Efficacy of buffered propionic acid against Salmonella Typhimurium in ground pork stored at 4°C and 10°C and inhibition of Listeria monocytogenes by buffered dry vinegar in ready-to-eat uncured turkey stored at 4°C

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Efficacy of buffered propionic acid against *Salmonella Typhimurium* in ground pork stored at 4°C and 10°C and inhibition of *Listeria monocytogenes* by buffered dry vinegar in ready-to-eat uncured turkey stored at 4°C

by

Mani Kumar Badvela

A dissertation submitted to the graduate faculty

in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

Major: Meat Science

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# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>CHAPTER 1</th>
<th>GENERAL INTRODUCTION</th>
<th>1</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Dissertation Organization</td>
<td>3</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>CHAPTER 2</th>
<th>LITERATURE REVIEW</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Importance of <em>Salmonella</em></td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>Characteristics, physiology and occurrence</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td><em>Salmonella</em> Typhimurium outbreaks linked to consumption of pork and products</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>Sources of <em>salmonella</em> spp contamination in pork and pork products</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>Effect of incubation temperature on growth of <em>salmonella</em> spp in meat products</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>Common chemical interventions for control of <em>Salmonella</em> Typhimurium in raw meats</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>Lactic acid</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>Acetic acid</td>
<td>11</td>
<td></td>
</tr>
<tr>
<td>Peroxy acetic acid</td>
<td>13</td>
<td></td>
</tr>
<tr>
<td>Propionic acid</td>
<td>14</td>
<td></td>
</tr>
<tr>
<td>Significance of <em>Listeria monocytogenes</em></td>
<td>15</td>
<td></td>
</tr>
<tr>
<td>Characteristics and physiology</td>
<td>16</td>
<td></td>
</tr>
<tr>
<td><em>Listeria monocytogenes</em> outbreaks linked to consumption of meat and poultry products</td>
<td>16</td>
<td></td>
</tr>
<tr>
<td>Commonly associated serovars</td>
<td>18</td>
<td></td>
</tr>
<tr>
<td>Effects of temperature, pH and water activity on <em>Listeria monocytogenes</em></td>
<td>19</td>
<td></td>
</tr>
<tr>
<td><em>Listeria monocytogenes</em> control strategies</td>
<td>21</td>
<td></td>
</tr>
<tr>
<td>Focus on post-lethality contamination</td>
<td>21</td>
<td></td>
</tr>
<tr>
<td>Cleaning and Sanitation Practices</td>
<td>22</td>
<td></td>
</tr>
<tr>
<td>Antimicrobial ingredients</td>
<td>24</td>
<td></td>
</tr>
<tr>
<td>Lactate-Diacetate Salts</td>
<td>24</td>
<td></td>
</tr>
<tr>
<td>Propionic acid and Propionate Salts</td>
<td>26</td>
<td></td>
</tr>
<tr>
<td>Cultured Sugar and Vinegar</td>
<td>27</td>
<td></td>
</tr>
<tr>
<td>Buffered Vinegar</td>
<td>28</td>
<td></td>
</tr>
<tr>
<td>Demand for Natural and Organic Foods</td>
<td>29</td>
<td></td>
</tr>
<tr>
<td>Significance of Low Sodium Foods</td>
<td>30</td>
<td></td>
</tr>
<tr>
<td>Summary of Literature and Objectives for Proposed Studies</td>
<td>31</td>
<td></td>
</tr>
<tr>
<td>References</td>
<td>34</td>
<td></td>
</tr>
</tbody>
</table>
CHAPTER 3 EFFICACY OF BUFFERED PROPIONIC ACID AGAINST SALMONELLA TYPHIMURIUM INOCULATED BY TWO DIFFERENT METHODS IN GROUND PORK STORED AT 4°C

Abstract
Introduction
Materials and methods
Results
Discussion
Acknowledgments
References

CHAPTER 4 EFFICACY OF BUFFERED PROPIONIC ACID AGAINST SALMONELLA TYPHIMURIUM IN GROUND PORK STORED AT 4°C AND 10°C

Abstract
Introduction
Materials and methods
Results
Discussion
Acknowledgments
References

CHAPTER 5 INHIBITION OF LISTERIA MONOCYTGENES BY BUFFERED DRY VINEGAR IN REDUCED SODIUM READY-TO-EAT UNCURED TURKEY STORED AT 4°C

Abstract
Introduction
Materials and methods
Results
Discussion
Acknowledgments
References

CHAPTER 6 GENERAL CONCLUSIONS AND RECOMMENDATIONS FOR FUTURE RESEARCH

ACKNOWLEDGMENTS

v
CHAPTER I GENERAL INTRODUCTION

Food safety continues to be of paramount importance to the food industry and regulatory agencies in the United States. In order to achieve the goal of a safer food supply, United States Department of Agriculture (USDA)’s Food Safety and Inspection Service (FSIS) has taken an aggressive enforcement approach to control foodborne pathogens such as *Salmonella* and *Campylobacter*. It is estimated that non-typhoidal *Salmonella* (NTS) causes approximately 1.2 million illnesses and 450 deaths annually in the United States and 5% of illnesses are attributed to consumption of pork products. Antimicrobial interventions are commonly used by the meat industry for controlling foodborne pathogens at various stages in production chain. Organic acids and their salts such as lactic acid, sodium lactate, acetic acid, sodium diacetate etc. are widely used antimicrobial interventions in raw and cooked meat products. As the regulations to control foodborne pathogens become tighter and tighter, it is necessary to develop and validate new effective antimicrobial ingredients to strengthen food safety. Recently, the meat industry has shown interest in antimycotic agents such as propionate and benzoate salts, which showed significant antimicrobial activity in cooked meat products. In 2013, USDA approved the use of sodium propionate, propionic acid and sodium benzoate in ready-to-eat (RTE) meat and poultry products. However, it appears that there have been no studies on the effect of buffered propionic acid on foodborne pathogens such as *Salmonella* spp. in raw ground meat and poultry products. Efficacy studies are required in order to gain regulatory approval for propionic acid as antimicrobial for raw meats and thus continuously improve food safety.
Listeria monocytogenes is another major foodborne pathogen that continues to be a serious threat to public health despite a decrease in number of cases annually. RTE meats such as deli meats and frankfurters without antimicrobials pose the greatest per-serving risk of illness/death from L. monocytogenes because they are often consumed directly from the refrigerator without reheating. FSIS has deemed L. monocytogenes an adulterant and has a zero-tolerance policy for the pathogen in RTE meats. To inhibit the growth of L. monocytogenes, FSIS has approved a variety of antimicrobial agents that can be added to RTE meat and poultry products and among them, lactates and diacetate are widely used. Consumer demand for natural and organic foods in the US is continuously increasing and there is a high priority for the meat industry to develop and use clean label ingredients. Another challenge faced by the processed meat industry is sodium reduction because high sodium intake may result in increased blood pressure and represents a risk factor for cardiovascular disease. In the past few years, the US food industry and the U.S. government has made several efforts to reduce the sodium content in processed foods. While sodium chloride imparts flavor and texture to foods, it also plays a critical role in food safety and hence, when developing low-sodium meats, precautions should be taken to avoid compromising on flavor, texture, shelf life, and safety. Recently, buffered vinegar has attracted considerable attention by the meat industry for inhibiting L. monocytogenes in RTE meats. Buffering the vinegar using sodium- or potassium-based alkali raises the pH and creates minimal impact on the functional properties of the processed meats. The advantage of using a potassium-based buffer is it does not contribute sodium in the final food product. Validation studies are needed to evaluate the efficacy of potassium-based and sodium-based buffered vinegars against L. monocytogenes in RTE meat and poultry products.
Dissertation Organization

This dissertation is organized into six chapters. The first chapter is a general introduction. The second chapter is a general literature review on two major foodborne pathogens associated with meat and poultry products i.e. *Salmonella enterica* serovar Typhimurium, a nontyphoidal *Salmonella* (ST) and *Listeria monocytogenes* (Lm). The literature review is a broad discussion of the importance and incidence of each pathogen, their sources of contamination in meat and poultry products, effect of storage temperature and chemical interventions adopted by the meat industry for pathogen control. Chapter 3 is a manuscript entitled “Efficacy of buffered propionic acid against *Salmonella* Typhimurium inoculated by two different methods in ground pork stored at 4°C.” Chapter 4 is a manuscript entitled “Efficacy of buffered propionic acid against *Salmonella* Typhimurium in ground pork stored at 4°C and 10°C.” Chapter 5 is entitled “Inhibition of *Listeria monocytogenes* by buffered dry vinegar in ready-to-eat uncured turkey stored at 4°C.” Chapters 3, 4 and 5 are organized in the style of the *Journal of Food Protection*. The sixth chapter gives overall summary of this research and recommendations for future research.
CHAPTER 2
LITERATURE REVIEW

Importance of *Salmonella* spp.

*Salmonella* and salmonellosis continue to be of concern to the food industry and regulatory agencies globally as it poses a unique threat to food safety. *Salmonella* bacteria can be classified as either “typhoidal” or “nontyphoidal” based on the serotype. Typhoidal *Salmonella* refers to certain *Salmonella* serotypes that cause typhoid fever or paratyphoid fever, including Typhi, Paratyphi A, Paratyphi B, and Paratyphi C. Nontyphoidal *Salmonella* (NTS) refers to all other *Salmonella* serotypes (CDC, 2015). This literature review is focused on nontyphoidal *Salmonella* serotype i.e. *Salmonella enterica* serovar Typhimurium. It is estimated that every year approximately 1.2 million illnesses, 19,000 hospitalizations and 450 deaths occur in the United States due to nontyphoidal *Salmonella* (Scallan *et al*., 2011) and 5% of illnesses due to NTS are attributed to pork products in the United States (Davies, 2011).

Characteristics, Physiology and Occurrence

*Salmonella* is a gram-negative, rod-shaped bacterium usually motile by peritrichous flagella. They are facultative anaerobic and chemoorganotrophic, having both a respiratory and a fermentative type of metabolism. Optimum temperature for growth is 37°C. *Salmonella* spp. is pathogenic for humans, causing enteric fevers, gastroenteritis and septicemia; may also infect many animal species besides humans. Some serovars are strictly host-adapted. (Bergey *et al*., 1994).
Primary manifestations of salmonellosis in humans are usually fever, diarrhea and abdominal cramps which develop 12 to 72 hours after infection. The illness usually lasts 4 to 7 days, and most individuals recover without antibiotic treatment. However, the diarrhea can be severe, and occasionally people with blood stream infection may require hospitalization. Dehydration often a problem and this must be treated. Elderly, infants, and those with a weakened immune system may have a more severe illness. In these patients, the infection may spread from the intestine to the blood stream and then to other body sites and can cause death unless treated promptly with antibiotics (CDC, 2015).

*Salmonella* exist in the intestinal tracts of humans and other animals, including birds, and is usually transmitted to humans by eating foods contaminated with animal feces. Foods commonly implicated as vehicles for salmonellosis include raw meats, poultry, eggs, milk and dairy products, sauces and salad dressing, cake mixes, fish, frog legs, shrimp, coconut, peanut butter, dried gelatin, cream filled desserts and toppings, cocoa, and chocolate (Lampel *et al.*, 1992).

*Salmonella Typhimurium (ST)* outbreaks linked to consumption of pork and pork products

In 2010, a foodborne outbreak occurred with consumption of pulled pork sold at the church festival in Hamilton county of Ohio resulting in 64 illnesses and investigation confirmed the outbreak pathogen was *Salmonella Typhimurium (ST)* (MWWR). In 2008, an outbreak in Denmark caused by *ST* (phage type U292), which resulted in 1054 cases and it was hypothesized that outbreak was originated from pigs because the interviewed patients had very high exposure to pork (Ethelberg *et al.*, 2008). In 2010, another foodborne outbreak caused by *ST* (phage type U323) was reported in Denmark resulting in a total of 172
foodborne cases. No single common food item was identified as the outbreak source, but repeated isolation of the outbreak strain from the slaughterhouse environment and in pork and products as well as patient interviews strongly suggested different pork products as the source of infection (Kuhn et al., 2013). In 2011, a ST outbreak in Denmark occurred due to consumption of contaminated smoked pork tenderloin with 22 laboratory-confirmed cases. This outbreak resulted in recall of the particular brand of smoked pork tenderloin from consumers (Wojcik et al., 2012). In 1999, Centers for Disease Control and Prevention (CDC) received reports from state health departments of Idaho, Minnesota and Washington regarding ST infections in employees and clients of small animal veterinary clinics and an animal shelter. The number of cases registered from each state was 20, 7 and 3 respectively (CDC, 2001). In 2013, a foodborne outbreak of uncommon 04 non-agglutinating ST linked to consumption of minced pork was reported in Saxony-Anhalt, Germany which resulted in 61 laboratory confirmed cases. In 2011, a large point-source outbreak of ST was reported by public health unit of Sydney which was linked to chicken, pork and salad rolls from a Vietnamese bakery. This outbreak resulted 83 laboratory confirmed cases (Norton et al., 2012). In 2004, an Easter outbreak of ST (DT 104A) associated with traditional pork salami was reported in Italy and this resulted in 63 laboratory confirmed cases. The investigators hypothesized that salami could have been sold in the market before the optimal fermentation period, because of the high demand for this particular item during Easter banquets in the Lazio region of Italy (Luzzi et al., 2007)
Sources of \textit{Salmonella} spp. contamination in pork and pork products

\textit{Salmonella} spp. contamination of pork can occur at two stages i.e. pre-harvest stage i.e. animal production on the farm and post-harvest stage i.e. carcass processing and cross-contamination (Alban and Stark, 2005; Funk \textit{et al.}, 2001). Pigs are healthy carriers of \textit{Salmonella} spp. (Borch \textit{et al.}, 1996). In a longitudinal study of \textit{Salmonella enterica} in growing pigs reared in multiple-site swine production systems, researchers isolated fifteen different serotypes of \textit{Salmonella enterica} and the most frequently isolated serotypes were \textit{S. Typhimurium} var Mbandaka and \textit{S. Typhimurium} var Copenhagen (Funk \textit{et al.}, 2001).

Berends \textit{et al.} (1997) reported live pigs carrying \textit{Salmonella} spp. were 3 to 4 times more likely to have the organism on their carcasses than are \textit{Salmonella}-free pigs. In their study, Berends \textit{et al.} (1997) reported that there is a strong correlation between the number of live pigs that carry \textit{Salmonella} spp. in their faeces and the number of contaminated carcasses at the end of the slaughter line. Live pigs that carry \textit{Salmonella} spp. are 3-4 times more likely to end up as a positive carcass than \textit{Salmonella}-free pigs. They estimated about 70\% of all carcass contamination results from the animals themselves being carriers, and 30\% because of a cross-contamination from other positive carcasses. It was estimated that approximately 5-30\% of the carcasses produced may contain \textit{Salmonella} spp. Schmidt \textit{et al.} (2012) characterized \textit{Salmonella enterica} contamination on pig carcasses in two large commercial pork processing plants in the US at three points – pre-scald, pre-evisceration, and after chilling, and the overall prevalences of \textit{Salmonella} were 91.2\%, 19.1\%, and 3.7\%, respectively. A total of 294 pre-scald carcasses had \textit{Salmonella} loads of >1.9 log CFU/cm$^2$. The authors isolated a total of 41 serotypes on pre-scald carcasses and the predominating serotypes were \textit{Salmonella enterica} serotypes Derby, Typhimurium, and Anatum. From the
24 serotypes isolated from pre-evisceration carcasses, serotypes Typhimurium and London were the most common. On final chilled carcasses, total 9 serotypes were identified and among them *Salmonella* serotypes Johannesburg and Typhimurium were the most frequently isolated. Algino *et al.*, (2009) studied *Salmonella* prevalence on pork carcasses in very small abattoirs of Wisconsin and they reported that *Salmonella* prevalences on skinned and unskinned prewash carcasses were 11.7 and 8.3% respectively. Corresponding values for chilled carcasses were 32.0 and 19.5% for 1-day chilled carcasses, and 11.4 and 14.7% for 2-day chilled carcasses. Lower *Salmonella* prevalence on prewash carcasses was significantly related to lower prewash carcass APC levels (odds ratio = 7.8 per change of 1.0 log CFU/cm²), while lower *Salmonella* prevalence on chilled carcasses was significantly related to 2-day chilling (odds ratio = 5.2). Davies (2011) opined that over recent decades, pork safety in the United States has substantially improved and due to management practices employed in modern industry some foodborne hazards such as with parasites have been virtually eliminated. The author also opined that the hypothesis that intensive pork production has increased risk for the major bacterial foodborne pathogens that are common commensals of the pig i.e. *Salmonella*, *Campylobacter*, *Listeria*, and *Yersinia enterocolitica* is not valid as the available evidence does not support it.

**Effect of incubation temperature on growth of *Salmonella* spp. in meat products**

Optimum temperature for growth of *Salmonella* spp is 35-37°C (Matches *et al.*, 1968). However, researchers have shown that they can survive and increase in numbers at much lower temperatures. Minimum growth temperature for *Salmonella* Derby, *Salmonella* Heidelberg and ST were determined by inoculating in broth tubes and incubated over a
temperature range of 1.1 to 12.3°C and growth was observed after 19 days of storage at 5.3°C, 6.2°C and 6.9°C respectively (Matches et al., 1968). Mackey et al., (1980) inoculated a mixture of Salmonella serotypes in ground beef and determined the growth rates by incubating at various chilled temperatures and mean generation times recorded were 8.1 h at 10 °C; 5.2 h at 12.5°C, and 2.9 h at 15°C. Growth did not occur at 7-8 °C. Wang et al., (2015) inoculated ground pork with Salmonella Derby, Salmonella Enteritidis and Salmonella Typhimurium at low and high inoculum levels (1-10 or 10-100 CFU/g) and stored at 10°C and results showed that Salmonella populations at both inoculum levels were increased by less than 1 log after 12 days of storage. Pradhan et al., (2012) showed that raw chicken breasts inoculated with ST at 4-5 log CFU/g and stored for 3 weeks at 4°C, did not show any significant change (p>0.05) in ST populations, and they indicated ST is sensitive to refrigerated temperature. Bailey et al., (2000) studied the microbiological profile of chilled and frozen chicken carcasses and reported that Salmonella-positive carcasses (1.5 log) when stored at various frozen and refrigerated temperatures including at 4°C for 2 weeks, did not show any change in Salmonella populations. Uhart et al., (2006) inoculated heat treated ground beef with Salmonella Typhimurium DT104 (5 log CFU/g and 8 log CFU/g) to be stored at 4°C and 8°C for 10 days and observed that both inoculum levels showed very slight or no difference in Salmonella populations at 4°C whereas, growth was seen at 8°C. Mbandi et al., (2001) inoculated sterile ground beef with Salmonella Enteritidis (3.5 log CFU/g) to be stored at 5°C and 10°C, and showed that Salmonella populations were undetected after 30 days at 5°C, whereas, a 4.5 log increase was seen after 20 days at 10°C. Sharma et al., (2012) inoculated fresh, boneless, uncooked chicken breast fillets with ST to be stored at 4°C for 7
days and showed that *Salmonella* populations dropped from 6.33 to 5.01 log CFU/g by the end of 7 days.

**Common chemical interventions for control of *Salmonella Typhimurium* in raw meats**

**Lactic acid**

Lactic acid is one of the most commonly used chemical interventions on red meat carcasses and fabricated products (Koohmaraie *et al.*, 2005; Wheeler *et al.*, 2014; Huffman, 2002). Lactic acid appears colorless to slightly yellowish color with good solubility in water and it occurs naturally in foods during fermentation processes (Jager, 1995). It is generally recognized as safe (GRAS) and USDA approved it as a processing aid on carcasses, primal, trimmings and variety meats (USDA-FSIS, 2013). Echeverry *et al.*, 2009 evaluated the effectiveness of 3.0% lactic acid against *E. coli* O157:H7 and ST (Phage type 104) inoculated (5 log) prior to packaging of mechanically tenderized and brine-enhanced beef strip loins and results showed lactic acid reduced internal pathogen loads by 3.0 log and 2.2 log respectively. King *et al.*, (2012) demonstrated that 2% lactic acid solution spray (40-50°C) when combined with water spray, resulted in 0.5 log reduction of *Salmonella* Hadar inoculated (6 log) on pork variety meats such as livers, intestines, hearts and stomachs. Killinger *et al.*, (2010) inoculated chicken wings with a *Salmonella* cocktail consisting of *Salmonella* Enteritidis (ATCC 13076), *Salmonella Typhimurium* (ATCC 14028), *Salmonella* Heidelberg (S9481) and *Salmonella* Kentucky (S94611), and rinsed with 2% lactic acid solution for 3 minutes. Results showed that lactic acid achieved a significant reduction (*p*<0.01) (below the detection limit) in *Salmonella* levels compared with the inoculated
control (5.78±0.09 log CFU/wing). The authors also conducted a field study where 20 chicken carcasses were immersed in 2% lactic acid solution for 3 minutes and this resulted in >2 log reduction of aerobic plate counts and coliform levels when compared with the untreated carcasses. Chaine et al., (2013) showed a 6 log/cm² reduction of Salmonella Enteritidis when inoculated on chicken skin and treated with steam at 100°C/8 sec followed by 5% lactic acid treatment. The lactic acid also showed persistent effect on Salmonella growth during storage (7 days at 4°C) which was significantly higher when the skin was not rinsed, reaching reductions of 3.8 log CFU/cm².

In another study (Wolf et al., 2012), beef trimmings were inoculated with a Salmonella cocktail consisting of Salmonella Typhimurium (ATCC 14028), Salmonella Enteritidis (phage type-13) and Salmonella Heidelberg Sheldon (3347-1) (5-6 log CFU/g), and were sprayed or dipped in 4.4% lactic acid solution and enumerated at 1 and 20 h after treatment. Trim was then ground and lactic acid effectiveness was measured at 1h, 24h, 72h and 7 days after grinding. Lactic acid dip reduced the Salmonella populations by 0.51 to 0.81 log CFU/g on beef trim and ground beef. Lactic acid spray treatment reduced the Salmonella populations by 0.5 log CFU/g on beef trim, but these reduced counts were not significant compared to the untreated control. Lactic acid was proven to be efficacious against Salmonella spp and other foodborne pathogens and is used in various areas of the red meat and poultry production chain as an antimicrobial intervention.

**Acetic acid**

After lactic acid, acetic acid is commonly used in meat processing systems as an antimicrobial intervention (Hardin et al., 1995; Hamby et al., 1987; Huffman, 2002;
Frederick et al., 1994). It has pungent odor and taste and it is a principal component in vinegars (Lopez et al., 2012). It is generally recognized as safe (GRAS) and USDA approved it as a processing aid on carcasses, primals, trimmings and variety meats (Frederick et al., 1994; USDA-FSIS, 2013). Frederick et al., (1994) showed that for pork cheeks, when sprayed with 2% acetic acid solution (25°C), the incidence of Salmonella decreased by 67% compared to untreated control, and there was significant (*p*<0.05) decrease in aerobic plate counts and coliforms. Dickson (1992) showed that lean and fat beef tissue surfaces inoculated with ST (ATCC 14028) and treated with 2% acetic acid, resulted in sub lethal injury (65%) of ST populations and residual effects of the acid resulted in 1 log reduction of ST on fat tissue over 4 hr. Carpenter et al., (2011) studied the efficacy of 2% acetic acid solution for Salmonella decontamination. They inoculated chicken skin and pork belly with Salmonella strains (consisting of Salmonella Enteritidis, Salmonella Typhimurium, Salmonella Thompson, Salmonella Hadar, Salmonella Copenhagen, Salmonella Heidelberg and Salmonella Montevideo) and stored the products at 8°C for 8 weeks. Decontamination results showed a reduction of 0.6 to 1 log/cm² compared to untreated control. Harris et al., (2012) inoculated beef trim with ST, treated the trim with 2% acetic acid, and sampled at three different points during production i.e. (i) immediately after treatment (20 min); (ii) immediately after grinding (6 hr); and (iii) 24 hours after grinding. Acetic acid treatment showed a drop of 0.6 log of ST by 24 hr after application in ground beef. Stivarius et al., (2002) inoculated lean beef trimmings with ST and treated with 5% acetic acid and then trimmings were ground, packaged and sampled at 0, 1, 2, 3 and 7 days of display. Results showed that before grinding acetic acid reduced ST populations by 1.47 log compared to control and across days of display, ST was reduced by 0.37 log CFU/g.
(1989) inoculated beef semitendinosus muscle with ST and later dipped for 15 sec in various concentrations of acetic acid (0, 1, 2 or 3%) held at 25, 40, 55 or 70°C. Results showed that 3% acetic acid when applied at 70 °C was most effective and reduced ST populations by 1.5 log/cm². Tamblyn et al., (1997) showed that a concentration of greater than 4% acetic acid was needed to kill ≥ 2 log of ST populations attached to broiler chicken skin. As shown in the literature, acetic acid was proven to be efficacious against Salmonella spp and other foodborne pathogens and is used in various areas of the red meat and poultry production chain as an antimicrobial intervention.

**Peroxyacetic acid (PAA)**

Peroxyacetic acid (PAA), also known as peracetic acid has widely gained acceptance by the meat industry (Wheeler et al., 2014). USDA FSIS has approved it for use as a carcass wash in beef or pork carcasses, parts, trim, and organs at a level not to exceed 400 ppm and in poultry parts, organs, and carcasses at a level not to exceed 1000 ppm (FSIS Directive 7120.1, Revision 33). King et al., (2005) reported that 1000 ppm of PAA reduced ST population by 1.3 log CFU/cm² on beef carcasses. Schmidt et al., (2014) showed a reduction in Salmonella enterica counts to 0.8 to 2.0 log CFU/cm² on the adipose surface of the beef cheek meat and 0.6 to 1.4 log CFU/cm² on the muscle surface of the cheek meat after dipping in 220 ppm of PAA for 1 min. Scott et al., (2015) reported that 700 ppm of PA (with 20 sec dip) reduced Salmonella enterica populations by 1.5 log CFU/ml on chicken wings. In another study, beef trimmings when dipped in 200 ppm PAA for 30 sec showed a reduction in ST populations by 0.2 to 0.7 log CFU/cm² (Geornaras et al., 2012). Ellebracht et al., (2005) showed that 200 ppm of PAA (with 15 sec dip) reduced ST populations by 1 log
CFU/cm² on fresh beef trim. PAA at 85 ppm in poultry chiller reduced *Salmonella* positive carcasses by 92% compared to a 57% reduction achieved by 30 ppm chlorine (Bauermeister *et al.*, 2008b). In conclusion, Peroxyacetic acid is an effective antimicrobial intervention for meat and poultry industry during pre and post harvesting application demonstrating bactericidal effect.

**Propionic acid**

Propionic acid effectiveness as a mold inhibitor is well documented (Willingham, 1941; Paster *et al.*, 1979). Propionic acid and propionate salts are widely used as mold inhibitors in bread, baked goods, tortillas, pizza crust etc. Literature shows that propionic acid is also effective against bacterial pathogens such as *E.coli*, *P.aeruginosa*, *S.aerus*, *Salmonella* spp (Eklund *et al.*, 1985). Recently, the meat industry has shown interest in propionate salts, which exhibited significant antilisterial activity in both media and actual meat matrices (Glass *et al.*, 2007a, b). Most recently, the U.S. Department of Agriculture has approved the use of sodium propionate, propionic acid and sodium benzoate in ready-to-eat (RTE) meat and poultry products (USDA 2013). Glass *et al.* (2007a) showed that 0.2% propionate and 0.1% benzoate showed equal efficacy in controlling *Listeria monocytogenes (Lm)* when compared to a combination of 1.6% lactate with 0.1% diacetate in RTE ham stored at 4°C for 12 weeks. In RTE uncured turkey, 0.2% propionate showed equal efficacy in controlling *Lm* when compared to a combination of 3.2% lactate with 0.2% diacetate. Gonzalez *et al.*, (2004) showed that *Lm* populations remained at the minimum level of detection for 12 weeks on frankfurters dipped in acidic calcium sulfate with propionic and lactic acid thus indicating its residual bactericidal effect. In cured deli-style turkey, liquid propionate or propionate-
benzoate ingredients limited $Lm$ growth to an increase of $<1$ log through 9 weeks storage at $4\, ^\circ C$ (Glass et al., 2013). These studies have shown that propionate is a promising alternative antimicrobial for the control of $Lm$ in RTE meat and poultry products. There have been no studies on the effect of buffered propionic acid on other foodborne pathogens such as $Salmonella$ spp. in raw ground meat and poultry products. More efficacy studies are required in order to gain regulatory approval for propionic acid as antimicrobial for raw ground meat and poultry products and thus improving food safety. The objectives of the study described in this thesis were to evaluate antimicrobial efficacy of buffered propionic acid on $Salmonella$ spp inoculated on ground pork and determine its impact on quality and sensory parameters of pork patties.

**Significance of *Listeria monocytogenes***

*Listeria monocytogenes* ($Lm$) is one of the major foodborne pathogens that continue to be a serious threat to public health despite a decrease in number of cases annually (CDC, 2015; Scallan et al., 2011). A serious food infection called “Listeriosis” is caused by eating food contaminated with *L. monocytogenes* (Schlech et al., 1983). It is estimated that in the United States, approximately 1600 illnesses and 260 deaths occur due to listeriosis (Scallan et al., 2011) and in 2013, the average annual incidence of listeriosis was 0.26 cases per 100,000 individuals in the United States (CDC, 2014). In 2011, a largest listeriosis outbreak in US history occurred resulting in 147 illnesses, 33 deaths and 1 miscarriage due to consumption of cantaloupes from a single farm (CDC, 2011a). Pregnant women and newborns, older adults and people with weakened immune systems are at greater risk of listeriosis infection (Jackson et al., 2010; Silk et al., 2012). Listeriosis in pregnant women may result in fetal
loss, premature labor, neonatal infection, and neonatal death (Jackson et al., 2010). Ready-to-eat (RTE) deli meats are considered as very high risk foods for causing listeriosis infection on both per serving and per annum basis. Frankfurters (not reheated), meat spreads, smoked sea food, cooked RTE crustaceans etc. are ranked high in relative risk per serving (USFDA, 2003; Crandall et al., 2015; Malley et al., 2015; Engel et al., 1990).

**Characteristics and Physiology**

*Listeria monocytogenes* is one of the six species in the genus *Listeria*. Other species include *Listeria inanovii, Listeria innocua, Listeria welshimeri, Listeria seeligeri, and Listeria grayi* (Rocourt & Buchrieser, 2007). *Lm* is a gram positive, non-sporoforming, non-acid fast, rod-shaped bacterium with rounded ends measuring 1.0 µ to 2.0 µ by 0.5 µ, and a facultative anaerobic that can be isolated from many environmental niches, for example, water, soil, decayed plant material etc. (Gray and Kilinger, 1966; Wexler & Oppenheim, 1979; Weis and Seeliger, 1975). *Lm* is widespread in nature and its ubiquitous distribution could be due to its resistance to many adverse environmental conditions (Junttila et al., 1988; Herbst et al., 2014). *Lm* can grow at wide range of temperature varying from 1.7 to 45.0 °C with optimum temperature reported to be in the range of 30 °C to 37 °C; it can survive in a wide range of pH from 4.7 to 9.2 and can tolerate high salt concentrations of up to 10% sodium chloride (Gray and Kilinger, 1966, Junttila et al., 1988; Petran and Zottola, 1989; Mcclure et al., 1991). Herbst et al., (2014) reported that this resistance spectrum allows *Lm* to overcome popular food conservation barriers such as acidification, low water activity or cooling, and is the prerequisite for its relevance as foodborne pathogen.
Listeria monocytogenes (Lm) outbreaks linked to consumption of meat and poultry products

Lm emerged as a problem in deli meats in the late 1980’s (FSIS, 1999). Both FSIS and FDA (Food and Drug Administration) worked with meat processing establishments to improve their procedures and emphasized “zero tolerance” (no detectable level permitted) for the pathogen in RTE meats and the rate of illnesses from Lm declined 44% between 1989 and 1993 (FSIS, 1999). In 1998, a major foodborne outbreak was reported in the US due to consumption of frankfurters that resulted in 14 deaths and four miscarriages or stillbirths (Mead et al., 2006). In 2001, an outbreak of febrile gastroenteritis associated with precooked sliced turkey was reported in Los Angeles county that sickened 16 people (Frye et al., 2002). In 2002, a multistate outbreak of listeriosis linked to turkey deli meat was reported in the US that resulted in 8 deaths and 3 miscarriages (Gottlieb et al., 2006). In 2005, a foodborne outbreak was reported in South Australia resulting in 4 cases and 2 deaths due to consumption of RTE meat and in 2009 another outbreak occurred resulting in 36 cases and 4 deaths due to consumption of chicken wraps sold on domestic airline flights across Australia (Popovic et al., 2014). It was reported that between 2001 and 2010 in Australia, there was not significant increase in the number of recalls due to Lm, but of the 96 reports of Lm linked food recalls, 61 involved processed RTE delicatessen meats (Popovic et al., 2014). One case of foodborne listeriosis was reported in Connecticut in 2008 linked to consumption of chicken salad (Marcus et al., 2009) and a multistate outbreak of Lm infection was reported in the US due to consumption of delicatessen turkey meat that resulted in 4 deaths and 3 miscarriages (Olsen et al., 2005). In 2011, six laboratory-confirmed cases were reported in Switzerland linked to consumption of cooked ham and investigation showed that the ham
was not contaminated in the production plant, but occurred in the premises of the co-packer’s slicing and packing facility (Hachler et al., 2013). In 2008, one of the worst cases of food contamination in Canadian history occurred due to consumption of *Lm* contaminated deli meats such as roast beef and corned beef that resulted in 20 deaths (Greenberg and Elliott, 2009). In 2010, 14 cases of laboratory-confirmed listeriosis were reported in Louisiana due to consumption of hog head cheese, a meat jelly made from swine heads and feet (CDC, 2011b). It appears that from the year 2011 to 2015, no listeriosis outbreaks due to meat and poultry products were reported in the US and this showed that RTE meat and poultry industry had made significant progress in controlling *Lm* thus saving human lives and monetary losses due to recalls.

**Commonly associated serovars**

Pan et al., (2009) reported that serovars 1/2a and 1/2b constitute majority of the *Lm* isolates recovered from foods and food processing environments although 4b strains cause the majority of listeriosis outbreaks. In another study by Pan et al., (2010), it was found that *Lm* serotype 1/2a formed a biofilm of greater density than serotype 4b and this could explain the greater prevalence of 1/2a in food processing facilities. In Ireland, between 2004 and 2007, PGFE patterns of 145 *Lm* isolates collected from food and food processing facilities showed that most common serotype was 1/2a (57.4%), 4b (14.1%), 1/2b (9.7%) and 1/2c (6.6%) (O’Connor et al., 2010). Numerous researchers have reported that majority of listeriosis outbreaks were caused by serotype 4b even though 1/2a is found most often in foods and food processing facilities (Aarnisalo et al., 2003; Wallace et al., 2003; Mead et al., 2006; Pan et al., 2010; Tresse et al., 2007). In a study on the distribution of serovars of *Lm* isolated
from 1363 listeriosis-affected patients in the UK, serovar 4b was found in 64% of cases, 1/2a in 15%, 1/2b in 10% and 1/2c in 4% (McLauchlin, 1990). Kathariou (2002) reported that even though serotype 4b is not commonly prevalent in foods, but has been involved in numerous outbreaks; this is a cause for concern. Using molecular subtyping studies researchers have suggested that Lm is composed of three distinct evolutionary lineages that differ in their ability to cause listeriosis (Piffaretti, 1989; Gray et al., 2004; Ward et al., 2004). It was found that lineage I consists of all strains that are associated with foodborne listeriosis in humans, primarily serotypes 1/2b and 4b (Wiedmann et al., 1997). Ragon et al., (2008) reported that serotype 4b has evolved from 1/2b and hence, serotype 1/2b is likely the original serotype for lineage I and also theorized that genetic evolution of 4b from 1/2b led to the increased virulence potential and an increased ability to cause human outbreaks. Wiedmann et al., (1997) also reported that lineage II contained some strains from human and animal listeriosis cases, but no strains from human listeriosis epidemics, while lineage III contained no human isolates.

**Effects of temperature, pH and water activity on Listeria monocytogenes**

*Lm* can grow at wide range of temperature varying from 1.7 to 45.0 °C with optimum temperature reported to be in the range of 30 °C to 37 °C, and it can survive in a wide range of pH from 4.7 to 9.2 (Gray and Kilinger, 1966; Juntila et al., 1988; Petran and Zottola, 1989). It has been reported that growth of *Lm* is reduced but not prevented at refrigerated temperatures of 0-5 °C thus causing a great concern for foods stored at refrigerated temperature (Kathariou, 2002; Zhu et al., 2005). It was shown that *Lm* grew at slow rates at 0.1 °C, but it grew much faster than other organisms at 15 °C (Grau and Vanderlinde, 1992).
As *Lm* can grow at refrigerated temperature, initially low levels of contamination can grow to high levels during storage at retail counters and also during consumer transport and storage, thereby increasing the risk of illness (Pouillot *et al*., 2015). In a quantitative risk assessment of listeriosis associated deaths due to *Lm* contamination of deli meats that were originated from manufacturer and retails, authors estimated that up to 41% of the estimated deaths due to *Lm* could be caused by home refrigerators with a temperature of above 10 °C and suggested that reducing storage temperature in home refrigerators to consistently below 7 °C would greatly reduce the risk of deaths due to listeriosis (Pradhan *et al*., 2010). The authors also suggested that reducing the storage temperature in distribution will have greatest impact on reducing listeriosis associated deaths, regardless of origin of contamination (Pradhan *et al*., 2010). In the quantitative risk assessment, the authors cited an example that the baseline of 13.2 deaths per year for elderly population would be increased to 27.2 with 3 °C increase in storage temperature (Pradhan *et al*., 2010). Pouillot *et al*., (2015) conducted a quantitative risk assessment of *Lm* in retail delicatessens and predicted that cases of listeriosis result from a sequence of following important events: (i) the contaminated RTE food supports the growth of *Lm*; (ii) improper retail and/or consumer storage temperature or handling results in the growth of *Lm*; and (iii) consumer of the RTE food is susceptible to listeriosis. Karina *et al*., (2011) showed that addition of 7.5% NaCl inhibited the growth of *Lm* at 20 and 30 °C and addition of lactic acid at 50 mM to obtain a pH of ≤ 5 inhibited the growth of *Lm*. Duffy *et al*., (1994) inoculated cooked beef and pork slices with *Lm*, vacuum packaged and stored at 0 or 5 °C; they reported that decreasing the pH from 6.9-5.9 and water activity from 0.993-0.960 increased the lag time of *Lm* and reduced the growth rate at 5 °C whereas, extent of
growth at 0 °C was considerably less than at 5 °C. Norrung (2000) reported that \( Lm \) can grow at water activity levels >0.92 and pH values of 4.5-9.2.

**Listeria monocytogenes control strategies**

**Focus on post-lethality contamination**

The heating step employed by RTE meat and poultry manufacturers for fully-cooked products is sufficient to kill \( Lm \) but recontamination can occur at various handling steps after the heating step, including slicing and packaging (Hwang and Tamplin, 2007; Jiang and Xiong, 2015). Sheen and Hwang (2008) investigated the transfer phenomenon of \( Lm \) from slicer to deli meat during mechanical slicing and found that more ham slices were contaminated with \( Lm \) when slicer blade was contaminated with higher levels of \( Lm \) (9 log CFU/blade). Lin *et al.*, (2006) reported that \( Lm \) can be transferred from a contaminated slicer onto meats and can survive or grow better on uncured, oven-roasted turkey than on salami or bologna with preservatives and higher \( Lm \) cell numbers inoculated on the slicer blade resulted in more \( Lm \)-positive sliced meat samples. Vorst *et al.*, (2006) studied the transfer of \( Lm \) during mechanical slicing of turkey breast, bologna and salami and concluded that slicers have the ability to harbor \( Lm \) and then transfer \( Lm \) to sliced products at a later point and this could pose a risk to consumers consuming these products, particularly immunocompromised individuals. In response to a nationwide outbreak of listeriosis associated with RTE meat and poultry products in late 1998 and early 1999, and a spate of recalls related to the pathogen, FSIS announced an action plan with three near-term initiatives; (i) to send a notice advising establishments to reassess their HACCP (Hazard Analysis and Critical Control Point) plans to ensure they are adequately addressing \( Lm \), (ii) FSIS will provide guidance to the meat
industry on practices that have been used successfully by other establishments to prevent the occurrence of \textit{Lm} in RTE meats, and (iii) FSIS will carry out educational efforts to those individuals who are at an increased risk of developing listeriosis (FSIS, 1999). In 2003, FSIS issued new regulations in order to reduce the risk of \textit{Lm} contamination in RTE meats (FSIS, 2003). Meat processors producing post-lethality exposed RTE product must meet the specific requirements of one of three alternative programs for addressing \textit{Lm}. In the first alternative, establishments control \textit{Lm} by using a post-lethality treatment of the product and an antimicrobial agent or process that suppresses or limits the growth of the pathogen. In the second alternative, establishments can use a post-lethality treatment or an antimicrobial agent or process that suppresses or limits the growth of the pathogen. In the third alternative, establishments control \textit{Lm} through sanitation procedures only (FSIS, 2003). The post-lethality intervention treatments used for either alternative 1 or 2 must be addressed by the establishment’s HACCP plan and the treatment must be a critical control point (CCP) for the HACCP plan. The sanitation procedures followed in alternative 3 must be validated and verified in accordance with 9 CFR 417.4 and sanitation in the post-lethality processing area must be maintained in accordance with 9 CFR 416 (FSIS, 2003).

\textbf{Cleaning and Sanitation Practices}

Initial efforts for controlling \textit{Lm} in RTE meat and poultry products were centered on cleaning and sanitation practices. FSIS issued sanitation guidelines for controlling \textit{Lm} in meat establishments. \textit{Lm} can grow in a damp environment, and can attach to surfaces that come into contact with raw or finished product, establish a niche and form biofilms. The sanitation programs should include cleaning and sanitizing procedures that have been proven effective,
segregation of raw and RTE processing areas, traffic control, employee hygiene, equipment flow and design etc. (FSIS, 2006). Somers and Wong (2004) studied the efficacy of two cleaning and sanitizer combinations on \( Lm \) biofilms on variety materials in the presence of RTE meat residue and observed that biofilms developed on all surfaces tested including stainless steel, rubber, wall and floor material. Cleaning efficacy was surface dependent and decreased with residue-soiled surfaces, biofilms developed on the brick and conveyor material were most resistant. Both detergents significantly \((p<0.05)\) removed or inactivated biofilm bacteria. Sanitizers further reduced biofilm numbers; however, the reduction was not significant for dual peracid sanitizer compared to hypochlorite. Cruz and Fletcher (2012) tested 21 commercially available sanitizers against 20 strains of \( Lm \), and results showed all tested sanitizers achieved a 5-log reduction of \( Lm \) cells in suspension at concentrations below the manufacturer’s recommended concentrations. Against biofilm, only the peroxyacetic acid, chlorine dioxide and acidified sodium chlorite gave 5-log decrease, within or close to the manufacturer’s recommended concentrations. Belessi et al., (2011) studied the efficiency of different sanitation methods on \( Lm \) biofilms formed under various environmental conditions. One sanitation procedure included biofilm formation on stainless steel coupons placed in tryptic soy broth with various concentrations of sodium chloride \((0.5, 7.5 \text{ and } 9.5\%)\) at different temperatures \((5 \text{ and } 20\degree C)\). The biofilms formed were exposed to water \((60\degree C)\) for 20 min or to 2% peroxyacetic acid for 1, 2, 3 and 6 min. The results showed that water caused no significant \((p>0.05)\) reductions in the attached populations whereas, the \( Lm \) populations decreased as the exposure time to peroxyacetic acid increased and could not be detected by culture after 6 min of exposure. Salt concentration in the growth medium had no marked impact on the resistance to peroxyacetic acid. Malley et al., (2015) reported that
complete elimination of postprocessing *Lm* contamination is challenging because of its presence in various environments outside processing plants and the organism can persist in food processing environments for years; therefore, a science-based strategies such as seek-and-destroy processes can be used for finding sites of persistent strains (niches) in food processing plants, with the goal of either eradicating or mitigating effects of *Lm* strains. Based on the literature, it is clearly evident that *Lm* control cannot depend solely on cleaning and sanitation practices, but additional hurdles need to be in place for effective control of *Lm* in RTE meat and poultry products.

**Antimicrobial Ingredients**

According to FSIS, an antimicrobial agent is defined as a substance in or added to an RTE product that has the effect of reducing or eliminating a microorganism, including a pathogen such as *Lm*, or that has the effect of suppressing or limiting growth of a pathogen, such as *Lm*, in the product throughout the shelf life of the product (FSIS, 2014). FSIS has approved a variety of antimicrobial ingredients for *Lm* control and periodically updates in the FSIS Directive 7120.1 (FSIS, 2016). Some of the commonly used antimicrobial ingredients for *Lm* control in RTE meat and poultry products are discussed below.

**Lactate-Diacetate Salts**

Among various antimicrobials, organic acid salts such as sodium or potassium lactate and sodium diacetate are widely used in RTE meat and poultry products and their efficacy is well documented. Hwang *et al.*, (2012) inoculated cooked ham with 3-log CFU/g of *Lm* and immersed samples in 0, 0.5, 0.75, 1.0, 1.25, 1.5, and 2.0% of lactic acid solution for 30 min,
then vacuum packaged, and stored the samples at 4, 8, 12 and 16 °C. Results showed that lactic acid immersion resulted in <0.7 log CFU/g immediate reduction of *Lm* on ham surfaces, indicating the immersion alone was not sufficient for reducing *Lm*. During storage, no growth of *Lm* occurred on ham treated with 1.5% lactic acid at 4 and 8 °C and with 2% lactic acid at all storage temperatures.

Lloyd *et al.*, (2009) evaluated the efficacy of lactate salts against *Lm* in turkey deli loaves by adding lactate to the raw product and as a postcook dip. The turkey loaves were tested at days 0, 7, 14, 21, 28, 42 and 56. Results showed no significant differences (p>0.05) among the organic acid treatments at any time points and all the antimicrobial treatments increased the lag phase of *Lm*, thus extending the shelf life of turkey loaves. Casco *et al.*, (2015) showed that 3.6% sodium lactate with 0.25% sodium diacetate extended the lag phase of *Lm*-inoculated turkey slices through 21 days of refrigerated storage whereas, combination of 3.6% sodium lactate with 0.75% sodium citrate and 0.25% sodium diacetate extended the lag phase through 42 days. Authors reported that organic acid dips prolonged the lag phase of *Lm* for 2 to 6 weeks on turkey product surfaces and can be useful antimicrobial agents for *Lm* control on postlethality exposed sliced deli products. Barmpalia *et al.*, (2004) formulated pork frankfurters with 1.8% sodium lactate or 0.25% sodium diacetate or combinations of 1.8% sodium lactate with 0.25 or 0.125% sodium diacetate and inoculated with *Lm* at 2 to 3 log CFU/cm² and left undipped or dipped for 2 min in 2.5% solution of lactic acid or acetic acid and stored at 10 °C for 40 days. Results showed that combination of 1.8% sodium lactate and 0.25% sodium diacetate provided complete inhibition of *Lm* growth throughout storage. For samples containing single antimicrobials and dipped in lactic acid or acetic acid, *Lm* growth was completely inhibited or reduced over 12 and 28 days, respectively, whereas final
populations were lower (p<0.05) than those in undipped samples of the same formulations. Bactericidal effects during storage (reductions of 0.6 to 1.0 log CFU/cm\(^2\) over 28 to 40 days) were observed in frankfurters containing combinations of SL and SD that were dipped in organic acid solutions. Lu et al., (2005) showed a decrease in Lm populations and increase of its generation time and lag phase on Lm-treated frankfurters after surface-treating with 6% sodium diacetate or 6% sodium lactate-sodium diacetate-potassium benzoate solutions at 1.1 °C. Surface treatment of frankfurters with 6% sodium diacetate was found to be more effective in inhibiting Lm growth among the treatments tested. Sijtsema et al., (2014) showed that 2% of sodium lactate (52%) and sodium diacetate (8%) blend controlled Lm for 90 days (2 log growth) in cured honey ham whereas, 2.4% of the blend controlled Lm for 90 days (2 log growth) in cured honey turkey. Golden et al., (2014) showed that 3.8% of lactate-diacetate blend delayed Lm growth up to 6 weeks in uncured turkey whereas, supplementation with 80 ppm of synthetic or natural nitrite delayed growth of Lm through 12 weeks.

**Propionic acid and Propionate Salts**

Recently, the meat industry has shown interest in propionate salts, which exhibited significant antilisterial activity in both media and actual meat matrices (Glass et al., 2007 a, b). Most recently, the U.S. Department of Agriculture has approved the use of sodium propionate, propionic acid and sodium benzoate in ready-to-eat (RTE) meat and poultry products (USDA 2013). Glass et al. (2007a) showed that 0.2% propionate and 0.1% benzoate showed equal efficacy in controlling Lm when compared to a combination of 1.6% lactate with 0.1% diacetate in RTE ham stored at 4°C for 12 weeks. In RTE uncured turkey, 0.2%
propionate showed equal efficacy in controlling *Lm* when compared to a combination of 3.2% lactate with 0.2% diacetate. Gonzalez *et al.*, (2004) showed that *Lm* populations remained at the minimum level of detection for 12 weeks on frankfurters dipped in acidic calcium sulfate with propionic and lactic acid thus indicating its residual bactericidal effect. In cured deli-style turkey liquid propionate or propionate-benzoate ingredients limited *Lm* growth to an increase of <1 log through 9 weeks storage at 4°C (Glass *et al.*, 2013). These studies have shown that propionate is a promising alternative antimicrobial for the control of *Lm* in RTE meat and poultry products.

**Cultured Sugar and Vinegar**

Golden *et al.*, (2014) showed that 1% cultured sugar-vinegar blend delayed *Lm* growth for 6 weeks at 4 °C in uncured deli-style turkey whereas, 1% cultured sugar-vinegar blend supplemented with 80 ppm of synthetic nitrite or natural nitrite source (cultured celery powder) delayed *Lm* growth for 12 weeks stored at 4 °C. Sullivan *et al.*, (2012) reported that ham treated with cultured sugar and vinegar blend and supplemented with either synthetic nitrite or nitrite from natural source showed no significant differences (p>0.05) in *Lm* inhibition. Weyker *et al.*, (2016) used Response Surface Methodology (RSM) to evaluate the effects of different levels of moisture (60-80%), pH (5.8-6.4) and cultured sugar-vinegar blend (2.5-5.0%) in *Lm*-challenged uncured turkey stored for 16 weeks at 4 °C and concluded that high concentrations of cultured sugar-vinegar are required to inhibit the growth of *Lm* in uncured products that have high moisture and high pH, and this was likely due to simple dilution of active ingredients in the moisture phase coupled with a lower concentration of the nonionized forms of the organic acids in cultured sugar-vinegar at higher pH. Sijtsema *et al.*, (2014) reported that 3% and 3.8% of cultured sugar-vinegar blend showed 2 log growth of
*Lm* after 90 days in cured honey ham whereas, control treatment without antimicrobial showed 2 log *Lm* growth in 45 days. In cured honey turkey, 3.4% of cultured sugar-vinegar showed complete inhibition of *Lm* during 90 days of incubation whereas, control without antimicrobial showed 2 log *Lm* growth in 35 days.

**Buffered Vinegar**

Vinegar has been used for centuries for a variety of purposes and has well-documented antimicrobial properties (Budak *et al.*, 2014). Although there are no standards of identity for vinegar, FDA guidelines indicate that natural vinegars normally contain in excess of 4 grams of acetic acid per 100 ml (USFDA, 1995). The low pH of vinegar (2.0-3.0) is a limiting factor for its application in RTE meat and poultry products as it can negatively affect physical and sensory characteristics. The advantages of buffering and drying the vinegar are three-fold – it reduces the pungent vinegar flavor to a mild vinegar flavor, it has less negative impact on the taste and flavor of the treated finished product and it can be used at lower application rates due to a more concentrated acetic acid. Lavieri *et al.*, (2014 a, b) reported dried vinegar as a potential bacteriostatic ingredient for inhibiting the growth of *Lm* inoculated into alternatively-cured frankfurters and alternatively cured ready-to-eat ham. Their research showed that inclusion of 1% dried vinegar when formulating both of these meat products prevented the growth of *Lm* for 14 weeks when stored at 4 ± 1°C. However, dried vinegar did not exhibit any bactericidal properties against *Lm* in their studies. Porto-Fett *et al.*, (2015) showed no change in *Lm* population in deli-style ham formulated with 1.5% buffered vinegar, with or without a stabilized solution of sodium chlorite, for up to 90 days of storage at 4 °C; whereas, 2.0 or 2.5% buffered vinegar reduced pathogen counts by
1.1 and 2.0 log CFU/slice respectively. Roast beef formulated with 1.0 or 1.5% buffered vinegar showed an increase of 2.2 to 2.4 log CFU/slice but they also found that roast beef formulated with 2.0 or 2.5% buffered vinegar decreased \( Lm \) counts by 0.7 and 1.2 log CFU/slice, respectively, when stored for 90 days at 4 °C. In another \textit{Listeria} challenge study on uncured turkey breast formulated with 3.0% buffered vinegar and surface treated with or without a stabilized solution of sodium chlorite in vinegar, Porto-Fett \textit{et al.}, (2014) observed counts decrease by approximately 0.7 to 1.3 log CFU/slice, respectively, when stored at 4°C for 90 days. However, when stored at 10°C, pathogen numbers increased by approximately 1.5 to 5.6 log CFU/slice after 48 days when formulated with 2.0 to 3.0% buffered vinegar and treated with or without 2% sodium chlorite in vinegar. McDonnell \textit{et al.} (2013) reported that 2.0% liquid buffered vinegar in sliced, uncured, deli-style turkey breast, alternative-cured boneless ham, and uncured roast beef delayed the growth of \( Lm \) until 6, 6 and 12 weeks of storage at 4°C, respectively. The authors speculated that significant inhibition of pathogen growth in roast beef compared to the turkey breast and boneless ham could be due to differences in pH and moisture content of the products.

**Demand for Natural and Organic Foods**

Consumer demand for natural and organic foods in the US is continuously increasing as evidenced by increasing sales of these products, which rose to $39.1 billion in 2014, and the organic market is experiencing double-digit growth of 11.3% (Organic Trade Association, 2015). These statistics are showing there is a tremendous growth for foods that are labeled organic, natural, no added preservatives, or minimally processed. Research studies have shown preferences for natural and organic foods based on concerns about pesticides,
antibiotics, hormones, genetic modifications, and chemical additives (Loo et al., 2010; Loo et al., 2011, Sofos, 2008). Hence, development of clean-label ingredients (e.g. without chemical-sounding names, any ingredients that says artificial, ingredients that consumers cannot understand etc.) represents a high priority for the meat industry. Some of the examples of clean-label ingredients currently used by meat industry are celery juice powder, a vegetable based nitrite source replacing synthetic nitrite, cultured sugar and vinegar blend, liquid and dry buffered vinegar, cherry juice powder, a natural source for ascorbic acid which is used as a cure accelerator etc. (Golden et al., 2014; Sullivan et al., 2012; Weyker et al., 2016; Sebranek et al., 2012). While replacing synthetic ingredients with clean-label ingredients in processed meats may look impressive, it could compromise microbiological safety and hence, more validation studies are needed to confirm the antimicrobial efficacy of clean-label ingredients.

**Significance of Low Sodium Foods**

Another challenge faced by the processed meat industry is sodium reduction because high sodium intake may result in increased blood pressure and is a risk factor for cardiovascular disease (Antman et al., 2014; Mozaffarian et al., 2014; Vandendriessche, 2008). The term “reduced sodium” may be used if the individual food contains at least a 25 percent reduction in sodium as compared to an appropriate reference food (USDA, 2007). In the past few years, the US food industry and the U.S. government has made many efforts to reduce the sodium content in processed foods (Doyle and Glass, 2010; Dunford et al., 2012). In 2008, the New York City Department of Health and Mental Hygiene started a voluntary ‘National Salt Reduction Initiative’ (NSRI) with the overall goal of reducing dietary salt consumption by
20% over five years (Clapp, 2014; Boon et al., 2010). To help the public reach this goal, the NSRI challenged food manufacturers to reduce the salt content of packaged and prepared foods by 25% over the same period. They developed a database containing 62 packaged and 25 restaurant food categories that contributed to salt intake, and established targets for sodium content to be achieved by the end of 2012 and 2014 (Clapp, 2014). Lunch meats fell into one of the processed food categories that were targeted. Since March 2011, 28 major food manufacturers (e.g. Kraft Heinz Company, Unilever, Campbell Soups) and leading restaurant chains (e.g. Subway, Starbucks) have agreed to pursue salt reduction targets in one or more food categories (Clapp, 2014). In 2013, it was announced that 21 companies met one or more of their voluntary commitments to reduce sodium content in pre-packaged or restaurant foods (Bloomberg and Farley, 2013). While sodium chloride imparts flavor and texture to foods, it also plays a critical role in food safety by reducing water activity, thereby diminishing the growth of spoilage and pathogenic microorganisms (Antman et al., 2014; Doyle and Glass, 2010). Hence, when developing low-sodium meats, precautions should be taken to avoid compromising on flavor, texture, shelf life, and safety.

Summary of Literature and Objectives for Proposed Studies

Foodborne pathogens such as *Salmonella Typhimurium* and *Listeria monocytogenes* continue to be of concern to the meat industry and regulatory authorities in the United States. Several foodborne outbreaks linked to consumption of *Salmonella Typhimurium* contaminated pork products were reported inside and outside of the US. It was reported that *Salmonella* contamination in pork can occur both at pre-harvest and post-harvest stages. Antimicrobials are commonly used for controlling foodborne pathogens at various stages in
meat production chain. Organic acids and their salts such as lactic acid, sodium lactate, acetic acid, sodium diacetate etc. are widely used in raw and cooked meat products. As the food safety regulations become stricter day by day, it is necessary to develop and validate new effective antimicrobial ingredients to strengthen food safety. USDA has approved the use of propionic acid and sodium propionate in ready-to-eat meat and poultry products, however it appears that there have been no studies on the effect of propionic acid on foodborne pathogens such as *Salmonella* spp. in raw ground meat and poultry products. Efficacy studies are required in order to gain regulatory approval for propionic acid as antimicrobial for raw meats and hence two research projects were undertaken to evaluate the antimicrobial efficacy of buffered propionic acid against *Salmonella Typhimurium* in ground pork. The objective of the first project was to determine effect of buffered propionic acid against *Salmonella Typhimurium* inoculated by two different methods in ground pork i.e. addition of propionic acid to ground pork followed by addition of *Salmonella* Typhimurium and addition of *Salmonella* Typhimurium to ground pork followed by addition of buffered propionic acid. Objective of the second project was to determine the efficacy of buffered propionic acid against *Salmonella Typhimurium* in ground pork stored at 4°C and 10°C.

Ready-to-eat meats such as deli meats and frankfurters without antimicrobials pose the greatest per-serving risk of illness/death from *L. monocytogenes* because they are often consumed directly from the refrigerator without reheating. In the past decade, several foodborne outbreaks linked to consumption of *Listeria monocytogenes* contaminated deli/ready-to-eat meats were reported both in the US and worldwide. Antimicrobials such as lactate-diacetate salts are widely used in ready-to-eat meat and poultry products for controlling *Listeria monocytogenes*. Consumer demand for natural and organic foods is
pushing the processed meat industry to use natural or clean-label ingredients. Sodium reduction is another challenge faced by the processed meat industry and there is a push from consumers as well as regulatory agencies to cut down sodium levels in processed meats. While sodium chloride imparts flavor and texture to foods, it also plays a critical role in food safety and hence, when developing low-sodium meats, precautions should be taken to avoid compromising on flavor, texture, shelf life, and microbiological safety. Recently, liquid and dry buffered vinegar has attracted considerable attention by the processed meat industry as a clean-label antimicrobial to control foodborne pathogens. Buffering the vinegar using sodium- or potassium-based alkali raises the pH and creates minimal impact on the functional properties of the processed meats. The advantage of using a potassium-based buffer is it does not contribute sodium in the final food product. It appears that there have been no studies on the antilisterial effect of potassium-based dry vinegar in ready-to-eat meat and poultry products and hence the third project was taken with the objective of evaluating the antilisterial efficacy of sodium-based and potassium-based buffered dry vinegar in reduced-sodium ready-to-eat uncured turkey stored at 4°C.
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CHAPTER 3. Effect of buffered propionic acid against Salmonella Typhimurium inoculated by two different methods in ground pork

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ABSTRACT

The objective of the study was to evaluate the antimicrobial efficacy of buffered propionic acid-based antimicrobials (BP-6 buffered to pH 6; BP-5 buffered to pH 5) against Salmonella Typhimurium (ST) inoculated by two methods in ground pork (1) Addition of antimicrobial to ground pork followed by inoculation with Salmonella Typhimurium (2) Addition of Salmonella Typhimurium to ground pork followed by addition of antimicrobial. This study was undertaken to check if there would be any difference in the antimicrobial efficacy of buffered propionic acid if added before or after Salmonella addition to ground pork. Ground pork treatments consisted of 0.2% BP-6, 0.2% BP-5 and an untreated control without any antimicrobial. The treatments were challenged with a 5-strain inoculum of ST with a target of 3-4 log CFU/g. Storage was at 4 °C for 4 weeks and ST populations were tested at weekly intervals. The study was conducted as a single replication. Results showed that in method-1, the initial ST populations in all the treatments were within the expected range. In method-2, the initial ST populations were lower (~ 1 log) than expected. In both methods of addition, a decline in ST populations was seen in the antimicrobial treatments as well as untreated control throughout 4 weeks. The probable reason for the decline could be the low incubation temperature (4 °C) that might have reduced survival. The propionic acid levels in both the antimicrobial treatments were within the expected range (0.09-0.1%). This study suggests that in method-1 and method-2, there was no difference in the log change of ST populations between the treatments throughout the testing period. However, 4 °C is not an optimum temperature for growth of ST because both antimicrobial treatments and untreated control resulted in restricted the growth of ST. Additional research has to be conducted to evaluate the efficacy of buffered propionic acid against ST in ground pork stored at temperatures optimal for ST growth.
INTRODUCTION

Foodborne infections associated with consumption of Salmonella spp-contaminated food products continue to be a serious threat to public health. It is estimated that non-typhoidal Salmonella (NTS) causes approximately 1.2 million illnesses and 450 deaths annually in the United States and 5% of illnesses are attributed to consumption of pork products (4, 21). In order to achieve the goal of a safer food supply, United States Department of Agriculture (USDA)’s Food Safety and Inspection Service (FSIS) has taken an aggressive enforcement approach to control foodborne pathogens such as Salmonella and Campylobacter. In February 2016, FSIS announced the finalization of new federal standards to reduce Salmonella and Campylobacter in ground chicken and turkey products and it is estimated that implementation of these standards will prevent an average of 50,000 illnesses annually (6). Pigs are healthy carriers of Salmonella spp and the most frequent serotype isolated from porcine meat is Salmonella Typhimurium (5, 14). Salmonella spp. contamination of pork can occur at two stages i.e. pre-harvest stage i.e. animal production on the farm and post-harvest stage i.e. carcass processing and cross-contamination (1, 7, 3). Berends et al. (3) reported live pigs carrying Salmonella spp. were 3 to 4 times more likely to have the organism on their carcasses than are Salmonella-free pigs. Antimicrobial interventions are commonly used by the meat and poultry industry for controlling foodborne pathogens at various stages in production chain. Organic acids and their salts such as lactic acid, sodium lactate, acetic acid, sodium diacetate etc. are widely used antimicrobial interventions in raw and cooked meat and poultry products and their efficacy is well documented (11, 12, 13, 15, 16, 18). Recently, propionic acid has attracted considerable attention by the meat industry. It is listed as “Generally Recognized as Safe” by the U.S. Food and Drug Administration (FDA) and its efficacy against molds is well documented (19, 23). In 2013, U.S. Department of Agriculture (USDA) approved the use of propionic acid, sodium propionate, and sodium benzoate as antimicrobials in ready-to-eat (RTE) meat and poultry products (22). There are several studies demonstrating the antimicrobial efficacy of propionate salts in various cooked meat
and poultry products. Glass et al. (8, 9) showed that 0.2% propionate and 0.1% benzoate showed equal efficacy in controlling \( Lm \) when compared to a combination of 1.6% lactate with 0.1% diacetate in RTE ham stored at 4°C for 12 weeks. In RTE uncured turkey, 0.2% propionate showed equal efficacy in controlling \( Lm \) when compared to a combination of 3.2% lactate with 0.2% diacetate. In cured deli-style turkey, liquid propionate or propionate-benzoate ingredients limited \( Lm \) growth to an increase of <1 log through 9 weeks storage at 4°C (10). However, it appears that there have been no studies on the antimicrobial effect of buffered propionic acid in raw meat and poultry products. Efficacy studies are required in order to gain regulatory approval for propionic acid as antimicrobial for raw ground meat and poultry products and thus improving food safety. An important aspect not to be overlooked while conducting \( ST \) challenge studies in ground meats is to mimic the real time \( Salmonella \) contamination during the meat grinding process i.e. using pre-\( ST \) contaminated raw material (e.g. pork trim). Hence, the objective of this study was to evaluate the antimicrobial efficacy of buffered propionic acid-based antimicrobials (BP-6 buffered to pH 6; BP-5 buffered to pH 5) against \( ST \) inoculated by two methods in ground pork (1) Addition of antimicrobial to ground pork followed by inoculation with \( ST \) (2) Addition of \( ST \) to ground pork followed by addition of antimicrobial. The treatments were stored at 4 °C for 4 weeks and \( ST \) populations were enumerated at weekly intervals.

MATERIALS AND METHODS

Ground pork treatments tested. For each method of addition, three treatments were tested – Untreated without any antimicrobial (negative control), 0.2% BP-5 (Liquid buffered propionic acid described as propionic acid buffered with sodium hydroxide to a pH of 4.8-5.2 (BactoCEASE® Liquid); Kemin Industries, Des Moines, IA) and 0.2% BP-6 (Liquid buffered propionic acid described as propionic acid buffered with sodium hydroxide to a pH of 6.1-6.5 (BactoCEASE®-6 Liquid); Kemin Industries, Des Moines, IA). The treatments for the first method of addition (i.e.
addition of antimicrobial followed by \textit{ST} inoculation) were manufactured in the meat laboratory at Iowa State University, Ames, IA, then transferred to Kemin Industries (Des Moines, IA) for inoculation. The inoculation and treatments for the second method of addition (i.e. addition of \textit{ST} followed by addition of antimicrobial) were done in the Bio-safety Level 2 (BSL-2) meat processing laboratory at Iowa State University, Ames, IA. The concentration of propionic acid is equal in both formulations. Pork trimmings (approximately 80\% lean and 20\% fat content) were obtained from commercial pork processor (Seaboard Foods, Mission, KS) and kept frozen (-17 °C) until use. The frozen pork trimmings were thawed at 4 °C before grinding.

**Manufacturing of ground pork treatments (method-1).** For each treatment, pork trimmings (11 lbs) were coarse-ground through a 12 mm plate (Biro MFG Co., Model 7.5 424852, Marblehead, OH). The coarse-ground meat was transferred to a paddle mixer (DVTS 50, Dupey Equipment Co., Clive, IA) and appropriate dosage (10 g) of buffered propionic acid (BP-5 or BP-6) was added drop-wise using a transfer pipet on different locations with intermittent mixing of the ground meat, then continuously mixed for three minutes to ensure even dispersion of the antimicrobial. After mixing, the product was fine-ground through a 6.35 mm plate and vacuum packaged (UV 2100, Multivac, Inc., Kansas City, MO) in high-barrier vacuum pouch. An untreated control batch was prepared containing no antimicrobial. The samples were transported to Kemin Industries, Des Moines, IA for \textit{ST} inoculation and testing.

**Manufacturing of ground pork treatments (method-2).** For each treatment, pork (11 lbs) was coarse-ground through a 12 mm plate (Biro MFG Co., Model 7.5 424852, Marblehead, OH) in the Iowa State University meat laboratory and transported to BSL-2 meat processing laboratory. The coarse-ground meat was transferred to a paddle mixer and 50 ml of \textit{ST} cocktail (prepared as per the procedure given below) was added drop-wise using a transfer pipet on different locations with intermittent rotation of the ground meat. Fifteen minutes was allowed for the \textit{ST} attachment followed by mixing for 2 minutes to ensure even dispersion of the inoculum. Appropriate dosage (10 g) of buffered propionic acid (BP-5 or BP-6) was added slowly drop-wise using a transfer pipet on
different locations with intermittent mixing and then continuously mixed for two minutes to ensure even dispersion of the inoculum and the antimicrobial treatment. After mixing, the product was fine-ground through a 6.35 mm plate and vacuum packaged in high-barrier vacuum pouches. An untreated control batch was prepared containing no antimicrobial. The samples were transported to Kemin Industries, Des Moines, IA for ST enumeration.

**ST Inoculum preparation.** The five strains of ST used in this study were ATCC 13311 (human feces, food poisoning), ATCC 14028 (animal tissue), ATCC BAA-215 (human stool), ATCC BAA-1603 (tomato) and ATCC 700720 (wild type strain isolated from natural source). One hundred micro liters of each strain from the stock culture cryovials (stored at -80 °C) containing 10% glycerol was aseptically transferred to 10 ml tryptic soy broth (TSB) (Bacto, BD Biosciences, Sparks, MD) and incubated at 37 °C for 18-20 h followed by a second transfer of 100 µl into 10 ml of TSB and incubated aerobically at 37°C for 18-20 h with agitation at 100 rpm in a shaker incubator (Model 1000 mini shaker, Troemner LLC, NJ). Upon completion of the incubation period, cells were harvested by centrifugation (2,500 x g, 20 min at 21 °C) and suspended in 9 ml of Butterfield’s phosphate buffer (pH-7.2 ± 0.1). Approximately equivalent populations of each isolate were combined to provide a 5-strain mixture of ST. Populations of each strain and the mixture were verified by spread plating on XLT-4 agar (Difco, BD, Sparks, MD) and incubated at 37 °C for 24 h.

**ST inoculation and testing (method-1).** Ground pork treatments were formed into patties by hand and surface-inoculated with a five-strain mixture of ST to provide approximately 6-log CFU per 100 g package (equivalent to 4-log CFU per ml rinse material). For each package, a total 1 ml liquid inoculum was added to the ground pork (100 g), hand massaged for 1 min and vacuum-packaged (VFTC 420, MPBS Industries, Los Angeles, CA) in gas-impermeable pouches (3 mil high-barrier Nylon/EVOH/PE vacuum pouches, Clarity™, Bunzl Processor Division, Kansas City, MO) and stored at 4 °C. ST populations were enumerated on rinse material obtained after massaging the contents of each package for about 1 minute in 100 ml of sterile Butterfield phosphate buffer. ST
populations were determined on triplicate samples at 0, 1, 2, 3 and 4 weeks by surface plating serial (1:10) dilutions of rinse material on XLT-4 agar (37°C, 24 h).

**ST testing (method-2).** For method-2, ground pork treatments were formed into patties and vacuum-packaged (VFTC 420, MPBS Industries, Los Angeles, CA) in gas-impermeable pouches (3 mil high-barrier Nylon/EVOH/PE vacuum pouches, Clarity™, Bunzl Processor Division, Kansas City, MO) and stored at 4 °C. **ST** populations were enumerated on rinse material obtained after massaging the contents of each package for about 1 minute in 100 ml of sterile butterfield phosphate buffer. **ST** populations were determined on triplicate samples at 0, 1, 2, 3 and 4 weeks by surface plating serial (1:10) dilutions of rinse material on XLT-4 agar (37°C, 24 h).

**Propionic acid analysis.** Uninoculated single sample of each treatment was analyzed by gas chromatography method at Kemin Industries, Inc (Des Moines, IA).

**RESULTS**

**ST populations in method-1.** The initial populations of **ST** in all the treatments were as expected (4.13-4.40 log CFU/ml rinse). All the treatments showed a decline (Fig.1) in **ST** populations throughout the testing period. Untreated control showed a decline of 0.10 to 0.36 log CFU/ml rinse. 0.2% BP-6 showed a decline of 0.55 to 1.07 log CFU/ml rinse whereas; 0.2% BP-5 showed a decline of 0.29-0.83 log CFU/ml rinse.
**Figure 1.** Average change in *Salmonella* Typhimurium populations on inoculated ground pork (method-1) stored at 4 °C for 4 weeks. Ground pork was treated with 0.2% BP-6 liquid and 0.2% BP-5 liquid and then inoculated with *Salmonella* Typhimurium with a target of approximately 4 log cfu /100g and analyzed for *Salmonella* Typhimurium populations. Untreated, ground pork served as a negative control (three samples per testing interval in one replication, n=1).

**ST populations in method-2.** The initial populations of ST in all the treatments were 1 log less than expected (3.34-3.39 log CFU/ml rinse). All the treatments showed a decline (Fig.2) in ST populations throughout the testing period. Untreated control showed a decline of 0.03 to 0.45 log CFU/ml rinse. 0.2% BP-6 showed a decline of 0.47 to 1.04 log CFU/ml rinse whereas; 0.2% BP-5 showed a decline of 0.26-0.82 log CFU/ml rinse.
Figure 2. Average change in *Salmonella* Typhimurium populations on inoculated ground pork (method-2) stored at 4 °C for 4 weeks. Ground pork was inoculated with *Salmonella* Typhimurium with a target of approximately 4 log cfu/100g and later treated with 0.2% BP-6 liquid and 0.2% BP-5 liquid. The treatments were analyzed for *Salmonella* Typhimurium populations. Untreated, inoculated ground pork served as a negative control (three samples per testing interval in one replication, n=1).

Propionic acid results. The propionic acid content of the antimicrobial treatments was within the expected range (data not shown).

DISCUSSION

Propionic acid and propionate salts have attracted considerable attention by the meat industry in the past few years since its approval by FSIS in 2013, as a antimicrobial for ready-to-eat meat and poultry products. As the FSIS regulations to control *Salmonella* and *Campylobacter* in raw meat and poultry products become tighter and tighter, it is necessary to develop and validate new effective antimicrobial ingredients to strengthen food safety. The literature shows that propionate salts are effective in controlling *Listeria monocytogenes* in various RTE meat and poultry products. However, there is not enough research showing the efficacy of propionates against *Salmonella* spp in raw meat and poultry products. Hence, the current study was undertaken to evaluate the antimicrobial efficacy of propionic acid in ground pork by mimicking the real time ST contamination occurring during meat grinding processes i.e. usage of pre-ST contaminated ground pork trimmings. In method-1, the
antimicrobial was added followed by ST addition and in method-2, ST addition was done first followed by antimicrobial addition. However, in both methods of addition, a decline in ST populations was seen in the antimicrobial treatments as well as the untreated control throughout 4 weeks with the probable reason the low incubation temperature (4 °C) that might have reduced survival. Pradhan et al. (20) showed that when raw chicken breasts were inoculated with ST at 4-5 log CFU/g and stored for 3 weeks at 4 °C, there was no significant change (p>0.05) in ST populations, and they indicated ST is sensitive to refrigerated temperature. Bailey et al. (2) studied the microbiological profile of chilled and frozen chicken carcasses and reported that Salmonella positive carcasses (1.5 log) when stored at various frozen and refrigerated temperatures including at 4 °C for 2 weeks, did not show any change in Salmonella populations. Matches et al. (17) studied the growth of Salmonella spp on irradiated and non-irradiated seafoods stored at various temperatures and reported that temperatures below 8 °C (e.g. 5 or 6 °C) completely inhibited the growth of Salmonella populations. They hypothesized that the very low incidence of Salmonellosis due to seafood consumption could be partly attributed to low storage temperatures (below 8 °C). Statistical analysis was not done as the study was conducted in single replication. The propionic acid levels in both the antimicrobial treatments were within the expected range. Future ST challenge studies should be conducted at higher temperatures that are optimum for growth of ST in order to validate the antimicrobial efficacy of buffered propionic acid.

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CHAPTER 4. Efficacy of buffered propionic acid against *Salmonella* Typhimurium in ground pork stored at 4°C and 10°C

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ABSTRACT

The objectives of the study are to evaluate the antimicrobial efficacy of two buffered propionic acid (BP) formulations (BP-6 buffered to pH 6; BP-5 buffered to pH 5) against *Salmonella Typhimurium* (*ST*) in ground pork stored at 4 °C and 10 °C for 3 weeks and to determine the effects on spoilage microflora, color, cook loss and sensory characteristics on uninoculated pork patties stored at 4 °C for 3 weeks. *ST* inhibition results at 4 °C showed no significant differences (*p*=0.9948) in the microbial change of *ST* populations between untreated control and buffered propionic acid treatments. All the treatments showed a drop of 0.1-0.4 log CFU/ml rinse in *ST* populations by the end of three weeks with sporadic growth in a few samples treatments at 4°C. *ST* results at 10°C showed that buffered propionic acid treatments resulted in a drop of 0.3-1.4 log and 0.2-1.8 log CFU/ml rinse respectively by the end of 3 weeks whereas, *ST* population in untreated control declined after one week and no significant *ST* microbial differences (*p*=0.9982) were seen throughout the testing period. Lactic acid bacteria results showed that untreated control and antimicrobial treatments reached >7 log CFU/ml rinse by the end of 2 and 3 weeks. Aerobic plate counts of untreated control and the antimicrobial treatments reached 7-8 log and 6-7 log CFU/ml rinse respectively by the end of 3 weeks. pH results showed significant differences (*p*<0.05) between the treatments at 0 and 1 week but no significant differences were seen at the remaining test intervals. Statistical analysis of the instrumental *L**, *a**, and *b** values showed significant differences (*p*<0.05) between the treatments at few testing intervals. The *a** values declined, as the color of the ground pork changed from bright red to pale red/brown and significant differences (*p*<0.05) were seen between the treatments at 1 and 2 weeks. Cook loss results showed no significant differences (*p*=0.1517) between untreated and antimicrobial treatments. Overall sensory scores showed no significant differences between the treatments at week-0 whereas; significant differences (*p*<0.05) were seen between some of the treatments at week-1 and week-2. Overall, this study demonstrated
that 4 °C is not an optimum temperature for *ST* growth because both untreated and antimicrobial treatments restricted the growth of *ST* in ground pork. At 10 °C, it is not possible to compare the *ST* inhibition results between the untreated and antimicrobial treatments because no growth of *ST* was seen throughout three weeks in buffered propionic acid formulations whereas; in untreated control *ST* growth was inhibited by the outgrowth of spoilage and non *ST* populations after one week. Both antimicrobial formulations delayed the growth of lactic acid bacteria and aerobic bacteria by one week compared to untreated control without negatively impacting the sensory parameters of ground pork stored at 4 °C for 3 weeks.
Food safety continues to be of paramount importance to the food industry and regulatory agencies in the United States. In order to achieve the goal of a safer food supply, United States Department of Agriculture (USDA)’s Food Safety and Inspection Service (FSIS) has taken an aggressive enforcement approach to control foodborne pathogens such as *Salmonella*. In February 2016, FSIS announced the finalization of new federal standards to reduce *Salmonella* in ground chicken and turkey products and it is estimated that implementation of these standards will prevent an average of 50,000 illnesses annually (7). It was stated that the new federal standards fulfill the major steps outlined in the *Salmonella* action plan, a blueprint created by the FSIS to address this pathogen of significant public health concern (7). It is estimated that non-typhoidal *Salmonella* (NTS) causes approximately 1.2 million illnesses and 450 deaths annually in the United States and 5% of illnesses are attributed to consumption of pork products (4, 23). *Salmonella* spp. contamination of pork can occur at two stages i.e. pre-harvest stage i.e. animal production on the farm and post-harvest stage i.e. carcass processing and cross-contamination (1, 8). Pigs are healthy carriers of *Salmonella* spp and the most frequent serotype isolated from porcine meat is *Salmonella* Typhimurium (6, 15). Berends et al. (3) reported a strong correlation between the number of live pigs that carry *Salmonella* spp. in their faeces and the number of contaminated carcasses at the end of the slaughter line. They estimated about 70% of all carcass contamination results from the animals themselves being carriers, and 30% because of a cross-contamination from other positive carcasses.

Antimicrobial interventions are commonly used by the meat and poultry industry for controlling foodborne pathogens at various stages in production chain. Organic acids and their salts such as lactic acid, sodium lactate, acetic acid, sodium diacetate etc. are widely used antimicrobial interventions in raw and cooked meat and poultry products and their efficacy is well documented (12, 13, 14, 16, 17, 20). Propionic acid is a three carbon carboxylic acid which is listed as “Generally Recognized as Safe” by the U.S. Food and Drug Administration (FDA) and its efficacy against molds
is well documented (21, 25). Propionic acid and propionate salts are widely used in bread, baked goods, tortillas, pizza crust etc. Literature shows that propionic acid is also effective against bacterial pathogens such as *E. coli*, *P. aeruginosa*, *S. auerus*, *Salmonella* spp, *Listeria monocytogenes* etc. (5). In 2013, U.S. Department of Agriculture (USDA) approved the use of propionic acid, sodium propionate, and sodium benzoate as antimicrobials in ready-to-eat (RTE) meat and poultry products (24). There are several studies demonstrating the antimicrobial efficacy of propionate salts in various cooked meat and poultry products. Glass et al. (9, 10) showed that 0.2% propionate and 0.1% benzoate showed equal efficacy in controlling *Lm* when compared to a combination of 1.6% lactate with 0.1% diacetate in RTE ham stored at 4°C for 12 weeks. In RTE uncured turkey, 0.2% propionate showed equal efficacy in controlling *Lm* when compared to a combination of 3.2% lactate with 0.2% diacetate. In cured deli-style turkey liquid propionate or propionate-benzoate ingredients limited *Lm* growth to an increase of <1 log through 9 weeks storage at 4°C (11). However, it appears that there have been no studies on the antimicrobial effect of buffered propionic acid in raw meat and poultry products.

Efficacy studies are required in order to gain regulatory approval for propionic acid as antimicrobial for raw ground meat and poultry products and thus improving food safety. The objectives of this study were to evaluate the antimicrobial efficacy of two buffered propionic acid formulations on *Salmonella Typhimurium* (*ST*) inoculated in ground pork stored at 4 °C and 10 °C and determine its impact on spoilage microflora (lactic acid bacteria and aerobic plate counts), color, cook loss and sensory characteristics of pork patties stored at 4 °C. *ST* inoculation and testing on ground pork was conducted at two temperatures (4 °C and 10 °C) because 4 °C was found to be inhibitory for *ST* growth based on previous *ST* challenge study on ground pork (data not shown) and hence it was hypothesized that *ST* will grow at 10 °C.
MATERIALS AND METHODS

Production of ground pork treatments. Seven treatment formulations of ground pork were manufactured in the meat laboratory of Iowa State University, Ames, IA. Treatments included an untreated control, 0.1%, 0.2% and 0.3% BP-5 (Liquid buffered propionic acid described as propionic acid buffered with sodium hydroxide to a pH of 4.8-5.2 (BactoCEASE® Liquid; Kemin Industries, Des Moines, IA) and 0.1%, 0.2% and 0.3% BP-6 (Liquid buffered propionic acid described as propionic acid buffered with sodium hydroxide to a pH of 6.1-6.5 (BactoCEASE®-6 Liquid; Kemin Industries, Des Moines, IA). The concentration of propionic acid is equal in both formulations. Pork trims (approximately 80% lean and 20% fat content) were obtained from commercial pork processor and kept frozen (-17 °C) until use. The frozen pork trims were thawed out at 4 °C before grinding. For each treatment, pork trim (11 lbs) was coarse-ground through a 12 mm plate using an electric meat grinder (Biro MFG Co., Model 7.5 424852, Marblehead, OH). The coarse ground meat was mixed using a paddle mixer (DVTS 50, Dupey Equipment Co., Clive, IA) and appropriate dosage of BactoCEASE-6 or BactoCEASE was added drop wise using a transfer pipet on different locations with intermittent rotation of the ground meat and then mixed for three minutes to ensure even dispersion of the antimicrobial. After mixing, the product was fine ground through a 6.35 mm plate and vacuum packaged (UV 2100, Multivac, Inc., Kansas City, MO) in high barrier vacuum pouches. An untreated control was prepared containing no antimicrobial. The treatments were transported to storage facility under refrigerated conditions for inoculation and testing. The study was independently replicated three times by manufacturing the treatments on three different days.

Inoculum preparation. The five strains of Salmonella Typhimurium (ST) used in this study were ATCC 13311 (human feces, food poisoning), ATCC 14028 (animal tissue), ATCC BAA-215 (human stool), ATCC BAA-1603 (tomato) and ATCC 700720 (wild type strain isolated from natural source). One hundred micro liter of each strain from the stock culture cryovials (stored at -80 °C)
containing 10% glycerol was aseptically transferred to 10 ml tryptic soy broth (TSB) (Bacto, BD Biosciences, Sparks, MD) and incubated at 37 °C for 18-20 h followed by a second transfer of 100 µl into 10 ml of TSB and incubated aerobically at 37°C for 18-20 h with agitation at 100 rpm in a shaker incubator (Model 1000 mini shaker, Troemner LLC, NJ). Upon completion of incubation period, cells were harvested by centrifugation (2,500 x g, 20 min at 21 °C) and suspended in 9 ml of butterfields phosphate buffer (pH-7.2 ± 0.1). Approximately equivalent populations of each isolate were combined to provide a 5-strain mixture of ST. Populations of each strain and the mixture were verified on XLT-4 agar (Difco, BD, Sparks, MD) after incubated at 37 °C for 24 h.

**Inoculation and testing.** Ground pork treatments were made into patties and surface inoculated with five strain mixture of ST to provide approximately 5-log CFU (colony forming units) per 100 g package (equivalent to 3-log CFU per ml rinse material when using 100 ml of rinse for testing). For each package, a total 1 ml liquid inoculum was added to the ground pork patty (100 g) and hand massaged for 1 min and vacuum-packaged (VFTC 420, MPBS Industries, Los Angeles, CA) in gas-impermeable pouches (3 mil high barrier nylon vacuum pouch with a water vapor transmission rate of 10 g/L/m²/24 h at 37.8 °C and 100% relative humidity and an oxygen transmission rate of 3000 cm³/L/m²/24 h at 23 °C and 1 atm), and stored at 4 °C and 10 °C. ST populations were enumerated on rinse material obtained after massaging the contents of each package for about 1 minute in 100 ml of sterile butterfield phosphate buffer. ST populations were enumerated on triplicate samples by surface plating serial (1:10) dilutions of rinse material on XLT-4 agar (37 °C, 24 h) at 0, 1, 2, and 3 weeks. ST study at 10 °C was conducted only in two replications instead of three replications due to lack of sufficient samples. For plotting the results, the ST populations of each treatment at each storage point were averaged for three replications (4 °C) and two replications (10 °C), and the change in ST population level from the initial (time 0) sampling was determined.

**pH, lactic acid bacteria (LAB) and aerobic plate counts (APC).** Changes in pH and populations of natural microflora were evaluated on uninoculated samples to determine the effect of
the experimental treatments on the growth of spoilage microorganisms that may ultimately affect the growth of ST. The pH of pork patty from each treatment (Inlab Expert Pro ISM probe; S220, Mettler Toledo Inc, Columbus, OH) was measured on the slurry obtained by removing 10 g of the uninoculated sample and homogenizing with 90 ml deionized water using a blender (Stomacher 400, A.J.Seward, London, England). To enumerate LAB and APC populations, the remaining portions of the uninoculated samples were rinsed with sterile Butterfield phosphate buffer (quantity equal to the weight of the pork patty), and the serial dilutions of the rinse material was plated on All Purpose Tween agar (APT agar; Difco, BD, Sparks, MD) with 0.002% bromocresol purple (25 °C, 48-72 h) and Plate Count Agar (Difco, BD, Sparks, MD; 37 °C, 48 h), respectively. Lactic acid bacteria and mesophilic APC populations were enumerated at 0, 1, 2 and 3 weeks.

**Active ingredient analysis.** Uninoculated single sample of each treatment for each replication was analyzed by gas chromatography method to verify the propionic acid content.

**Instrumental color measurement.** For each treatment, two round patties (100 g each) were shaped by hand and placed onto 13.3 cm$^2$ expanded polystyrene meat trays (GENPAK 1S, Instawares, Edwardsville, KS) that were lined with meat tray pads (#341108NC, Instawares, Edwardsville, KS). The trays were covered with polyethylene cling wrap (Instawares, Edwardsville, KS) and stored in a cardboard box in refrigerator (4 °C) for 3 weeks. Commission Internationale de l’Eclairage (CIE) $L^*$, $a^*$, $b^*$ values (lightness, redness, yellowness, respectively) were measured on duplicate samples of each treatment every week using a Hunterlab ColorFlex® Colorimeter (Hunter Associates Laboratory; Reston, VA), with Illuminant D65, 10° standard observer, and 1.25” viewing area and port. Three random measurements were taken per patty, and the six readings were averaged for each treatment replicate.

**Sensory and cook loss.** Informal sensory evaluation on cooked patties was conducted with 5-8 untrained panelists who are familiar with sensory aspects of meat products treated with propionic acid at 0, 1 and 2 weeks during each replication (except replicate-1 where week-2 sensory could not be done due to lack of sufficient samples) on a 9 point hedonic scale to the nearest 1 point, where
1=dislike extremely, 2=dislike very much, 3=dislike moderately, 4=dislike slightly, 5=neither like nor dislike, 6=like slightly, 7=like moderately, 8=like very much, and 9=like extremely. The panelists were asked to evaluate the characteristics like flavor, texture, odor and color of the samples. Unsalted crackers and water were provided to panelists to cleanse their palate between samples. In order to avoid stress on the panelists four treatments were subjected to sensory on one day and three treatments on another day. The pork patties were cooked on a grill to an internal temperature of 165 °F and were blindly labeled and presented to 5 to 8 panelists. Cook loss was determined by weighing the patties before and after cooking.

**Statistical analysis.** The microbiological data was reported as average values and standard deviations (log CFU/ml rinse) for triplicate samples and three independent trials (n=3) for each test formulation at 4 °C and two independent trials (n=2) for each test formulation at 10 °C. Log differences between the antimicrobial treatments and the untreated control were analyzed by multifactor analysis of variance (ANOVA) using the STATGRAPHICS® Centurion XV software package (Statpoint Technologies, Inc; Warrenton, VA). Color, cook loss and sensory results were subjected to multifactor analysis of variance. Means were separated by using least significant differences (p<0.05). All statistically significant differences in the study were reported at p <0.05 level.

**RESULTS**

**Inhibition of Salmonella Typhimurium at 4 °C.** Untreated control (Fig.1) showed a drop of 0.30 ± 0.18 log CFU/ml rinse by the end of 3 weeks. 0.1%, 0.2% and 0.3% BP-6 treatments showed a drop of -0.37 ± 0.28, -0.27 ± 0.18 and -0.12 ± 0.12 log CFU/ml rinse respectively whereas, BP-5 at 0.1%, 0.2% and 0.3% showed a log change of -0.24 ± 0.26, -0.01 ± 0.11 and -0.12 ± 0.19 log CFU/ml rinse respectively by the end of 3 weeks. Statistical analysis of the log change in ST populations showed no significant differences (p=0.9948) between the untreated and antimicrobial treatments.
Inhibition of *Salmonella* Typhimurium at 10 °C. Untreated control (Fig. 2) showed a log change of $-0.15 \pm 0.22$ log CFU/ml rinse at the end of week-1 but no ST populations were seen at weeks 2 and 3 due to outgrowth of non ST populations. By the end of 3 weeks, 0.1%, 0.2% and 0.3% BP-6 treatments showed a drop of $-0.81 \pm 0.29$, $-0.76 \pm 0.22$ and $-0.94 \pm 0.32$ log CFU/ml rinse respectively whereas, BP-5 at 0.1%, 0.2% and 0.3% showed a drop of $-1.03 \pm 0.17$, $-0.84 \pm 0.41$ and $-0.94 \pm 0.60$ log CFU/ml rinse respectively. Statistical analysis of the log change in ST populations showed no significant differences ($p=0.9982$) between the antimicrobial treatments.

**Figure 1.** Average change in *Salmonella* Typhimurium populations on inoculated ground pork stored at 4 °C for 3 weeks. Ground pork was treated with 0.1%, 0.2% and 0.3% BP-6 liquid and 0.1%, 0.2% and 0.3% BP-5 liquid and then inoculated with *Salmonella* Typhimurium with a target of approximately 3 log colony forming units (CFU)/ml rinse and analyzed for *Salmonella* Typhimurium populations. Untreated, ground pork served as a negative control. Error bars represent the mean ± standard deviation of three replications (three samples per testing interval in three replications, n=3).
Figure 2. Average change in *Salmonella* Typhimurium populations on inoculated ground pork stored at 10 °C for 3 weeks. Ground pork was treated with 0.1%, 0.2% and 0.3% BP-6 liquid and 0.1%, 0.2% and 0.3% BP-5 liquid and then inoculated with *Salmonella* Typhimurium with a target of approximately 3 log colony forming units (CFU)/ml rinse and analyzed for *Salmonella* Typhimurium populations. Untreated, ground pork served as a negative control. Error bars represent the mean ± standard deviation of three replications (three samples per testing interval in two replications, n=2).

**Lactic acid bacteria counts and pH.** Lactic acid bacteria counts (Fig. 3) at 0-time for the untreated control and antimicrobial treatments ranged from 2.5-4.5 and 1.4-3.4 log CFU/ml rinse. At the end of 3 weeks, counts increased to 7.59 ± 0.54 log CFU/ml rinse for untreated control. 0.1%, 0.2% and 0.3% BP-6 showed 6.96 ± 0.46, 6.94 ± 0.43 and 6.81 ± 0.56 log CFU/ml rinse respectively. BP-5 at 0.1%, 0.2% and 0.3% showed 6.90 ± 0.52, 6.57 ± 0.35 and 6.30 ± 0.37 log CFU/ml rinse respectively. Significant differences (p<0.05) in pH results (Fig. 4) were seen between the treatments at 0 and 1 week. At weeks-0 and 1, untreated control differed significantly (p<0.05) with 0.2%, 0.3% BP-5 and 0.3% BP-6. No significant differences were seen between the treatments at 2 and 3 weeks.

**Aerobic plate counts.** The initial APC counts (Fig. 5) for the untreated was 3.26 ± 1.02 log CFU/ml rinse. By the end of 3 weeks, counts reached 7.08 ± 1.33 log CFU/ml rinse. Initial counts for the antimicrobial treatments ranged from 1.2-3.4 log CFU/ml rinse. By the end of 3 weeks, BP-6 at 0.1%, 0.2% and 0.3% showed 5.98 ± 1.97, 6.00 ± 1.91, and 5.86 ± 1.85 log CFU/ml rinse.
respectively. BP-5 at 0.1%, 0.2% and 0.3% showed 6.34 ± 1.32, 6.12 ± 1.02, and 5.73 ± 1.23 log CFU/ml rinse respectively. These results showed that buffered propionic acid treatments did not inhibit the growth of mesophilic aerobic bacteria but delayed the growth better than untreated control.

Figure 3. Average log counts of Lactic acid bacteria in uninoculated ground pork samples stored at 4°C for 3 weeks from three replications. Ground pork was treated with BP-6 at 0.1%, 0.2% and 0.3%; BP-5 at 0.1%, 0.2% and 0.3% and analyzed for Lactic acid bacteria counts. Ground pork without antimicrobial served as an untreated control. Error bars represent the mean ± standard deviation of three replications (two samples per testing interval in each replication, n=3)
Figure 4. pH results of uninoculated ground pork samples stored at 4°C for 3 weeks from three replications. Ground pork was treated with BP-6 at 0.1%, 0.2% and 0.3%; BP-5 at 0.1%, 0.2% and 0.3% and analyzed for pH. Ground pork without antimicrobial served as an untreated control. Error bars represent the mean ± standard deviation of three replications (two samples per testing interval in each replication, n=3).
Figure 5. Average log counts of Aerobic plate counts in uninoculated ground pork samples stored at 4°C for 3 weeks from three replications. Ground pork was treated with BactoCEASE-6 at 0.1%, 0.2% and 0.3%; BactoCEASE at 0.1%, 0.2% and 0.3% and analyzed for Aerobic plate counts. Ground pork without antimicrobial served as an untreated control. Error bars represent the mean ± standard deviation of three replications (two samples per testing interval in each replication, n=3)

Instrumental color. Instrumental color measurement results are shown in Tables 1-3. Statistical analysis of $L^*$ (lightness) values showed no significant differences between the treatments at week-0 and week-3 while significant ($p<0.05$) differences were seen between some of the treatments at week-1 and week-2. The $a^*$ (redness) values declined, as the color of the ground pork changed from bright red to pale red/brown and significant differences ($p<0.05$) were seen between the treatments throughout the testing period. No significant differences were seen in $b^*$ (yellowness) values across the testing period except at week-0 and week-1 significant differences ($p<0.05$) were seen between some of the treatments.
**Cook loss.** Statistical analysis of cook loss values (Table 4) showed no significant differences ($p=0.2360$) between the treatments.

**Sensory.** No significant differences were observed between the treatments (Table 5) at week-0. At week-1, significant differences ($p<0.05$) were seen between 0.1% BP-6 and 0.1% BP-5 with former having a higher score. At week-2, 0.1% BP-6 differed significantly with 0.3% BP-6 and 0.3% BP-5 by having lower score.

**Propionic acid.** The results of propionic acid in the buffered propionic acid treatments were in the expected range.

### Table 1. Instrumental color ($L^*$ values) of ground pork treatments

<table>
<thead>
<tr>
<th>Treatment</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated control</td>
<td>58.33&lt;sup&gt;a&lt;/sup&gt;</td>
<td>59.00&lt;sup&gt;a&lt;/sup&gt;</td>
<td>58.48&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>0.1% BP-6</td>
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<td>62.82&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>60.58&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>63.61&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>0.2% BP-6</td>
<td>59.67&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>64.14&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>66.04&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>0.1% BP-5</td>
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<td>66.87&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.48</td>
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<sup>a,b,c</sup> Within each column, means with different superscripts are significantly different ($p<0.05$)

### Table 2. Instrumental color ($a^*$ values) of ground pork treatments

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<th>Treatment</th>
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<th>2</th>
<th>3</th>
<th>SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated control</td>
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<td>8.30&lt;sup&gt;d&lt;/sup&gt;</td>
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<sup>a,b,c,d</sup> Within each column, means with different superscripts are significantly different ($p<0.05$)
Table 3. Instrumental color ($b^*$ values) of ground pork treatments

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<th>Treatment</th>
<th>Weeks (w)</th>
<th>SEM</th>
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<td></td>
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<tr>
<td>Untreated control</td>
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<td>16.34b</td>
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<td>15.46ab</td>
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a, b Within each column, means with different superscripts are significantly different ($p<0.05$)

Table 4. Cook loss (%) of ground pork treatments

<table>
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<th>Weeks (w)</th>
<th>SEM</th>
</tr>
</thead>
<tbody>
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<tr>
<td>Untreated control</td>
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<tr>
<td>0.1% BP-6</td>
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<td>0.2% BP-5</td>
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<td>0.3% BP-5</td>
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Table 5. Sensory scores of ground pork treatments

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<tr>
<td>Untreated control</td>
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<td>6.14ab</td>
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<td>0.1% BP-6</td>
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<td>6.76b</td>
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<td>0.2% BP-6</td>
<td>6.86a</td>
<td>6.33ab</td>
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<tr>
<td>0.3% BP-6</td>
<td>7.05a</td>
<td>6.33ab</td>
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<td>0.1% BP-5</td>
<td>6.28a</td>
<td>6.00a</td>
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<tr>
<td>0.3% BP-5</td>
<td>6.39a</td>
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DISCUSSION

Propionic acid and propionate salts have attracted considerable attention by the meat industry in the past few years since its approval by FSIS in 2013, as antimicrobial for ready-to-eat meat and poultry products. As the FSIS regulations to control Salmonella and Campylobacter in raw meat and poultry products become tighter and tighter, it is necessary to develop and validate new effective antimicrobial ingredients to strengthen food safety. Literature showed propionate salts are effective in controlling Listeria monocytogenes in various RTE meat and poultry products. However, there is not
enough literature showing the efficacy of propionates against *Salmonella* spp in raw meat and poultry products and hence the current study was undertaken to evaluate the antimicrobial efficacy of propionic acid in ground pork. The results from 3 reps, showed 4 °C is not an optimum temperature for growth of *ST* as no growth was seen in untreated control by the end of 3 weeks. These results were supported by previous studies evaluating the survival *Salmonella* in meats during low storage temperatures. Pradhan et al. (22) showed that when raw chicken breasts were inoculated with *ST* at 4-5 log CFU/g and stored for 3 weeks at 4 °C, did not show any significant change (*p*>0.05) in *ST* populations and they indicated *ST* is more sensitive to refrigerated temperature. Bailey et al. (2) studied the microbiological profile of chilled and frozen chicken carcasses and reported that *Salmonella* positive carcasses (1.5 log) when stored at various frozen and refrigerated temperatures including at 4 °C for 2 weeks, did not show any change in *Salmonella* populations. Matches et al. (18) studied the growth of *Salmonella* spp on irradiated and non-irradiated seafoods stored at various temperatures and reported that temperatures below 8 °C (e.g. 5 or 6 °C) completely inhibited the growth of *Salmonella* populations and they hypothesized that very low incidence of Salmonellosis due to seafood consumption could be partly attributed to low storage temperatures (below 8 °C). At 10 °C, BP-6 and BP-5 treatments showed a drop in *ST* populations throughout the testing period. By the end of 3 weeks, BP-6 and BP-5 showed a drop of 0.3-1.4 log and 0.2-1.8 log CFU/ml rinse respectively. *ST* population in untreated control declined after one week due to outgrowth of non-*ST* populations and no populations were seen at 2 and 3 weeks. Matches et al. (19) observed that at lower temperatures, psychrotrophic saprophytes can grow very rapidly, outcompeting *Salmonella* populations. The hypothesis in this study was *ST* will grow at 10 °C but it was not assumed that spoilage microflora will out compete *ST* populations in untreated control when stored at abused temperature like 10 °C. A method to knock out the competing spoilage microflora in untreated control would have helped in allowing *ST* growth but this was out of scope of this study. Hence, the antimicrobial efficacy of both antimicrobials against *ST* at 10 °C could not be compared with untreated control as *ST* populations were out competed by spoilage microflora.
Results of Lactic acid bacteria and aerobic plate counts showed that antimicrobial treatments extended the shelf life of ground pork by one week when stored at 4 °C. pH results showed that treating ground pork with BP-5 (0.2% and 0.3%) and BP-6 (0.3%) can result in a pH drop by 0.2 to 0.4 units whereas, no significant drop was seen with the remaining antimicrobial treatments. The color of the ground pork changed from initial bright red to pale red/brown during the storage for 3 weeks and significant differences were seen between the antimicrobial treatments and untreated control. This is predicted because buffered propionic acid does not help in color retention and antioxidants should be added for color protection which was out of scope of this study. Antimicrobial treatments showed no negative impact on sensory and cook loss of ground pork. The propionic acid levels in all the treatments were within the expected range. Overall, this study demonstrated that 4 °C is not an ideal temperature for ST growth as it completely inhibited ST in the untreated control. At 10 °C, both BP-6, and BP-5 treatments showed no growth of ST throughout three weeks but the results could not be compared with untreated control due to outgrowth of competing saprophytes which have a lower optimum growth temperature and can grow more rapidly by utilizing nutrients more effectively than ST (19). Additional research must be conducted to determine the efficacy of buffered propionic acid for controlling ST in other raw meat and poultry products.

**Acknowledgements**

We thank Mike Holtzbauer (ISU), Steve Bryant (ISU) and Jeff Mitchell (ISU) for their painstaking efforts in getting the ground pork treatments manufactured as per the requirements. This research was sponsored by the Kemin Industries Inc, Des Moines, IA.
REFERENCES

CHAPTER 5. Inhibition of *Listeria monocytogenes* by Buffered Dry Vinegar in Reduced-Sodium Ready-to-Eat Uncured Turkey stored at 4°C

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Key words: *Listeria monocytogenes*, vinegar, low sodium, turkey

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ABSTRACT

A reduced sodium ready-to-eat (RTE) uncured turkey was manufactured with buffered dry vinegar treatments to validate the inhibition of *Listeria monocytogenes* and spoilage microflora, and to determine the effects on sensory and quality attributes. Samples were stored at 4 °C for 12 weeks, and the study was independently replicated three times. Two different 5-strain inoculum of *L. monocytogenes* obtained from different sources were used for evaluating the efficacy of the buffered dry vinegar treatments. The results showed that 0.6% and 0.8% buffered dry vinegar sodium-based (BDV-SB) and potassium–based buffered dry vinegar (BDV-PB) at 0.7% and 0.9% controlled *L. monocytogenes* for 12 weeks. Untreated control product containing no buffered dry vinegar showed >1 log increase in *L. monocytogenes* populations counts at the end of 2 weeks. Statistical analysis confirmed that the dry vinegar treatments inhibited (*P*<0.05) the growth of *L. monocytogenes* compared to the untreated control. No significant differences (*P>*0.05) were seen in the inhibition of *L. monocytogenes* between the two different 5-strain inocula. Instrumental color results showed no significant differences between the treatments. Purge loss results showed no significant differences between the dry vinegar treatments but significant differences were seen between untreated control and dry vinegar treatments at few testing intervals. The overall results indicated that the dry vinegar ingredients (6.66 mM – 8.83 mM acetic acid in finished product) were effective in inhibiting *L. monocytogenes* obtained from multiple sources in reduced-sodium RTE uncured turkey stored at 4°C without adversely impacting the quality attributes.
INTRODUCTION

*Listeria monocytogenes* is one of the major foodborne pathogens that continue to be a serious threat to public health despite a decrease in number of cases annually (4, 20). Consuming food contaminated with *L. monocytogenes* can result in a serious infection which could lead to fetal loss in pregnant women, fatal to elderly and people with weakened immune systems (21, 22). *L. monocytogenes* is one of the top five pathogens contributing to domestically acquired foodborne illnesses resulting in death (20). Among selected categories of Ready-to-Eat (RTE) meat and poultry products, deli meats and frankfurters without antimicrobials pose the greatest per-serving risk of illness/death from *L. monocytogenes* because they are often consumed directly from the refrigerator without reheating (6, 21, 28). To inhibit the growth of *L. monocytogenes*, USDA-FSIS has approved a variety of antimicrobial agents that can be added to RTE meat and poultry products and among them, lactates and diacetate are widely used (6, 9, 15, 29). It is estimated that if all listeria-prone deli products were reformulated with a growth inhibitor that 96% of the predicted listeriosis illnesses associated with RTE products sold at the retail deli could be prevented (25).

Demand for natural and organic foods in the US is continuously increasing as evidenced by increasing sales of these products, which rose to $39.1 billion in 2014, and the organic market is experiencing double-digit growth of 11.3% (17). Research studies have shown preferences for natural and organic foods based on concerns about pesticides, antibiotics, hormones, genetic modifications, and chemical additives (13, 14). Hence, development of clean label ingredients (e.g. without chemical-sounding names, any ingredients that says artificial, ingredients that consumers cannot understand etc.) to inactivate *L. monocytogenes* and to inhibit its growth in RTE meats represents a high priority
for the meat industry. Another challenge faced by the processed meat industry is sodium reduction because high sodium intake results in increased blood pressure and a risk factor for cardiovascular disease \((1, 16)\). The term “reduced sodium” may be used if the individual food contains at least a 25 percent reduction in sodium as compared to an appropriate reference food \((26)\). In the past few years, the US food industry and the U.S. government has made many efforts to reduce the sodium content in processed foods \((7, 8, 23)\). In 2008, the New York City Department of Health and Mental Hygiene started a voluntary ‘National Salt Reduction Initiative’ (NSRI) with the overall goal of reducing dietary salt consumption by 20% over five years \((5, 10)\). To help the public reach this goal, the NSRI challenged food manufacturers to reduce the salt content of packaged and prepared foods by 25 % over the same period. They developed a database containing 62 packaged and 25 restaurant food categories that contributed to salt intake, and established targets for sodium content to be achieved by the end of 2012 and 2014 \((5)\). Lunch meats fell into one of the processed food categories that were targeted. Since March 2011, 28 major food manufacturers (e.g. Kraft Heinz Company, Unilever, Campbell Soups) and leading restaurant chains (e.g. Subway, Starbucks) have agreed to pursue salt reduction targets in one or more food categories \((5)\). In 2013, it was announced that 21 companies met one or more of their voluntary commitments to reduce sodium content in pre-packaged or restaurant foods \((2)\). While sodium chloride imparts flavor and texture to foods, it also plays a critical role in food safety by reducing water activity, thereby diminishing the growth of spoilage and pathogenic microorganisms \((1, 7)\). Hence, when developing low-sodium meats, precautions should be taken to avoid compromising on flavor, texture, shelf life, and safety.
Buffered vinegar has attracted considerable attention by the meat industry for inhibiting *L. monocytogenes* in RTE meat and poultry products. Non-buffered vinegar has limited usage in RTE meat and poultry products because of its low pH that could denature the meat proteins thereby impacting the water retention and textural characteristics (24). Buffering the vinegar using sodium or potassium based alkali raises the pH and creates minimal impact on the functional properties of the processed meat and poultry products. The advantage of using a potassium based buffer is it does not contribute sodium in the final food product but excess use can impart bitter or metallic taste. Also, when compared to sodium salt, potassium salt has to be used at higher application rate owing to its high molecular weight. The current study highlights the antimicrobial efficacy of two buffered dry vinegar-based ingredients; one with sodium-based (BDV-SB) and the other with potassium-based (BDV-PB). The objectives of this study were to validate the inhibition of *L. monocytogenes* (two 5-strain inoculum obtained from different sources) and spoilage microflora (aerobic mesophilic populations and lactic acid bacteria) on reduced sodium RTE uncured turkey manufactured with the two different dry vinegar ingredients, stored at 4 °C for up to 12 weeks, and to determine the effect of the dry vinegar treatments on quality attributes such as color and purge.

**MATERIALS AND METHODS**

**Production of sliced turkey treatments.** Seven treatment formulations of sliced, reduced-sodium, uncured, deli-style turkey breast were manufactured in the meat laboratory of Iowa State University, Ames, IA. Treatments included an untreated control, 0.4%, 0.6% and 0.8% BDV-SB, sodium-based buffered dry vinegar (Dry vinegar described as white distilled vinegar buffered with sodium carbonate, sodium bicarbonate, and/or sodium
hydroxide to a pH of 5.7-6.1, 67.2% acetic acid (BactoCEASE® NV Dry); Kemin Industries, Des Moines, IA) and 0.5%, 0.7% and 0.9% BDV-PB, potassium-based buffered dry vinegar (Dry vinegar described as white distilled vinegar buffered with potassium hydroxide to a pH of 5.7-6.1, 58% acetic acid (BactoCEASE® NVK Dry); Kemin Industries, Des Moines, IA). The application rates of the two dry vinegar ingredients were adjusted based on the actual acetic acid concentration to provide equivalent concentrations of acetic acid in the products. Turkey breasts were purchased from Turkey Valley Farms (Marshall, MN) and kept frozen until use. The turkey breasts were thawed at 4 °C for 3 days before use. Turkey breasts were coarse-ground (Biro MFG Co., Model 7.5 424852, Marblehead, OH) through a kidney plate and 10% of the coarse-ground product was subsequently fine-ground through a 0.3 cm plate. For each treatment, 10.2 kg of coarse-ground turkey and 1.1 kg of fine-ground turkey was used to achieve effective protein binding and adhesion. The ground turkey was enhanced to 40% of original weight by adding 4.5 kg of brine solution (Table 1) containing water, salt (1.4%), dextrose, sodium phosphate, potato starch, and dry vinegar and was tumbled under vacuum for 30 min (DVTS 50, Dupey Equipment Co., Clive, IA). After tumbling, the breast meat was stuffed (Risco vacuum stuffer, Model 1040C, Stoughton, MA) into plastic casings (15 cm diameter x 50 cm length) (Dupey Equipment Co., Clive, IA) and cooked in a smoke house (Thermal processor, Maurer-Atmos, Reichenau, Germany) using a three step process – 1 hour at 60 °C, 1 hour at 65.5 °C and finish until the internal temperature reached 75.5 °C (168 °F). After cooking, the turkey logs were transported to 4 °C cooler overnight and later casings were removed, the turkey logs were sliced (Bizerba, SE 12D, Piscataway, NJ), with the individual slices weighing approximately 25 g ± 0.5 g each. Four slices were then vacuum packaged (UV 2100, Multivac, Inc., Kansas City, MO) using high barrier vacuum
pouches [Cryovac Sealed Air Corporation, B2175, Duncan, SC; oxygen transmission rate of 3-6 cc at 4.4 °C (m², 24 hrs atm @ 4.4° C, 0% RD) and water vapor transmission rate of 0.5-0.6 g at 37.7 °C (100% RD, 650 cm², 24 hrs]. The desired concentration of salt in the final product was 1.4%. The sliced product was transported to Kemin Industries (Des Moines, IA) under refrigerated conditions for inoculation and testing. The study was independently replicated three times by manufacturing the treatments on three different days.

**Table 1.** Composition of brine solutions used to manufacture reduced-sodium, ready-to-eat deli-style turkey breast containing different levels of buffered dry vinegar

<table>
<thead>
<tr>
<th>Treatments a,b</th>
<th>Water (lb)</th>
<th>Salt (lb)</th>
<th>Dextrose (lb)</th>
<th>Sodium phosphate (lb)</th>
<th>Potato Starch (lb)</th>
<th>Buffered Dry Vinegar (lb)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated control</td>
<td>8.22</td>
<td>0.49</td>
<td>0.49</td>
<td>0.10</td>
<td>0.70</td>
<td>0.00</td>
</tr>
<tr>
<td>0.4% BDV-SB</td>
<td>8.08</td>
<td>0.49</td>
<td>0.49</td>
<td>0.10</td>
<td>0.70</td>
<td>0.14</td>
</tr>
<tr>
<td>0.6% BDV-SB</td>
<td>8.01</td>
<td>0.49</td>
<td>0.49</td>
<td>0.10</td>
<td>0.70</td>
<td>0.21</td>
</tr>
<tr>
<td>0.8% BDV-SB</td>
<td>7.94</td>
<td>0.49</td>
<td>0.49</td>
<td>0.10</td>
<td>0.70</td>
<td>0.28</td>
</tr>
<tr>
<td>0.5% BDV-PB</td>
<td>8.00</td>
<td>0.49</td>
<td>0.49</td>
<td>0.10</td>
<td>0.70</td>
<td>0.18</td>
</tr>
<tr>
<td>0.7% BDV-PB</td>
<td>7.97</td>
<td>0.49</td>
<td>0.49</td>
<td>0.10</td>
<td>0.70</td>
<td>0.25</td>
</tr>
<tr>
<td>0.9% BDV-PB</td>
<td>7.91</td>
<td>0.49</td>
<td>0.49</td>
<td>0.10</td>
<td>0.70</td>
<td>0.31</td>
</tr>
</tbody>
</table>

*BDV-SB and BDV-PB designate dry vinegar buffered with and without sodium-containing ingredients, respectively. Both buffered dry vinegar ingredients were supplied by Kemin Industries, Inc. (Des Moines, IA).*

*b Corresponding increasing concentrations of BDV-SB and BDV-PB ingredients provided equivalent acetic acid concentrations in the final RTE turkey breast product.*

**Inoculum preparation.** Two different 5-strain inoculum of *L. monocytogenes* were used in this study and inoculated on different sets of turkey samples separately, thus resulting in two parallel challenge studies for each replication. The purpose of using two different 5-
strain inoculum was to check if there is any difference in the antimicrobial efficacy of dry vinegar ingredients against different strains. Inoculum-1 consisted of LM 101 (hard salami isolate, serotype 4b), LM 108 (hard salami isolate, serotype 1/2a), LM 310 (goat’s milk cheese isolate, serotype 4), FSL-C1-109 (deli turkey isolate associated with illness, serotype 4b) and V7 (raw milk isolate, serotype 1). These strains were provided by Dr. Kathleen Glass (Food Research Institute, University of Wisconsin, Madison, WI). Inoculum -2 consisted of H7762 (frankfurter isolate, serotype 4b), H7764 (deli turkey isolate, serotype 1/2a), H7769 (serotype 4b), H7976 (source not known) and Scott A (clinical isolate, serotype 4b) and these strains were obtained from Dr. James Dickson (Dept. of Animal Science, Iowa State University Ames, IA). One hundred micro liters of each strain from the stock culture cryovials (stored at -80 °C) containing 10% glycerol was aseptically transferred to 10 ml tryptic soy broth (TSB;Bacto, BD, Sparks, MD) and incubated at 37 °C for 18-20 h. A transfer of the overnight culture was made by transferring 100µl into 10 ml of fresh TSB in an Erlenmeyer flask and incubated at 37 °C for 18-24 h. Cells were harvested by centrifugation (1,174 X g, 20 min at 21 °C) and suspended in 4.5 ml 0.1% buffered peptone water (pH 7.2). Approximately equivalent populations of each isolate were combined to provide a 5-strain mixture of *L. monocytogenes*. Populations of each strain and the mixture were verified by plating on trypticase soy agar (BBL, BD, Sparks, MD) and modified Oxford agar (Listeria Selective Agar base, Difco, BD, Sparks, MD).

**Inoculation and testing.** Uncured turkey was surface inoculated with *L. monocytogenes* Inoculum-1 or Inoculum-2 to provide approximately 5-log CFU per 100-g package (equivalent to 3-log CFU per ml of rinse material when using 100 ml rinse for testing). For each package containing 4 slices, a total of 1.005 ml of liquid inoculum was
added by distributing 0.335 ml over the surface of each slice excluding the top one, and slices were stacked such that the inoculum was between the slices. Inoculated products were vacuum packaged (C100 Multivac, Sepp Haggemuller KG, Wolfertschwenden, Germany) in gas-impermeable pouches (3 mil high barrier nylon vacuum pouch with a water vapor transmission rate of 10 g/L/m2/24 h at 37.8 °C and 100% relative humidity and an oxygen transmission rate of 3000 cm^3/L/m^2/24 h at 23 °C and 1 atm) and stored at 4 °C for up to 12 weeks. Triplicate inoculated samples for each treatment were assayed for changes in *L. monocytogenes* populations, and duplicate uninoculated samples were assayed for changes in lactic acid bacteria and pH at 0, 2, 4, 6, 7, 8, 9, 10, 11 and 12 weeks.

*L. monocytogenes* populations were determined in rinse material obtained after adding 100 ml of sterile Butterfield phosphate buffer to the package and massaging the contents externally by hand for approximately two minutes. Serial (1:10) dilutions of rinse material were spread plated on duplicate plates of modified Oxford agar and incubated at 37 °C for 48 h. The acceptance criterion for an effective dry vinegar treatment in this study was that it should not show >1 log increase in *L. monocytogenes* counts throughout the testing period. For plotting the results, the *L. monocytogenes* counts of each treatment at each storage point were averaged for three replications, and the change in *L. monocytogenes* population level from the initial (time 0) sampling was determined.

**pH, lactic acid bacteria (LAB) and aerobic plate counts (APC).** Changes in pH and populations of natural microflora were evaluated on uninoculated samples to determine the effect of the experimental treatments on the growth of spoilage microorganisms that may ultimately affect the growth of *L. monocytogenes*. The pH of turkey slices from each
treatment (Inlab Expert Pro ISM probe; S220, Mettler Toledo Inc, Columbus, OH) was measured on the slurry obtained by removing 10 g of the uninoculated sample and homogenizing with 90 ml deionized water using a blender (Stomacher 400, A.J. Seward, London, England). To enumerate LAB and APC populations, the remaining portions of the uninoculated samples were rinsed with sterile Butterfield phosphate buffer (quantity equal to the weight of the turkey slices), and the serial dilutions of the rinse material was plated on All Purpose Tween agar (APT agar; Difco, BD, Sparks, MD) with 0.002% bromocresol purple (25 °C, 48-72 h) and Plate Count Agar (Difco, BD, Sparks, MD; 37 °C, 48 h), respectively. Mesophilic APC populations were enumerated at 0, 4, 8 and 12 weeks.

**Proximate and active ingredient analysis.** Triplicate uninoculated samples of each treatment for each replication were analyzed at Kemin Industries, Inc for moisture (5 h, 100 °C, AOAC 950.46), water activity (Aqualab, Model series-3, Decagon Devices, Inc. Pullman, WA) and pH. Duplicate samples of each treatment for each replication were analyzed for protein (AOAC 990.03), fat (AOAC 960.39), and sodium content (ICP-AOAC-965.17/985.01 mod.) by Eurofins Scientific (Des Moines, IA). Acetic acid was analyzed by Gas Chromatography method at Kemin Industries, Inc for duplicate samples of each treatment at 0 week and 12 weeks for each replication.

**Instrumental color measurement.** Commission Internationale de l'Eclairage (CIE) $L^*$, $a^*$, $b^*$ values (lightness, redness, yellowness, respectively) were measured on each treatment using a Hunterlab ColorFlex® Colorimeter (Hunter Associates Laboratory; Reston, VA), with Illuminant D65, 10° standard observer, and 1.25” viewing area and port. Color was measured on duplicate uninoculated turkey samples for each treatment after removing the slices from the package at four different times post-processing (0, 4, 8, and 12 weeks).
**Purge loss (water holding capacity).** Purge loss was determined on duplicate samples of each treatment at four different times post-processing (0, 4, 8 and 12 weeks) by a weight difference method. Each pre-packaged treatment sample was measured to determine gross weight. The samples were removed from the package, blotted dry with paper towels for 10 seconds, and a net sample weight was recorded. The package was dried with a paper towel and reweighed to determine net packaging weight. Differences were calculated to determine percent purge loss as shown below:

\[
\text{Purge loss (\%)} = \left[\frac{(\text{Gross weight (with packaging)} - \text{packaging weight} - \text{sample weight})}{\text{Gross weight}}\right] \times 100.
\]

**Statistical analysis.** The microbiological data was reported as average values and standard deviations (log CFU/ml rinse) for triplicate samples and three independent trials (n=3) for each test formulation. Differences between the experimental treatments and the untreated control for each 5-strain inoculum as well as between the two 5-strain inoculum were analyzed by multifactor analysis of variance (ANOVA) using the STATGRAPHICS© Centurion XV software package (Statpoint Technologies, Inc; Warrenton, VA). Color and purge loss results were subjected to multifactor analysis of variance (ANOVA). All statistically significant differences in the study were reported at \( P < 0.05 \) level.

**RESULTS**

**Proximate and active ingredient results.** No appreciable differences were observed in the results for pH, moisture, water activity, fat and protein among the treatments (data not shown). The pH values of the treatments ranged from 6.27 ± 0.04 to 6.34 ± 0.06. Moisture contents ranged from 75.23 ± 0.96 to 75.93 ± 0.84%. Water activity of the
treatments ranged from 0.9796 ± 0.0027 to 0.9840 ± 0.0043. Fat and protein content ranged from 0.50 ± 0.07 to 0.62 ± 0.12% and 17.48 ± 0.74 to 18.33 ± 0.85% respectively. Sodium and potassium content (Table 2) were in the range of 0.62 ± 0.06 to 0.83 ± 0.04 and 0.25 ± 0.01 to 0.54 ± 0.02 respectively. The acetic acid results (Table 2) of the dry vinegar treatments were in the expected range of 0.27 to 0.54%

Table 2. Sodium, potassium and acetic acid results of reduced-sodium, ready-to-eat deli-style turkey breast containing different levels of buffered dry vinegar

<table>
<thead>
<tr>
<th>Treatment a,b</th>
<th>Sodium (%)</th>
<th>Potassium (%)</th>
<th>Acetic acid (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated control</td>
<td>0.66 ± 0.04</td>
<td>0.25 ± 0.01</td>
<td>0.02 ± 0.01</td>
</tr>
<tr>
<td>0.4% BDV-SB</td>
<td>0.74 ± 0.04</td>
<td>0.25 ± 0.01</td>
<td>0.30 ± 0.01</td>
</tr>
<tr>
<td>0.6% BDV-SB</td>
<td>0.77 ± 0.03</td>
<td>0.25 ± 0.01</td>
<td>0.43 ± 0.03</td>
</tr>
<tr>
<td>0.8% BDV-SB</td>
<td>0.83 ± 0.04</td>
<td>0.25 ± 0.01</td>
<td>0.57 ± 0.03</td>
</tr>
<tr>
<td>0.5% BDV-PB</td>
<td>0.62 ± 0.06</td>
<td>0.42 ± 0.01</td>
<td>0.35 ± 0.03</td>
</tr>
<tr>
<td>0.7% BDV-SB</td>
<td>0.63 ± 0.04</td>
<td>0.50 ± 0.02</td>
<td>0.49 ± 0.03</td>
</tr>
<tr>
<td>0.9% BDV-SB</td>
<td>0.63 ± 0.03</td>
<td>0.54 ± 0.02</td>
<td>0.52 ± 0.05</td>
</tr>
</tbody>
</table>

a BDV-SB and BDV-PB designate dry vinegar buffered with and without sodium-containing ingredients, respectively. Both buffered dry vinegar ingredients were supplied by Kemin Industries, Inc. (Des Moines, IA).

b Corresponding increasing concentrations of BDV-SB and BDV-PB ingredients provided equivalent acetic acid concentrations in the final RTE turkey breast product.

Inhibition of L. monocytogenes (5-strain inoculum-1 and 2). Results from three replications (Table 3) showed that all dry vinegar treatments significantly ($P < 0.05$) inhibited the growth of L. monocytogenes compared with the untreated control. The untreated control showed an average log increase of $1.11 ± 0.36$ and $3.00 ± 0.58$ log CFU/ml
rinse by the end of 2 and 4 weeks, respectively. The acceptance criterion for an effective dry vinegar treatment in this study was that it should not show >1 log increase in *L. monocytogenes* counts throughout the testing period. The 0.4% BDV-SB showed an average increase of 0.89 ± 1.07 log CFU/ml rinse at the end of 8 weeks. The 0.5% BDV-PB showed an average increase of 0.36 ± 0.65 log CFU/ml rinse at the end of 9 weeks. The higher application levels of BDV-SB (0.6% and 0.8%) and BDV-PB (0.7% and 0.9%) consistently showed <1 log increase in *L. monocytogenes* counts throughout the testing period. No significant differences were seen between 0.6% BDV-SB and 0.7% BDV-PB as well as 0.8% BDV-SB and 0.9% BDV-PB thus indicating no difference in the antimicrobial activity between sodium and potassium salts of dry vinegar.
Table 3. Pooled average change in *Listeria monocytogenes* (5-strain inoculum -1 and 2) levels on inoculated reduced-sodium, uncured turkey breast stored at 4 °C for 12 weeks. RTE turkey breast formulated with BDV-SB (0.4, 0.6 and 0.8%) or BDV-PB (0.5, 0.7, and 0.9%) was inoculated with *Listeria monocytogenes* to a target of 3 log CFU/ml rinse. Untreated, inoculated RTE turkey breast served as a negative control. Changes in *L. monocytogenes* population levels were determined during vacuum-packaged storage. Error bars represent the mean ± standard deviation of three replications (three samples per testing interval in each replication, n=3)\(^1,2\)

<table>
<thead>
<tr>
<th>Week</th>
<th>Untreated</th>
<th>0.4% BDV-SB</th>
<th>0.6% BDV-SB</th>
<th>0.8% BDV-SB</th>
<th>0.5% BDV-PB</th>
<th>0.7% BDV-PB</th>
<th>0.9% BDV-PB</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0 ± 0</td>
<td>0 ± 0</td>
<td>0 ± 0</td>
<td>0 ± 0</td>
<td>0 ± 0</td>
<td>0 ± 0</td>
<td>0 ± 0</td>
</tr>
<tr>
<td>2</td>
<td>1.11 ± 0.36(^a)</td>
<td>0.01 ± 0.37(^b)</td>
<td>-0.13 ± 0.24(^b)</td>
<td>-0.06 ± 0.15(^b)</td>
<td>-0.02 ± 0.11(^b)</td>
<td>-0.05 ± 0.14(^b)</td>
<td>-0.27 ± 0.13(^b)</td>
</tr>
<tr>
<td>4</td>
<td>3.00 ± 0.58(^a)</td>
<td>-0.19 ± 0.22(^b)</td>
<td>-0.22 ± 0.29(^b)</td>
<td>-0.36 ± 0.23(^b)</td>
<td>-0.12 ± 0.21(^b)</td>
<td>-0.18 ± 0.31(^b)</td>
<td>-0.42 ± 0.36(^b)</td>
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<tr>
<td>6</td>
<td>4.88 ± 0.73(^a)</td>
<td>0.43 ± 0.59(^b)</td>
<td>-0.24 ± 0.32(^b)</td>
<td>-0.40 ± 0.14(^c)</td>
<td>-0.03 ± 0.26(^c)</td>
<td>-0.35 ± 0.15(^c)</td>
<td>-0.54 ± 0.14(^c)</td>
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<tr>
<td>7</td>
<td>5.36 ± 0.60(^a)</td>
<td>0.33 ± 0.49(^b)</td>
<td>-0.19 ± 0.33(^b)</td>
<td>-0.35 ± 0.20(^c)</td>
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<td>-0.60 ± 0.28(^c)</td>
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<td>8</td>
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<td>0.89 ± 1.07(^b)</td>
<td>-0.05 ± 0.61(^c)</td>
<td>-0.37 ± 0.15(^c)</td>
<td>0.35 ± 0.50(^c)</td>
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<td>9</td>
<td>5.75 ± 0.19(^a)</td>
<td>1.37 ± 1.30(^b)</td>
<td>-0.20 ± 0.34(^c,d)</td>
<td>-0.59 ± 0.30(^c,d)</td>
<td>0.36 ± 0.65(^c)</td>
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<td>10</td>
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<td>0.05 ± 0.55(^c,d)</td>
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<tr>
<td>11</td>
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<td>1.37 ± 1.42(^b)</td>
<td>-0.02 ± 0.64(^c,d)</td>
<td>-0.58 ± 0.24(^d)</td>
<td>0.42 ± 0.89(^b,c)</td>
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<td>-0.73 ± 0.19(^d)</td>
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<tr>
<td>12</td>
<td>5.92 ± 0.05(^a)</td>
<td>1.89 ± 1.84(^b)</td>
<td>0.08 ± 0.77(^c,d)</td>
<td>-0.51 ± 0.25(^c,d)</td>
<td>0.71 ± 1.00(^c)</td>
<td>-0.54 ± 0.32(^d)</td>
<td>-0.84 ± 0.32(^d)</td>
</tr>
</tbody>
</table>

\(^a,b\) Within each row, means with different superscripts are significantly different (p<0.05)

1 BDV-SB and BDV-PB designate dry vinegar buffered with and without sodium-containing ingredients, respectively. Both buffered dry vinegar ingredients were supplied by Kemin Industries, Inc. (Des Moines, IA).

2 Corresponding increasing concentrations of BDV-SB and BDV-PB ingredients provided equivalent acetic acid concentrations in the final RTE turkey breast product.
Lactic acid bacteria counts and pH. Lactic acid bacteria counts (Fig. 1) at 0-time for all the treatments were less than levels detectable by direct plating (<1 log CFU/ml rinse). At the end of 12 weeks, counts increased to 8.54 ± 0.47 log CFU/ml rinse for untreated control samples and significant differences were seen compared to dry vinegar treatments. The 0.4%, 0.6% and 0.8% BDV-SB showed 5.53 ± 3.75, 2.94 ± 3.16 and 4.22 ± 3.26 log CFU/ml rinse, respectively, and no significant differences were seen among the three treatments. The BDV-PB treatments at 0.5%, 0.7% and 0.9% showed 5.10 ± 3.62, 4.07 ± 3.30 and 4.46 ± 3.33 log CFU/ml rinse, respectively, and no significant differences were seen among the three treatments. The BDV-SB treatments at 0.6% and 0.8% differed significantly compared to the 0.5% and 0.9% BDV-PB treatments. No significant differences were seen between 0.4% BDV-SB and 0.7% BDV-PB treatments. These results showed that both dry vinegar ingredients did not inhibit the spoilage microbes. The pH results (Fig. 2) of the dry vinegar treatments showed no significant differences except 0.4% BDV-SB was significantly lower than the other dry vinegar treatments at week-12; whereas, untreated control samples were significantly lower than dry vinegar treatments from 8-12 weeks.
Figure 1. Average log populations of lactic acid bacteria in uninoculated, uncured RTE turkey breast samples stored at 4°C for 12 weeks. RTE turkey breast products formulated with BDV-SB (0.4, 0.6 and 0.8%) and BDV-PB (0.5, 0.7 and 0.9%) were analyzed for lactic acid bacteria levels during storage. Untreated turkey breast samples served as a negative control. Error bars represent the mean ± standard deviation of three replications (two samples per testing interval in each replication, n=3).
**Figure 2.** pH determinations of uninoculated, uncured, RTE turkey breast samples stored at 4°C for 12 weeks. Turkey breast samples were formulated with BDV-SB (0.4, 0.6, and 0.8%) or BDV-PB (0.5, 0.7, and 0.9%) and analyzed for pH. Turkey breast without dry vinegar ingredients served as an untreated control. Error bars represent the mean ± standard deviation of three replications (two samples per testing interval in each replication, n=3).

**Aerobic plate counts.** The initial mesophilic aerobic count (Fig. 3) for the untreated control slices was 1.69 ± 1.07 log CFU/ml rinse. By the end of 12 weeks, counts reached 7.43 ± 0.63 log CFU/ml rinse. The APC for untreated control samples differed significantly with the vinegar treatments across all the testing intervals. Initial counts for the dry vinegar treatments were <1 log CFU/ml rinse. By the end of 12 weeks, BDV-SB at 0.4%, 0.6% and 0.8% showed 3.73 ± 2.40, 1.63 ± 1.41, and 2.20 ± 1.91 log CFU/ml rinse respectively. BDV-PB at 0.5%, 0.7% and 0.9% showed 1.56 ± 0.61, 1.70 ± 1.00, and 2.90 ± 1.76 log CFU/ml rinse respectively. No significant differences in APC were seen among the dry
vinegar treatments across all the testing intervals except 0.4% BDV-SB, which was higher than the other dry vinegar treatments at week-12. These results showed that dry vinegar treatments did not inhibit the growth of mesophilic aerobic bacteria but delayed the growth better than untreated control.

**Figure 3.** Average log populations of mesophilic aerobic populations in uninoculated, uncured RTE turkey breast samples stored at 4°C for 12 weeks. Turkey breast products were formulated using BDV-SB (0.4, 0.6 and 0.8%) and BDV-PB (0.5, 0.7 and 0.9%) and analyzed for aerobic plate counts throughout storage. Turkey breast formulated with no dry vinegar ingredients served as an untreated control. Error bars represent the mean ± standard deviation of three replications (two samples per testing interval in each replication, n=3).

**Instrumental color.** No differences ($P > 0.05$) were observed for $L^*$ (76.81-78.68), $a^*$ (1.57-2.83) and $b^*$ (11.93-14.60) values among the 7 treatments.

**Purge loss.** Purge loss values ranged from 1.4 – 5.4%. Statistical analysis of purge loss values showed no significant differences among the dry vinegar treatments but significant differences were seen between untreated and few dry vinegar treatments. There were no significant differences among untreated, 0.4% and 0.8% BDV-SB treatments.
whereas significant \((P < 0.05)\) differences were observed between untreated and the remaining dry vinegar treatments at weeks 0 and 8. The 0.9% BDV-PB treatment resulted in significantly \((P < 0.05)\) greater purge when compared to 0.4% and 0.6% BDV-SB and the untreated control at week 8.

**DISCUSSION**

Vinegar has been used for centuries for a variety of purposes and has well documented antimicrobial properties \((3)\). Although there are no standards of identity for vinegar, FDA guidelines indicate that natural vinegars normally contain in excess of 4 grams of acetic acid per 100 ml \((27)\). The low pH of vinegar \((2.0-3.0)\) is a limiting factor for its application in RTE meat and poultry products as it can negatively affect physical and sensory characteristics. The advantages of buffering and drying the vinegar are three-fold – it reduces the pungent vinegar flavor to a mild vinegar flavor, it has less negative impact on the taste and flavor of the treated finished product and it can be used at lower application rates due to a more concentrated acetic acid. This study demonstrated that BDV-SB at 0.6% and 0.8%, and BDV-PB at 0.7% and 0.9% controlled \(L.\) monocytogenes for 12 weeks in reduced-sodium RTE uncured turkey breast \((\text{approximately 76}\% \text{ moisture, pH 6.30 and 0.66}\% \text{ sodium})\) stored at 4°C. Lavieri et al. \((11, 12)\) reported dried vinegar as a potential bacteriostatic ingredient for inhibiting the growth of \(L.\) monocytogenes inoculated into alternatively-cured frankfurters and alternatively cured ready-to-eat ham. Their research showed that inclusion of 1% dried vinegar when formulating both of these meat products prevented the growth of \(L.\) monocytogenes for 14 weeks when stored at 4 ± 1°C. However, dried vinegar did not exhibit any bactericidal properties against \(L.\) monocytogenes in their
studies. Porto-Fett et al. (18) showed no change in *L. monocytogenes* population in deli-style ham formulated with 1.5% buffered vinegar, with or without a stabilized solution of sodium chlorite, for up to 90 days of storage at 4 °C; whereas, 2.0 or 2.5% buffered vinegar reduced pathogen counts by 1.1 and 2.0 log CFU/slice respectively. Roast beef formulated with 1.0 or 1.5% buffered vinegar showed an increase of 2.2 to 2.4 log CFU/slice but they also found that roast beef formulated with 2.0 or 2.5% buffered vinegar decreased *L. monocytogenes* counts by 0.7 and 1.2 log CFU/slice, respectively, when stored for 90 days at 4°C. In another *Listeria* challenge study on uncured turkey breast formulated with 3.0% buffered vinegar and surface treated with or without a stabilized solution of sodium chlorite in vinegar, Porto-Fett et al. (19) observed counts decrease by approximately 0.7 to 1.3 log CFU/slice, respectively, when stored at 4°C for 90 days. However, when stored at 10°C, pathogen numbers increased by approximately 1.5 to 5.6 log CFU/slice after 48 days when formulated with 2.0 to 3.0% buffered vinegar and treated with or without 2% sodium chlorite in vinegar. McDonnell et al. (15) reported that 2.0% liquid buffered vinegar in sliced, uncured, deli-style turkey breast, alternative-cured boneless ham, and uncured roast beef delayed the growth of *L. monocytogenes* until 6, 6 and 12 weeks of storage at 4°C, respectively. The authors speculated that significant inhibition of pathogen growth in roast beef compared to the turkey breast and boneless ham could be due to differences in pH and moisture content of the products. It is to be noted that liquid buffered vinegar has a lower concentration of acetic acid when compared to dry vinegar and hence higher application rates were used for liquid buffered vinegar.

In the current study, dry vinegar with sodium (BDV-SB) and potassium-based dry vinegar (BDV-PB) showed similar antimicrobial efficacy against the two 5-strain inoculum
of *L. monocytogenes* used for the challenge study. Variations were seen in pH and spoilage microflora among the three replications and this could be due to differences in the raw material quality and processing conditions such as slicing. In spite of differences in the growth of spoilage microflora across the replications, the dry vinegar treatments showed consistent inhibition of *L.monocytogenes* indicating their robustness in antimicrobial efficacy. To our knowledge, there is no published literature showing the antimicrobial efficacy of potassium-based dry vinegar, thus this finding could be of significant importance for enhancing the safety of low sodium/reduced sodium RTE uncured turkey breast. Additional research must be conducted to determine the efficacy of these dry vinegar-based ingredients for controlling *L.monocytogenes* in a broader range of RTE products, and also the impact of slightly to moderately higher storage temperatures, as the current data will not be sufficient for validating this technology in other RTE products.

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REFERENCES


CHAPTER 6
GENERAL CONCLUSIONS AND FUTURE RESEARCH

Salmonella Typhimurium and Listeria monocytogenes continue to be serious threats to public health despite a decrease in number of cases annually. Salmonella Typhimurium is a pathogen of concern particularly in raw meat and poultry products whereas, Listeria monocytogenes is a cause for concern in ready-to-eat meat and poultry products. Recently, United States Department of Agriculture (USDA)’s Food Safety and Inspection Service (FSIS) has taken an aggressive enforcement approach to control Salmonella spp. in raw meat and poultry products and this created a significant challenge to meat processors to comply with the recent regulations. In order to address these challenges, there is a need to develop and validate new effective antimicrobial ingredients to inhibit or kill Salmonella spp and ensure safe products for consumers. In 2013, USDA approved the use of propionic acid in ready-to-eat (RTE) meat and poultry products but not in raw meat and poultry products. Efficacy studies are required in order to gain regulatory approval for propionic acid as antimicrobial for raw meats.

Hence, two research studies were undertaken to evaluate the antimicrobial efficacy of buffered propionic acid in ground pork. Two buffered propionic acid formulations (BP-5; pH-5 and BP-6; pH-6) were evaluated in the current studies. The first research study in ground pork showed that there was no difference at 4 °C in the growth of Salmonella Typhimurium added to ground pork before or after the addition of buffered propionic acid. However, 4 °C is not an ideal temperature for growth of Salmonella Typhimurium because no growth was seen in untreated controls without the antimicrobial. Based on these results, we hypothesized that abuse temperatures like 10 °C should be used to evaluate the growth of
Salmonella Typhimurium. In the second study, Salmonella Typhimurium results at 10 °C showed that buffered propionic acid treatments resulted in a decline of 0.3-1.4 log and 0.2-1.8 log CFU/ml rinse respectively by the end of 3 weeks. However, Salmonella Typhimurium population in untreated controls also declined after one week due to outgrowth of non-ST populations and this was not expected. A method to inhibit the competing spoilage microflora in untreated control would have helped in allowing Salmonella Typhimurium growth but this was beyond the scope of the current study. Hence, the antimicrobial efficacy of buffered propionic acid formulations against Salmonella Typhimurium at 10 °C could not be compared with untreated control as Salmonella Typhimurium populations were outcompeted by spoilage microflora. Additional validation studies have to be conducted to evaluate the antimicrobial efficacy of buffered propionic acid formulations by storing raw ground meat at temperatures optimal for Salmonella spp growth such as above 20 °C, and also appropriate methods have to be used to inhibit the competing microflora in untreated controls.

The third research project was undertaken to address two challenges faced by ready-to-meat processors i.e. need for natural antimicrobials for controlling Listeria monocytogenes and sodium reduction in luncheon meats. Two buffered dry vinegar ingredients (sodium based and potassium based) were evaluated for their anti listerial efficacy in reduced sodium deli-style turkey. Two different 5-strain inoculum of L. monocytogenes obtained from different sources were used for evaluating the efficacy and it was hypothesized that there should be no difference in the antimicrobial efficacy against different strains of L. monocytogenes. The results showed that 0.6% and 0.8% buffered dry vinegar sodium-based and potassium–based buffered dry vinegar at 0.7% and 0.9% controlled Listeria
monocytogenes for 12 weeks. No significant differences ($P > 0.05$) were seen in the inhibition of *L. monocytogenes* between the two different 5-strain inocula. The overall results indicated that the dry vinegar ingredients were effective in inhibiting *L. monocytogenes* obtained from multiple sources in reduced-sodium RTE uncured turkey stored at $4^\circ$C without adversely impacting the quality attributes. Currently, there has been no published literature showing the antimicrobial efficacy of potassium-based dry vinegar, thus this finding is of significant importance for enhancing the safety of low sodium/reduced sodium RTE uncured turkey breast. Additional validation studies have to be conducted to determine the efficacy of these dry vinegar-based ingredients for controlling *L. monocytogenes* in wide variety of RTE products, and also to assess the impact of slightly-to-moderately higher storage temperatures, as the current data is not be sufficient for validating this technology in other RTE products.
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