levels, as well as an antimicrobial agent that suppresses growth of the pathogen. This may allow processors to operate under the first alternative of the FSIS final rule *(207).* In addition, since spraying with the mixture may result in more than 2-log reduction of the pathogen, plants using this post-lethality treatment may be subject to less frequent FSIS verification testing *(210).* However, processors need to validate the effectiveness of these antimicrobials for specific RTE meat or poultry products and evaluate their effect on sensory properties in-plant.

Table 5.1. The pH values of frankfurters (LSD = 0.14*) that were sprayed for 10 s with distilled water (DW), solutions of lactic acid (LA; 5%), sodium lauryl sulfate (SLS; 0.5%), mixture of lactic acid and sodium lauryl sulfate (LASLS), or left untreated (control), and inoculated before or after treatment, vacuum-packaged and stored for 90 days at 4°C.

Storage (days)	Antimicrobial treatments						
	Untreated control	Spraying before inoculation		Spraying after inoculation			
		DW	LASLS	DW	LA	SLS	LASLS
0	6.04 ± 0.04	6.03 ± 0.04	5.81 ± 0.10	6.03 ± 0.09	5.75 ± 0.03	6.02 ± 0.03	5.83 ± 0.03
7	6.07 ± 0.08	6.07 ± 0.05	5.91 ± 0.07	6.13 ± 0.02	5.91 ± 0.08	6.13 ± 0.05	5.89 ± 0.07
14	6.11 ± 0.08	6.08 ± 0.13	5.95 ± 0.03	5.96 ± 0.27	5.94 ± 0.08	6.00 ± 0.18	5.91 ± 0.05
21	6.06 ± 0.13	6.07 ± 0.10	5.90 ± 0.10	6.13 ± 0.11	5.84 ± 0.07	6.17 ± 0.04	5.86 ± 0.10
28	6.07 ± 0.10	6.19 ± 0.11	5.94 ± 0.09	6.23 ± 0.07	5.96 ± 0.07	6.16 ± 0.11	6.01 ± 0.03
35	6.08 ± 0.07	6.11 ± 0.03	5.88 ± 0.02	6.12 ± 0.09	5.79 ± 0.07	6.03 ± 0.11	5.81 ± 0.11
42	6.13 ± 0.05	6.07 ± 0.09	5.96 ± 0.04	6.14 ± 0.11	5.84 ± 0.08	6.07 ± 0.04	5.92 ± 0.07
52	6.00 ± 0.10	5.95 ± 0.16	5.94 ± 0.06	6.01 ± 0.08	5.86 ± 0.09	6.04 ± 0.17	5.92 ± 0.07
62	5.93 ± 0.25	5.63 ± 0.38	5.86 ± 0.22	5.97 ± 0.13	5.99 ± 0.11	5.79 ± 0.47	5.98 ± 0.17
77	5.71 ± 0.20	5.84 ± 0.04	5.89 ± 0.02	5.70 ± 0.10	5.86 ± 0.05	5.81 ± 0.08	5.95 ± 0.11
90	5.51 ± 0.15	5.39 ± 0.19	5.75 ± 0.22	5.69 ± 0.22	5.69 ± 0.20	5.60 ± 0.11	5.82 ± 0.11

*Least significant difference.

Table 5.2. Growth kinetics of *Listeria monocytogenes* on frankfurters that were sprayed for 10 s with distilled water, solutions of lactic acid (5%), sodium lauryl sulfate (0.5%), mixture of lactic acid and sodium lauryl sulfate, or left untreated, and inoculated before or after treatment, vacuum-packaged and stored for 90 days at 4°C.

Antimicrobial treatment	Initial reduction (log CFU/cm^2)[a]	Lag phase duration (days)	Growth rate (log CFU/cm^2/day)[b]	Y_0 (log CFU/cm^2)[c]	Y_{end} (log CFU/cm^2)[d]	R^2
Untreated (control)	– [C]	13.85 - 15.18	0.049 - 0.056	4.58 - 4.70	7.00 - 7.27	0.91 - 0.94
Distilled water (before inoculation)	0.1 ± 0.0 [C]	12.83 - 15.91	0.076 - 0.092	4.54 - 4.64	7.03 - 7.09	0.95 - 0.96
Lactic acid (5%) + sodium lauryl sulfate (0.5%, before inoculation)	1.8 ± 0.4 [B]	36.04 - 43.96	0.086 - 0.166	2.99 - 3.20	– [e]	0.91 - 0.93
Distilled water (after inoculation)	1.3 ± 0.2 [B]	16.71 - 24.60	0.102 - 0.109	3.24 - 3.52	7.18 - 7.41	0.87 - 0.91
Lactic acid (5%)	1.8 ± 0.5 [B]	39.14 - 41.01	0.063 - 0.089	2.76 - 3.00	–	0.85 - 0.94
Sodium lauryl sulfate (0.5%)	2.0 ± 0.4 [AB]	9.57 - 15.96	0.083 - 0.116	2.64 - 2.66	7.03 - 7.45	0.93 - 0.95
Lactic acid (5%) + sodium lauryl sulfate (0.5% after inoculation)	2.8 ± 0.2 [A]	25.14 - 38.68	0.084 - 0.91	1.53 - 1.79	–	0.91 - 0.93

[a] Mean ± standard deviation. Different superscripts indicate significant differences ($P < 0.05$).
[b] Maximum specific rate of the potential growth of *L. monocytogenes (10).*
[c] Lower asymptote of sigmoid curve estimated by the Baranyi et al. model *(10).*
[d] Upper asymptote of sigmoid curve estimated by the Baranyi et al. model *(10).*
[e] The stationary phase was not reached.

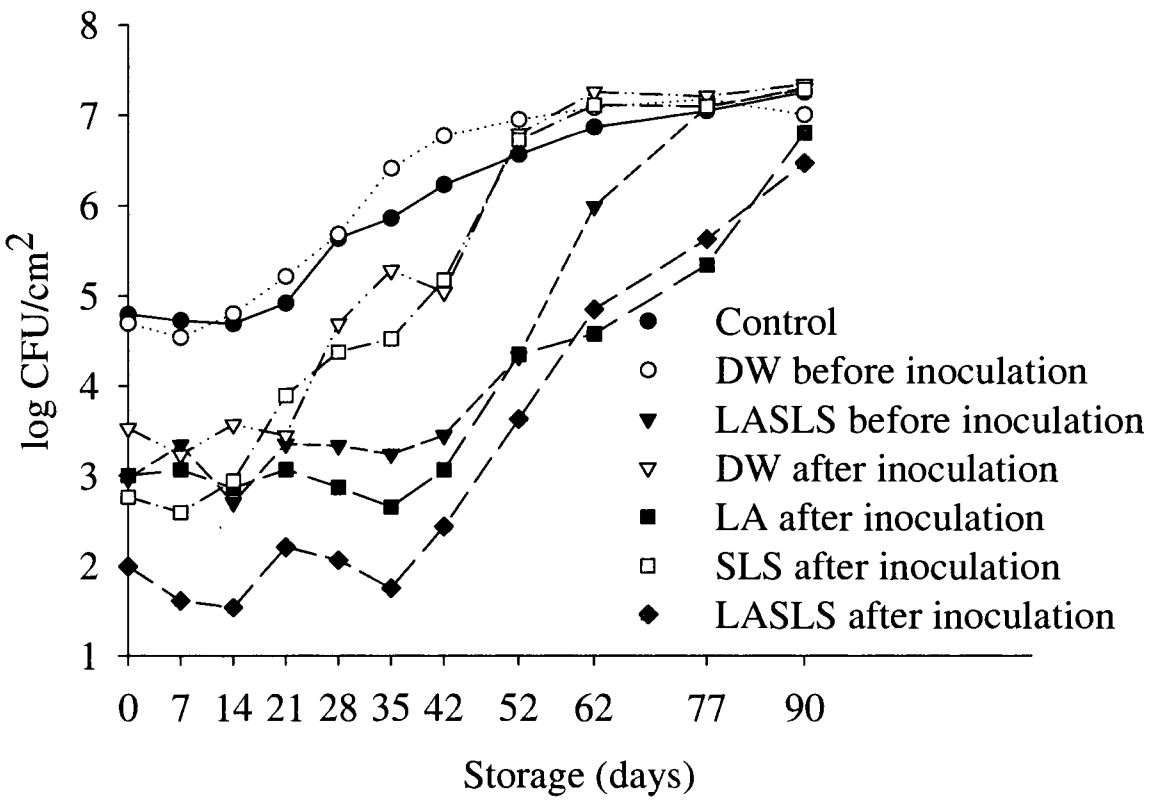


Figure 5.1 (Appendix Table 4). Numbers of *Listeria monocytogenes* (PALCAM agar) on frankfurters that were sprayed for 10 s with distilled water (DW), solutions of lactic acid (LA; 5%), sodium lauryl sulfate (SLS; 0.5%), mixture of lactic acid and sodium lauryl sulfate (LASLS), or left untreated (control), and inoculated before or after treatment, vacuum-packaged and stored for 90 days at 4°C.

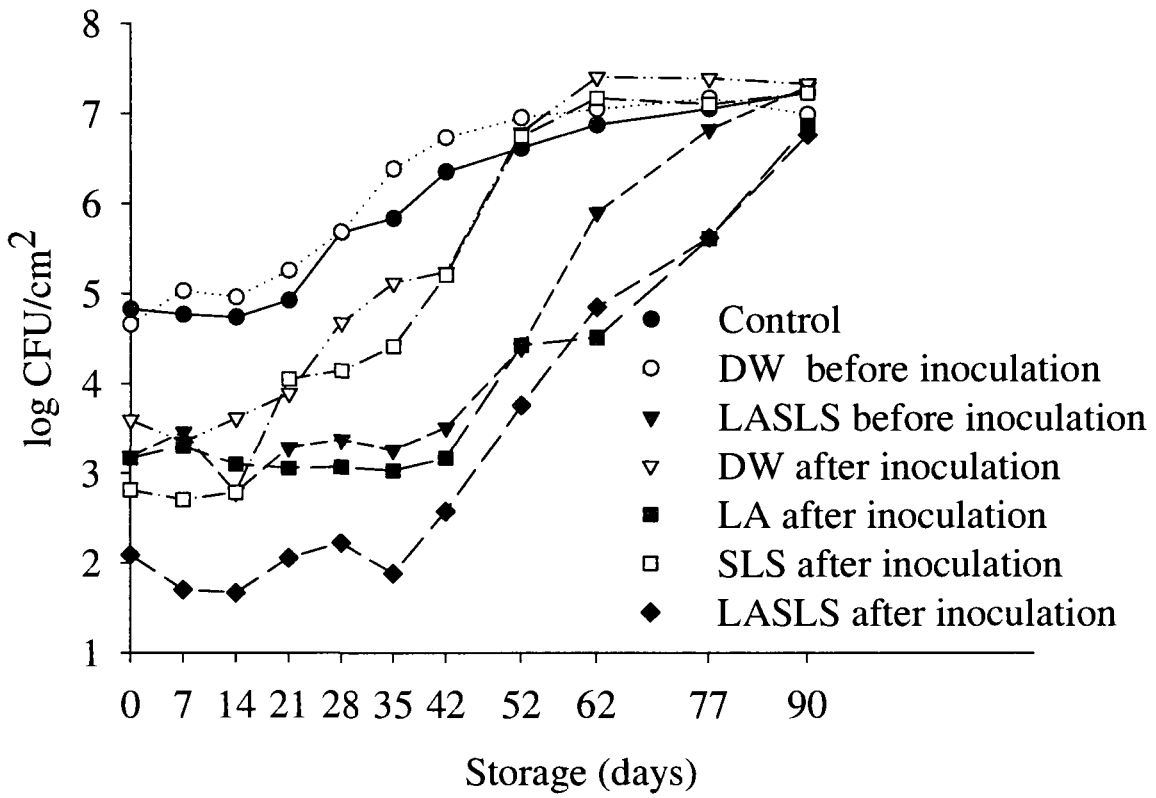


Figure 5.1 (Appendix Table 5). Total microbial counts (TSAYE) on frankfurters that were sprayed for 10 s with distilled water (DW), solutions of lactic acid (LA; 5%), sodium lauryl sulfate (SLS; 0.5%), mixture of lactic acid and sodium lauryl sulfate (LASLS), or left untreated (control), and inoculated before or after treatment, vacuum-packaged and stored for 90 days at 4°C.

CHAPTER 6

Evaluation of Changes in *Listeria monocytogenes* Populations on Frankfurters at Different Stages from Manufacturing to Consumption

Abstract

This study evaluated the fate of inoculated *Listeria monocytogenes* on frankfurters stored under conditions simulating those that may be encountered between manufacturing and consumption. Frankfurters with or without 1.5% potassium lactate and 0.1% sodium diacetate (PL/SD) were inoculated (1.8 ± 0.1 log CFU/cm^2) with a 10-strain composite of *L. monocytogenes,* vacuum-packaged, and stored under conditions simulating pre-distribution storage (24 h, 4°C), temperature abuse during transportation (7 h, 7°C followed by 7 h, 12°C), and storage before purchase by consumers (60 d, 4°C; SBP). At 0, 20, 40, and 60 d of SBP, samples were exposed to conditions simulating delivery from stores to homes or food establishments (3 h, 23°C), and then opened or held vacuum-packaged at 4 or 7°C for 14 d (SHF). Pathogen counts remained relatively constant on frankfurters with PL/SD regardless of product age and storage conditions; however, they increased ($P < 0.05$) on product without antimicrobials. In vacuum-packaged samples, during SHF at 4°C, the pathogen grew faster ($P < 0.05$) on older product (20 d of SBP) compared to product that was fresh (0 d of SBP); a similar trend was observed in opened

packages. At 7°C the fastest growth (0.35 ± 0.02 log CFU/cm^2/d) was observed on fresh product in opened packages; in vacuum-packages, growth rates on fresh and aged products were similar. By day-40 of SBP, the pathogen reached high numbers and increased slowly or remained unchanged during SHF. This information may be valuable in *L. monocytogenes* risk assessments and in development of guidelines for storage of frankfurters between package opening and product consumption.

Introduction

The United States Food Code provides specific food handling guidelines, including recommendations for time and temperature control of potentially hazardous foods, to be followed by retail and food service establishments such as restaurants, grocery stores, and institutions such as nursing homes *(60)*. Specifically, the guidelines suggest that vacuum-packaged, commercially–manufactured, ready-to-eat (RTE) foods that are opened in a food establishment need to be clearly marked with a date if they are intended to be stored for more than 24 h before sale or consumption, and should be stored for no longer than 7 d at or below 5 °C *(60, 61)*.

The U.S. Department of Agriculture Food Safety and Inspection Service (USDA-FSIS) also provides advice for safe food handling by consumers. A Cold Storage Chart developed by the agency provides time and temperature limits for safe storage of food products, including RTE meats. Specifically, the agency recommends storage of frankfurters at 40°F (4.4°C) in opened packages for ≤ 7 d or in vacuum-packages for ≤ 14 d *(211)*. Even though, according to a web-based survey, a majority of consumers store

frankfurters within these recommended time limits *(31)*, 59% of consumers store refrigerated foods at 5 to 10°C *(99)*.

It is generally accepted that the target microorganism for control in refrigerated RTE meat and poultry products should be *Listeria monocytogenes* because of its virulence and ability to grow at refrigeration temperatures *(40, 60, 160)*. However, the infectious dose of the pathogen remains unknown and, therefore, there is no general consensus on performance standards or a specified allowable maximum increase in numbers of *L. monocytogenes (217)* during storage of foods. Nevertheless, the Food and Drug Administration (FDA) and USDA-FSIS safe time and temperature storage recommendations aim to limit growth to no more than a 1-log unit *(60)*. However, this recommendation does not imply presence of any *L. monocytogenes* in foods, and does not establish an acceptable level for the pathogen. As a basis for the recommendations, the agencies used various data sources such as peer-reviewed journal articles, growth modeling programs and science-based reports such as the 2003 listeriosis risk assessment *(40, 59, 60)*. However, available data have several limitations. For example, most of the pathogen growth models were based on data collected in liquid media, and did not consider factors such as presence of spoilage microflora and chemicals that may be present in processed foods and affect growth of the pathogen *(40)*. In addition, most of the published studies on growth kinetics of *L. monocytogenes* in foods were conducted under constant storage conditions, and did not account for temperature fluctuations to which foods may be exposed between manufacturing and consumption.

A published quantitative assessment of relative risk to public health from foodborne listeriosis has emphasized the importance of time/temperature control during

storage of RTE foods *(59)*. Because of this, the National Advisory Committee on Microbiological Criteria for Foods (NACMCF) provided federal food safety agencies with information for potential establishment of safety-based consume-by date labels (SBDL) for refrigerated RTE foods *(160)*. The Committee concluded that application of SBDL at multiple points in the food chain may significantly reduce the risk of foodborne listeriosis. However, there is a need for microbial challenge studies that may be used to validate a SBDL *(160)*. Therefore, the objective of this study was to evaluate the fate of *L. monocytogenes* on inoculated frankfurters, formulated with or without lactate/diacetate antimicrobials, and stored under variable conditions to which the product may be exposed during the time between manufacturing and consumption.

Materials and Methods

Preparation of frankfurters

Two types of frankfurters (with and without 1.5% potassium lactate and 0.1% sodium diacetate; PL/SD) were manufactured using 60% pork and 40% beef. Fresh pork (pork shoulder, 28-30% fat) and beef (beef shoulder, 22-24% fat) were ground through a 0.79-cm grinder plate, and ice, sodium chloride, dextrose, corn syrup solids, polyphosphate (Heller, Inc., Bedford Park, IL), sodium nitrite, sodium erythrobate, and spices (AC Legg Co., Birmingham, AL) were added before emulsification according to the formulation described by Samelis et al. *(168)*. The emulsion was prepared under vacuum (0.5 bar) in a vacuum bowl chopper (RMF, Kansas City, MO) and extruded using a meat stuffer (Handmann, model VF 50, Biberach/Riss, Germany) into 22-mm peelable cellulose casings (Nojax® Viskase Co., Inc., Darien, IL) and linked at 7.3 ± 0.3

cm lengths. The linked product was weighed and cooked in a humidity-controlled smokehouse (Alkar, DEC Intl. Inc., Lodi, WI). The cooking cycle consisted of the following stages: 10 min at 43°C and 68% relative humidity (RH) followed by 20 min at 63°C and 50% RH; hot smoking for 30 min; cooking for 20 min at 63°C and 50% RH followed by 20 min at 71°C and 51% RH; and steam cooking at 76°C and RH 100% until the internal temperature of the product reached 71.1°C. Liquid smoke (Zesti Smoke, Monterrey, TN) was applied during the cooking cycle. At the end of the cycle, frankfurters were showered with tap water (23 ± 2°C) for 20 min and then kept in a walk-in refrigerator at 4°C for 16 to 18 h, until used. The following day, frankfurters were reweighed to determine the cooking yield, peeled, and taken to the microbiology laboratory for inoculation, vacuum-packaging and storage.

Preparation of inoculum

The inoculum consisted of the following 10 strains of *L. monocytogenes* of food, environmental and clinical origin: N1-225 and N1-227 [serotype 4b, associated with a 1998–1999 listeriosis outbreak traced to hot dogs; *(34)*], 558 (serotype 1/2, pork meat isolate), NA-1 (serotype 3b, pork sausage isolate), N-7150 (serotype 3a, meat isolate), R2-500 and R2-501 [serotype 4b, associated with a 2000 listeriosis outbreak traced to soft cheese; *(36)*], and R2-763, R2-764 and R2-765 [serotype 4b, isolates associated with a 2002 listeriosis outbreak traced sliceable turkey deli meats; *(37)*] *(67)*. Strains N1-225, N1-227, R2-500, R2-501, R2-763, R2-764, and R2-765 were kindly provided by Dr. M. Wiedmann (Cornell University, Ithaca, NY). Cultures were stored in an ultra-freezer (-70°C) in tryptic soy broth (TSB; Difco, Becton Dickinson, Sparks, MD) supplemented with 0.6% yeast extract (YE; Acumedia, Lansing, MI) and 20% glycerol until used to

prepare working cultures. Cultures of each strain activated in TSBYE at 30°C for 22 h were then subcultured under the same conditions. Cells of each strain were harvested (individually) by centrifugation (Eppendorf model 5810 R, Brinkman Instruments Inc., Westbury, NY; 4,629 × *g*, 15 min, 4°C), resuspended in 10 ml of phosphate buffered saline (PBS, pH 7.4; 0.2 g 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) and washed by centrifugation as described above. After washing, each culture pellet was resuspended in autoclave-sterilized frankfurter extract (10% wt/wt in distilled water) and stored for habituation at 4°C for 72 h, as described by Lianou et al. *(117).* Before inoculation, habituated cultures were combined and serially diluted in frankfurter extract to approximately 4 log CFU/ml.

Inoculation of frankfurters

Each frankfurter was surface-inoculated with 0.2 ml of the 10-strain composite of *L. monocytogenes.* The inoculum was spread with a sterile bent glass rod over the frankfurter surface (in a manner allowing maximum surface coverage) under a biological safety cabinet to obtain a target inoculum level of approximately 1.8 log CFU/cm^2. Inoculated frankfurters were held at 4 °C for 15 min to allow for cell attachment before being vacuum-packaged (Hollymatic Corp., Countryside, IL) in groups of 10 or three frankfurters per bag (zip-top type bags, Zip Vak 15.2 x 20.3 cm, nylon/EVA copolymer, Winpak Winnipeg, MB, Canada). Packages that contained three frankfurters were used to simulate vacuum-packaged storage, whereas packages that contained 10 frankfurters were used to simulate storage of opened packages at home or food service establishments after purchase following vacuum-packaged storage.

Foods may be exposed to a broad range of time/temperature combinations and may be temperature abused between manufacturing and consumption *(160)*. Therefore, conditions used in this study were chosen to simulate a potential temperature abuse scenario. After vacuum-packaging, samples were stored at 4°C for 24 h to simulate in-plant storage of the product before distribution (Figure 6.1). Then, they were stored for 7 h at 7°C, followed by 7-h storage at 12°C to simulate potential temperature abuse during transportation from a plant to retail. After that, samples were stored at 4 °C for up to 60 d to simulate storage before purchase (SBP) at retail. On d 0, 20, 40 and 60 of SBP, groups of packaged samples were exposed to conditions simulating temperature abuse during delivery from stores to homes or food service (3 h at 23°C); based on the Audits International report *(9)*, the time between removal of product from store display until placement in home refrigeration was between 10 min and 5 h 27 min. These packages were then opened or held sealed at 4 or 7°C for 14 d to simulate storage at consumer homes or food service establishments (SHF). Storage at 4°C was chosen to represent storage at retail and food service; 7°C represented the temperature of consumer refrigerators *(160)*.

Microbiological analysis

Samples were analyzed before and after the simulated storage at a processing plant, transportation from the plant to stores and from stores to consumer homes/food service, during the SBP (d 0, 20, 40 and 60) and SHF (d 0, 2, 4, 7 and 14) (Figure 6.1). At each sampling time, packages were opened aseptically and two frankfurters from each package were transferred into sterile 24-oz bags (Whirl-Pak, Nasco, Modesto, CA) containing 50 ml of maximum recovery diluent (MRD; Oxoid, Remel Inc, Lenexa, KS;

(117)). The MRD is an isotonic solution which contains low concentrations of peptone (0.1%) and sodium chloride (0.85% of NaCl) and helps to maintain the viability of bacteria during sample preparation. Packages that initially contained 10 frankfurters were zip-closed and placed back in the incubator (4 or 7°C) for subsequent samplings, whereas when two out of three frankfurters were used (vacuum-packaged storage during SBP or SHF), the remaining frankfurter was discarded. The 24-oz bags with two frankfurters and 50 ml MRD were shaken to detach the cells *(168)* and ten-fold serial dilutions of the rinsate were prepared in 0.1% buffered peptone water (Difco). Aliquots of appropriate dilutions were surface-plated onto PALCAM agar (Difco) and tryptic soy agar with 0.6% yeast extract (TSAYE) for pathogen and total microbial populations, respectively. The PALCAM agar and TSAYE plates were incubated at 30°C for 48 h and at 23°C for 72 h, respectively. Bacterial colonies were enumerated and counts were expressed as $\log_{10}$ CFU/cm^2.

Physical and chemical analyses

Samples analyzed microbiologically (2 frankfurters in a bag with 50 ml of MRD) were also used for the pH analysis after pummeling (2 min; Masticator, IUL Instruments, Barcelona, Spain). The pH values were measured using a pH meter fitted with a glass electrode (Denver Instruments, Arvada, CO; *(117)*). Water activity (a_w) values (AquaLab model series 3, Decagon Devices, WA) were determined at d-0. The fat and moisture contents of frankfurters were determined according to the AOAC International official methods (960.39 and 950.46.B, respectively) *(8)*.

Statistical analysis

The experiment was repeated twice each time using a different batch of frankfurters; three samples of each treatment were analyzed at each sampling time. Microbial colony forming unit (CFU) counts were converted to log-units and then data were analyzed as a randomized complete block design with a factorial arrangement of the treatments using the GLM procedure of SAS *(172)*. The analysis assessed the main effects of product formulation, storage temperature, type of packaging, SBP, and SHF as well as two-way interactions (product formulation × storage temperature, product formulation × type of packaging, product formulation × SBP, product formulation × SHF, storage temperature × type of packaging, storage temperature × SBP; storage temperature × SHF; type of packaging × SBP; type of packaging × SHF, and SHF × SBP). Growth rates were the slopes of the lines fitted to curves by simple linear regression. Differences in analysis of variance were considered significant at $P < 0.05$.

Results and Discussion

Physico-chemical characteristics

The cooking yield (approximately 92%) and fat content (15.2 to 15.9%) of frankfurters were not affected by the presence of PL/SD in the formulation; however, the moisture content of samples with antimicrobials was slightly higher (61.7%) than that of samples without antimicrobials (58.9%). The initial water activity of frankfurters with and without PL/SD was 0.971 ± 0.004 and 0.974 ± 0.005, respectively, while corresponding initial pH values were 6.07 ± 0.23 and 6.18 ± 0.17, respectively (Table 6.1). The pH of PL/SD samples remained constant during refrigerated vacuum-packaged

storage as previously reported *(168)*. In samples without PL/SD, the initial pH did not change during SBP or during SHF after 0 d of SBP; however, it decreased ($P < 0.05$) by the end of SHF following 20, 40 and 60 d of SBP (Table 6.1). These findings were similar to those of Lianou et al. *(117)* who showed that the pH of sliced cured ham stored aerobically at 7°C was stable for the first 4 d of storage and, in some instances, decreased only after 8 to 12 d. The decrease in pH was positively correlated with increases in total microbial populations and may have resulted from accumulation of microbial metabolites on the surface of frankfurters *(117, 168)*. Some types of spoilage microflora, however, may not produce acids and therefore, may not decrease product pH.

***L. monocytogenes* during simulated transportation and SBP**

The initial level of *L. monocytogenes* was 1.8 ± 0.1 log CFU/cm^2, and counts remained relatively constant on frankfurters with PL/SD regardless of product history and storage conditions (Figures 6.2 and 6.3). The growth inhibitory effect of PL/SD on *L. monocytogenes* has been documented, with some variation in effectiveness depending on concentrations of the antimicrobials, types of RTE meats, and storage conditions *(74, 117, 168)*. However, under certain conditions, PL/SD treatments may not be able to completely suppress the growth of the pathogen. For example, Barmpalia et al. *(12)* showed that *L. monocytogenes* was able to grow (0.009 and 0.084 log CFU/cm^2/day at 4 and 10°C, respectively) on sliced pork bologna formulated with 1.8% of sodium lactate and 0.25% of sodium diacetate.

Pathogen levels and total microbial counts on frankfurters without PL/SD did not change ($P \geq 0.05$) during the 24 h simulated storage at a processing plant (4°C) and simulated transportation conditions from a plant to stores (7 h at 7°C followed by 7 h at

12°C) (Table 6.2). The lack of microbial growth during these process steps was expected because of the low temperature and the relatively short time (approximately 38 h) which elapsed from the time of inoculation. This time was not sufficient for significant microbial growth, especially considering that bacterial cells usually require time to adjust their metabolism to a new environment before initiation of cell division, when the growth medium or conditions change (apparently, the habituation of cells before product inoculation did not affect the lag phase). Also, cell counts did not increase during the temperature abusive step simulating transportation from stores to consumer homes or food service establishments (3 h at 23°C). Similarly, Klepzig et al. *(103)* demonstrated almost no growth of *L. monocytogenes* on iceberg lettuce during storage for 3 h at 25 °C (samples were stored at 6 °C before the cooling was interrupted). In that study, an increase in pathogen numbers occurred only after 6 h of storage at the abusive temperature.

In the present study, *L. monocytogenes* grew continuously on frankfurters without PL/SD (0.08 log CFU/cm^2/d) during SBP, and increased by more than 5 log-cycles by d-60 (Figure 6.2). The obtained growth rate was within the range of those reported in previous studies when vacuum-packaged frankfurters were stored at 4 or 4.4°C *(14, 79)*.

***L. monocytogenes* during SHF**

On d-0 of SBP and SHF (after simulated transportation to homes/food service), numbers of the pathogen on frankfurters without PL/SD were 1.9 ± 0.1 log CFU/cm^2 and increased steadily during storage of the fresh product under all conditions (Figure 6.3). The highest growth rate (0.35 log CFU/cm^2/d) was observed on samples stored at 7°C in opened packages (Table 6.3); as expected, the pathogen grew slower ($P < 0.05$) at 4°C

compared to 7°C. Given that, according to USDA-FSIS *(211)* frankfurters may be safely stored at 4.4°C for 7 or 14 d (in opened or vacuum-packages, respectively) and considering differences between growth rates at 4 and 7°C obtained in this study, it was evident that by the end of the recommended storage period the higher storage temperature may have allowed a more than 10-fold higher pathogen level in frankfurters without antimicrobials. These observations confirm that improper food storage conditions at consumers' homes or food service establishments may substantially increase the risk of foodborne listeriosis *(59, 217)*.

The pathogen grew slower ($P < 0.05$) on vacuum-packaged frankfurters compared to product stored in opened packages at either temperature (Table 6.3). Generally, *L. monocytogenes* is capable of growing under aerobic and anaerobic conditions *(167)*. However, conflicting results have been reported as to which atmospheric conditions favor growth. For example, Buchanan et al. *(25)* reported that atmospheric conditions did not affect the growth kinetics of *L. monocytogenes* in broth media at 19 to 37°C; however, at 5°C, the growth rate tended to be higher under anaerobic conditions compared to that in presence of oxygen. In contrast, Peterson et al. *(150)* demonstrated inhibition of *L. monocytogenes* in cold smoked salmon by vacuum-packaging, compared to aerobic packaging. Suppression of pathogen growth by vacuum-packaging also was reported in fresh lamb pieces and minced lamb stored at 5°C *(70)*. Fenlon *(64)* reported that growth of *Listeria* spp. in silage was enhanced by microaerophilic conditions. In order to generate energy from nutrients in the presence of oxygen, facultative anaerobes such as *Listeria* carry out aerobic respiration, whereas when oxygen is not available, bacteria use other electron acceptors such as nitrate or fumarate *(221)*. However, oxygen allows for a

higher adenosine triphosphate (ATP) yield compared to other acceptors *(221)*. Therefore, growth in the presence of oxygen (as on the fresh product, in the present study, stored in opened packages) may result in faster cell division and increase of the population.

Growth rates during SHF following 20 d of SBP were 0.19 to 0.21 log CFU/cm^2/d and were not affected ($P \geq 0.05$) by storage conditions (Table 6.3). The pathogen levels did not increase after 7 d of storage of aged product at 4 or 7°C in opened packages (Figure 6.3); therefore, in these two cases, growth rates were estimated as slopes between d-0 and -7. The lack of pathogen growth after 7 d of SHF may be due to competition with spoilage microflora, which in cured RTE meats is represented predominantly by *Lactobacillus* spp. *(11, 20)*. Similarly, Lianou et al. *(117)* reported significant growth of lactic acid bacteria on aerobically stored cured ham after 4 to 8 d of storage at 7°C.

When aged (20 d of SBP), vacuum-packaged frankfurters were stored at 4°C, pathogen growth rates were higher ($P < 0.05$) compared to those on fresh product (0 d SBP) (Table 6.3). The faster growth on the aged product could be explained by the possibility that, during SBP, the pathogen adjusted to the chemical composition of the frankfurters and new environmental conditions, and then initiated its growth and grew exponentially during SHF. In other words, during the first 20 days of storage, the pathogen population was mostly in lag phase, whereas between day 20 and day 40, the population was in logarithmic growth phase. While at 4°C, the pathogen grew faster on aged products, but the opposite was observed at 7°C, especially in opened packages. When aged product was stored at 7°C, the spoilage microflora began to dominate, inhibiting growth of the pathogen, which may be supported by the comparison of total microbial counts and counts of *L. monocytogenes* (Figures 6.3 and 6.4).

Even though at 7°C the pathogen grew slower on aged product compared to product that was fresh, it cannot be suggested that aged product is safer for consumption because *L. monocytogenes* started its growth during SBP and had already increased by 1.6 log CFU/cm^2. Considering possible contamination levels of RTE foods sold at retail *(82, 115)*, such an increase may result in pathogen levels of more than 1,000 cells per g, which is likely to cause listeriosis *(56)*. By 40 and 60 d of SBP, numbers of the pathogen increased by approximately 4 to 5 log-cycles (Figure 6.3). Growth rates of *L. monocytogenes* during SHF did not follow a particular trend and were generally lower than on fresher samples (Table 6.3), which was probably because the bacterium was close to the stationery phase and because of competition with spoilage microflora.

Total microbial counts

During SBP of frankfurters formulated without PL/SD, total microbial populations (TSAYE) were not different ($P \geq 0.05$) from populations of *L. monocytogenes* (PALCAM agar) at any tested period (Figure 6.2). Also, during the SHF of fresh product at all conditions or after 20 d of SBP during the SHF at 4°C under either atmospheric conditions, total microbial counts were not different ($P \geq 0.05$) from pathogen counts (Figures 6.3 and 6.4). However, when samples were stored at 7°C (regardless of package type) after 20 d of SBP or at either temperature after 40 and 60 d of SBP, total microbial counts were higher ($P < 0.05$) than counts of *L. monocytogenes*. Similarly, Barmpalia et al. *(11)* reported that spoilage bacteria outgrew the pathogen only after 28 to 40 d of storage of vacuum-packaged frankfurters at 10°C.

On product formulated with PL/SD, total microbial counts did not increase ($P \geq 0.05$) during SBP and, in most instances, were stable during SHF. The populations

increased ($P < 0.05$) only after 40 d of SBP during SFH at 7°C in opened or vacuum-packages and after 60 d of SBP at 7°C in opened packages. For all treatments, variation in total microbial counts increased with storage time. Therefore, potassium lactate and sodium diacetate, at concentrations used in this study, may suppress growth of *L. monocytogenes*, allowing for growth of spoilage microflora. Therefore, frankfurters formulated with the antimicrobials and exposed to a slightly abusive temperature (7°C) for a week, after 40 days of storage at the simulated retail conditions (4°C), may appear spoiled and possibly be discarded by consumers, as most of them describe spoilage as a major food safety concern *(66)*. Unlike frankfurters with PL/SD, fresh samples (less than 20 d of age) without the antimicrobials contained high numbers of *L. monocytogenes* at the end of SFH, whereas levels of spoilage microflora were low and product looked acceptable.

Conclusions

Results indicated that in some instances, levels of *L. monocytogenes* may increase above the performance criterion (1.0 log CFU/cm^2) on frankfurters stored within the safe time and temperature storage conditions *(60, 211)* if product is formulated without antimicrobials and stored for 20 d before SHF. Therefore, based on the growth rates obtained in this study, if a 1-log increase of *L. monocytogenes* is used as the criterion, fresh frankfurters without PL/SD (after 0 d of SBP) may be stored at 4°C in opened or vacuum-packages for 6 and 13 d, respectively. This is similar to current storage recommendations, which suggest that frankfurters can be safely stored at 4.4°C in opened packages for no more than a week or in vacuum-packages for no more than two weeks *(60, 61, 211)*. However, if product without antimicrobials was contaminated at

packaging, held under control of the manufacturer for 2 weeks and then stored unopened for 7 days at the retail/consumer level, pathogen numbers may increase by more than 1 log-cycle (according to data from this study, >1.6-log increase would be expected in 21 days even when held at constant 4°C). The results of this study can potentially be used in establishing safe time and temperature storage recommendations for frankfurters and in *L. monocytogenes* risk assessments. In addition, these results may be useful in efforts to consider implementation of safety-based consume-by date labels or in establishment of guidelines for length of storage of frankfurters before consumption.

Table 6.1. The pH values of *L. monocytogenes*-inoculated frankfurters formulated without or with 1.5% potassium lactate and 0.1% sodium diacetate (PL/SD) and stored in opened or vacuum-packages at 4 or 7°C.

Storage period (d) before purchase at 4°C	Storage period (d) at home or food service at 4 or 7°C	Stored at 4°C				Stored at 7°C			
		Opened packages		Vacuum-packages		Opened packages		Vacuum-packages	
		No PL/SD	PL/SD	No PL/SD	PL/SD	No PL/SD	PL/SD	No PL/SD	PL/SD
Initial		6.18 A a	6.07 A a	6.18 A a	6.07 A a	6.18 A a	6.07 A a	6.18 A a	6.07 A a
0	0	6.13 A a	6.06 A a	6.13 A a	6.06 A a	6.13 A a	6.06 A a	6.13 A a	6.06 A a
	2	6.23 A a	6.12 A a	6.18 A a	6.12 A a	6.20 A a	6.12 A a	6.23 A a	6.12 A a
	4	6.09 A a	6.00 A a	6.06 A a	6.04 A a	6.06 A a	6.19 A a	6.09 A a	6.02 A a
	7	6.12 A a	6.05 A a	6.11 A a	6.04 A a	6.12 A a	6.05 A a	6.12 A a	6.05 A a
	14	6.19 A a	6.15 A a	6.22 A a	6.16 A a	6.19 A a	6.09 A a	6.18 A a	6.17 A a
20	0	6.14 A a	6.11 A a	6.14 A a	6.11 A a	6.14 A a	6.11 A a	6.14 A a	6.11 A a
	2	6.18 A a	6.09 A a	6.18 A a	6.11 A a	6.15 A a	6.09 A a	6.13 A a	6.11 A a
	4	6.25 A a	6.14 A a	6.22 A a	6.16 A a	6.15 A a	6.16 A a	6.23 A a	6.14 A a
	7	6.09 A a	6.19 A a	6.11 A a	6.17 A a	6.05 A a	6.17 A a	6.22 A a	6.18 A a
	14	5.86 A a	5.99 A a	5.74 B a	5.96 A a	5.70 BC a	6.01 A a	5.70 B a	6.07 A a
40	0	6.20 A a	6.07 A a	6.20 A a	6.07 A a	6.20 A a	6.07 A a	6.20 A a	6.07 A a
	2	6.00 A a	6.12 A a	5.96 A a	6.02 A a	5.90 A a	6.14 A a	6.09 A a	6.12 A a
	4	6.01 A a	6.04 A a	6.00 A a	6.11 A a	5.86 B a	6.11 A a	5.74 B a	6.12 A a
	7	6.03 A a	6.13 A a	6.24 A a	6.18 A a	5.82 B a	6.16 A a	6.12 A a	6.19 A a
	14	5.94 A a	6.08 A a	6.04 A a	6.17 A a	5.46 C b	5.78 B b	5.68 B b	6.15 A a
60	0	6.23 A a	6.26 A a	6.23 A a	6.26 A a	6.23 A a	6.26 A a	6.23 A a	6.26 A a
	2	5.58 B b	6.07 A a	5.81 B a	6.13 A a	5.55 BC b	5.96 A a	5.69 B b	6.05 A a
	4	5.43 B b	6.03 A a	5.92 A a	6.08 A a	5.68 BC b	5.95 A a	6.09 A a	6.01 A a
	7	5.69 B b	6.11 A a	5.82 B a	6.14 A a	5.50 C b	5.88 A a	5.96 A a	5.80 B a
	14	5.41 B b	5.97 A a	5.76 B a	6.03 A a	5.40 C b	5.78 B a	5.42 B b	6.01 A a

Within a column, means lacking a common uppercases letter are significantly different ($P < 0.05$).
Within a row, means lacking a common lowercase letter are significantly different ($P < 0.05$).

Table 6.2. Counts (mean ± standard deviation) of *L. monocytogenes* (PALCAM agar) and total microbial populations (TSAYE) on inoculated frankfurters formulated without 1.5% potassium lactate and 0.1% sodium diacetate at different stages from manufacturing to consumption.

Storage period	Microbial counts (log CFU/cm^2)	
	PALCAM	TSAYE
Initial*	1.8 ± 0.1 D	1.7 ± 0.1 D
24 h at 4°C	1.7 ± 0.2 D	1.7 ± 0.2 D
7 h at 7 followed by 7 h at 12°C	1.6 ± 0.2 D	1.9 ± 0.1 D
3 h at 23°C	1.9 ± 0.1 D	1.8 ± 0.2 D
20 d of SBP	3.2 ± 0.5 C	3.3 ± 0.5 C
3 h at 23°C	3.5 ± 0.2 C	3.5 ± 0.2 C
40 d of SBP	5.3 ± 0.5 B	5.3 ± 0.5 B
3 h at 23°C	5.7 ± 0.5 B	5.7 ± 0.4 B
60 d of SBP	7.1 ± 0.5 A	7.0 ± 0.3 A
3 h at 23°C	6.7 ± 0.5 A	6.6 ± 0.6 A

Within a column, means lacking a common uppercase letter are significantly different ($P < 0.05$).
*Microbial populations (*L. monocytogenes* and total microbial counts) on samples analyzed within 1 h after inoculation
SBP: simulated storage before purchase (60 d, 4°C)
SHF: storage at 4 or 7°C for 14 d to simulate storage at consumer homes or food service establishments

Table 6.3. Growth rates of *L. monocytogenes* on frankfurters formulated without 1.5% potassium lactate and 0.1% sodium diacetate during storage for 14 d at 4 or 7°C in opened or vacuum-packages after 0, 20, 40 and 60 d of vacuum-packaged storage at 4°C.

Storage in vacuum-packages at 4°C (d)	Pathogen counts after the simulated storage before purchase (SBP; log CFU/cm^2)	Simulated home or foodservice storage temperature (SHF; °C)	Growth rates (log CFU/cm^2/d)	
			Opened packages	Vacuum-packages
0	1.9 ± 0.1	4	0.16 ± 0.00 aB	0.08 ± 0.03 bB
		7	0.35 ± 0.02 aA	0.24 ± 0.01 bA
20	3.5 ± 0.2	4*	0.21 ± 0.08 aB	0.19 ± 0.02 aA
		7*	0.21 ± 0.00 aB	0.21 ± 0.03 aA
40	5.7 ± 0.5	4	0.03 ± 0.01 aCD	0.02 ± 0.02 aB
		7	0.03 ± 0.02 aCD	0.08 ± 0.02 aB
60	6.7 ± 0.5	4	-0.03 ± 0.03 aD	0.05 ± 0.08 bB
		7	0.07 ± 0.02 aC	0.06 ± 0.06 aB

*Growth rates were estimated using data points obtained during the first 7 d of storage, since pathogen numbers peaked at d-7 and did not increase by d-14, presumably because of competition with the spoilage microflora

Within a column, means lacking a common uppercase letter are significantly different ($P < 0.05$).

Within a row, means lacking a common lowercase letter are significantly different ($P < 0.05$).

SBP: simulated storage before purchase (60 d, 4°C)

SHF: storage at 4 or 7°C for 14 d to simulate storage at consumer homes or food service establishments

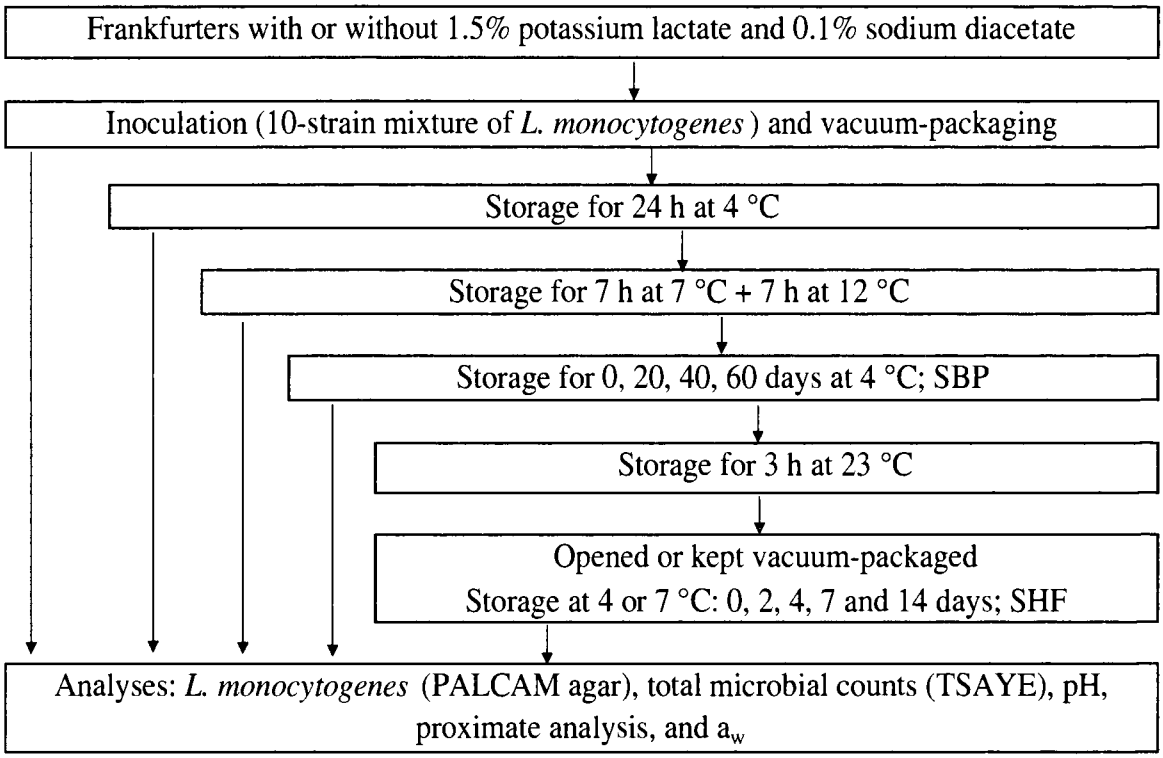


Figure 6.1. Frankfurters (with or without 1.5% potassium lactate and 0.1% sodium diacetate) were inoculated with a 10-strain mixture *of L. monocytogenes* (to a target inoculum level of 1.8 ± 0.1 log CFU/cm^2) and stored under conditions simulating in-plant storage before transportation to retail (24 h at 4°C), temperature abuse during transportation (7 h at 7°C followed by 7 h at 12°C), storage before purchase (up to 60 d at 4°C; SBP), temperature abuse during transportation from retail to consumer homes or food service establishments (3 h at 23°C), and storage at consumer homes or food service establishments (4 or 7°C for up to 14 d; SHF). Microbial, chemical and physical analyses were performed as described in the text.

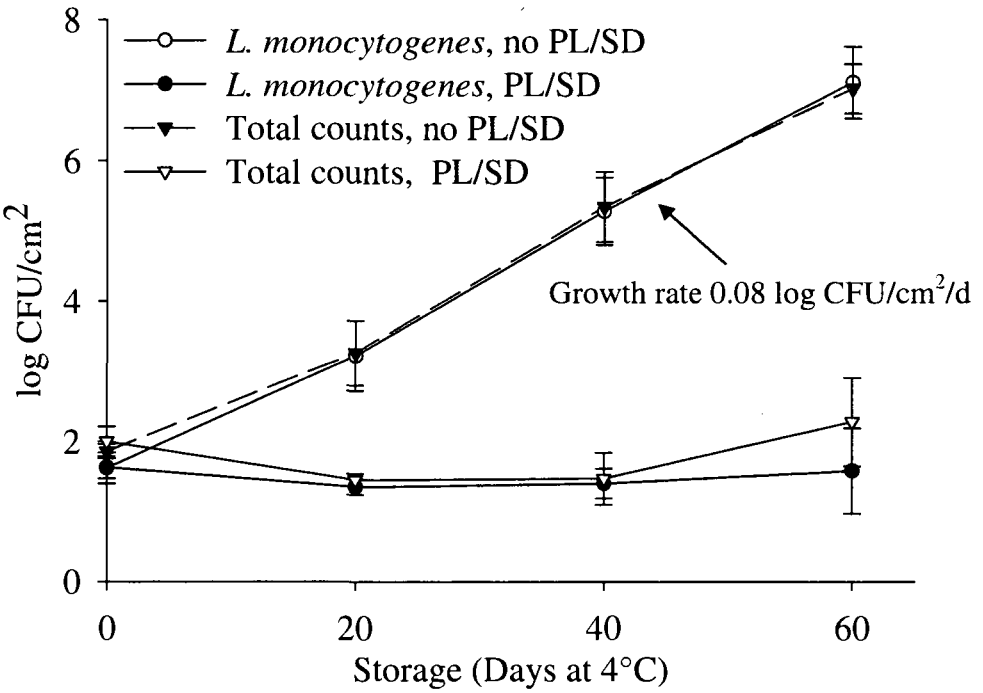
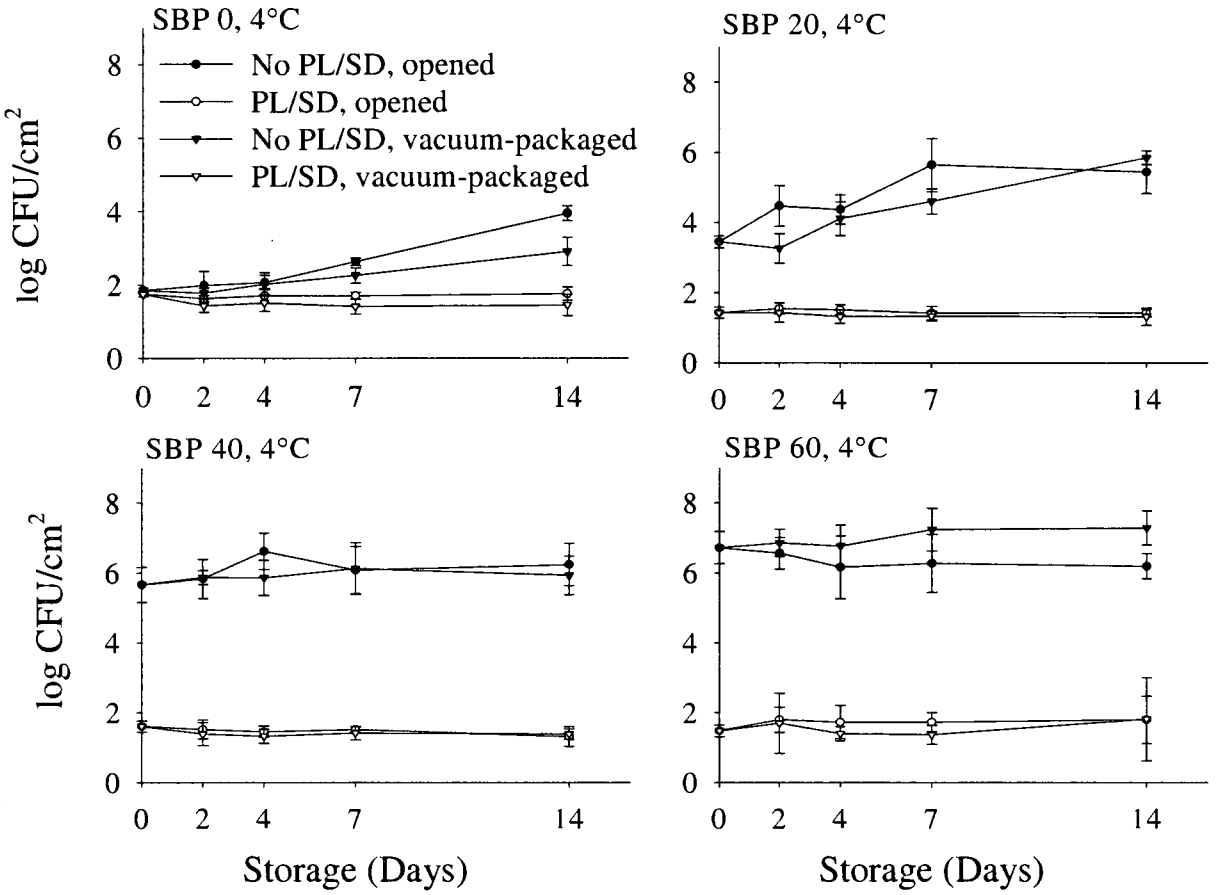


Figure 6.2 (Appendix table 6). *L. monocytogenes* (PALCAM agar) and total microbial (TSAYE) populations on inoculated frankfurters formulated with or without 1.5% potassium lactate and 0.1% sodium diacetate (PL/SD), vacuum-packaged and stored at 4°C for 60 d.


SBP 0, 4°C
No PL/SD, opened
PL/SD, opened
No PL/SD, vacuum-packaged
PL/SD, vacuum-packaged
SBP 20, 4°C
SBP 40, 4°C
SBP 60, 4°C
log CFU/cm2
log CFU/cm2
0
2
4
6
8
0
2
4
7
14
Storage (Days)
Storage (Days)

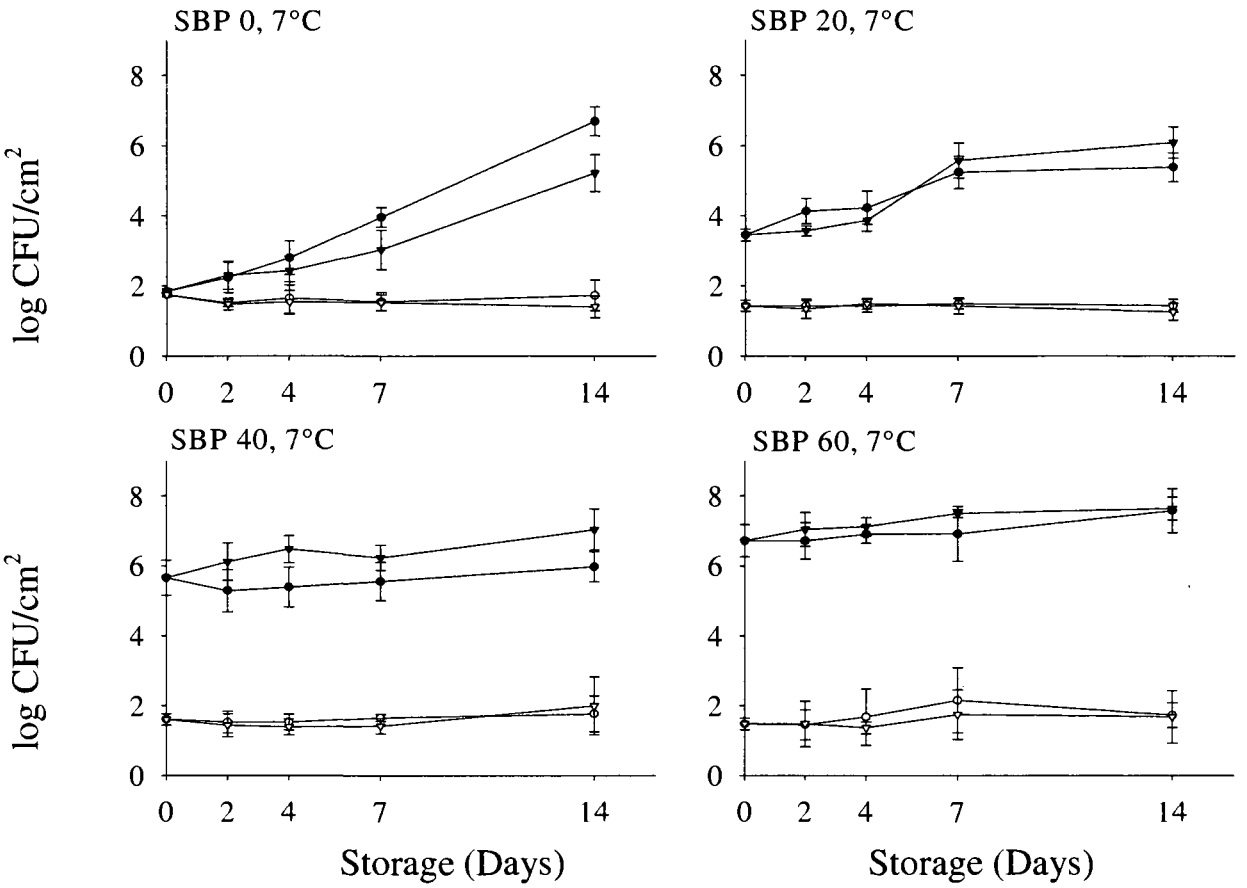
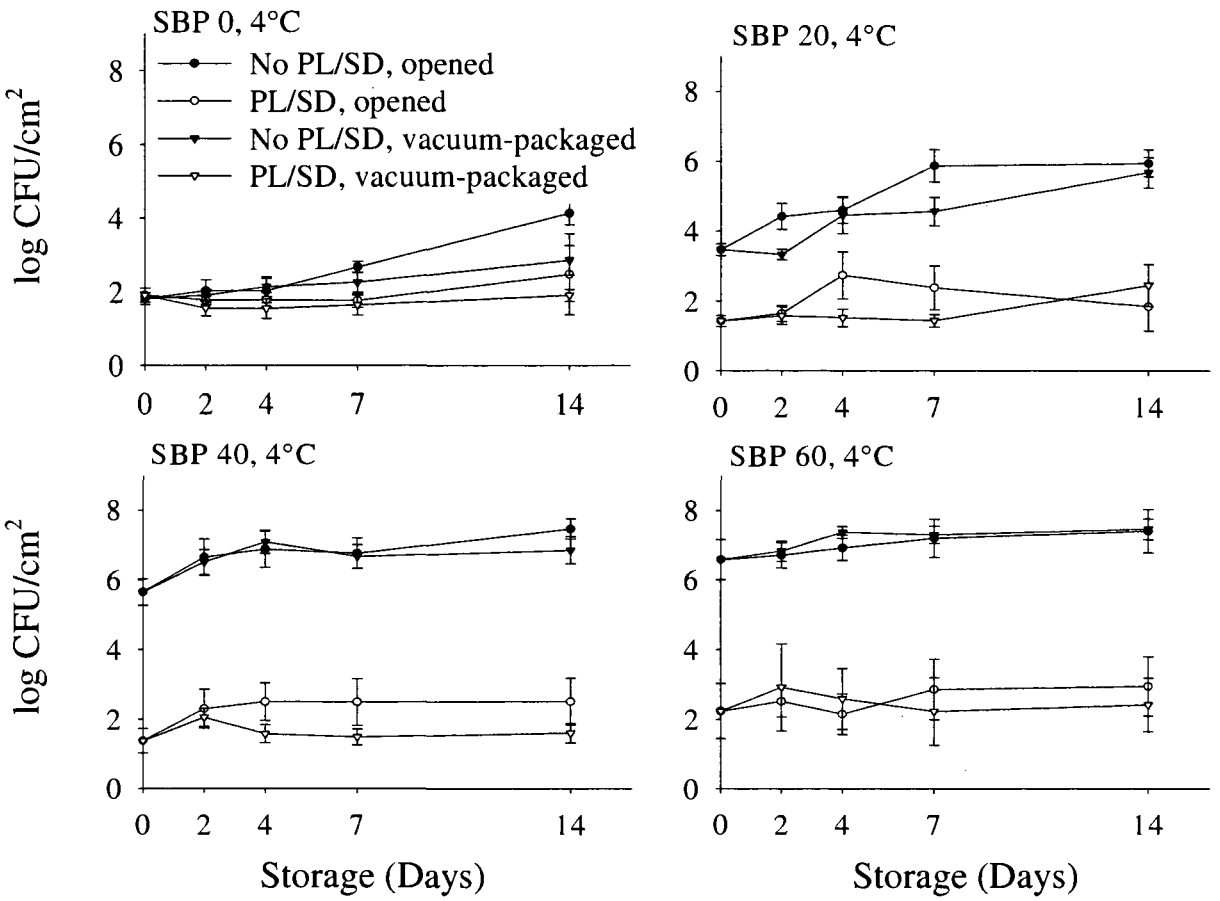


Figure 6.3 (Appendix Table 6). *L. monocytogenes* populations (PALCAM agar) on inoculated frankfurters formulated with or without 1.5% potassium lactate and 0.1% sodium diacetate (PL/SD) that were stored for 14 d in opened or vacuum-packages at 4 or 7°C, after 0, 20, 40 and 60 d of simulated storage before purchase (SBP).


SBP 0, 4°C
No PL/SD, opened
PL/SD, opened
No PL/SD, vacuum-packaged
PL/SD, vacuum-packaged
SBP 20, 4°C
SBP 40, 4°C
SBP 60, 4°C
log CFU/cm2
log CFU/cm2
0
2
4
6
8
0
2
4
7
14
Storage (Days)
Storage (Days)

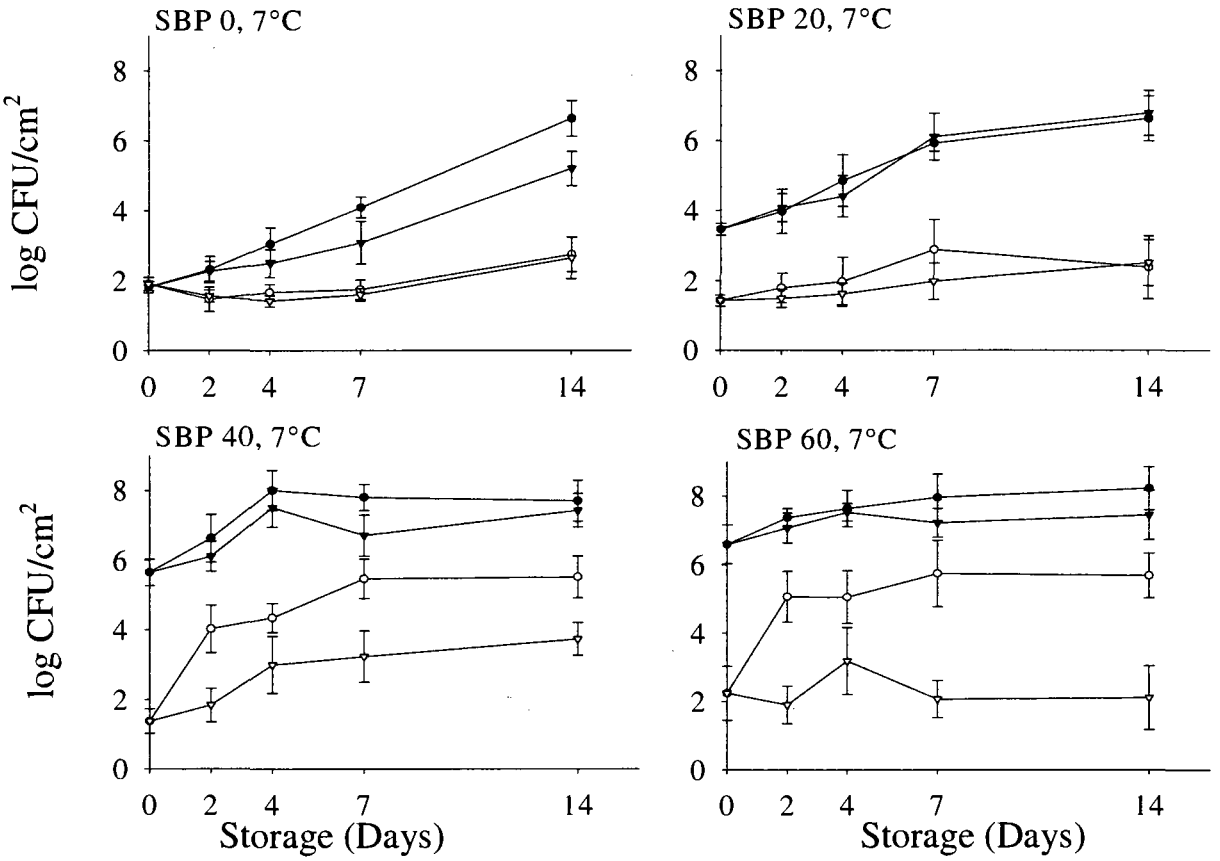


Figure 6.4 (Appendix Table 7). Total microbial populations (TSAYE) on inoculated frankfurters formulated with or without 1.5% potassium lactate and 0.1% sodium diacetate (PL/SD) that were stored for 14 d in opened or vacuum-packages at 4 or 7°C, after 0, 20, 40 and 60 d of simulated storage before purchase (SBP).

CHAPTER 7

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APPENDIX

Appendix Table 1 (Figure 4.1). Survivors (PALCAM agar; log CFU/cm^2) of *L. monocytogenes* on frankfurters by dipping in distilled water (DW) or lactic acid (0, 1.0, 1.5, 2.0, and 3.0%; LA) at 4, 25 or 55°C.

Dipping time (sec)	Lactic acid concentration (%)	Temperature of the solution (°C)		
		4	25	55
0 (Untreated)	—	4.4±0.1 aA	4.4±0.1 aA	4.4±0.1 aA
15	0 (DW)	ND*	3.4±0.2 bcA	3.3±0.1 bA
30		3.3±0.1 bA	3.5±0.2 bA	3.3±0.1 bA
60		ND	3.3±0.1 bcA	3.3±0.1 bA
120		3.3±0.1 bA	3.3±0.4bcA	3.3±0.3 bA
15	1	ND	3.3±0.0 bcA	2.8±0.3 bcA
30		3.8±0.0 abA	3.3±0.1 bcAB	2.7±0.1 bcdB
60		ND	3.2±0.0 bcA	2.3±0.0 cdefB
120		3.6±0.3 bA	3.3±0.4 bcA	2.2±0.3 cdefgB
15	1.5	ND	3.5±0.2 bA	2.5±0.0 cdeB
30		ND	3.2±0.0 bcA	2.6±0.0 cdeA
60		ND	ND	ND
120		ND	ND	ND
15	2	ND	3.3±0.2 bcA	2.3±0.3 cdefB
30		3.7±0.0 bA	3.0±0.4 bcdB	2.4±0.3 cdeB
60		ND	3.0±0.4 bcdA	2.0±0.3 defgB
120		3.6±0.0 bA	2.8±0.4 bcdB	1.7±0.5 fgC
15	3	ND	3.0±0.1 bcdA	2.2±0.3 cdefgB
30		3.6±0.1 bA	2.9±0.2 bcdB	1.9±0.2 efgC
60		ND	2.8±0.1 bcdA	1.9±0.1 efgB
120		3.2±0.1 bA	2.4±0.0 dA	1.6±0.5 gB

*ND: Not done

Within a column, means lacking a common lowercase letter are significantly different ($P < 0.05$). Within a row, means lacking a common uppercase letter are significantly different ($P < 0.05$).

Appendix Table 2 (Figure 4.2). Survivors (tryptic soy agar with 0.6% yeast extract; TSAYE; log CFU/cm^2; mean ± standard deviation) of *L. monocytogenes* on frankfurters by dipping in distilled water (DW) or lactic acid (0, 1.0, 1.5, 2.0, and 3.0%; LA) at 4, 25 or 55°C.

Dipping time (sec)	Lactic acid concentration (%)	Temperature of the solution (°C)		
		4	25	55
0 (Untreated)	—	4.3±0.2 aA	4.3±0.2 aA	4.3±0.2 aA
15	0 (DW)	ND	3.3±0.0 bA	3.1±0.2 bcA
30		3.4±0.1 bA	3.3±0.2 bA	3.2±0.2 bA
60		ND	2.8±0.2 bcdA	3.1±0.2 bcA
120		3.4±0.2 bA	3.0±0.2 bcA	3.1±0.3 bcA
15	1	ND	3.0±0.0 bcA	2.6±0.4 bcdA
30		3.7±0.0 bA	3.0±0.1 bcB	2.7±0.2 bcdB
60		ND	2.8±0.1 bcdA	2.2±0.1 defA
120		3.6±0.4 bA	2.8±0.0 bcdB	2.1±0.3 defC
15	1.5	ND	3.3±0.0 bA	2.9 ± 0.2 bcA
30		ND	3.3±0.0 bA	2.7 ± 0.2 bcdA
60		ND	ND	ND
120		ND	ND	ND
15	2	ND	3.1±0.3 bcA	2.2±0.2 defB
30		3.8±0.1 b	2.8±0.3 bcdA	2.4±0.4 cdeA
60		ND	2.8±0.4 bcdA	1.8±0.1 efB
120		3.7±0.0 b	2.6±0.3 cdA	1.6±0.6 fB
15	3	ND	2.9±0.2 bcdA	2.1±0.3 defB
30		3.6±0.1 b	3.0±0.4 bcA	1.9±0.2 efB
60		ND	2.7±0.1 bcdA	1.7±0.1 fB
120		3.3±0.0 b	2.3±0.0 dA	1.6±0.3 fB

*ND: Not done

Within a column, means lacking a common lowercase letter are significantly different ($P < 0.05$). Within a row, means lacking a common uppercase letter are significantly different ($P < 0.05$).

Appendix Table 3. Surviving total microbial populations (tryptic soy agar with 0.6% yeast extract; TSAYE; log CFU/cm^2; mean ± standard deviation) on inoculated frankfurters that were left undipped or dipped in distilled water (DW) or lactic acid (1.0, 2.0, and 3.0%) at 25, 40, or 55°C for 15 or 30.

Dipping time (s)	Lactic acid concentration (%)	Temperature of dipping solution (°C)		
		25	40	55
0 (Undipped)	—	4.7±0.4 aA	4.7±0.4 aA	4.7±0.4 aA
15	0 (DW)	3.5±0.2 bcA	3.5±0.5 bA	3.7±0.2 bA
	1	3.7±0.1 bA	3.1±0.3 bcA	3.0±0.2 cdA
	2	3.3±0.4 bcA	2.6±0.1 B	2.0±0.5 efB
	3	3.4±0.7 bcA	2.6±0.4B	1.9±0.1 efC
30	0 (DW)	3.5±0.3 bcA	3.5±0.5 bA	3.2±0.3 bcA
	1	3.6±0.1 bA	2.9±0.3 bcB	2.5±0.1 deB
	2	3.3±0.2 bcA	2.7±0.2 cAB	2.2±0.3 efB
	3	2.9±0.7 cA	2.5±0.4 cA	1.8±0.3 fB

Within a column, means lacking a common lowercase letter are significantly different ($P < 0.05$). Within a row, means lacking a common uppercase letter are significantly different ($P < 0.05$).

Appendix Table 4 (Figure 5.1). Numbers of *Listeria monocytogenes* (PALCAM agar; log CFU/cm^2; mean ± standard deviation) on frankfurters that were sprayed for 10 s with distilled water, solutions of lactic acid, sodium lauryl sulfate, mixture of lactic acid and sodium lauryl sulfate, or left untreated, and inoculated before or after treatment, vacuum packaged and stored for 90 days at 4°C.

Storage period (days)	Untreated (control)	Distilled water, before inoculation	Lactic acid (5%) + sodium lauryl sulfate (0.5%), before inoculation	Distilled water, after inoculation	Lactic acid (5%), after inoculation	Sodium lauryl sulfate (0.5%), after inoculation	Lactic acid (5%) + sodium lauryl sulfate (0.5%), after inoculation
0	4.8±0.1 eA	4.7±0.1 eA	3.0±0.4 dB	3.5±0.1 cdB	3.0±0.5 cdB	2.8±0.0 dCB	2.0±0.1 dC
7	4.7±0.2 eA	4.5±0.1 eA	3.3±0.2 cdB	3.2±0.3 dB	3.1±0.4 cdB	2.6±0.2 dCB	1.6±0.7 dC
14	4.7±0.1 eA	4.8±0.1 eA	2.7±0.3 dB	3.6±0.5 cdB	2.9±0.2 dB	2.9±0.4 cdB	1.5±0.3 dC
21	4.9±0.3 eA	5.2±0.4 edA	3.4±0.5 cdB	3.4±0.3 cdB	3.1±0.3 cdBC	3.9±0.3 bcdB	2.2±0.2 cdC
28	5.6±0.6 dA	5.7±0.4 cdA	3.3±0.3 cdCD	4.7±0.2 bcAB	2.9±0.5 dDE	4.4±0.6 bcBC	2.1±0.4 cdE
35	5.9±0.5 cdAB	6.4±0.1 bcB	3.2±0.6 cdCD	5.3±0.7 bAB	2.7±0.2 dD	4.5±0.7 bBC	1.7±0.6 dD
42	6.2±0.4 bcdAB	6.8±0.3 abA	3.4±0.5 cdCD	5.0±1.2 bBC	3.1±0.4 cdD	5.2±0.6 bAB	2.4±0.7 cdD
52	6.6±0.4 abcA	6.9±0.1 abA	4.3±0.7 cB	6.8±0.3 aA	4.3±0.4 bcB	6.7±0.6 aA	3.6±0.8 bcB
62	6.9±0.3 abABC	7.1±0.1 abAB	6.0±0.3 bABC	7.3±0.1 aA	4.6±0.6 bC	7.1±0.4 aAB	4.8±1.2 abBC
77	7.0±0.1 aA	7.2±0.1 aA	7.1±0.3 abA	7.2±0.2 aA	5.3±0.5 baB	7.1±0.1 aA	5.6±0.5 aB
90	7.3±0.2 aA	7.0±0.3 abA	7.3±0.2 aA	7.3±0.2 aA	6.8±0.8 aA	7.3±0.4 aA	6.5±0.9 aA

Mean values with different lowercase letters in the same column are significantly different ($P < 0.05$).
Mean values with different uppercase letters in the same row (within the same type of response variable) are significantly different ($P < 0.05$).

Appendix Table 5 (Figure 5.2). Total microbial counts (tryptic soy agar with 0.6% yeast extract; TSAYE; log CFU/cm^2; mean ± standard deviation) on frankfurters that were sprayed for 10 s with distilled water, solutions of lactic acid, sodium lauryl sulfate, mixture of lactic acid and sodium lauryl sulfate, or left untreated, and inoculated before or after treatment, vacuum packaged and stored for 90 days at 4°C.

Storage period (days)	Untreated (control)	Distilled water, before inoculation	Lactic acid (5%) + sodium lauryl sulfate (0.5%), before inoculation	Distilled water, after inoculation	Lactic acid (5%), after inoculation	Sodium lauryl sulfate (0.5%), after inoculation	Lactic acid (5%) + sodium lauryl sulfate (0.5%), after inoculation
0	4.8±0.1 fA	4.7±0.1 dA	3.2±0.5 dB	3.6±0.2 cdB	3.2±0.4 cB	2.8±0.1 dCB	2.1±0.2 cC
7	4.8±0.1 fA	5.0±0.7 cdA	3.5±0.2 cdB	3.3±0.2 dB	3.3±0.2 cB	2.7±0.2 cdCB	1.7±0.6 cC
14	4.7±0.1 fA	5.0±0.3 cdA	2.8±0.3 dB	3.6±0.5 cdB	3.1±0.2 cB	2.8±0.2 cdB	1.7±0.1 cB
21	4.9±0.3 fAB	5.3±0.3 cdA	3.3±0.5 cdCD	3.9±0.7 cdBC	3.1±0.3 cCD	4.0±0.5 bcABC	2.1±0.2 cD
28	5.7±0.6 eA	5.7±0.4 bcA	3.4±0.3 cdCD	4.7±0.2 bcAB	3.1±0.4 cCD	4.1±0.5 bBC	2.2±0.6 cD
35	5.8±0.4 edAB	6.4±0.2 abA	3.3±0.4 dD	5.1±0.7 bBC	3.0±0.5 cD	4.4±0.6 bC	1.9±0.5 cE
42	6.3±0.3 cdA	6.7±0.3 aA	3.5±0.5 cdB	5.2±0.4 bA	3.2±0.3 cB	5.2±0.5 bA	2.6±0.8 cB
52	6.6±0.4 bcA	6.9±0.1 aA	4.4±0.6 cB	6.8±0.3 aA	4.4±0.3 bcB	6.7±0.6 aA	3.8±0.8 bcB
62	6.9±0.4 abcAB	7.0±0.1 aA	5.9±0.3 bABC	7.4±0.3 aA	4.5±0.6 bcC	7.2±0.4 aA	4.8±1.2 abBC
77	7.1±0.2 abA	7.2±0.2 aA	6.8±0.2 baA	7.4±0.4 aA	5.6±0.8 abA	7.1±0.1 aA	5.6±0.6 abA
90	7.2±0.3 aA	7.0±0.3 aA	7.3±0.2 aA	7.3±0.2 aA	6.9±0.8 aA	7.2±0.4 aA	6.8±1.1 aA

Mean values with different lowercase letters in the same column are significantly different ($P < 0.05$).
Mean values with different uppercase letters in the same row (within the same type of response variable) are significantly different ($P < 0.05$).

Appendix table 6 (Figure 6.2). *L. monocytogenes* (PALCAM agar) and total microbial (TSAYE) populations (log CFU/cm^2) on inoculated frankfurters formulated with or without 1.5% potassium lactate and 0.1% sodium diacetate (PL/SD), vacuum-packaged and stored at 4°C for 60 d.

Storage (d)	PALCAM agar		TSAYE	
	No PL/SD	PL/SD	No PL/SD	PL/SD
0	1.7±0.1 dA	1.7±0.1 aA	1.8±0.1 cA	1.8±0.1 bA
20	3.3±0.5 cA	1.4±0.1 aB	3.2±0.5 cA	1.5±0.1 bB
40	5.3±0.5 bA	1.4±0.2 aB	5.3±0.5 bA	1.5±0.4 bB
60	7.0±0.3 aA	1.6±0.6 aB	7.1±0.5 aA	2.1±0.6 aB

Mean values with different lowercase letters in the same column are significantly different ($P < 0.05$).
Mean values with different uppercase letters in the same row (within each plating medium) are significantly different ($P < 0.05$).

Appendix Table 7 (Figure 6.3). *L. monocytogenes* populations (PALCAM agar; log CFU/cm^2; mean ± standard deviation) on inoculated frankfurters formulated with or without 1.5% potassium lactate and 0.1% sodium diacetate (PL/SD) that were stored for 14 d in opened or vacuum-packages at 4 or 7°C, after 0, 20, 40 and 60 d of simulated storage before purchase (SBP).

Storage period (d) before purchase at 4°C	Storage period (d) at home or food service at 4 or 7°C	Stored at 4°C				Stored at 7°C			
		Opened packages		Vacuum-packages		Opened packages		Vacuum-packages	
		No PL/SD[b]	PL/SD	No PL/SD	PL/SD	No PL/SD	PL/SD	No PL/SD	PL/SD
Initial*		1.8±0.1 gA	1.7±0.1 aA	1.8±0.1 fA	1.7±0.1 aA	1.8±0.1 hA	1.7±0.1 aA	1.8±0.1 hA	1.7±0.1 abA
0	0	1.9±0.1 gA	1.8±0.1 aA	1.9±0.1 fA	1.8±0.1 aA	1.9±0.1 hA	1.8±0.1 aA	1.9±0.1 ghA	1.8±0.1 abA
	2	1.9±0.4 gA	1.6±0.1 aA	1.8±0.1 fA	1.4±0.2 aA	2.1±0.5 hA	1.5±0.1 aA	2.2±0.4 gfA	1.5±0.2 abA
	4	2.0±0.2 fgBC	1.7±0.2 aC	1.9±0.3 fBC	1.5±0.2 aC	2.8±0.5 gA	1.7±0.5 aC	2.4±0.4 gAB	1.5±0.3 abC
	7	2.7±0.1 fBC	1.7±0.1 aD	2.3±0.2 fC	1.4±0.2 aD	4.0±0.3 efA	1.6±0.3 aD	3.0±0.6 fB	1.5±0.2 abD
	14	4.0±0.2 eC	1.8±0.2 aE	2.9±0.4 eD	1.5±0.3 aE	6.7±0.4 bA	1.7±0.4 aE	5.2±0.5 dB	1.4±0.3 bE
20	0	3.5±0.2 eA	1.4±0.2 aB	3.5±0.2 eA	1.4±0.2 aB	3.5±0.2 fA	1.4±0.2 aB	3.5±0.2 efA	1.4±0.2 abB
	2	4.5±0.6 dA	1.6±0.2 aD	3.3±0.4 eD	1.4±0.3 aD	4.1±0.4 eAB	1.4±0.2 aD	3.6±0.2 efBC	1.4±0.3 bD
	4	4.4±0.4 dA	1.5±0.2 aB	4.1±0.5 dA	1.3±0.2 aB	4.2±0.5 eA	1.4±0.2 aB	3.9±0.3 eA	1.5±0.2 abB
	7	5.7±0.8 bcA	1.4±0.2 aC	4.6±0.4 dB	1.3±0.1 aC	5.2±0.5 dA	1.5±0.1 aC	5.6±0.5 dA	1.4±0.2 abC
	14	5.5±0.6 cAB	1.4±0.1 aC	5.9±0.2 cAB	1.3±0.3 aC	5.4±0.4 dB	1.5±0.2 aC	6.1±0.5 cdA	1.3±0.2 bC
40	0	5.7±0.5 bcA	1.6±0.2 aB	5.7±0.5 cA	1.6±0.2 aB	5.7±0.5 cdA	1.6±0.2 aB	5.7±0.5 cdA	1.6±0.2 abB
	2	5.8±0.6 bcAB	1.5±0.3 aC	5.9±0.2 cA	1.4±0.3 aC	5.3±0.6 dB	1.5±0.3 aC	6.1±0.5 cdA	1.4±0.3 abC
	4	6.6±0.6 abA	1.5±0.2 aC	5.9±0.5 cAB	1.3±0.2 aC	5.4±0.6 dB	1.5±0.2 aC	6.5±0.4 bcA	1.4±0.2 bC
	7	6.1±0.7 bA	1.5±0.2 aB	6.1±0.7 cA	1.4±0.2 aB	5.6±0.6 cdA	1.7±0.2 aB	6.2±0.4cA	1.4±0.2 abB
	14	6.2±0.6 abB	1.3±0.3 aD	5.9±0.6 cB	1.4±0.2 aD	6.0±0.4 cB	1.8±0.5 aCD	7.1±0.6 bA	2.0±0.8 aC
60	0	6.7±0.5 aA	1.5±0.2 aB	6.7±0.5 bA	1.5±0.1 aB	6.7±0.5 bA	1.5±0.2 aB	6.7±0.5 bcA	1.5±0.2 abB
	2	6.6±0.5 abA	1.8±0.4 aB	6.8±0.4 abA	1.7±0.9 aB	6.7±0.5 bA	1.5±0.4 aB	7.1±0.5 bA	1.5±0.7 abB
	4	6.2±0.9 abB	1.7±0.5 aC	6.8±0.6 abA	1.4±0.2 aC	6.9±0.3 bA	1.7±0.8 aC	7.1±0.3 abA	1.4±0.2 bC
	7	6.3±0.8 abB	1.7±0.3 aCD	7.3±0.6 aA	1.4±0.3 aD	7.2±0.8 abA	2.2±0.9 aC	7.5±0.1 a	1.8±0.7ab CD
	14	6.2±0.4 abB	1.8±0.7 aC	7.3±0.5 aA	1.8±1.2 aC	7.6±0.6 aA	1.7±0.4 aC	7.6±0.3 aA	1.7±0.8 abC

Mean values with different lowercase letters in the same column are significantly different ($P < 0.05$).
Mean values with different uppercase letters in the same row (within each plating medium) are significantly different ($P < 0.05$).
*Microbial populations on samples analyzed within 1 h after inoculation.

Appendix Table 8 (Figure 6.4). Total microbial counts (tryptic soy agar with 0.6% yeast extract; TSAYE; log CFU/cm^2; mean ± standard deviation) on inoculated frankfurters formulated with or without 1.5% potassium lactate and 0.1% sodium diacetate (PL/SD) that were stored for 14 d in opened or vacuum-packages at 4 or 7°C, after 0, 20, 40 and 60 d of simulated storage before purchase (SBP).

Storage period (d) before purchase at 4°C	Storage period (d) at home or food service at 4 or 7°C	Stored at 4°C				Stored at 7°C			
		Opened packages		Vacuum-packages		Opened packages		Vacuum-packages	
		No PL/SD[b]	PL/SD	No PL/SD	PL/SD	No PL/SD	PL/SD	No PL/SD	PL/SD
Initial*		1.7±0.1 hA	1.8±0.1 bcA	1.7±0.1 hA	1.8±0.1 bcA	1.7±0.1 jA	1.8±0.1 efA	1.7±0.1 hA	1.8±0.1 deA
0	0	1.8±0.2 hA	1.9±0.2 bcA	1.8±0.2 ghA	1.9±0.2 bA	1.8±0.2 jA	1.9±0.2 efA	1.8±0.2 ghA	1.9±0.2 deA
	2	1.9±0.3 hAB	1.8±0.1 bcAB	1.9±0.2 ghAB	1.6±0.2 bcB	2.2±0.4 jA	1.5±0.3 fB	2.2±0.3 ghA	1.6±0.2 eB
	4	2.0±0.3 hB	1.8±0.2 bcBC	2.1±0.3 ghB	1.6±0.3 bcBC	2.9±0.5 iA	1.7±0.2 efBC	2.4±0.4 gAB	1.4±0.2 eC
	7	2.7±0.1 gBC	1.8±0.2 bcDE	2.3±0.3 gCD	1.6±0.3 bcE	4.1±0.3 gA	1.8±0.3 efDE	3.1±0.6 fB	1.6±0.2 eE
	14	4.1±0.3 eC	2.3±1.0 ab	2.9±0.4 fD	1.9±0.1 bF	6.6±0.5 dA	2.6±0.6 dDE	5.2±0.5 dB	2.5±0.6 bcDEF
20	0	3.5±0.2 fA	1.4±0.2 cB	3.5±0.2 eA	1.4±0.2 cB	3.5±0.2 hA	1.4±0.2 fB	3.5±0.2 fA	1.4±0.2 eB
	2	4.4±0.4 eA	1.6±0.2 cC	3.3±0.2 efB	1.6±0.2 bcC	4.0±0.6 ghA	1.8±0.4 efC	4.1±0.4 eA	1.5±0.3 eC
	4	4.6±0.4 eA	2.5±0.7 abB	4.5±0.5 dA	1.5±0.3 bcC	4.9±0.8 fA	1.8±0.6efC	4.4±0.6 eA	1.6±0.3 eC
	7	5.9±0.5 dA	2.2±0.6 bCD	4.6±0.4 dB	1.4±0.2 cE	5.9±0.2 eA	2.7±0.8 dC	6.1±0.7 cA	1.9±0.4 deDE
	14	6.0±0.4 dB	1.7±0.6 bcE	5.7±0.5 cC	2.3±0.6 abD	6.6±0.7 dA	2.2±0.8 deED	6.8±0.7 bA	2.3±0.6 cdD
40	0	5.7±0.4 dA	1.4±0.4 cC	3.5±0.2 eB	1.4±0.2 cC	3.5±0.2 hB	1.4±0.2 fC	3.5±0.2B	1.4±0.2 eB
	2	6.7±0.5 bcA	2.1±0.5 bcD	6.5±0.4 bAB	2.0±0.2 bD	6.6±0.7 dAB	3.9±0.9 cC	6.1±0.5 cB	1.8±0.5 deD
	4	6.9±0.5 bcC	2.4±0.6 abE	7.1±0.3 abBC	1.6±0.3 bcF	8.0±0.6 abA	4.4±0.5 bcD	7.5±0.6 aAB	2.7±0.8 bcE
	7	6.8±0.4 bcB	2.3±0.7 abE	6.7±0.4 bB	1.5±0.2 bcF	7.9±0.2 abcA	5.4±0.6 aC	6.7±0.6 bB	3.0±0.8 bD
	14	7.5±0.3 aA	2.4±0.7 abE	6.9±0.4 bB	1.6±0.3 bcF	7.7±0.6 abcA	5.5±0.6 aC	7.4±0.5 aAB	3.8±0.5 aD
60	0	6.6±0.6 cA	2.0±0.8 bcC	3.5±0.2 eB	1.4±0.2 cC	3.5±0.2 hB	1.4±0.2 fC	3.5±0.2 fB	1.4±0.2 eC
	2	6.7±0.4 bcB	2.4±0.5 abD	6.8±0.3 bB	2.6±1.3 aD	7.4±0.3 cA	4.8±0.8 bC	7.1±0.4 abAB	1.8±0.5 deE
	4	6.9±0.4 bcB	2.0±0.5 bcE	7.4±0.1 abAB	2.4±0.8 abE	7.6±0.6 bcA	4.8±0.8 bC	7.5±0.3 aA	3.0±0.9 bD
	7	7.2±0.6 abB	2.6±1.0 abD	7.3±0.3 abB	1.9±0.9 bE	8.0±0.7 abA	5.5±1.1 aC	7.2±0.4 abB	1.9±0.5 deE
	14	7.4±0.6 abB	2.8±0.9 aD	7.5±0.3 aB	2.2±0.7 abE	8.2±0.7 aA	5.5±0.7 aC	7.5±0.7 aB	1.9±0.8 deE

Mean values with different lowercase letters in the same column are significantly different ($P < 0.05$).
Mean values with different uppercase letters in the same row (within each plating medium) are significantly different ($P < 0.05$).
*Microbial populations on samples analyzed within 1 h after inoculation.