CONTROL OF *LISTERIA MONOCYTOGENES* IN FURTHER PROCESSED MEAT AND POULTRY USING ORGANIC ACID POST-COOK DIPS

by

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ABSTRACT

Most major outbreaks attributed to *Listeria monocytogenes* (LM) involve post-processing contamination of turkey and ham deli slices, and frankfurters. This study evaluated the antilisterial effects of organic acids applied as post-cook dips to turkey and ham deli slices and beef frankfurters. Two trials were conducted to analyze LM levels. Efficacy was determined by prolongation of the lag phase and overall LM levels at the end of storage.

Treatments included sodium lactate (SL; 3.6%), potassium lactate (PL; 3.6%), sodium citrate (SC; 0.75%), a combination of SL and sodium diacetate (SDA; 0.25%), and a combination of SL/PL/SDA. Products were formulated with 1.5% sodium chloride and 0.45% sodium tripolyphosphate. Ham deli loaves and beef frankfurters were also formulated with 156 ppm and 6.25% sodium nitrite, respectively. Products were surface inoculated with 10^4-10^5 log CFU/mL of streptomycin resistant (1,500 μg/mL) LM strain Scott A before immersion treatment. Positive and negative controls were immersed in sterile water. Products were stored in Whirlpak™ bags under refrigerated temperatures (~4˚C) for sampling at 0, 7, 14, 21, 28, 42, and 56 d.

The SL/SDA combination applied to turkey extended the lag phase until d21. When applied to ham, the lag phase extended through d14. For both products, LM concentrations rose less than one log (0.7 log CFU/g) throughout the 56 d storage period. The SL/PL/SDA applied to turkey extended the lag phase throughout d 42 with decreasing LM concentrations after d 21. An overall decrease (0.1 log CFU/g) was noted by 56 d. When applied to ham, treatment with a SL and both combination treatments extended the lag phase through d 14. Lag phase in the inoculated control lasted until d 7.

Single lactic acid treatments and the SL/SDA combination treatment applied to frankfurters extended the lag phase throughout 28 d post-processing. Lag phase was also extended throughout d 28 for control frankfurters. Treatment with SL/PL/SDA extended the lag phase throughout 21 d. The lag phase for the inoculated control persisted until d 28. Overall growth was limited within the range of 1.0 log CFU/g for all organic acid treatments. Combination organic acid dipping solutions prolonged the LM lag phase for 2 to 6 weeks on treated turkey deli loaves. Combination dipping solutions and sodium lactate extended the lag phase for an additional week beyond the inoculated control when applied to ham. Single and combination lactic acid treatments prolonged the LM lag phase for 3 to 4 weeks on treated beef frankfurters, but the same results were noted on untreated frankfurters.

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CHAPTER I

INTRODUCTION

According to the Centers for Disease Control (CDC) estimates collected through 1997, listeriosis, the foodborne illness resulting from ingestion of *Listeria monocytogenes* (LM), affects approximately 2,500 people annually with approximately 500 deaths (CDC 2005). Although vulnerable groups typically include those with developing and compromised immune systems such as children, critically ill individuals, transplant patients, and the elderly, the affliction of pregnant women generates the largest regulatory concern due to the high percentage of maternal susceptibility to this disease and infant mortality. Of the reported cases of listeriosis, approximately 30% occurred during pregnancy (CDC 2005). Approximately 50% of these cases resulted in neonate mortality (CFSP 2005). Studies conducted by the CDC show that pregnant women are twenty times more likely than healthy adults to contract listeriosis (CDC 2005).

Adaptations to environmental stressors, such as extremes of temperature, pH, salinity, and water activity, establish LM as a potent foodborne pathogen**.** *Listeria monoyctogenes* is a gram positive, bacilli-shaped, and facultatively anaerobic bacteria (Davidson and others 2005). In comparison to gram negative bacteria, gram positive bacteria have a thicker layer of peptidoglycan in their cell walls responsible for a more fortified intracellular environment. Ubiquitous in nature, occurring in soil and decaying vegetation, contamination of food products is typically introduced by proliferation in the processing environment from cross contamination with soil, animal manure, or contaminated water. Psychrophilic capabilities, defined as the ability to flourish in cold temperatures, promote its residence in processing environments as low as -1.5˚C (Lundén

2004). Consequently, most recent LM outbreaks have been attributed to refrigerated, ready- to- eat (RTE) foods such as deli meats, including sliced ham and turkey, Mexican-style soft cheeses, and frankfurters (Davidson and others 2005). Based on observations from recent outbreaks, Walls and others (2004) described LM-susceptible foods as often stored at refrigeration for extended time periods, having a potential for contamination, and providing nutritive support for the microorganism.

Current industry success for combating LM includes good manufacturing practices, environment sanitation, managing HACCP throughout manufacturing, pasteurization, inclusions in product formulation, and post processing treatments. Current research focuses on the addition of organic acids as formulations and post-cook marinades to processed meat products with the intent of decreasing contamination growth of LM and prolonging the bacterial lag phase. The ingredients most widely studied include sodium lactate (SL), potassium lactate (PL), sodium citrate (SC), and sodium diacetate (SDA). The actions of these additives are most commonly applied in conjunction with industry controls such as sodium chloride (NaCl), sodium tripolyphosphate (STPP), and sodium nitrite (SN) (Davidson and others 2005). The effects of treatment with organic acids can be described as bacteriostatic or bacteriocidal. Bacteriostatic ingredients prolong the lag phase of bacterial growth during which the microorganism is adjusting to the extracellular environment. This is the initial phase of the bacterial growth curve followed by the logarithmic growth phase during which LM has the potential to double in number every hour (Doyle and others 1997). Bacteriocidal ingredients actually reduce bacterial numbers by initiating cell death.

Combined antimicrobials can achieve the same effects as their single counterparts at lower concentrations. This compilation of ingredient strengths and mechanisms can be visualized as presenting multiple streams of attack, a technique referred to as "multi-hurdle technology." Doyle and others (1997) referred to this method as "deoptimizing" the conditions required for marinade ingredients to achieve antimicrobial effectiveness, thereby visualized as lowering the bar. The purpose of this experiment is to determine the inhibitory effect of organic acid marinades, applied alone and in combination, to further processed meat and poultry products as post-cook dips. This will be determined by the extension of the lag phase, and therefore shelf life, as measured by fluctuating LM levels.

CHAPTER II

LITERATURE REVIEW

Disease Characteristics

Listeria monocytogenes poses a major concern due to its relatively long incubation time, mortality, and severity of symptoms. Of thirteen serovars, 95% are human isolates of which serovars ½ a, ½ b, and 4b are most implicated in foodborne illness. With regards to the bacterial classification scheme, a strain is a microbial population descended from a pure culture (Prescott 1999). Microorganisms can be grouped into serovars within strains based on antigen characteristics (Prescott 1999). Out of these implicated serovars, 33 to 50% of infections result from serovar 4b which has been involved in all LM outbreaks since 1981 (Doyle and others 1997). Although it is difficult to pinpoint an exact infective dose, most LM infections result following ingestion of >100 CFU/g (Doyle and others 1997). Gastrointestinal symptoms, such as nausea and diarrhea, can occur within a few hours. More severe symptoms such as meningitis, septicemia, neural disorders, and late term abortion may take days or weeks to develop. Infection of the central nervous system results in stiff neck, nervous conditions, confusion, incoordination, and convulsions. Pregnant women suffer from flu-like symptoms with the potential for miscarriage, still birth, premature birth, or infection potential to the neonate (Doyle and others 1997, BFHD 2002). Mortality occurs in 20 to 30% of nonpregnant, susceptible populations (CFSP 2005). Of healthy adults, 2 to 6% are asymptomatic carriers (Doyle and others 1997).

Infection

The infection process, typically occurring after oral ingestion, encompasses several organ systems by cell-to-cell spread. The process can be divided into phases including invasion, lysis and intracellular multiplication, intracellular motility, and cell-to-cell spread. *Listeria monocytogenes* initially crosses the intestinal barrier and induces phagocytosis in resident macrophages. Once encapsulated in host cells, LM frees itself from the vacuoles within thirty minutes and is released into the cytosol to initiate logarithmic replication. The pathogen begins accumulation of host actin microfilaments collected from the cytoplasm. The microfilaments conglomerate to create a "comet tail" that LM uses during motility. Tail size has been used as an indicator of speed since longer tails have been photographed on faster moving cells. Upon contact with the plasma membrane, LM emits surface protrusions with new bacterial cells attached to each end. These are distributed to neighboring cells by phagocytosis into double membraned vacuoles during cell-to-cell spread. Lysis follows encapsulation to initiate the new cycle with the entire procession occurring over five hours. *Listeria monocytogenes* cells can then enter the bloodstream for circulation to the lymph nodes. Most are killed by T-cell immunity, specifically neutrophils and mononuclear phagocytes, on route to the typical target organs, the liver and spleen. Further circulation facilitates infection of the blood, brain, or placenta (Doyle and others 1997).

Defense Mechanisms

Listeria monocytogenes employs several defense mechanisms, such as genetics, surface proteins, and mobility enhancements, during invasion which are responsible for their survival and proliferation in the host environment during the infection process. Hly

encodes Act A, Internalin A, Internalin B, and Listeriolysin O (LLO) which are responsible for actin-based motility, transcription of Internalin proteins, and cellular perforation, respectively, during host cell invasion. During host phagocytosis, LLO initiates escape from the phagosome by porulation of the cell membrane caused by acidification of the host cell environment. Upon penetration, LM may utilize host actin microfilaments to drive motility. DNA transcription during multiplication is maintained by the prf A gene which encodes 14 base pair attachment regions for its similarly named regulation protein. Internalin proteins are characterized by leucine rich regions which bond with host cell peptidoglycan membranes to facilitate LM attachment during cell-to-cell spread. Internalin B aids in the infection of liver hepatocytes facilitating spread of circulating LM to new organ systems (Cary and others 2000).

Biofilms

The formation of biofilms allows LM to proliferate in processing niches, a major source of contamination of RTE foods. Biofilms serve as a protective barrier against antimicrobials and detergents. Poor equipment sanitation and moist conditions allow for the accumulation of soils and formation of bacterial biofilms on hydrophilic surfaces such as glass and stainless steel (Chmielewski and Frank 2003).

Samelis and Metaxopoulos (1999) conducted a study investigating the sources of contamination in a sausage processing plant which fabricated sausages from raw materials. Of the raw turkey, pork, and lard trimmings, roughly 50% of lots received tested positive for LM and other *Listeria* species. Cross contamination occurred in vacuum tumblers due to continuous batch processing with infrequent sanitation. Further

investigation of products processed within the plant uncovered high contamination levels in mechanically deboned turkey and ham meat prior to processing. *Listeria* was also present in tumbled meats that had been cooked below 70˚C and country sausages that were processed at 65 to 68˚C, but not in oven cooked and emulsion-type sausages processed at 72 to 75˚C. Post processing contamination occurred in cutting equipment used to slice the sausages indicating the need for a post-processing intervention step.

Biofilm formation is initiated when planktonic cells, those suspended in solution or the environment, attach to a surface and initiate proliferation (Takhistov 2007). The initial biofilm attachment is reversible and maintained by surface interactions, but subsequent secretion of exopolysaccharides cements the cells to the surface (Lundén 2004). Attachment surfaces on processing machinery typically include grooves or corrugated surfaces. Niche size determines whether bacteria will align along the corners or disperse evenly over the attachment surface. Nutrient availability determines the spatial arrangement of cells, assuming a branched network during periods of high nutrition and clumped network during low nutrition. This arrangement allows cells to either take advantage of high nutrition by expanding their range or communicate to initiate nutrient conservation. Bridging between cells in close proximity facilitates networking allowing cells to function collectively in response to environmental stressors (Takhistov 2007).

Inhibition Methods of Organic Acid Ingredients

Lactic Acid Salts: Sodium and Potassium Lactate

The popularity of lactates as antimicrobials is enhanced by their positive contribution to numerous sensory attributes including palatability, moisture retention,

visual appeal, and preservation. Lactic acid salts, such as SL and PL, are produced during the complex of "acid with the hydroxide or carbonate of a metal counterion (Naidu 2000)." Lactic acid is also produced through the commercial fermentation of carbohydrates to produce crystalline calcium lactate. This is further purified by sulfuric acid or hydrolysis of lactonitrile (Shelef 1994). The FSIS recently established the use of sodium and potassium lactates at concentrations of 4.8% of the final product formulation. Lactate use is restricted in baby foods (FSIS 2006).

In a study regarding the antimicrobial efficacy of three lactic acid salts, SL, calcium lactate, and PL applied to pork liver sausage, LM was decreased using a concentration of ~2 to 3% of each. This effect was enhanced by the presence of 3% sodium chloride (Weaver and Shelef 1993). Banks and others (1998) noted lower pH and aerobic plate counts in whole muscle pork injected with STPP and SL. Williams and Phillips (1998) noted that the bacteriostatic abilities of SL increased with higher concentrations, but an acidic aftertaste, increased pH, and metallic off flavors formed when added above 2%. Stekelenburg (2003) found that a solution of 2 to 3% formulation, consisting of 5 to 6% PL and 4% SDA, retarded the growth of LM inoculated onto frankfurters after cooking. Growth inhibition using the combination treatment was maintained throughout the shelf life, whereas PL added alone allowed LM growth.

Many studies note enhanced benefits of lactic acids when applied in combination with acetic acid or its salts and are addressed jointly in this section and under the heading for SDA. Rozum and Maurer (1997) found that SL injected in cooked chicken breasts lost its preservative effect on aerobic bacteria inhibition after the first week. While researching the antilisterial effects of SL applied to vacuum packaged, refrigerated

frankfurters, Samelis and others (2001) noticed lag phase extension from 30 to 50 d. The lag phase was prolonged throughout the 120 d storage period when 0.25% SL was combined with 0.25% SA, SDA, or Glucona-d-lactone (GDL). During a trial period of six weeks, Zhu and others (2005) noted bacteriostatic effects when combinations of SL with SDA and SL with potassium benzoate were applied to turkey ham formulations. Sensory characteristics of meat treated with SL were minimal and included a slight increase in firmness and saltiness. Nuńez de Gonzales (2003) and others explored the effectiveness of acidified calcium sulfate frankfurter formulations treated with a lactic acid post cook dip. When vacuum packaged, the bacteriostatic effect was maintained for up to twelve weeks of storage. These studies support the extension of shelf life when using lactates singly and in combination.

The use of lactic acid to decrease pH has been confirmed in many studies, but much debate surrounds whether inhibition is primarily due to acidification or the lactate moiety. In an inhibitory study conducted by Buchanan and others (1994), the inhibitory effect of SL was greater, in comparison to acetic acid/SA, when applied at a lower pH. Houtsma and others (1996) similarly found that pH and temperature were influential to the activity of SL when compared to sodium chloride during in vivo inhibition (cited by Davidson and others 2005). Schelef (1994) disputed lactic acid inhibition caused by acidity with regard to the low concentration of undissociated acid molecules when lactates are applied to meat products. Shelef instead proposed inhibition was associated with anion toxicity and substrate level inhibition of metabolism. Increased concentrations of the lactate moiety would shift the production of pyruvate to lactate therefore inhibiting cellular energy forming processes. Similarly, this may also couple with ATP production

resulting in high intracellular concentrations of energy and inhibition of the pathway. Comparison studies between SL and PL applied to LM grown on tryptic soy broth displayed interchangeable inhibition characteristics demonstrating inhibition by the lactate moiety. The interchangeability of SL and PL with regards to LM cultured in vitro credit inhibition by the lactate moiety.

The relationship between SL and decreasing water activity has been widely compared with the mechanism exhibited by sodium chloride. Research conducted by Chen and Shelef (1992) noted a decrease in water activity for effective LM inhibition in cooked meat product (cited by Davidson and others 2005). Comparison studies regarding water activity between SL and sodium chloride found susceptibility variation between bacteria, but noted an increased sensitivity of gram positive bacteria, including LM, to the lactate moiety (Naidu and others 2000). These results were debated by Houstma and others (1993) who observed that SL and sodium chloride exhibit the same effects on water activity within concentrations ranging from 0.089 to 0.89 mol/L (cited by Naidu and others 2000). This potentially indicates that salt and lactates, regardless of the lactate moiety, have the ability to reduce water activity. Based on these studies, lactates demonstrate a limited effect on water activity.

Citric Acid Salts: Sodium Citrate

Citric acid and its salts have been shown to inhibit spoilage through chelation of metal ions, related to the enzymatic browning of food, and lowering pH. Due to its production during the oxidative processes of the tricarboxylic acid cycle, citric acid and its associated salts are mildly alkaline (Naidu and others 2000). Citric acid and its salts are currently generally regarded as safe food additives at levels used for antimicrobial

effectiveness. It is commonly applied as an additive to citrus based foods including ice cream, sauces, preserves, and canned produce (Davidson and others 2005).

The antilisterial effectiveness has been widely debated. Buchanan and Golden (1994) studied the combination of sodium citrate and citric acid applied to LM in BHI broth at levels of 0.1, 0.5, 1.0, and 2.0 M and at pH values from 4 to 7. At pH ranges near neutral, 5 to 6, low levels of citric acid had a protective effect towards LM. When compared to the control treated with hydrochloric acid, higher concentrations of citric acid exhibited an anion effect with inhibition attributed to the high amount of the undissociated acid. Research cited by Davidson and others (2005) and conducted by Cole and others (1990) discussed a temperature-dependent effect on pH when LM inhibition was achieved in trypticase soy yeast extract broth. When applied as a post cook dip, citric acid was not inhibitory towards LM at refrigerated temperatures (cited by Davidson and others 2005). Al-Holy and others (2006) researched the antimicrobial effects of nisin and citric acid, alone and in combination, against *Listeria innocua* when applied to BHI broth and hummus. In both the broth and hummus, citric acid failed to inhibit *Listeria* growth and in aerobic plate counts. When applied at 0.2% with 500 IU/mL of nisin in BHI broth *Listeria* was completely eliminated. At 0.3% and 1000 IU/g nisin applied to hummus the combination maintained a 1.5-log reduction in *Listeria* numbers over a period of 6 d . Dél Rio and others (2007) tested the effects of acidified sodium chlorite, 2% citric acid, 220 ppm peroxy acids, and 12% trisodium phosphate dipping solutions on LM, *Staphylococcus aureus, Bacillus cereus, Salmonella* Enteriditis, *Escherichia coli,* and *Yersinia enterocolitica*. Following fifteen minutes of dipping in individual treatments, raw chicken legs were packed into storage bags and held at refrigerated storage for a

period of 5 d. Treatment with citric acid displayed the greatest effectiveness against gram positive bacteria overall with 1.97 CFU/g log reduction. Samples treated with citric acid and ascorbic acid maintained the lowest pH throughout the end of treatment. Limited research, beyond comparison with curing agents, support the antimicrobial success of citric acid applied singly. Based upon these studies, its antilisterial effects are increased when citric acid is applied in combination.

Acetic Acid Salts: Sodium Diacetate

Sodium diacetate, a salt of acetic acid, is commonly associated with the growth hindrance of yeasts, molds, and bacteria. This effect is favorable towards its application in starchy foods, condiments and sauces, and fruit based goods (Davidson and others 2005). Aside from its cost efficiency and GRAS status applied by the FDA, its use is limited by the production of undesirable acidic and bitter off flavors (Naidu 2000). There is currently no limit to the acceptable daily intake of acetic acid, the Food Safety and Inspection Service recently set the standards for sodium diacetate use at 0.25% of the product formulation (FSIS 2006).

Combination treatments of SL and SDA potentially prolong the LM lag phase for 4 to 12 weeks. Bacteriocidal decreases of 1 to 2.5 logs have also been reported. In frankfurters held at refrigerated storage and treated with combinations of SL, SDA, and SA , Bedie and others (2001) noted growth inhibition over the 90-d storage period with a pH decrease in SDA treatments. In commercial wieners formulated with pork, turkey and beef, and commercial bratwurst formulated with beef and pork, Glass and others (2001) found SL and SDA post cooked dips exhibited no antilisterial effects, but extended the lag phase for four and twelve weeks, respectively, when applied for formulation. Goode

and others (2004) extended the lag phase of modified atmosphere packed turkey frankfurters using treatments of SL, SDA, sodium acetate, and their combinations. A combination of SL and SDA achieved the greatest extension of lag phase at four weeks. Barmpalia and others (2004) also studied the effects of SL, SDA, and GDL towards LM inhibition in formulations of pork bologna at refrigerated temperatures. The SL and SDA combination were found to be the most effective with SL, SDA, and GDL following. A similar study conducted by Geornaras and others (2006a) noted the same effects in smoked sausage. Uhart and others (2004) noted a 1 to 2 log reduction of three Listerial strains inoculated on beef frankfurters when a SL/SDA post cook dip was applied. When a three-combination dip including pediocin was used, a 2.5-log reduction was achieved. Mbandi and others (2006) found that in vitro treatment with a combination of SDA and SL resulted in the upregulation or downgrade of six proteins. Oxidoreductase, responsible for cell homeostasis and F_0F_1 ATP synthase electron transfer, and lipoproteins, involved in stress resistance, signaling, protein export and folding, and substrate binding and folding, increased. DNA binding protein, a transcription regulator, alpha amylase, required for metabolism of carbon and energy sources to usable forms, and two sec A proteins, involved in translocation and protein export, were suppressed by SDA.

Combinations of PL and SDA have also been associated with prolonging the lag phase for up to eight weeks. Williams and others (2004) noted a pH decrease when SDA was applied with PL, STPP, and salt to whole muscle steaks. A similar study conducted by Steckelenburg (2001) noted a synergistic effect between PL and SDA. Barmpalia and others (2004) found that growth was greatly inhibited in frankfurters prepared with a formulation of PL and SDA, and treated with lactic or acetic acid post cook dips. In a

comparison study of the antilisterial effects of SDA, potassium benzoate, potassium sorbate, and SL applied to frankfurters alone and in combination, Lu and others (2005) noted the greatest effect was achieved with SDA treatments under refrigerated storage. Barmpalia and others (2004) found that growth was greatly inhibited in frankfurters prepared with a formulation of PL and SDA, and treated with lactic or acetic acid post cook dips. Of frankfurters treated with PL and SDA, Knight and others (2006) noted growth inhibition in vacuum packaged frankfurters after eight weeks of unopened storage with no negative sensory attributes. Lianou and others (2006a) conducted a study approximating home storage conditions at elevated temperatures (~7˚C) of commercial turkey breast inoculated after the heating process and formulated with a mixture of PL and SDA. Microbial numbers were inhibited throughout the twelve-d testing period compared to turkey breast formulated without antimicrobials. A subsequent study conducted with ham achieved the same results (Lianou 2007c).

Curing Agents

Sodium Chloride

Sodium chloride is a halide salt formed by the union of the cationic sodium ion and anionic chloride ion (Naidu 2000). Risks to health and palatability limit the intake of NaCl to 2.8 to 8.4 g daily therefore hindering most of its antimicrobial benefits (Davidson and others 2005). Due to these restrictions, NaCl is often combined with other antimicrobial agents at lower temperatures to achieve the same benefits at lower concentrations. Based on these additions the effects are variable with respect to changes in temperature, pH, substrate, processing time, and contamination level (Naidu 2000).

Antimicrobial mechanisms of NaCl are primarily attributed to the reduction of water activity and effects induced in conjunction with other antimicrobial agents (Naidu 2000, Davidson and others 2005). The resulting metabolic changes noted in bacteria typically include cellular plasmolysis, dormancy, respiratory inhibition, enzymatic inhibition, limited oxygen solubility, and altered glucose utilization. Cellular defenses are designed to combat this through the accumulation of osmoprotectants, such as amino acids, solutes, and ions. During this process energy is shifted from active transport to sodium exclusion. This prevents the influx of glucose and its metabolism for energy production (Naidu 2000, Davidson and others 2005).

Conner and others (1986) researched the effects of temperature, NaCl, and pH on cabbage juice containing two strains of LM, Scott A and LDLC81-861. At NaCl concentrations equal to or above 2% stored at 30˚C, the populations of both strains decreased. At these conditions, pH was shown to decrease from 5.6 to 4.1. When maintained at refrigeration temperatures of 5˚C and at a 5% salt concentration, the population of LM Scott A decreased by 90% over the 70 d storage period. LM strain LDLC 81-861 remained viable at these conditions with NaCl levels at or below 3.5%. Patchett and others (1992) found that LM cultivated in-vitro and exposed to 5% NaCl accumulated increased amounts of potassium and glycine betaine. At 7.5% NaCl, cells contained increased potassium, glycine, glycine betaine, alanine, and proline. Of these solutes, glycine betaine, glycine, and proline were found to promote growth on media formulated with 4% NaCl. No growth occurred at higher NaCl concentrations. Peterson and others (1993) researched the effects of NaCl concentrations at 3.5% and 6% on cold processed, smoked salmon. At 10˚C, LM growth was inhibited for two weeks when

treated with 6% NaCl before populations rose to $1x10^8$ CFU/g. This effect was more noticeable at 5˚C with LM remaining below 100 CFU/g when treated with 6% NaCl. Rajkowski and others (1994) tested the effects of polyphosphate at concentrations of 0.5% and 1.0% and NaCl at 0.5% and 4.5% on LM and *Staphylococcus aureus* concentrations in inoculated ultra-high temperature (UHT) pasteurized milk. The polyphosphate treatment alone at both concentrations failed to significantly inhibit either bacteria. The addition of 2% NaCl increased the inhibitory effect of polyphosphate to increase generation times and prolong the lag phase. These studies indicate that high concentrations of NaCl are needed to prevent LM growth.

Many studies document increased intrinsic resistance of LM to environmental stressors with increasing concentrations of NaCl. Yen and others (1991) evaluated the effects of various levels of curing ingredients on a mixture of nine strains of LM inoculated on ground pork stored in baby food jars and cooked in a water bath to an internal temperature of 60˚C. Thermal resistance was increased by treatment with NaCl at concentrations from 0% to 3%. In vitro studies conducted by Briandet and others (1999) on the Scott A strain of LM exposed to salt concentrations of $1.5x10^{-1}$ and $1.5x10^{-3}$ M and temperatures of 15˚C and 37˚C demonstrated that LM adhesion to stainless steel increased with increasing salt concentrations and temperature. This was supported by Jensen and others (2007) using in-vitro research conducted on eighteen strains of LM maintained at two temperatures, 5˚C and 37˚C, and salinities, 0.5% and 5% NaCl. These concentrations were selected to approximate the average salinity of RTE foods which ranges from 2 to 5%. Thirteen of the eighteen strains also displayed aggregation behavior when exposed to salt. Faleiro and others (2003) explored the effects of 2% NaCl and pH

ranging from 5.1 to 6.2 on four strains of LM. Of the strains selected, two were meat isolates and two were isolated from Portuguese cheese. Cheese isolates tended to be more resistant to both factors. This resistance was decreased when products were maintained at refrigerated storage temperatures. Osmotolerance and adaptation to NaCl concentrations as high as 20% occurred following exposure to a pH of 5.5 for two hours and incubation at 3.5% NaCl. Shekarforoush and others (2003) examined the potential for thermal resistance during research documenting the effects of NaCl, pH, and acid type on the thermal destruction of microwave heated LM in broth. At the various concentrations of NaCl tested, 0.5, 1.5, and 3.0%, no significant impact on thermal destruction was noted.

Sodium Nitrite

The binding properties of sodium nitrite are utilized to add value and antimicrobial protection to cured meats. Binding to myoglobin produces nitrosamyoglobin responsible for a characteristic pink color, smoky flavor, and reduced fat oxidation. Nitrous acid and nitrogen oxides complex with bacterial amino groups to attack the glucose dehydrogenase enzyme and prevent glucose degradation. Acceptable daily intake for sodium nitrite is limited to 0.2 mg/kg body weight and completely restriction in baby food (Davidson and others 2005).

The gray area known as the "Perigo Effect" relates to nitrite complexes formed during heating, such as nitrite binding to cysteine, iron, and sulfhydryl groups, allowing it to have a greater inhibitory effect. These factors, known as Perigo type factors (PTF), were initially researched by Perigo in the late 1960's following observation of the dissipation of nitrite following thermal processing. The antimicrobial presence of nitrite in cured meats without thermal processing can be attributed to a decrease in water

activity, pH reduction due to a high concentration of undissociated nitrous acid, and interference with the electron transport chain (Davidson and others 2005). The role of nitrite as an electron acceptor allows for inhibition of active transport, oxygen uptake, and oxidative phosphorylation at the electron transport chain. These reactions are more notable under favorable conditions of temperature and acidic pH (Lück and Jager 1997).

Undissociated nitrite is also associated with the inhibition of LM in cured products. Whiting and Masana (1994) noted the effects of 300 μg/mL nitrite and pH on LM survival in uncooked, fermented meat products held at 37˚C over a 21 d storage period. The time, in days, to induce a four-log reduction in LM numbers was noted. At pH 5.0, a 4-log reduction occurred after 21 d. At pH 4.0, a 4-log reduction occurred in <1 d. Additional nitrite had no effect on LM concentrations, favoring the hypothesis of initial inhibition by diminishing undissociated nitrite. Duffy and others (1994) evaluated the effects of water activity, acidity, nitrite, and ascorbate on the growth of LM strain Murray B in cooked, vacuum packaged meats stored at 0˚C and 5˚C. Duffy found that 3 μM of undissociated nitrite doubled the lag phase.

Ngutter and Donnelly (2003) explored the survivability of LM to 100 to 200 ppm of sodium nitrite formulation in cooked frankfurters. Following approximately 98% injury, LM was able to either fully recover or proliferate with new cell growth when transferred to Listeria recovery broth. Recovery was not possible following 99% damage. Nyachuba and others (2007) evaluated LM survival on nitrite containing RTE meat products including beef frankfurters, beef bologna, smoked ham, and smoked salmon. Following inoculation with a five-strain LM cocktail, products were vacuum packaged and held under refrigerated storage over an eight-week observation period. At

concentrations of 100 to 200 ppm, *Listeria* populations grew from <100 CFU/g to 1×10^4 --1×10^5 CFU/g by the end of storage. Although nitrite exposure initially damaged 83 to 93% of the LM population, it can be hypothesized that bacterial populations were able to repair and proliferate by the end of the testing period. Nyachuba concluded that early storage detection of LM in nitrite containing RTE foods can be misleading.

Sodium Tripolyphosphate

Sodium tripolyphosphate, produced by the neutralization of phosphoric acid with sodium alkali metal ions, is an industry control added to emulsions, to enhance the binding of processed meats, and antimicrobial defense (Naidu 2000, Davidson and others 2005). Daily STPP intake is limited to 0.5% of the body weight (Davidson and others 2005). Its antimicrobial mechanisms include metal ion chelation, protein interaction, and buffering capacity. These effects are supported by conditions of high acidity (Naidu 2000, Davidson and others 2005). Complex of these strongly electronegative polyanions with metal cations, such as Ca^{2+} and Mg^{2+}, induces cation deficiency resulting in instability of bacterial transport mechanisms and cell membrane structure (Naidu 2000, Davidson and others 2005). Gram positive bacteria are primarily affected by the necessity of bivalent cations for peptidoglycan formation of the cell wall (Naidu 2000). Zenner and Shelef noted that this effect is hindered when an abundance of these metals exists. Further studies showed that detriment to cell morphology included mineral leakage and potential lysis, membrane damage, and loss of osmoregulatory abilities (Davidson and others 2005). Zaika and others confirmed these changes and cell elongation in LM (1997). In addition, growth was inhibited due to an inhibition of cell division (Naidu 2000).

Few studies detail the use of STPP as a sole method of antimicrobial prevention. In a previously mentioned study conducted by Duffy and others (1994), 0.3% STPP was found to favor LM growth by increasing the pH on vacuum packaged, refrigerated cooked meats. In a study conducted by Hwang and Beauchat (2005), raw chicken skin was inoculated with *Salmonella* spp. or *Listeria monocytogenes* and treated with polyphosphates. Lower populations were achieved with 1% trisodium phosphate or 1% lactic acid than 10% STPP. The antimicrobial benefits of polyphosphates in whole muscle meats are questionable due to the production of pyrophosphatases and polyphosphatases which nullify the compounds by degradation to less reactive forms (Davidson and others 2005).

Post-Processing Treatments

Recent studies have shown that organic acid marinades applied post-processing may be used as an effective means to reduce bacterial contamination and extend product shelf life. Samelis and others (2001) studied the effects of post-processing dipping solutions on sliced, vacuum packaged bologna inoculated with LM and held at 4˚C for 120 d. Slices were treated with various chemicals including 2.5% and 5% lactic acid, acetic acid, SDA, and 5% to 10% SL. Control slices without treatment achieved LM concentrations exceeding 7 log CFU/cm^2 by d 20. Significant LM growth did not occur at either concentration of acetic acid or 5% SDA throughout the storage period. Growth occurred using all other treatments including both concentrations of SL after 20 to 35 storage days. A similar study was performed by Kain and others (2001) using LM inoculated pork bologna. Kain noted that the use of 5% SDA inhibited LM growth throughout the 90-d storage period. Of all acids tested, acetic acid maintained its

antilisterial activity throughout the storage period. Treatment with organic acid dipping solutions were also shown to inhibit all other bacteria potentially extending the shelf life of RTE products.

Geornaras and others (2005) studied the effects of post-processing antimicrobial solutions on a ten-strain culture of LM inoculated on vacuum packaged bologna and ham. Product was treated using various chemicals including 2.5% acetic acid and 2.5% lactic acid. Products were vacuum packaged and stored at 10˚C for a 48-d storage period. Day 0 pathogen reductions for slices treated with lactic acid, acetic acid, or 5% potassium benzoate ranged from 0.4 to 0.7 log CFU/ cm^2. All treatments displayed substantial listericidal effects, but received negative responses during sensory evaluation. Geornaras concluded that sensory characteristics could be improved using lower antimicrobial concentrations with the same listericidal effects. A similar study conducted by Geornaras and others (2006a) using vacuum packaged smoked sausage. In addition to the previously mentioned treatments, product was formulated with and without 1.5% PL and 0.05% sodium diacetate. Treatment with either lactic or acetic acid resulted in a 0.4 to 1.5 log CFU/cm^2 reduction. Growth was inhibited by immersion treatments formulated without antimicrobials for 4.9 to 14.8 d. Growth occurred by the end of storage for all treatments.

Ingredient Antimicrobial Mechanisms: Overview

Chelation

The binding of metal ions by chelators disrupts the structural integrity of the cell wall and allows for permeability. In other cases, induced vulnerability by chelators allows other bacterial agents such as lysozymes to access the cell. Accumulation of high solute

concentrations within the bacterial cell can prevent this (Davidson and others 2005). Please see the aforementioned sections regarding the actions of SC and STPP.

Water Activity

The available water content of food, and therefore microbial growth, is called water activity (A_w). Potentially hazardous foods, those supporting microbial growth, typically have an A_w of .85 or higher on a 0 to 1 scale (NRAEF 2006). An A_w below this level induces osmotic shock resulting in inhibited growth and prolongation of the lag phase. Intracellular activity shifts towards maintaining a homeostatic balance which is often accomplished by the accumulation of osmoprotectants such as solutes, ions, and amino acids. Extremophiles, bacteria adapted to environmental extremes, also accumulate polyols, such as glycerol, that add protection of enzymes and serve as potential nutrients (Davidson and others 2005).

Most foodborne pathogens do not survive below a water activity of 0.84. Spoilage bacreria, yeasts, molds, do not survive below .90, .88, and .80, respectively (Jay 2005). Arranging the treatment acids in order from the lowest water activity to the highest (Table 1), the order would be SL/PL/SDA (.80), SL/SD (.85), SL (.86), PL (.87), and SC (.90). Based on this, it can be expected that the organic acid treatments will effectively inhibit most pathogenic and spoilage microorganisms.

Acidity

The efficacy of organic acids depends upon the strength of both acid and bacteria. Concentration and pH of the acid contribute to penetration of the bacteria. Solfos and others discussed the contribution of acid components upon complete dissociation of the acid within the intracellular pH with regard that the proton and anion both participate in

the role (Davidson and others 2005). This can be attributed to the relationship between pH, the concentration of hydrogen ions and hydroxide ions in a solution, and pKa, the dissociation constant (Campbell and Farrell 2006). Greater acid strengths are associated with a smaller pKa. At pH values lower than the pKa, the undissociated form of the acid predominates in solution. At pH values greater than the pKa, the dissociated, or unprotonated, form predominates in solution (Campbell and Farrell 2006).

In this experiment, the pKa values ranging from highest to lowest were associated with triprotic citric acid (5.41), acetic acid (4.76), the diprotic form of citric acid (4.75), lactic acid (3.86), and the monoprotic form of citric acid (3.09) (Lück and Jager 1997). Arranging these by greatest to least acid strength, the order would be the monoprotic form of citric acid, lactic acid, the diprotic form of citric acid, acetic acid, and the triprotic form of citric acid. The pH of the solutions, ordered from strongest acid to weakest acid, that were used in this experiment range from 5.32, 5.63, 6.25, 6.35, and 8.26 for the combination of SL/SDA, the combination of SL/PL/SDA, sodium lactate, potassium lactate, and sodium citrate, respectively (Table 1).

Based upon the lipophilic potential of weak acids, treatments which include either SL, PL, or their combination should be the most effective at penetrating the LM cell membrane and dissociating in contact with the more acidic intracellular pH (Lück and Jager 1997, Davidson and others 2005). The accumulation of protons causes strain on proton motive force and cellular energy is exhausted in their expulsion (Davidson and others 2005). Based on finding a large content of ATP in exhausted cells, research by Gould, as cited by Davidson and others (2005), stipulated that the probability of the lack

of oxygen consumption, hindering the final electron acceptor of the electron transport chain.

TABLE 1. *Post-cook dipping solution pH and water activity(A_w) for each treatment and control*

Treatment	pH	A_w
SL[1]	6.25	0.86
PL[2]	6.35	0.87
SC[3]	8.26	0.90
SL/SD[4]	5.32	0.85
SL/PL/SDA[5]	5.63	0.80
CONTROL[6]	7.00	1.00

[1] Sodium lactate (3.6%) and water
[2] Potassium lactate (3.6%) and water
[3] Sodium citrate (0.75%) and water
[4] Sodium lactate (3.6%) and sodium diacetate (0.25%)
[5] Sodium lactate (3.6%), potassium lactate (3.6%), sodium diacetate (0.25%) and water
[6] Sterile water

Cytoplasmic Damage

In the event that a weak antimicrobial lacks the potential for lethality it is still capable of establishing vulnerability towards the actions of other antimicrobials. Interactions with certain functional groups, such as phenols, alcohols, organic acids, esters, and isothiozolones can trigger cytoplasmic damage. This is typically seen as a loss of structural integrity, including leakage or lysis, and a loss of functionality, such as a disruption of proton motive force or enzymatic inhibition (Davidson and others 2005). Leakage of cell constituents proceeds from structurally important metals, such as potassium, to free phosphates, amino acids, and genetic material. The disruption of proton motive force targets major transport and energy functions of the cell such as oxidative phosphorylation, active transport, and synthesis of ATP (Davidson and others 2005). The electron transport chain is compromised when proton rich species, such as

organic acids and esters, contrast with the negatively charged, alkaline environment of the cell. Enzymatic inhibition primarily occurs at the thiol residues of the sulfur containing amino acids, cysteine and methionine (Davidson and others 2005).

Resistance

The prevalent use of organic acids in bacterial prevention methods presents a major concern due to the potential for bacterial resistance. Resistance can be classified as innate, apparent, or acquired. This concern is due to the lack of published knowledge regarding mechanisms of antimicrobial action and the microbial response to exposure. While exposure to high levels of antimicrobials has a greater likelihood of destroying most bacterial cells, many microorganisms have the potential to adapt to these extremes using innate resistance. Due to this potential for survival and proliferation, more research is needed to address the combination of antimicrobials applied to site specific microbial needs (Davidson and Harrison 2002).

Innate resistance is natural resistance determined by genetic contribution. This includes cellular barriers such as the thick peptidoglycan layer of gram positive bacteria. Continual exclusion of protons and solutes from the cell is maintained through pumping mechanisms powered by electrochemical gradients (Davidson and others 2005). Genes such as bsh, responsible for bile salt dehydrogenase, and gad A, responsible for glutamate decarboxylase, may also aid in resistance to environmental extremes such as salinity and low pH (Garner and others 2006).

Apparent resistance is measured by bacterial responses to fluctuations in environmental conditions such external factors of pH and salinity (Davidson and Harrison 2002). Garner and others (2006) conducted research regarding the ability of LM

cells grown in NaCl, SDA, SL, or a combination of the three to invade intestinal epithelial (Caco-2) cells. The highest invasion potential was observed in LM grown in SL or salt. Survivability in synthetic gastric acid was reduced when the cells were grown in salt, SDA, or SL. This demonstrates that virulence associated with pathogens can vary with food content. Briandet and others (1999) found that temperature and pH changes altered physiological and chemical properties of the cell surface. Bacteria demonstrated high mobility near neutral pH and low mobility in acidic pH. This was related to the temperature dependent production of flagella and distribution of charges to surface functional groups. It is suggested that supplemented media protonated negatively charged groups, such as phosphates, carboxylates, and proteins that are distributed throughout the cell wall, causing reduction to the electrophoretic mobility of the cell (Briandet and others 1999).

Acquired resistance is best described as stress adaptation, known as "stress hardening," to environmental stresses such as pH and compromised homeostasis (Lou and others 1997). In LM exposed to ethanol, Lou and Yousef (1997) noted resistance to subsequently applied environmental stressors such as temperature, salinity, starvation, and hydrogen peroxide. Kouassi and Shelef (1995) conducted a comparison study regarding the in vitro effects of SL, citrate, and acetate on LM stored under aerobic and anaerobic conditions and at 37˚C. Aerobic storage and treatment with these antimicrobials enhanced LLO secretion. This was prevented through the inclusion of sorbate which contributes to LLO blocking. O' Driscoll and others (1996) found that acid adaptation exposure of LM to pH 5.5 for one hour protected it during subsequent exposure to pH 3.5. Upon examination of surviving cells, O' Driscoll noted the

proliferation of several mutants with increased pathogenicity inside inoculated mouse livers. It was concluded that sublethal exposure to pH may favor the selection of mutant strains. In a study evaluating the thermal inactivation of LM, Murphy and others (2004) observed a 53% to 75% increase in the D-values, the time required at a given temperature to reduce a bacterial population by one log cycle, with no effect on Z-values, the temperature required to reduce a bacterial population by one log cycle, of LM and *Salmonella* exposed to SL. Duran and Sofos (2006) noted that bacterial growth rate plays a role in determining susceptibility to antimicrobial concentration. Slow growing strains such as Scott A were effectively inhibited by lower concentrations of antibiotics.

Acid adapted LM also demonstrated resistance to heat, salinity, crystal violet staining, and ethanol exposure. Okereke and Thompson (1996) found that LM Scott A cells adapted to pH 5.5 in vitro were 150 to 7,500 times more acid tolerant to pH 3.3 than unadapted cells grown at a pH of 7.2. Acid adapted cells were also more antibiotic resistant to Nisin. Gahan and others (1999) attributed LM acid tolerant response to the accumulation of resistance proteins and chromosomal signaling loci necessary for stress response. Phan-Thanh and others (2000) noticed a greater LM susceptibility to organic acids, in comparison with weaker acids, due to their stronger ability to decrease intracellular pH. Conte and others (2000) supported the observation of increased invasiveness of acid adapted LM using in-vitro cell models. Koutsoumanis and others (2003) observed that acid tolerance following sublethal exposure to mild acids was dependent upon external factors such as salinity, osmotic shock, and temperature.

Food Processing

Comminution

Comminution is the reduction of whole muscle meat into smaller particles for incorporation into meat products. The main benefits of this process are tenderness, ingredient distribution, and product uniformity. Course and fine comminution were employed for the purposes of this study. Course comminuted products are composed of visual meat particles as applied to the fabrication of turkey and ham deli loaves. As deli loaves are coarsely comminuted products composed of cubed meat and ground meat, meat grinders are typically responsible for comminution of the ground portion. Fine comminuted products, such as frankfurters, achieve a pasty consistency similar to emulsion. A bowl chopper is typically used in this instance to chop the raw ingredients and incorporate the formulation into a paste ready for stuffing. This property earns frankfurters the title of batter-type sausages. Larger scale frankfurter operations would implement an emulsion mill which achieves a finer comminution in a shorter amount of time (Aberle and others 2001).

Curing and Marination

Curing is the application of salt, nitrite, seasonings or other ingredients to initiate positive sensory, preservatory, or antimicrobial characteristics in processed meats. For the sake of this experiment, the application of NaCl and STPP were applied as industry binding controls to ensure the stabilization of comminuted meats. Sodium nitrite was applied to ham deli loaves and frankfurters to impart a smoky flavor and “cure pink” color (Aberle and others 2001).

Several methods are available for the application of curing agents and marinades on processed meat products. Pickling, involves a long term immersion of product within the liquid curing solution. This method is less favorable due to the time requirements and potential for microbial growth and spoilage. Dry rubbing involves successive applications of dry curing ingredients across cut surfaces of the meat followed by a long period of refrigeration. The average curing rate for this method is approximately 2.5 cm/week. A currently used method, injection, is also a cause of industry concern. Injection is achieved by direct injection of curing solution into the meat products using hollow needles. Injection can be further subdivided by injection site into artery pumping, stitch pumping, and multiple injection. Cure is injected into the arteries, thick sections and joints of the meat using a single needle, and simultaneously throughout the cut using multiple needles, respectively (Aberle and others 2001).

Mechanical agitation can be used to ensure even dissemination of curing agents throughout the product. Tissue disruption caused by tumbling or massaging enhances distribution of salt to salt soluble proteins. A protein exudate is formed which aids in binding of comminuted meat particles. For the purposes of this experiment, this was accomplished by tumbling. Application of a vacuum prevents the accumulation of air bubbles, and thus foam production, which would create air pockets during encasement of the finished product. Cook shrinkage is also limited by enhanced moisture binding during tumbling (Aberle and others 2001).

Physical disruption of the meat surface by maceration also improves cure distribution. This process is often applied to whole muscle meats with a high amount of connective tissue or leaf fat that are used for course comminution. During maceration,

rotating blades score the meat surface to facilitate cure deposition within the surface indentations. For the purpose of this experiment, maceration was applied to cubed pork for ham deli loaves (Aberle and others 2001).

Emulsions

Fine comminution of muscle, salt, water, and fat produces a meat batter often referred to as an emulsion. A true emulsion is formed by the combination of two immiscible liquids where particles of one liquid are freely suspended throughout the other. The most easily visualized emulsion is oil in water in which oil droplets would compose the dispersion phase. Due to the size of dispersed fat in meat emulsions, 50 μm in diameter, in contrast to that typically exhibited in liquid emulsions, 1 to 5 μm, meat emulsions do not fit the typical specifications of emulsion identity. For the purposes of this study, meat emulsions will still be identified as such. In relation to meat, fat droplets are emulsified and suspended through a gel-like protein matrix.

Emulsifying agents stabilize emulsions through amphipathic interactions. Typical hydrophobic interactions generally force the aggregation of fat particles. Emulsifying agents envelop fat particles using a monolayer system similar to that displayed during micelle formation or the bilayer of cellular plasma membranes. Each molecule of emulsifying agent is typically composed of a hydrophilic, or water-loving head, and a hydrophobic, or water-repulsed, tail. These molecules orient favorably to allow the dispersal of both mediums. STPP applied as a binding medium frees salt soluble proteins allowing them to coat the fat particles and serve as the emulsifying agent (Aberle and others 2001).

LM Regulations

A risk based assessment conducted by the FSIS (2006) for LM contamination in RTE meat products focuses on three alternatives for LM control applied post-processing and before packaging. The first alternative addressing potential LM contamination focuses on control through the application of post-lethality treatments and antimicrobial agents and processes. The second alternative focuses on using either of the aforementioned options singly. Previous studies have demonstrated that post-processing heat treatments including steam pasteurization, hot water pasteurization, radiant heating, high pressure processing, and ultraviolet treatment can achieve 1 to 7 log CFU/g reductions depending on the method, temperature, product, and duration of treatment. Establishments are also required to list verification procedures for post-lethality methods in HACCP plants, sanitation standard operating procedures, and prerequisites. Verification can be accomplished by listing the results of published studies and implementing successful methods listed in that research. Unpublished research may also be cited pending a successful trial of LM test methods.

Antimicrobial agents and processes are primarily used to enhance product shelf life. Selection typically favors agents that produce a detrimental bacterial environment. An example of commonly selected agents are those that are capable of reducing the water activity of foods below 0.85 on a scale of 0 to 1, a condition uninhabitable by LM and a majority of bacteria. Antimicrobial processes serve to prevent bacterial growth and development. Freezing is an antimicrobial process for products held under frozen temperatures throughout their shelf life until being thawed by the consumer. Examples of frozen, RTE foods include frozen, fully cooked chicken nuggets, fish sticks, or

microwaveable dinners. Processing facilities implementing these methods are required to provide supporting documentation proving that the desired effect was achieved on treated products prior to packaging.

Alternative three, in comparison to previously mentioned methods, implements sanitation to control LM in the post lethality environment. Sanitation methods must be detailed in HACCP, SSOP, and prerequisites. Testing of food contact surfaces in the post-lethality environment is required with follow-up testing to measure effectiveness of the sanitation plan. Detailed verification research must also be provided regarding the reason for selection of the program methods, testing details including sample sites and frequency, hold and test procedures in the event of a LM positive sample, and test results. Examples of foods typically processed under alternative three include refrigerated, packaged deli meats and frankfurters. Of the previous methods, alternative three receives the greatest FSIS scrutiny.

CHAPTER III

LISTERIA MONOCYTOGENES IN PROCESSED TURKEY

Research indicates an increased prevalence of LM on RTE and machine processed turkey meat. Studies cited by Lawrence and Gilmour (1995) cited LM occurrence in 12-27% of retail cooked and RTE turkey meat. In certain cases, LM levels ranged as high as 700 CFU/g. Although the infective dose of LM varies according to victim susceptibility, research by Doyle (1999) places the level at >100 CFU/g. According to several studies, LM occurs in 90% of turkey frankfurters, 76% of ground turkey, and 38% of whole muscle turkey parts (Hayes and others 1991, Versalovic and others 1991, Ryser and others 1996, cited by Ramos and others 1999) . This presents an increased risk associated with processed turkey meat.

The majority of recent turkey implicated recalls have been associated with LM contamination of RTE turkey deli slices and frankfurters. 7-Eleven Inc. issued a statewide recall of Big Eats® Stacked Turkey and Ham Club Sandwiches discovered to be contaminated with LM by New York state food inspectors (Chabris 2003). Gottlieb and others (2006) found that of a 2002 outbreak involving 188 people spanning nine states and a recall of over 1.3 million kilograms of meat, 54 people were infected with the same LM strain. Disease traceback found that infection had been contracted nosocomially through the consumption of turkey deli slices consumed at hospital lunch counters. In 2006, Honey Baked Foods, Inc., aided by the USDA-FSIS, recalled over 20,000 kilograms of fully cooked ham and turkey products distributed nationwide through internet, catalog, and telephone sales. This risk was discovered during company microbiological testing (Eamich 2006). In 2007, Diestel Turkey Ranch, aided by the

USDA-FSIS, issued a recall of over 3,000 kilograms of RTE turkey breast distributed to six states. Although no illness was reported, the safety risk was discovered during routine microbiological testing (Eamich 2007).

These recalls have prompted increased regulatory scrutiny of poultry processing facilities. Lawrence and Gilmour (1995) determined through genetic amplification that of 289 LM strains collected from a poultry processing facility and associated products over a six-month testing span, all were representative of A and B genetic profiles. Of profile A, isolated from the raw processing section, and profile B, collected from cooked products and cooking facilities, both profiles were present in either section up to one year later. This provides evidence of the potential for cross contamination between processing areas. Ojeniyi and others (2000) discovered LM prevalence during processing in a Denmark turkey processing plant ranged from 25.9% to 41.4%. Routine cleaning and disinfection decreased this risk by 6.4%. *Listeria monocytogenes* was still present in RTE and raw meats at levels of 7.3% and 17.4%, respectively. These findings potentially support LM post-processing survival in 7.3% of products. Research by Olsen and others (2005) correlated a 1989 outbreak attributed to turkey frankfurters, and resulting in the USDA zero-tolerance policy for LM in RTE foods, with a 2000 outbreak responsible for 30 cases of illness ranging across 11 states. The implicated strain was involved in both cases stemming from the same processing plant. This research provides that the contamination risk had persisted, with the potential for intermittent affectation of the product, over a period of 11 years.

Findings note an increased potential for LM contamination resulting from processing machinery. Lin and others (2005) tested blade contamination on uncured oven

roasted turkey breast, salami, and antimicrobial formulated bologna. A commercial slicer was inoculated with a five-strain LM cocktail at a concentration of 10^3 CFU/mL. Five consecutive slices per product were vacuum packaged and held at refrigerated storage conditions throughout the thirty day sampling period. Slices were sampled in groups of 25 g, two slices, and the entire package of five slices. Contamination levels ranged from 90%, 9% and 3% of turkey, salami, and bologna, respectively, for all test groups. An additional study was conducted on roast turkey breast sampled at 60 and 90 d post-slicing using a 10^1 to 10^2 CFU/g inoculum. At lower concentrations, LM presence was not detected until 60 d post-processing. Positive test samples increased by 90 d post-processing.

The larger contamination levels associated with roast turkey are potentially attributed to a higher water activity and pH associated with the product. Although the bologna demonstrated comparable qualities, PL and SDA included in the formulation potentially minimized LM concentrations. Keskinen and others (2008) observed the transfer of weak and strong biofilm producing LM strains from grade 304 stainless steel knife blades to roast turkey breast. After inoculation with (2) three-strain cocktails, consisting of three weak biofilm producing strains and three strong biofilm producing strains, the knives were stored at room temperature and 78% relative humidity for 6 and 24 h. Of sixteen consecutive slices, LM log concentrations decreased by 3 to 5 log CFU/slice. Comparison studies determined that strong biofilm-producing strains demonstrated greater survival and transferability.

Positive results associated with organic acids applied to turkey products support the use of these treatments as a post-processing intervention. Islam and others (2002)

tested the ability of organic acid marinades applied to turkey frankfurters as post-cook dipping solutions to reduce LM growth and populations. Products were treated by dipping in sodium benzoate, sodium propionate, potassium sorbate, and sodium diacetate at concentrations of 15, 20, and 25% weight/volume before surface inoculation with a 10^6 CFU/mL concentration of a five-strain LM cocktail. Frankfurters were stored in sterile stomacher bags at 4, 13, and 22˚C with timepoint testing at 3, 7, 10, and 14 d of storage. At all concentrations for each treatment, initial microbial populations were reduced by 1 to 2 logs. After 14 d of storage, LM concentrations were 3.5 to 4.5 logs less in treated frankurters compared to untreated frankfurters. Signs of slime production and spoilage were noted within 7 d at 22˚C for untreated frankfurters compared to spoilage after 14 d for treated frankfurters.

Lianou and others (2007) studied the inhibitory effects of PL and SDA formulations on uncured turkey breast inoculated with a ten strain LM cocktail under post processing conditions. The product was vacuum packaged and maintained at 4˚C for sampling at 5, 15, 25, and 50 d. Remaining slices from the package were resealed in delicatessen bags using rubber bands and stored at 7˚C to simulate aerobic home storage conditions. Proliferation of LM in products formulated without antimicrobials held under post-processing storage conditions resulted in microbial concentrations ranging from 4.6 to 7.4 CFU/cm^2 after 3 d at aerobic home storage conditions. The growth rate for products formulated with antimicrobials was .14 to .16 log CFU/cm^2/day compared to .25 to .51 log CFU/cm^2/day for products formulated without antimicrobials. Although this research demonstrates the ability of organic acid treatments to decrease microbial growth rates, growth was also shown to decrease as a result of product age. Carroll and others

(2007) evaluated the effects of the addition of organic acid marinades to turkey deli loaf formulations on extention of LM lag phase. Sodium tripolyphosphate, SL, SDA, SC, and a combination of SL and SDA were added at treatment concentrations of 0.45%, 3%, 0.25%, 0.75%, respectively. An industry control of 1.5% salt was added in all solutions. Turkey deli slices were inoculated with a 10^3 concentration of a three-strain LM cocktail and vacuum packaged for storage at refrigerated temperatures. Product was sampled at weekly timepoints throughout the 77-d storage period. Based on aerobic plate count concentrations of $>10^6$ CFU/g, STPP, SL, SC, SDA, and the combination treatment extended the lag phase for 14, 20, 40, 70, and 74 d, respectively. Collectively, the use of organic acids applied as post-processing interventions are shown to extend shelf life and product quality.

The purpose of this study was to evaluate the inhibitory capabilities of organic acid marinade treatments applied as post cook dips to turkey deli loaf slices. Efficacy was gauged by bacteriostatic effects on LM growth and bacteriocidal reduction of microbial populations. To accomplish this, currently used organic acid ingredients, including SL, PL, SC, and SDA were applied singly and as combination treatments, SL/SDA and SL/PL/SDA, to cured turkey slices formulated with NaCl and STPP. In doing so, this study evaluated both single and multi-hurdle approaches to post-processing LM control by organic acids.

CHAPTER IV

MATERIALS AND METHODS

Preliminary Growth and Antibiotic Resistance

Two strains of *L. monocytogenes* (LM), Scott A and Brie I, were obtained from a preliminary experiment conducted Auburn University and cultivated to strengthen the culture. Frozen strains, stored in 1.5-µL microcentrifuge tubes (VWR Intl., West Chester, PA) in a -80˚C freezer (Harris, Asheville, NC), were thawed for inoculation. One-milliliter of each frozen culture was allocated by 1000-µL pipette (Eppendorf Scientific, Hamburg, Germany) into 9.0 mL of sterile BHI broth (Oxoid, Basingstoke/Hampshire, England). The media tubes were incubated (Fisher Scientific, Pittsburgh, PA) overnight at 37˚C. Turbid cultures were transferred to fresh media and the incubation process was repeated. Five 1:10 serial dilutions were allocated into tubes containing 9.0 mL of sterile phosphate buffered saline (PBS) solution (Pierce Perbio Thermo Scientific, Bonn, Germany). Each dilution was plated in duplicate using 15- x 100-mm plates (VWR Intl., West Chester, PA) on BHI agar (Difco, Lawrence, KS) and incubated at 37˚C. Plates were enumerated by computer using Q count software (Spiral Biotech, Norwood, MA) to obtain a preliminary estimate of the colony forming units (CFU)/mL.

Streptomycin resistance was induced through exposure of LM to antibiotic in media. Both bacterial strains were gradually exposed to increasing concentrations of streptomycin in treated BHI broth and BHI agar (Killinger-Mann 2005). A stock solution of streptomycin was prepared by mixing streptomycin Sulfate salts (Sigma-Aldrich, St. Louis, MO) and sterile distilled water in a 1-L flask (Pyrex, Lowell, MA) using a combination hotplate/stir unit (VWR Intl.-Henry Troemner, West Chester, PA). The

solution was filter sterilized into 250-mL media bottles (Pyrex, Lowell, MA) using 60 mL syringes (Fisher Scientific, Pittsburgh, PA) fitted with 20-µm syringe filters (VWR Intl.,West Chester, PA). This was added to sterilized media under a laminar flow hood (Microzone Corp. Model FFM-2-4-WA, Ontario, Canada) based on the desired concentration of exposure. This was calculated using the formula:

(Concentration 1) (Volume 1) = (Concentration 2) (Volume 2)

Pending survival at the initial exposure concentrations, 0.2 µg/ml, 1.6 µg/ml, 6.4 µg/ml, and 12.8 µg/ml, a gradual doubling of the dosage ensued in both broth and agar until a resistance level of 1,500 µg/ml was reached.

Desired cultures were subsequently frozen in sterile 1.5-mL microcentrifuge tubes. The strains were initially incubated in 10 mL of BHI broth with 10% glycerol solution at 37˚C (Killinger-Mann 2005). From this, 300 uL was added to 700 uL of glycerol in the microcentrifuge tubes using a 1000-µL pipette and vortexed using a scientific touch mixer model 231 (Fisher Scientific, Basingstoke/Hampshire, England) prior to freezing. A sample of each strain was subsequently thawed and regrown to determine bacterial survival following the procedure.

Resistant strains were grown on BHI slants and validated for identity by the USDA - Agricultural Research Service (Athens, GA). Strains were streaked on modified oxford (MOX) agar (Remel, Lenexa, KS) for the cultivation of characteristic small, black colonies. A sterile loop was used to collect five to ten colonies for beta hemolysis testing (Berrang 2007). This procedure involved streaking for isolation on horse blood overlay plates. At least two colonies were collected from positive samples for confirmatory testing including a gram stain, CAMP test, rhamnose acid formation, positive catalase

reaction, and tumbling motility under a wet mount (Berrang 2007). The Scott A strain tested positive for retention of LM characteristics and was used in this experiment. This contrasts with the strain used for the Auburn University experiment which was conducted with a positive Brie 1 strain. Strains were spread plated according to the Association of Official Analytical Chemists (AOAC) methods. Media was sterilized throughout the experiment using a Cat 2007 autoclave at 121˚C for 15 minutes at 15 psi (Tuttnauer, Hauppauge, NY).

Product Preparation

Preparation of meat products involved incorporation of non-iodized salt (1.5%; Morton, Chicago, IL) and sodium tripolyphosphate (0.45%, Innophos, Cranbury, NJ). Controls were calculated based on industry standards. Marinade was included (20% weight/weight marinade) with water comprising a portion of the inclusion weight. Water was added in a 7:3 water to ice ratio to maintain a low temperature during processing. Industry controls and additives were blended using a handheld biohomogenizer (ESGE m13333-1281-O, Bartlesville, OK). Prior to marination, 20% of the turkey lobe meat (Patuxent Farms, Columbia, MD) was sectioned into ground meat using a Waring grinder (Waring Products, Inc., Torrington, CT) while the remaining meat was sectioned by hand into ~2.54-cm^3 cubes. All meat used was maintained at 4˚C throughout transport and processing. Ingredients were incorporated through vacuum tumbling (Hollymatic Corp., Countryside, IL) for one hour at 25 mm Hg, 14 RPM, and 4˚C. This was followed by equilibration at ~4˚C for 3 h.

Deli loaves were fabricated under industry simulation regarding equipment and practice. Turkey deli loaves were manually stuffed into 10.16-cm diameter fibrous

cellulose casing (EZ Peel fibrous casing, Viskase Companies, Inc., Willowbrook, IL**)** by using a hand crank manual stuffer (Koch, Kansas City, MO). Each loaf was approximately 63.5-cm long. Loaves were trucked into the single truck smokehouse (Alkar, Lodi, WI**)** and smoked (Table 2). The loaves were removed and chilled overnight to an internal temperature of ~4˚C. The loaves were hand peeled then sectioned into ~2-mm slices by slicing machine (9512 12" Max Manual Meat Slicer, Univex Corp.; Salem, NH**).** The blade wa**s** calibrated by caliper (Marathon Watch Co., Ontario, Canada)**.** Slices fell from the slicing blade onto a table lined with sterile foil and were placed in a sterile metal pan covered with sterile foil for transport. Ten slices were individually weighed by laboratory scale and averaged to obtain an approximate slice weight to be used in later calculations.

TABLE 2. *Dry bulb temperatures, wet bulb temperatures, time, and smoke setting for each step used in the turkey deli loaf smoke cycle*

Step	Dry Bulb Temp (˚C)	Wet Bulb Temp (˚C)	Time (min)	Smoke
1	53.9	-17.8	30	off
2	65.6	46.1	120	off
3	76.7	58.9	60	off
4	85.0	68.9	Until 71.1˚C internal temp	off
5	-17.8	-17.8	15 (cold shower)	off

Inoculation

The culture was passed at least 3 d before inoculation to allow for enumeration and strengthening as previously described. The first of five 1:10 serial dilutions was passed from inoculated BHI broth into a sterile test tube with 9.0 mL of PBS solution. Each dilution was spread in duplicate 15- x 100-mm plates on untreated BHI agar and incubated overnight at 37˚C. Plates were scanned by computer using Q Count software

with an acceptable enumeration range between 25 to 250 CFU. The duplicate plates were subsequently averaged and multiplied by the appropriate dilution factor. The estimated CFU/mL for stock collected from frozen culture was required to surface inoculate each slice at the desired level of 10^4 to 10^5 CFU/g.

Each slice was inoculated with stock culture using 10 µL aliquots dispensed from a sterilized, calibrated 200-µL pipetter (Eppendorf Scientific, Hamburg, Germany) equipped with sterilized pipette tips. Disposable, sterile 10-µL inoculating loops (VWR Intl., West Chester, PA) were used to spread the inoculum across the surface of the meat. This was performed on a single side of the slice. Inoculated slices were set aside for two minutes to allow for bacterial attachment. Transfer of slices to segregated stations was conducted using ethanol sterilized forceps. Inoculation was conducted beneath a Class II type A or A/B3 biological safety cabinet (SafeAire Fischer Hamilton, Two Rivers, WI**)** lined with sterile sheets of disposable foil. The foil sheets were replaced after each round of inoculation.

Immersion Treatment

The following treatments (Table 3), single and combined organic acids, were applied to the inoculated slices to be held at refrigerated (Norlake Scientific, Hudson, WI) storage temperatures of ~4˚C for the purpose of prolonging the lag phase of LM.

1. Sodium lactate (3.6%) (Fischer Scientific, Pittsburgh, PA)
2. Potassium lactate (3.6%) (City Chemicals, West Haven, CT)
3. Sodium citrate (0.75) (Fischer Scientific, Pittsburgh, PA)
4. Sodium lactate (3.6%) and sodium diacetate (0.25%) (Spectrum Chemical Manufacturing Corporation, Gardena, CA)

5. Sodium lactate (3.6%), potassium lactate (3.6%), and sodium diacetate (0.25%)

6. Positive control – inoculated slice dipped in sterile water

7. Negative control – non-inoculated slice dipped in sterile water

TABLE 3. *Immersion treatment formulations including percent of ingredient used, ingredient percentage in formulation, and water percentage in formulation*

Treatment	% Used	% of Formulation	% Water in Formulation
SL	3.6	18.0	82.0
PL	3.6	18.0	82.0
SC	0.75	3.8	96.2
SL/SDA	3.85[1]	19.3	80.7
SL/PL/SDA	7.45[2]	37.3	62.7

[1] Calculated by adding percent of SL and SDA (0.25)
[2] Calculated by adding SL, PL, and SDA percentages

Slices were dipped for approximately one minute and removed with sterile forceps for drying on sterile foil lined drying racks. Excess marinade was allowed to dry for at least 10 minutes with intermediate flipping of the slices. Dry slices were packaged in groups of three, according to treatment, using 708 g Whirlpak bags (Nasco, Fort Atkinson, WI) and stored at ~4˚C until their appropriate sampling times. It is important to note that three slices were allocated to each of the seven timepoints per each of the five treatments totaling up to 105 slices. Two slices per timepoint were allotted for a positive and negative control, providing an additional 28 slices.

Testing

Packaged slices were removed from refrigerated storage for sampling on d 0, 7, 14, 21, 28, 42, and 56. Sterile forceps were used to divide the slices into individual filtered stomacher bags. Each bag was filled with 50 mL of sterile PBS and homogenized,

using a Stomacher 400 Circulator (Seward, West Sussex, UK), for two minutes at 245 rotations per minute (RPM). Five 1:10 serial dilutions were made beginning with the first dilution removed from the diluent in the filter bag. Each test tube was vortexed prior to plating on streptomycin treated BHI agar plates. Samples were incubated for ~48 hours at 35˚C prior to enumeration. Plates were enumerated, as described previously, using Q-count software. Duplicate plates were averaged and calculated by dilution to obtain an estimated CFU/g. The average slice weight was considered with 50 mL of PBS added to create the first dilution. Each calculated sample CFU/g was average to find the treatment CFU/g calculation. These were plotted using Microsoft Excel software.

Statistical Analysis

Statistical analysis was performed using the Proc GLM procedure of SAS (SAS, Cary, NC). Means were grouped by treatment and separated using Duncan's Multiple Range Test. Statistical significance was determined using an alpha of 5%. As there were no trial x treatment interactions, data, consisting of two trials and three replications, was pooled by treatment.

CHAPTER V

RESULTS AND DISCUSSION

Despite the establishment of the USDA zero-tolerance policy for LM contamination on RTE foods, LM has remained a persistent contaminant on 12 to 27% of retailed RTE meat products (Lawrence and Gilmour 1995). In reference to specific foods, Ramos and others (1999) correlated high levels of contamination proportional to the amount of machination required to process meats. Isolates collected from processing facilities indicate the potential for LM survival on processing machinery, niches, and fixtures for several years (Lawrence and Gilmour 1995, Olsen 2005). Major outbreaks attributed to LM in the poultry industry have centered around post-processing contamination of turkey deli slices. Due to the frequency of contamination occurring post-processing and before packaging, this study was conducted to investigate the viability of organic acid application as a post-processing intervention against streptomycin resistant LM strain Scott A. The effectiveness of these treatments was determined by analysis of LM levels over time, specifically the bacteriostatic and bacteriocidal effects of organic acid dipping solutions towards the prolongation of the bacterial lag phase.

The combination treatments, SL/SDA and SL/PL/SDA, were the most successful in extending the LM lag phase post-processing (Tables 4 and 5, Figure 1). Despite a slight increase (0.2 log CFU/g) at d 14, treatment with SL/SDA extended the lag phase until d 21. Intermittent increases occurred thereafter on d 42 and 56. Treatment with SL/PL/SDA extended the lag phase throughout d 42 with decreasing LM concentrations noted after d 21. An overall decrease (0.1 log CFU/g) from initial levels was noted by the

end of the 56 d storage period. Treatment with SL or PL extended the lag phase throughout d 7. For sodium lactate treated products, LM growth occurred logarithmically from d 14 to 28 and dropped to <0.5 log CFU/g until d 56. For potassium lactate treated products, growth rate decreased steadily between d 14 and 28 with a decrease in LM populations on d 42. Growth resumed on d 56. Sodium citrate levels, followed by the inoculated control, rose immediately after d 0. Growth on products treated with sodium citrate rose by roughly 1.0 log CFU/g between d 0 and 7 and 2.0 CFU/g between d 7 and 14. Growth decreased to 0.4 log CFU/g by d 28 and ceased on d 42. Growth resumed again on d 56. Growth on inoculated control products rose by roughly 0.7 log CFU/g from d 0 to 7. A large increase (2.0 log CFU/g) followed between d 7 to 14. A small decrease in growth rate (0.2 log CFU/g) occurred on d 28. Increasing growth occurred from d 42 through 56.

TABLE 4. *Levels of LM average log CFU/g and superscripts by day for sodium lactate, potassium lactate, sodium citrate, sodium lactate and sodium diacetate combination, and sodium lactate, potassium lactate, and sodium diacetate combination treatments on turkey deli loaves*

Treatment	D 0	D 7	D 14	D 21	D 28	D 42	D 56
SL/PL/SDA	5.4^{a}	5.0^{b}	5.0^{d}	5.0^{c}	4.9^{d}	4.5^{b}	5.3^{c}
SL	5.3^{a}	5.0^{b}	5.8^{c}	6.6^{a}	7.7^{b}	7.9^{a}	8.3^{ab}
SL/SDA	5.4^{a}	4.9^{b}	5.1^{d}	4.8^{c}	5.7^{c}	5.0^{b}	6.1^{c}
PL	5.3^{a}	5.1^{b}	6.7^{b}	7.9^{a}	8.3^{ab}	8.2^{a}	8.6^{ab}
CONTROL	5.2^{a}	5.9^{a}	7.7^{a*}	7.8^{a}	7.6^{b}	7.7^{a}	8.1^{b}
SC	5.1^{a}	6.3^{a}	8.3^{a*}	8.4^{a}	8.8^{a}	8.8^{a}	9.2^{a}

* Estimated log CFU/g because values were outside countable range

TABLE 5. *Listeria monocytogenes CFU/g by day, represented as gains and losses, for sodium lactate (SL), potassium lactate (PL), sodium citrate (SC), sodium lactate and sodium diacetate (SDA) combination, and sodium lactate, potassium lactate, and sodium diacetate treatments on turkey deli loaves*

Treatment	Initial CFU/g	D7	D14	D21	D28	D42	D56	Overall Increase
SL	5.3	-0.3	+0.3	+0.8	+1.1	+0.2	+0.4	+3.0
PL	5.3	-0.2[2]	+1.6	+1.2	+0.4	-0.1	+0.4	+3.3
SC	5.1	+1.2[1]	+2.0	+0.1	+0.4	0	+0.4	+4.1
SL/SDA	5.4	-0.5	+0.2	-0.3	+0.9	-0.7	+1.1	+0.7
SL/PL/SDA	5.4	-0.4	0	0	-0.1	-0.4	+0.8	-0.1
CONTROL	5.2	+0.7	+1.8	+0.1	-0.2	+0.1	+0.4	+2.9

[1] + denotes increase
[2] - denotes decrease

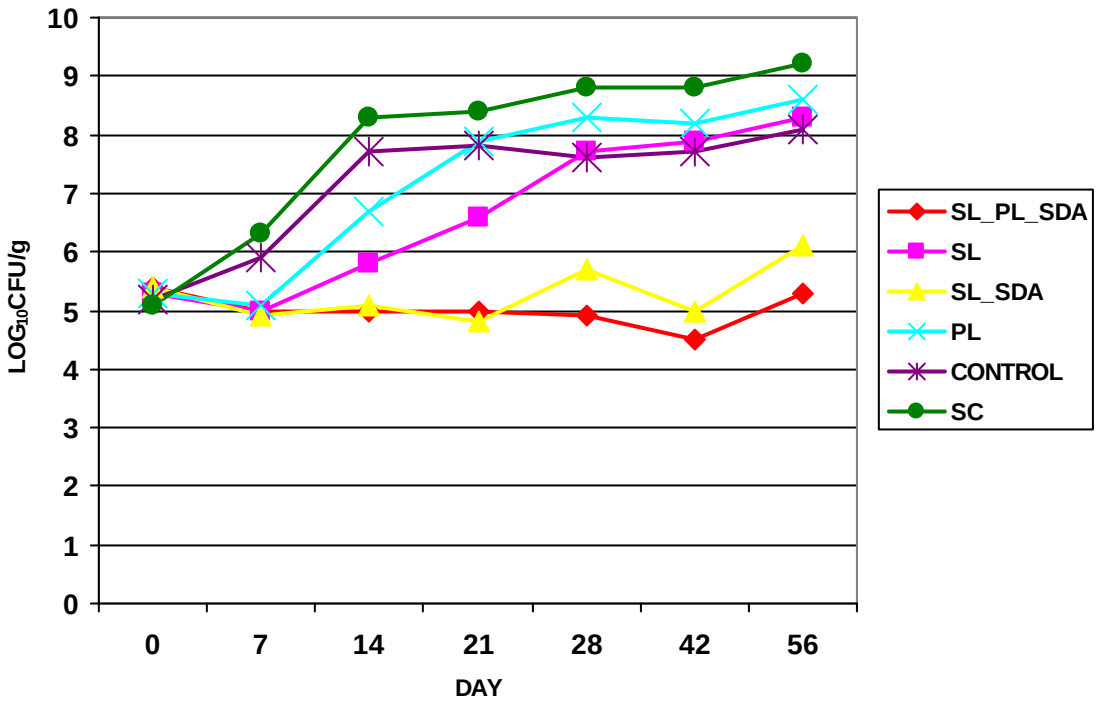

FIGURE 1. *Pooled LM results for d 0 through 56 for the sodium lactate (SL), potassium lactate (PL), sodium citrate (SC), sodium lactate and sodium diacetate (SDA) combination treatment, and sodium lactate, potassium lactate, and sodium diacetate combination treatments applied to turkey deli slices*

While most related research supports antimicrobial success in poultry formulations, limited knowledge regards their application as post-cook dipping solutions.

Lianou and others (2006a) found that commercial lactate and diacetate combinations formulated in cured turkey breast demonstrated reduced LM growth rates (0.14 to 0.16 CFU/cm^2/day) compared to untreated turkey breast (0.25 to 0.51 CFU/cm^2/day). Carrol and others (2007) found that a combination treatment of 3% SL and 0.75% SDA applied to inoculated, vacuum packaged turkey deli slices extended the lag phase throughout 63 d of storage.

Combination dipping treatments applied to frankfurters have proven successful in extending the lag phase for at least three weeks post-processing. Doyle (1999a) cited research regarding the antilisterial effects of 1% lactic, acetic, tartaric, or citric acids on inoculated frankfurters. A 1 to 2 log decrease in LM populations was initially noted, but surviving bacteria proliferated by the end of the 90 d storage period. Increasing SDA concentrations to 5% exhibited bacteriocidal effects and prolonged shelf life throughout 90 d at refrigerated storage. Samelis and others (2002) noted that combination treatments of 0.25% SL with 0.25% SA, SDA, or GDL applied to frankfurters extended the lag phase throughout 120 d at refrigerated storage. Glass and others (2001) found that SL and SDA post-cooked dips applied to turkey, pork, and beef combination frankfurters and pork and beef combination bratwurst sausages, prolonged the lag phase for four and twelve weeks, respectively. Palumbo and Williams (2002) found that inoculated, vacuum packaged frankfurters treated with a combination of 2.5% lactic acid and 2.5% acetic acid dip suppressed LM growth throughout 90 d at refrigerated storage temperatures. Uhart and others (2004) found that combination dipping treatments of 3% SDA and 6% SL reduced LM populations by 1 to 2 logs during the three-week sampling period.

Two modes of action have been proposed for the antimicrobial mechanisms of lactate and diacetate. As previously discussed, success has been attributed to penetration of the undissociated acid molecule through the bacterial cell membrane. Weak acids, such as sodium lactate and potassium lactate, are favored for intracellular permeability due to the predominantly hydrophobic fraction and its interactions with the cellular lipid bilayer. Once inside, the weak acid may dissociate upon contact with the more acidic intracellular pH and increase the acidity or cause substrate level inhibition of metabolism. This may result in cell damage, death, or exhaustion due to shifting of energy and resources to exclude the acid.

CHAPTER VI

CONCLUSION

Based on the success of combination treatments applied to turkey deli loaves, multi-hurdle technology applied as organic acid post-cook dips can be used to successfully prolong the lag phase of LM for three to six weeks in turkey deli slices packaged under aerobic storage conditions and held at refrigerated temperatures. Current LM programs developed for RTE meats focus on contamination stemming from improper product processing and poor ingredient quality, poor sanitation or biofilm formation on processing machinery, or cross contamination between processing areas or facilities. Food safety inspection service regulations for processing facilities producing RTE products require the development of interventions detailing LM control measures in HACCP, SSOP, and GMP systems. These treatments can be applied to the first and second alternatives, regarding LM control through the use of antimicrobial agents and processes, when developing post-processing intervention guidelines.

CHAPTER VII

LISTERIA MONOCYTOGENES IN PROCESSED HAM

Research indicates an increased prevalence of LM on RTE ham products. Four Star Meat Products Co. Inc., aided by the FSIS, recalled 323 kilograms of RTE boneless ham distributed to a Tennessee warehouse (Dement 2004). Fortner Foods Commissary, aided by the FSIS, issued a recall for RTE ham distributed to pizza outlets in three Tennessee cities (Cohen 2005b). In perhaps one of the largest recalls associated with RTE food products, ConAgra Foods, aided by the FSIS, issued a nationwide recall of Armour Lunch Makers® meals amounting over 1.2 million kilograms of ham, turkey, and bologna (Cohen 2005a).

These recalls have prompted increased regulatory scrutiny of pork processing facilities. Samelis and Metaxopoulos (1999) noted contamination in 51% and 49% of incoming shipments of raw meat during two trips to European meat processing plants. The greatest risk was associated with turkey necks and legs, and pork trimmings and lard. Contamination was primarily noted in tumbled meats due to continuous processing with improper sanitation methods between batches. This incidence was present in 23.3% and 6.7% of vacuum packaged RTE meats and 40% and 10% of sausages indicating that contamination had occurred post processing. Heat survivors were also noted in RTE meats cooked by broiler as opposed to oven cooking. Peccio and others (2003) analyzed 68 swab and food samples collected from a pork meat processing plant and noted seven LM strains over the course of the yearly sampling period. Strains were isolated from raw materials, partially processed meats combined with nitrite and spices, finished sausages, and ‘clean’ processing equipment. Analysis of strains collected from other plants in the

area yielded two genetically identical strains indicating that contamination was transported by pork meat suppliers. This contamination risk had persisted in both processing environments for at least one year. Both studies discuss the increased potential for post-processing LM contamination introduced by machinery.

Positive results associated with organic acids applied to ham products as post-cook marinades support the use of these treatments as a post-processing intervention. Glass and others (1999) studied the inhibitory properties of lactate and diacetate combination dipping solutions on ham inoculated with a 10^2 CFU/mL concentration of a five-strain LM cocktail. Ham was dipped for five seconds, before inoculation, in a solution composed of 3% lactate and 1.5% diacetate. Slices were then stored in gas impermeable packages and maintained at refrigerated temperatures throughout the 60-d sampling period. At these ingredient concentrations, LM growth was delayed for 30 d post-processing. Using a 6% lactate and 3% diacetate solution, growth was delayed for 45 d. Samelis and others (2001) applied treatments of 2.5% and 5% acetatic and lactic acid, their salts sodium acetate or SDA, 5% and 10% PL, 5% potassium sorbate, or 5% potassium benzoate as post-cook dips to pork bologna inoculated with a 10^3 to 10^4 CFU/mL concentration of a ten-strain LM cocktail. Treated slices were vacuum packaged and maintained at refrigerated storage tempatures for 120 d. Treatment with both concentrations of acetic acid, 5% SDA, or 5% potassium benzoate maintained the bacterial lag phase throughout the 120-d storage period. Treatment with 5% lactic acid maintained the lag phase for 90 d. Both concentrations of SL maintained the lag phase for 20 to 35 d of storage. A similar study conducted by Geornaras and others (2004) evaluated 2.5% acetic acid, 2.5% lactic acid, 5% potassium benzoate, 0.5% Nisipalin, and

combinations of organic acids with Nisipalin applied to ham and pork bologna as post cook dipping solutions. The inhibitory effects were tested against a 3 to 4 log/cm^2 inoculum of a ten-strain LM cocktail. Ham samples were vacuum packaged and maintained at 10˚C. Control LM populations rose from 3.4 log CFU/cm^2 to over 7 log CFU/mL after 8 d of storage. Microbial populations on both products for all treatments decreased by 0.7 to 1.0 log CFU/cm^2 by the end of the 48-d storage period. Based on these studies, the application of antimicrobial dipping solutions has been shown to prolong the lag phase, including bacteriocidal capabilities, for at least 30 d post-processing.

The purpose of this study was to evaluate the inhibitory capabilities of organic acid marinade treatments applied as post cook dips to ham deli loaf slices. Efficacy was be gauged by bacteriostatic effects on LM growth and bacteriocidal reduction of microbial populations. To accomplish this, currently used organic acid ingredients, including SL, PL, SC, and SDA were be applied singly and as combination treatments, SL/SDA and SL/PL/SDA, to cured ham slices formulated with sodium nitrite, NaCl and STPP. In doing so, this study evaluated both single and multi-hurdle approaches to post-processing LM control by organic acids.

CHAPTER VIII

MATERIALS AND METHODS

Preliminary Growth and Antibiotic Resistance

Two strains of *L. monocytogenes* (LM), Scott A and Brie I, were obtained from a preliminary experiment conducted Auburn University and cultivated to strengthen the culture. Frozen strains, stored in 1.5-µL microcentrifuge tubes (VWR Intl., West Chester, PA) in a -80˚C freezer (Harris, Asheville, NC), were thawed for inoculation. One-milliliter of each frozen culture was allocated by 1000 µL pipette (Eppendorf Scientific, Hamburg, Germany) into 9.0 mL of sterile BHI broth (Oxoid, Basingstoke/Hampshire, England)**.** The media tubes were incubated (Fisher Scientific, Pittsburgh, PA) overnight at 37˚C. Turbid cultures were transferred to fresh media and the incubation process was repeated. Five 1:10 serial dilutions were allocated into tubes containing 9.0 mL of sterile phosphate buffered saline (PBS) solution (Pierce Perbio Thermo Scientific, Bonn, Germany). Each dilution was plated in duplicate using 15- x 100-mm plates (VWR Intl., West Chester, PA) on BHI agar (Difco, Lawrence, KS) and incubated at 37˚C. Plates were enumerated by computer using Q count software (Spiral Biotech, Norwood, MA) to obtain a preliminary estimate of the CFU/mL.

Streptomycin resistance was induced through exposure of LM to antibiotic in media. Both bacterial strains were gradually exposed to increasing concentrations of streptomycin in treated BHI broth and BHI agar (Killinger-Mann 2005). A stock solution of streptomycin was prepared by mixing streptomycin Sulfate salts (Sigma-Aldrich, St. Louis, MO) and sterile distilled water in a 1-L flask (Pyrex, Lowell, MA) using a combination hotplate/stir unit (VWR Intl.-Henry Troemner, West Chester, PA). The

solution was filter sterilized into 250 mL media bottles (Pyrex, Lowell, MA) using 60 mL syringes (Fisher Scientific, Pittsburgh, PA) fitted with 20-µm syringe filters (VWR Intl.,West Chester, PA). This was added to sterilized media under a laminar flow hood (Microzone Corp. Model FFM-2-4-WA, Ontario, Canada) based on the desired concentration of exposure. This was calculated using the formula:

(Concentration 1) (Volume 1) = (Concentration 2) (Volume 2)

Pending survival at the initial exposure concentrations, 0.2 µg/ml, 1.6 µg/ml, 6.4 µg/ml, and 12.8 µg/ml, a gradual doubling of the dosage ensued in both broth and agar until a resistance level of 1,500 µg/ml was reached.

Desired cultures were subsequently frozen in sterile 1.5-mL microcentrifuge tubes (Killinger-Mann 2005). The strains were initially incubated in 10 mL of BHI broth with 10% glycerol solution at 37˚C. From this, 300 uL was added to 700 uL of glycerol in the microcentrifuge tubes using a 1000-µL pipette and vortexed using a scientific touch mixer model 231 (Fisher Scientific, Basingstoke/Hampshire, England) prior to freezing. A sample of each strain was subsequently thawed and regrown to determine bacterial survival following the procedure.

Resistant strains were grown on BHI slants and validated for identity by the USDA - Agricultural Research Service (Athens, GA). Strains were streaked on modified oxford (MOX) agar (Remel, Lenexa, KS) for the cultivation of characteristic small, black colonies. A sterile loop was used to collect five to ten colonies for beta hemolysis testing (Berrang 2007). This procedure involved streaking for isolation on horse blood overlay plates. At least two colonies were collected from positive samples for confirmatory testing including a gram stain, CAMP test, rhamnose acid formation, positive catalase

reaction, and tumbling motility under a wet mount (Berrang 2007). The Scott A strain tested positive for retention of LM characteristics and was used in this experiment. This contrasts with the strain used for the Auburn University experiment which was conducted with a positive Brie 1 strain. Strains were spread plated according to the Association of Official Analytical Chemists (AOAC) methods. Media was sterilized throughout the experiment using a Cat 2007 autoclave at 121˚C for 15 minutes at 15 psi (Tuttnauer, Hauppauge, NY).

Product Preparation

Preparation of meat products involved incorporation of non-iodized salt (1.5%; Morton, Chicago, IL) and sodium tripolyphosphate (0.45%; Innophos, Cranbury, NJ). Sodium nitrite (156 ppm; A.C. Legg, Calera, AL**)** was additionally added to the ham deli loaves. Controls were calculated based on industry standards. Marinade was included on a 20% weight by weight marinade basis with water comprising a portion of the inclusion weight. Water was added in a 7:3 water to ice ratio to maintain a low temperature during processing. Industry controls and additives were blended using a handheld biohomogenizer (ESGE m13333-1281-O, Bartlesville, OK). Prior to marination, 20% of the ham lobe meat (TTU G.W. Davis Meat Lab, Lubbock, TX) was sectioned into ground meat using a Waring grinder (Waring Products, Inc., Torrington, CT) while the remaining meat was sectioned by hand into ~2.54 cm^3 cubes. The cubed meat was tenderized using a Hobart tenderizer (Hobart, Troy, OH) to improve cure distribution based on poor bindability experienced during a prelimary trial. All meat used was maintained at 4˚C throughout transport and processing. Ingredients were incorporated through vacuum

tumbling (Hollymatic Corp., Countryside, IL) for two hours at 25 mm Hg and 4˚C.This was followed by equilibration at ~4˚C for three hours.

Deli loaves were fabricated under industry simulation regarding equipment and practice. Ham deli loaves were manually stuffed into 10.16-cm diameter fibrous cellulose casing (EZ Peel fibrous casing, Viskase Companies, Inc., Willowbrook, IL**)** by using a hand crank manual stuffer (Koch, Kansas City, MO). Each loaf was approximately 63.5 cm long. Loaves were trucked into the single truck smokehouse (Alkar, Lodi, WI**)** and smoked (Table 6). Natural smoke was added using an Alkar smoke generator (Lodi, WI). The loaves were removed chilled overnight to an internal temperature of ~4˚C. The loaves were hand peeled then sectioned into ~3.5-mm slices by slicing machine (9512 12" Max Manual Meat Slicer, Univex Corp.; Salem, NH**).** The blade wa**s** calibrated by caliper (Marathon Watch Co., Ontario, Canada)**.** Slices fell from the slicing blade onto a table lined with sterile foil and were placed in a sterile metal pan covered with sterile foil for transport. Ten slices were individually weighed by laboratory scale and averaged to obtain an approximate slice weight to be used in later calculations.

TABLE 6. *Dry bulb temperatures, wet bulb temperatures, time, and smoke setting for each step used in the ham deli loaf smoke cycle*

Step	Dry Bulb Temp (˚C)	Wet Bulb Temp (˚C)	Time (min)	Smoke
1	53.9	-17.8	30	off
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The culture was passed at least 3 d before inoculation to allow for enumeration and strengthening as described previously. The first of five 1:10 serial dilutions was passed from inoculated BHI broth into a sterile test tube with 9.0 mL of PBS solution. Each dilution was spread in duplicate 15- x 100-mm plates on untreated BHI agar and incubated overnight at 37˚C. Plates were scanned by computer using Q Count software with an acceptable enumeration range between 25 to 250 CFU. The duplicate plates were subsequently averaged and multiplied by the appropriate dilution factor. The estimated CFU/mL for stock collected from frozen culture was required to surface inoculate each slice at the desired level of 10^4 to 10^5 CFU/g.

Each slice was inoculated with stock culture using 10-µL aliquots dispensed from a sterilized, calibrated 200-µL pipetter (Eppendorf Scientific, Hamburg, Germany) equipped with sterilized pipette tips. Disposable, sterile 10-µL inoculating loops (VWR Intl., West Chester, PA) were used to spread the inoculum across the surface of the meat. This was performed on a single side of the slice. Inoculated product was set aside for two minutes to allow for bacterial attachment. Transfer of slices to segregated stations was conducted using ethanol sterilized forceps. Inoculation was conducted beneath a Class II type A or A/B3 biological safety cabinet (SafeAire Fischer Hamilton, Two Rivers, WI**)** lined with sterile sheets of disposable foil. The foil sheets were replaced after each round of inoculation.

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4. Sodium lactate (3.6%) and sodium diacetate (0.25%) (Spectrum Chemical Manufacturing Corporation, Gardena, CA)
5. Sodium lactate (3.6%), potassium lactate (3.6%), and sodium diacetate (0.25%)
6. Positive control – inoculated slice dipped in sterile water
7. Negative control – non-inoculated slice dipped in sterile water

Slices were dipped for approximately one minute and removed with sterile forceps for drying on sterile foil lined drying racks. Excess marinade was allowed to dry for at least 10 minutes with intermediate flipping of the slices. Dry slices were packaged in groups of three, according to treatment, using 708 g Whirlpak bags (Nasco, Fort Atkinson, WI) and stored at ~4˚C until their appropriate sampling times. It is important to note that three slices were allocated to each of the seven timepoints per each of the five treatments totaling up to 105 slices. Two slices per timepoint were allotted for a positive and negative control, providing an additional 28 slices.

Testing

Packaged slices were removed from refrigerated storage for sampling on d 0, 7, 14, 21, 28, 42, and 56. Sterile forceps were used to divide the slices into individual filtered stomacher bags. Each bag was filled with 50 mL of sterile PBS and homogenized, using a Stomacher 400 Circulator (Seward, West Sussex, UK), for two minutes at 245 RPM. Five 1:10 serial dilutions were made beginning with the first dilution removed from the diluent in the filter bag. Each test tube was vortexed prior to duplicate plating on streptomycin treated BHI agar plates. Samples were incubated for ~48 hours at 35˚C prior to enumeration. Plates were enumerated, as described previously, using Q-count software. Duplicate plates were averaged and calculated by dilution to obtain an estimated CFU/g. The average slice weight was considered with 50 mL of PBS added to create the first dilution. Each calculated sample CFU/g was average to find the treatment CFU/g calculation. These were plotted using Microsoft Excel software.

Statistical Analysis

Statistical analysis was performed using the Proc GLM procedure of SAS (SAS, Cary, NC). Means were grouped by treatment and separated using Duncan's Multiple Range Test. Statistical significance was determined using an alpha of 5%. Although two trials, consisting of three replications each, were conducted, only Trial 2 data were used.

CHAPTER IX

RESULTS AND DISCUSSION

Despite the establishment of the USDA zero-tolerance policy for LM contamination on RTE foods, LM has remained a persistent contaminant on 12 to 27% of retailed RTE meat products (Lawrence and Gilmour 1995). In reference to specific foods, Ramos and others (1999) correlated high levels of contamination proportional to the amount of machination required to process meats. In two studies conducted at a European meat processing facility, Samelis and Metaxopoulos (1999) noted that LM contamination from incoming shipments of raw pork trimmings and lard was transferred during vacuum tumbling to 23.3% and 6.7% of vacuum packaged RTE meats. Peccio and others (2003) isolated LM raw and cooked meat products and cleaned processing equipment and fixtures. Major outbreaks due to LM in the pork industry have centered around the contamination of ham deli slices. Due to the frequency of contamination occurring post-processing and before packaging, this study was conducted to investigate the viability of organic acid application as a post-processing intervention against streptomycin resistant LM strain Scott A. The effectiveness of these treatments was determined by analysis of LM levels over time, specifically the bacteriostatic and bacteriocidal effects of organic acid dipping solutions towards the prolongation of the bacterial lag phase.

Based on the extension of the lag phase, both combination treatments and sodium lactate applied alone were the most successful at prolonging LM growth on ham deli slices (Table 7 and 8, Figure 2). Treatment with a SL/SDA combination extended the lag phase through d 14. Slight decreases ($\leq$0.5 log CFU/g) were noted on d 28 and 56. Treatment with a SL/PL/SDA combination extended the lag phase through d 14.

Populations of LM increased by 1.3 log CFU/g by d 21 with small increases (≤ 0.3 log CFU/g) noted thereafter. The lag phase was extended throughout d 7 in the inoculated control with an increase of ~1.0 log CFU/g by d 14 and a decrease by 0.7 log CFU/g d 21. Growth rate approached 1.0 log CFU/g thereafter until d 56. Sodium lactate extended the lag phase until d 14. An increase of 0.8 log CFU/g occurred between d 14 and 21. A small decrease in LM populations (0.2 log CFU/g) was noted on D28, but growth rate proceeded steadily (0.5 log CFU/g) from d 28 through 56. Sodium citrate and PL did not extend the lag phase. In products treated with SC, growth began immediately after inoculation, but the rate decreased steadily and stopped from d 14 through 28. Growth resumed again until d 42, but LM populations (0.2 Log CFU/g) decreased between d 42 and 56. Growth in products treated with potassium lactate increased immediately after inoculation. Growth ceased between d 7 and 14, but resumed until d 21. A decrease in LM levels (0.3 log CFU/g) occurred between d 21 and 28. Growth rate proceeded steadily (0.5 log CFU/g) from d 28 through 56.

TABLE 7. *Levels of LM average log CFU/g by day for sodium lactate, potassium lactate, sodium citrate, sodium lactate and sodium diacetate combination, and sodium lactate, potassium lactate, and sodium diacetate combination treatments on ham deli loaves, Trial 2*

Treatment	D 0	D 7	D 14	D 21	D 28	D 42	D56
SL/PL/SDA	4.5^{a}	4.3^{bc}	4.0^{c}	5.3^{ab}	5.4^{a}	5.7^{a}	6.5^{a}
SL	4.5^{a}	4.5^{ab}	4.4^{b}	5.2^{ab}	5.0^{a}	5.5^{a}	6.0^{a}
SL/SDA	4.3^{a}	4.3^{bc}	4.3^{bc}	4.7^{c}	4.4^{a}	5.5^{a}	5.0^{a}
PL	4.3^{a}	4.5^{ab}	4.5^{b}	5.0^{b}	4.7^{a}	5.4^{a}	6.1^{a}
CONTROL	4.3^{b}	3.9^{a}	5.2^{a}	4.5^{c}	4.7^{a}	5.6^{a}	6.5^{a}
SC	4.4^{a}	5.0^{a}	5.5^{a}	5.5^{a}	5.5^{a}	6.0^{a}	6.1^{a}

TABLE 8. *Listeria monocytogenes CFU/g by day, represented as gains and losses, for sodium lactate (SL), potassium lactate (PL), sodium citrate (SC), sodium lactate and sodium diacetate (SDA) combination, and sodium lactate, potassium lactate, and sodium diacetate treatments on ham deli loaves, Trial 2*

Treatment	Initial CFU/g	D7	D14	D21	D28	D42	D56	Overall Increase
SL	4.5	0	-0.1	+0.8	-0.2	+0.5	+0.5	+1.5
PL	4.2	+0.3[1]	0	+0.5	-0.3	+0.7	-0.7	+1.9
SC	4.4	+0.6	+0.5	0	0	+0.4	-0.2	+1.3
SL/SDA	4.3	0	0	+0.4	-0.3	+1.1	-0.5	+0.7
SL/PL/SDA	4.5	-0.2[2]	-0.3	+1.3	+0.1	+0.3	+0.1	+1.3
CONTROL	4.0	-0.1	+1.3	-0.7	+0.2	+0.9	+0.9	+2.5

[1] + denotes increase
[2] - denotes decrease

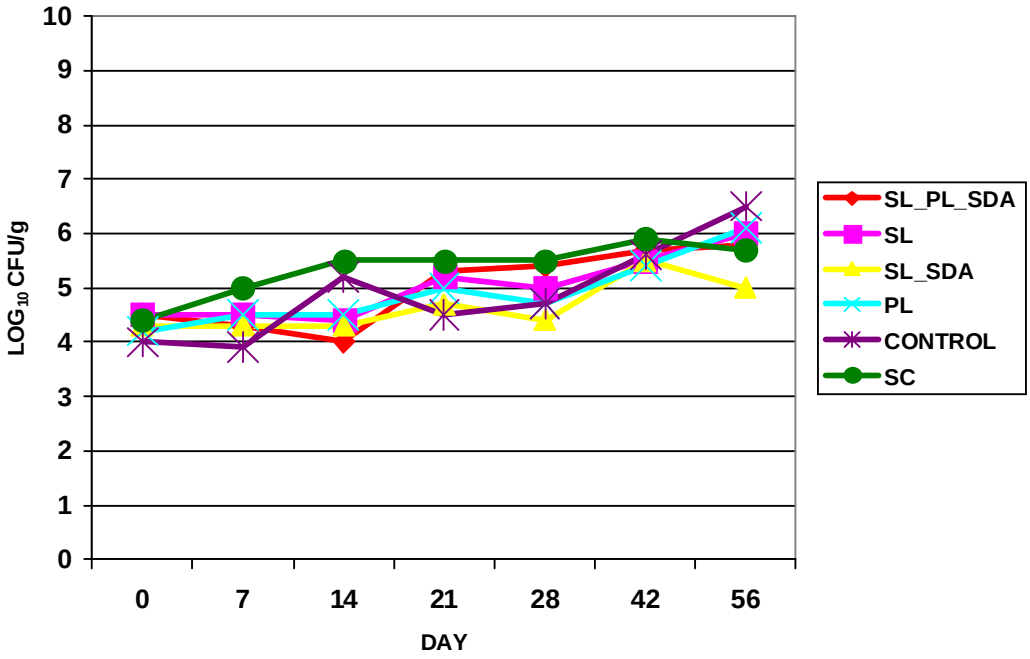

FIGURE 2. *LM levels for d 0 through 56 for the sodium lactate (SL), potassium lactate (PL), sodium citrate (SC), sodium lactate and sodium diacetate (SDA) combination treatment, and sodium lactate, potassium lactate, and sodium diacetate combination treatments applied to ham deli slices, Trial 2*

Although few studies regard the effects of combination post-processing dipping solutions applied to ham, single applications of organic acids have proven successful in extending the lag phase of vacuum packaged products for at least two weeks post-

processing. Samelis and others (2001) found that growth was limited throughout the 90-d storage period of vacuum packaged pork bologna using dipping solutions of 2.5% or 5% acetic acid, and 5% SDA. Solutions of 5% or 10% SL extended the lag phase throughout 20 to 35 d of storage. Geornaras and others (2006a) found that immersion treatments of 2.5% acetic acid or 2.5% lactic acid extended the lag phase of vacuum packaged smoked sausage for 4 to 14 d. Although no log reductions were noted at the end of this study, immersion treatments with lactates were successful in prolonging the lag phase of aerobically stored, refrigerated ham for 7 to 14 d.

Two modes of action have been proposed for the antimicrobial mechanisms of lactate and diacetate. As previously discussed, success has been attributed to penetration of the undissociated acid molecule through the bacterial cell membrane. Weak acids, such as SL and PL, are favored for intracellular permeability due to the predominantly hydrophobic fraction and its interactions with the cellular lipid bilayer. Once inside, the weak acid may dissociate upon contact with the more acidic intracellular pH and increase the acidity or cause substrate level inhibition of metabolism. This may result in cell damage, death, or exhaustion due to shifting of energy and resources to exclude the acid

Growth limitations of LM in smoked products, ham deli slices and frankfurters, versus unsmoked products, turkey deli slices, might be attributed to undissociated nitrite in the formulation. Duffy and others (1994) noted a prolonged lag phase when undissociated nitrite was studied in vacuum packaged smoked meats. Ngutter and Donnelly (2003) noted that LM injured by sodium nitrite formulations of 100 to 200 ppm recovered and proliferated on cooked frankfurters by the end of the testing period.

Two methods of action have been proposed for the antimicrobial mechanisms of sodium nitrite. The Perigo effect, complex of nitrites with functional groups such as cysteine, iron, and sulfhydryl groups, has been attributed to cellular metabolic malfunction (Davidson and others 2005). Sodium nitrite serving as an electron acceptor may also interfere with the electron transport chain, oxygen uptake, and oxidative phsophorylation (Davidson and others 2005).

CHAPTER X

CONCLUSION

Further research is required to elucidate the success of post-processing organic acid treatments. Due to mechanical constrains, vacuum packaging could not be implemented in this study. Although the conditions implemented closely approximate aerobic home storage conditions, it would be useful to determine the effects of post-processing organic acid treatments on vacuum packaged products. Potential effects of undissociated nitrite should be measured and addressed in future studies. The results from the first trial were discarded due to technical interference with the processed product. An additional trial will be performed to combine the results of two ham trials.

Current LM programs developed for RTE meats focus on contamination stemming from improper product processing and poor ingredient quality, poor sanitation or biofilm formation on processing machinery, or cross contamination between processing areas or facilities. Food safety inspection service regulations for processing facilities producing RTE products require the development of interventions detailing LM control measures in HACCP, SSOP, and GMP systems. These treatments can be applied to the first and second alternatives, regarding LM control through the use of antimicrobial agents and processes, when developing post-processing intervention guidelines. Pending further success of combination treatments applied to ham deli loaves, multi-hurdle technology applied as organic acid post-cook dips can be used to successfully prolong the lag phase of LM for one week beyond the inoculated control in ham deli slices packaged under aerobic storage conditions and held at refrigerated temperatures. Based on these

results, it may not be necessary, or cost efficient, to implement this as a post-processing intervention on RTE ham deli slices.

CHAPTER XI

LISTERIA MONOCYTOGENES IN PROCESSED BEEF

Research indicates an increased prevalence of LM on RTE beef products such as frankfurters. Smith Packing Co., Inc., aided by the FSIS, recalled 400 pounds of Honest John's hot dogs distributed county-wide in New York (Blake 2000). John Morrell & Co. recalled roughly 63,000 kilograms of beef, chicken, and pork franks distributed nationwide to retail stores and military commissaries (Long 2002). Hi-Grade Meats, aided by the FSIS, recalled over 2,400 kilograms of hot dogs distributed to statewide convenience stores (Eamich 2004). Although much has been done to create safer frankfurters, these major cases testament a lingering risk for *Listeria* contamination to the RTE food industry.

These recalls have prompted increased regulatory scrutiny of beef processing facilities. A study conducted by Fenlon and others (1996) regarding the sources of food contamination found that 21 of 23 minced beef samples tested positive for LM, as opposed to a lower percentage of contamination noted in whole beef carcasses. This indicates that the contamination noted in this study resulted during processing. In a survey of six Norwegian processing plants, Nesbakken and others (1996) analyzed 133 LM isolates collected from fresh meat, deli meat, and the processing environment. Genetic separation isolated these samples into clusters A and B. Isolate B was primarily collected from deboned, raw meat. The majority of isolate A samples were collected from RTE deli meats. This demonstrates that the majority of contamination, noted primarily in RTE and machine processed meats, resulted from contact with the processing environment. One species of cluster A was collected at four of the six plants sampled and

had been recovered from the majority of Norwegian outbreak patients. This demonstrates the potential for regional cross-contamination between processing plants. Barros and others (2007) analyzed the prevalence of *Listeria spp.* and LM in eleven Brazilian processing facilities. Of 443 samples collected from food products, equipment, and processing installations, 38.1% tested positive for *Listeria spp.* Of this percentage, 12.6% was attributed to LM. Of all *Listeria spp.* recovered, 51.4% occurred on equipment, 35.4% on installations, and 30.2% from products. Based on this study, it can be determined that at least 86.8% of the contamination in products occurred from contact with the processing environment.

Positive results associated with organic acids applied to beef frankfurters as post-cook marinades support the use of these treatments as a post-processing intervention. Uhart and others (2004) studied the inhibitory effects of organic acid dipping solutions on beef frankfurters inoculated with a 10^7 CFU/mL concentration of a four-strain LM cocktail. Following cooking and peeling, frankfurters were dipped for five minutes in either 6000 AU of pediocin, 3% SDA, 6% SL, or a combination of the three. Samples were vacuum packaged and stored at refrigerated storage for sampling at 0, 2, and 3 weeks. Combination treatments with SDA and SL resulted in a 1-log reduction after two weeks of storage, and a 1 to 2 log reduction by the end of the sampling period. Nuńez de Gonzalez and others (2004) studied the antilisterial effects of post-process dipping solutions on frankfurters inoculated with a 10^8 CFU/mL concentration of a four-strain LM cocktail. Frankfurters were formulated with or without 3.3% PL and immersed in treatments of either a 1:2 ratio commercially available mixture of acidified calcium sulfate, 3.3% PL, or 3.4% SL. Treated frankfurters were vacuum packaged and stored for

twelve weeks at 4.5˚C. Aerobic plate counts were maintained at the minimum concentration of 2 log CFU per frankfurter for those treated with or without PL and dipped in acidified calcium sulfate or lactic acid throughout the twelve-week storage period. Geornaras and others (2006b) evaluated the antilisterial effects of 1.5% PL and 0.05% SDA formulations in commercially manufactured frankfurters treated with dipping solutions of 2.5% acetic acid, 2.5% lactic acid, 5% potassium benzoate, or 0.5% nisipalin. Frankfurters were inoculated with a 3 to 4 log CFU/cm^2 concentration of a 10-strain LM cocktail prior to treatment. Vacuum packaged samples were maintained at 10˚C for 48 d. Dipping treatments of acetic acid, lactic acid, or potassium benzoate reduced initial LM concentrations by 1.0 to 1.8 log CFU/cm^2. Products treated with dipping solutions, but formulated without PL and SDA in formulation, initially reduced LM concentrations but significant growth occurred by the end of the testing period. Sensory attributes were not significantly affected by dipping treatments. Based on these findings, treatment of frankfurters with organic acid dipping solutions should prolong the lag phase for at least three weeks post-processing with additional bacteriocidal effects.

The purpose of this study was to evaluate the inhibitory capabilities of organic acid marinade treatments applied as post cook dips to beef frankfurters. Efficacy was gauged by bacteriostatic effects on LM growth and bacteriocidal reduction of microbial populations. To accomplish this, currently used organic acid ingredients, including SL, PL, SC, and SDA were applied singly and as combination treatments, SL/SDA and SL/PL/SDA, to cured beef frankfurters formulated with sodium nitrite, frankfurter seasonings, NaCl and STPP. In doing so, this study evaluated both single and multi-hurdle approaches to post-processing LM control by organic acids.

CHAPTER XII

MATERIALS AND METHODS

Preliminary Growth and Antibiotic Resistance

Two strains of *L. monocytogenes* (LM), Scott A and Brie I, were obtained from preliminary experiment conducted Auburn University and cultivated to strengthen the culture. Frozen strains, stored in 1.5-µL microcentrifuge tubes (VWR Intl., West Chester, PA) in a -80˚C freezer (Harris, Asheville, NC), were thawed for inoculation. One milliliter of each frozen culture was allocated by 1000 µL pipette (Eppendorf Scientific, Hamburg, Germany) into 9.0 mL of sterile BHI broth (Oxoid, Basingstoke/Hampshire, England)**.** The media tubes were incubated (Fisher Scientific, Pittsburgh, PA) overnight at 37˚C. Turbid cultures were transferred to fresh media and the incubation process was repeated. Five 1:10 serial dilutions were allocated into tubes containing 9.0 mL of sterile phosphate buffered saline (PBS) solution (Pierce Perbio Thermo Scientific, Bonn, Germany). Each dilution was plated in duplicate using 15- x 100- mm plates (VWR Intl., West Chester, PA) on BHI agar (Difco, Lawrence, KS) and incubated at 37˚C. Plates were enumerated by computer using Q count software (Spiral Biotech, Norwood, MA) to obtain a preliminary estimate of the CFU/mL.

Streptomycin resistance was induced through exposure of LM to antibiotic in media (Killinger-Mann 2005). Both bacterial strains were gradually exposed to increasing concentrations of streptomycin in treated BHI broth and BHI agar. A stock solution of streptomycin was prepared by mixing streptomycin Sulfate salts (Sigma-Aldrich, St. Louis, MO) and sterile distilled water in a 1-L flask (Pyrex, Lowell, MA) using a combination hotplate/stir unit (VWR Intl.-Henry Troemner, West Chester, PA).

The solution was filter sterilized into 250-mL media bottles (Pyrex, Lowell, MA) using 60-mL syringes (Fisher Scientific, Pittsburgh, PA) fitted with 20-µm syringe filters (VWR Intl.,West Chester, PA). This was added to sterilized media under a laminar flow hood (Microzone Corp. Model FFM-2-4-WA, Ontario, Canada) based on the desired concentration of exposure. This was calculated using the formula:

(Concentration 1) (Volume 1) = (Concentration 2) (Volume 2)

Pending survival at the initial exposure concentrations, 0.2 µg/ml, 1.6 µg/ml, 6.4 µg/ml, and 12.8 µg/ml, a gradual doubling of the dosage ensued in both broth and agar until a resistance level of 1,500 µg/ml was reached.

Desired cultures were subsequently frozen in sterile 1.5-mL microcentrifuge tubes. The strains were initially incubated in 10 mL of BHI broth with 10% glycerol solution at 37˚C (Killinger-Mann 2005). From this, 300 uL was added to 700 uL of glycerol in the microcentrifuge tubes using a 1000-µL pipette and vortexed using a scientific touch mixer model 231 (Fisher Scientific, Basingstoke/Hampshire, England) prior to freezing. A sample of each strain was subsequently thawed and regrown to determine bacterial survival following the procedure.

Resistant strains were grown on BHI slants and validated for identity by the USDA - Agricultural Research Service (Athens, GA). Strains were streaked on modified oxford (MOX) agar (Remel, Lenexa, KS) for the cultivation of characteristic small, black colonies. A sterile loop was used to collect five to ten colonies for beta hemolysis testing (Berrang 2007). This procedure involved streaking for isolation on horse blood overlay plates. At least two colonies were collected from positive samples for confirmatory testing including a gram stain, CAMP test, rhamnose acid formation, positive catalase

reaction, and tumbling motility under a wet mount (Berrang 2007). The Scott A strain tested positive for retention of LM characteristics and was used in this experiment. This contrasts with the strain used for the Auburn University experiment which was conducted with a positive Brie 1 strain. Strains were spread plated according to the Association of Official Analytical Chemists (AOAC) methods. Media was sterilized throughout the experiment using a Cat 2007 autoclave at 121˚C for 15 minutes at 15 psi (Tuttnauer, Hauppauge, NY).

Product Preparation

Preparation of meat products prior to cooking involved incorporation of non-iodized salt (1.5%;Morton, Chicago, IL) and sodium tripolyphosphate (0.45%; Innophos, Cranbury, NJ). Sodium nitrite (28.35 g (6.25%)/ 11.33 kilograms; A.C. Legg, Calera, AL) was also added to the frankfurters. Flavoring was added using A.C. Legg's Frank and Bologna Seasoning (A.C. Legg, Calera, AL) at a concentration of 396.9 grams per 11.33 kilograms of meat. Controls were calculated based on industry standards. Marinade was included on a 20% weight by weight marinade basis with water comprising a portion of the inclusion weight. Ice was added on a 1:1 ratio to maintain emulsion stability during processing. It is important to note that 50% of the water in the recipe was added to liquefy the marinade while the remaining 50% was added as ice during the emulsion process. Industry controls and additives were blended using a handheld biohomogenizer (ESGE m13333-1281-O, Bartlesville, OK). All meat used was maintained at 4˚C throughout transport and processing.

Frankfurters were fabricated under industry simulation regarding equipment and practice. Beef frankfurters were fabricated using batch frankfurter manufacturing to

incorporate the dry ingredients listed in the previous section, ice, water, seasoning, and 50/50% lean trim (Cargill, Plainview, TX) with 93/7% lean beef (Cargill, Plainview, TX) to make 70% lean frankfurters. This was done in stepwise fashion proceeding through grinding, comminution, stuffing, linking, cooking-smoking, and peeling (Savell 2000). Meat and trim were separately chopped using a bowl chopper (Koch C3527 N1219, Kansas City, MO). Lean trim was prechopped using the bowl chopper before processing and was removed for addition in a later processing step. Ground beef was then added to the bowl chopper with the marinade mixture and mixed for 1 to 2 minutes. Lean trim was finally added and the mixture was chopped for an additional 4 to 8 minutes until a pasty consistency was achieved. Ice was added throughout the process to ensure emulsion stability; it was important to maintain the temperature of the mixture at or less than 10˚C.

The emulsion was then transferred to a stuffing machine (Albert Handtmann Maschinenfubrik VF50 88400, Biberach, Germany) and extruded through a stuffing horn (~2.54-cm diameter) into EZ Peel casings (EZ Peel plastic casing, Viskase Companies, Inc., Willowbrook, IL). This was formed into one large link to be sized appropriately after cooking and to allow for ease of peeling. The frankfurters were individually trucked into the single truck smokehouse (Alkar, Lodi, WI**)** and smoked (Table 9). Natural smoke was added to the frankfurters and ham using an Alkar smoke generator (Lodi, WI). The frankfurters were removed and chilled overnight to an internal temperature of ~4˚C. Frankfurters were hand peeled and sectioned into ~15.24 cm long portions over sterile foil using sterile cutting equipment. Frankfurters were stored in a sterile metal pan covered with sterile foil for transport. Ten frankfurters were individually weighed by

laboratory scale and averaged to obtain an approximate frank weight to be used in later calculations.

TABLE 9. *Dry bulb temperatures, wet bulb temperatures, time, and smoke setting for each step used in the beef frankfurter smoke cycle*

Step	Dry Bulb Temp (˚C)	Wet Bulb Temp (˚C)	Time (min)	Smoke
1	60.0	-17.8	30	off
2	65.6	-17.8	20	smoke
3	73.9	54.4	15	smoke
4	79.4	67.2	10	smoke
5	82.2	66.7	Until 73.9˚C internal temp	off
6	-17.8	-17.8	15 (cold shower)	off

Inoculation

The culture was passed at least 3 d before inoculation to allow for enumeration and strengthening as previously described. The first of five 1:10 serial dilutions was passed from inoculated BHI broth into a sterile test tube with 9.0 mL of PBS solution. Each dilution was spread in duplicate 15- x 100-mm plates on untreated BHI agar and incubated overnight at 37˚C. Plates were scanned by computer using Q Count software with an acceptable enumeration range between 25 to 250 CFU. The duplicate plates were subsequently averaged and multiplied by the appropriate dilution factor. The estimated CFU/mL for stock collected from frozen culture was required to surface inoculate each frank at the desired level of 10^4 to 10^5 CFU/g.

Each was inoculated with stock culture using 10-µL aliquots dispensed from a sterilized, calibrated 200-µL pipetter (Eppendorf Scientific, Hamburg, Germany) equipped with sterilized pipette tips. Disposable, sterile 10-µL inoculating loops (VWR Intl., West Chester, PA) were used to spread the inoculum across the visible surface of the frank. Inoculated franks were set aside for two minutes to allow for bacterial

attachment. Transfer of franks to segregated stations was conducted using ethanol sterilized forceps. Inoculation was conducted beneath a Class II type A or A/B3 biological safety cabinet (SafeAire Fischer Hamilton, Two Rivers, WI**)** lined with sterile sheets of disposable foil. The foil sheets were replaced after each round of inoculation.

Immersion Treatment

The following treatments, consisting of both single and combined organic acids, were applied to the inoculated frankfurters to be held at refrigerated (Norlake Scientific, Hudson, WI) storage temperatures of ~4˚C for the purpose of prolonging the lag phase of LM.

1. Sodium lactate (3.6%) (Fischer Scientific, Pittsburgh, PA)
2. Potassium lactate (3.6%) (City Chemicals, West Haven, CT)
3. Sodium citrate (0.75) (Fischer Scientific, Pittsburgh, PA)
4. Sodium lactate (3.6%) and sodium diacetate (0.25%) (Spectrum Chemical Manufacturing Corporation, Gardena, CA)
5. Sodium lactate (3.6%), potassium lactate (3.6%), and sodium diacetate (0.25%)
6. Positive control – inoculated slice dipped in sterile water
7. Negative control – non-inoculated slice dipped in sterile water

Dipping occurred inside the biological safety cabinet where rounds of franks were dipped collectively into single use beakers filled with equal amounts of marinade. Franks were dipped for approximately one minute and removed with sterile forceps for drying on sterile foil lined drying racks. Excess marinade was allowed to dry for at least 10 minutes with intermediate flipping of the frankfurters. Dry franks were packaged in groups of

three, according to treatment, using 708 g Whirlpak bags (Nasco, Fort Atkinson, WI) and stored at ~4˚C until their appropriate sampling times. It is important to note that three frankfurters were allocated to each of the seven time points per each of the five treatments totaling up to 105 franks. Two franks per time point were allotted for a positive and negative control, providing an additional 28 frankfurters.

Testing

Packaged frankfurters were removed from refrigerated storage for sampling on d 0, 7, 14, 21, 28, 42, and 56. Sterile forceps were used to divide the slices into individual filtered stomacher bags. Each bag was filled with 50 mL of sterile PBS and homogenized, using a Stomacher 400 Circulator (Seward, West Sussex, UK), for two minutes at 245 RPM. Five 1:10 serial dilutions were made, starting with the first dilution removed from the diluent in the filter bag, using sterile PBS. Each test tube was vortexed prior to duplicate plating on streptomycin treated BHI agar plates. Samples were incubated for ~48 hours at 35˚C prior to enumeration. Plates were enumerated, as described previously, using Q count software. Duplicate plates were averaged and calculated by dilution to obtain an estimated CFU/g. The average slice weight was considered with 50 mL of PBS added to create the first dilution. Each calculated sample CFU/g was average to find the treatment CFU/g calculation. These were plotted using Microsoft Excel software.

Statistical Analysis

Statistical analysis was performed using the Proc GLM procedure of SAS (SAS, Cary, NC). Means were grouped by treatment and separated using Duncan's Multiple Range Test. Statistical significance was determined using an alpha of 5%. As there were

no trial x treatment interactions, data, consisting of two trials and three replications, were pooled by treatment.

CHAPTER XIII

RESULTS AND DISCUSSION

Despite the establishment of the USDA zero-tolerance policy for LM contamination on RTE foods, LM has remained a persistent contaminant on 12 to 27% of retailed RTE meat products (Lawrence and Gilmour 1995). In reference to specific foods, Ramos and others (1999) correlated high levels of contamination proportional to the amount of machination required to process meats. Nesbakken and others (1996) found that contamination of with two prominent *Listeria* strains resulted from contact with the processing environment. Identical strains found in area processing plants and outbreak patients indicated the potential for contamination. Of 38.1% of *Listeria* positive samples collected by Barros and others (2007) from Brazilian processing facilities, at least 86.8% were contaminated from contact with the processing environment. Major outbreaks attributed to LM in the beef industry are centered around post-processing contamination of frankfurters. Due to the frequency of contamination occurring post-processing and before packaging, this study was conducted to investigate the viability of organic acid application as a post-processing intervention against streptomycin resistant LM strain Scott A. The effectiveness of these treatments was determined by analysis of LM levels over time, specifically the bacteriostatic and bacteriocidal effects of organic acid dipping solutions towards the prolongation of the bacterial lag phase.

Lactic acid dipping solutions prolonged the lag phase for four weeks post-processing with limited growth by the end of the 56-d storage period overall. Sodium lactate, PL, and the combination of SL and SDA were successful in extending the lag phase throughout 28 d of storage (Tables 10 and 11, Figure 3). A small increase (0.1 log

CFU/g) was noted on d 21 for the SL treatment. LM levels decreased (0.2 log CFU/g) between d 21 and 28. Growth resumed (0.2 log CFU/g) between d 28 and 42, but ceased completely thereafter. Increase (0.2 log CFU/g) occurred between d 7 and 14 of the SL/SDA combination treatment. A small decrease occurred between d 42 and 56. Sodium citrate inhibited the lag phase for 7 d post-processing. Small increases (<0.5 log CFU/g occurred between d 7 and 42. *Listeria monocytogenes* levels decreased (0.2 log CFU/g) between d 42 and 56. The SL/PL/SDA combination treatment extended the lag phase throughout 21 d. Intermittent growth (<0.5 log CFU/g) was noted on d 14, 28, and 56. A decrease in LM levels occurred on d 21 and 28. Lag phase was extended throughout 28 d in the control slices with a slight increase (0.2 log CFU/g) from initial values on d 14. Growth approached 1.0 log CFU/g between d 28 to 56. Overall growth was limited within the range of 0.3 to 0.6 log CFU/g for all treatments.

TABLE 10. *Levels of LM average log CFU/g and superscripts by day for sodium lactate, potassium lactate, sodium citrate, sodium lactate and sodium diacetate combination, and sodium lactate, potassium lactate, and sodium diacetate combination treatments on beef frankfurters*

Treatment	D 0	D 7	D 14	D 21	D 28	D 42
SL/PL/SDA	4.8^{a}	4.6^{a}	4.7^{a}	4.3^{ab}	4.6^{a}	4.0^{b}
SL	4.7^{a}	4.4^{a}	4.3^{a}	4.4^{ab}	4.2^{ab}	4.4^{ab}
SL/SDA	4.6^{a}	4.4^{a}	4.6^{a}	4.3^{b}	4.2^{ab}	4.4^{ab}
PL	4.5^{a}	4.5^{a}	4.5^{a}	4.3^{ab}	4.3^{ab}	4.7^{ab}
CONTROL	4.5^{a}	4.2^{a}	4.4^{a}	4.1^{ab}	3.9b	4.2^{ab}

TABLE 11. *Listeria monocytogenes CFU/g by day, represented as gains and losses, for sodium lactate (SL), potassium lactate (PL), sodium citrate (SC), sodium lactate and sodium diacetate (SDA) combination, and sodium lactate, potassium lactate, and sodium diacetate treatments on beef frankfurters*

Treatment	Initial CFU/g	D7	D14	D21	D28	D42	D56	Overall Increase
SL	4.7	-0.3	-0.1	+0.1	-0.2	+0.2	0	-0.3
PL	4.5	0	0	-0.2	0	+0.4	+0.2	+0.4
SC	4.4	-0.2	+0.1	+0.2	+0.2	+0.3	-0.2	+0.4
SL/SDA	4.6	-0.2	+0.2	-0.3	-0.1	+0.2	-0.2	-0.4
SL/PL/SDA	4.8	-0.2	+0.1	-0.4	+0.3	-0.6	+0.2	-0.6
CONTROL	4.5	-0.3	+0.2	-0.3	-0.2	+0.3	+0.8	+0.5

[1] + denotes increase
[2] - denotes decrease

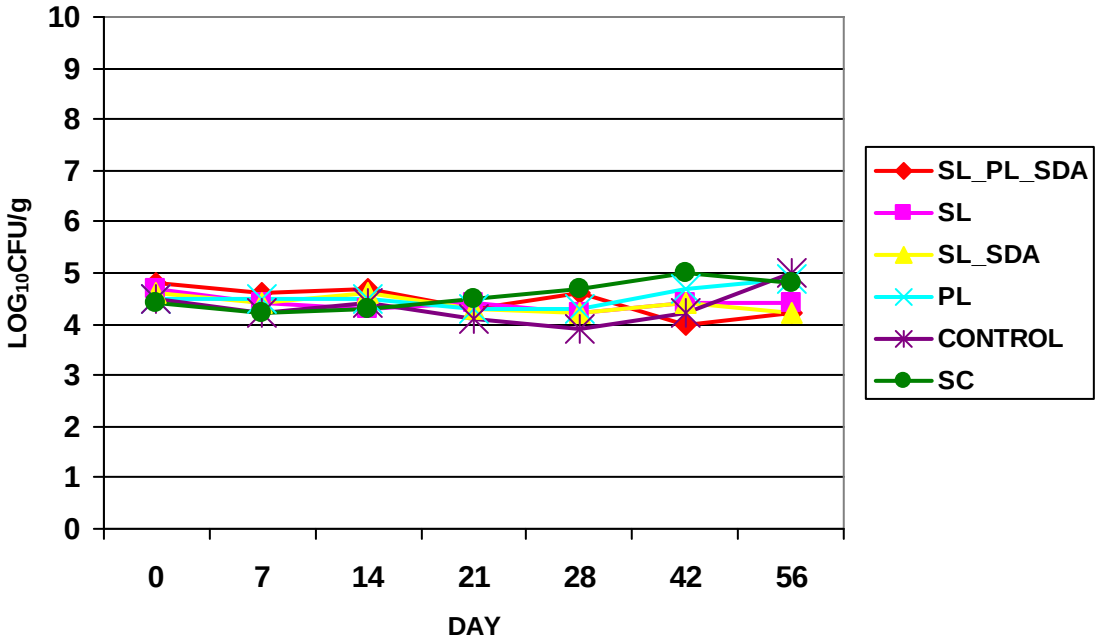

FIGURE 3. *Pooled LM results for d 0 through 56 for the sodium lactate (SL), potassium lactate (PL), sodium citrate (SC), sodium lactate and sodium diacetate (SDA) combination treatment, and sodium lactate, potassium lactate, and sodium diacetate combination treatments applied to beef frankfurters*

Organic acid dipping solutions including lactic acid ingredients applied to frankfurters have proven successful in extending the lag phase for at three weeks post-processing. Glass and others (2001) found that SL and SDA post-cooked dips applied to

turkey, pork, and beef combination frankfurters and pork and beef combination bratwurst sausages, prolonged the lag phase for four and twelve weeks, respectively. Samelis and others (2002) noted that combination treatments of 0.25% SL with 0.25% SA, SDA, or GDL applied to frankfurters extended the lag phase throughout 120 d at refrigerated storage. Palumbo and Williams (2002) found that inoculated, vacuum packaged frankfurters treated with a combination of 2.5% lactic acid and 2.5% acetic acid dip suppressed LM growth throughout 90 d at refrigerated storage temperatures. Uhart and others (2004) found that combination dipping treatments of 3% SDA and 6% SL reduced LM populations by 1 to 2 log cycles during the three-week sampling period. Although no log reductions were noted during the 56-d storage period, the results of the current study coincide with the use of organic acids to prolong the lag phase for at least three weeks post-procesing.

Two modes of action have been proposed for the antimicrobial mechanisms of lactates. As previously discussed, success has been attributed to penetration of the undissociated acid molecule through the bacterial cell membrane. Weak acids, such as sodium lactate and potassium lactate, are favored for intracellular permeability due to the predominantly hydrophobic fraction and its interactions with the cellular lipid bilayer. Once inside, the weak acid may dissociate upon contact with the more acidic intracellular pH and increase the acidity or cause substrate level inhibition of metabolism. This may result in cell damage, death, or exhaustion due to shifting of energy and resources to exclude the acid

Growth limitations of LM in smoked products, ham deli slices and frankfurters, versus unsmoked products, turkey deli slices, might be attributed to undissociated nitrite

in the formulation. Duffy and others (1994) noted a prolonged lag phase when undissociated nitrite was studied in vacuum packaged smoked meats. Ngutter and Donnelly (2003) noted that LM injured by sodium nitrite formulations of 100 to 200 ppm recovered and proliferated on cooked frankfurters by the end of the testing period.

Due to the similarities between the overall growth and lag phase extension between control and treated frankfurters, and in comparison to products formulated with increasing nitrite levels ranging from none in turkey to increasing amounts in ham and frankfurters, growth was affected by the presence of undissociated nitrite in the product.Two methods of action have been proposed for the antimicrobial mechanisms of sodium nitrite. The Perigo effect, complex of nitrites with functional groups such as cysteine, iron, and sulfhydryl groups, has been attributed to cellular metabolic malfunction (Davidson and others 2005). Sodium nitrite serving as an electron acceptor may also interfere with the electron transport chain, oxygen uptake, and oxidative phsophorylation (Davidson and others 2005).

CHAPTER XIV

CONCLUSION

Further research is required to elucidate the success of post-processing organic acid treatments. Due to mechanical constrains, vacuum packaging could not be implemented in this study. Although the conditions implemented closely approximate aerobic home storage conditions, it would be useful to determine the effects of post-processing organic acid treatments on vacuum packaged products. Potential effects of undissociated nitrite should be measured and addressed in future studies.

Current LM programs developed for RTE meats focus on contamination stemming from improper product processing and poor ingredient quality, poor sanitation or biofilm formation on processing machinery, or cross contamination between processing areas or facilities. Food safety inspection service regulations for processing facilities producing RTE products require the development of interventions detailing LM control measures in HACCP, SSOP, and GMP systems. While lactic acid dipping solutions can be used to successfully extend the lag phase throughout three to four weeks post-processing of inoculated frankfurters held under aerobic home storage conditions, the same results were noted for both treated and untreated frankfurters. This suggests that it may not be necessary, or cost efficient, to implement this program as a post-processing intervention for RTE beef frankfurters.

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