Evaluation of Lactic Acid and Sodium Metasilicate against Pathogens of Concern on Fresh and Deli Meats

by

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Abstract

Lactic acid and sodium metasilicate have been used in meat processing facilities as antimicrobial compounds. Their uses vary from carcasses rinses to ready to eat product applications at a variety of concentrations and temperatures. Utilizing these antimicrobials in different stages during meat processing may assist in the reduction of the risk of pathogenic microorganisms. The purpose of this study was to determine optimum concentrations and temperatures of application of lactic acid and sodium metasilicate for pathogen reduction on beef bottom round muscles. Using this information, optimal concentrations and temperatures were then applied to fresh pork and processed deli meats in addition to fresh beef. Four consecutive studies were conducted. In the first study, lactic acid (LA) was applied at 1, 2, 3, and 4% (LA1, LA2, LA3, and LA4) and sodium metasilicate (SM) was applied to fresh beef bottom rounds at 2, 3, 4, and 5% (SM2, SM3, SM4, and SM5) levels. In the next study, LA4 (v/v), SM4 (w/v), the combination of the two solutions (LASM), and distilled water (control) were applied to fresh beef bottom rounds at 4, 25, and 60 °C. During the third study, LA4, SM4, and distilled water were applied to fresh beef and pork lean muscle and roast beef, ham, and turkey deli meats that were manufactured at the Lambert Powell Meat Laboratory without the use of antimicrobial solutions. All data were analyzed using the PROC MIXED procedure of SAS and Tukey pairwise comparisons, where appropriate ($P > 0.05$). LA and SM reduced ($P < 0.05$) the bacterial load of all the meat samples. Temperature of

application had no effect ($P > 0.05$) on bacterial counts in any of the treatments. LA or SM alone were more effective ($P < 0.05$) in reduction of microbes than when used together (LASM). The control treatment resulted in higher microbial counts regardless of inoculum or species than either the LA or SM treatments ($P < 0.01$). Treatments including a hot water dip decreased the bacterial load of samples in comparison to those that did not receive the post packaging lethality treatment ($P < 0.01$). Regardless of hot water dip treatments, there were no differences among treatment groups in regards to microbial counts ($P > 0.73$). SM4 and LA4 were determined to be the lowest concentrations most effective against all microorganisms. Meat processors can apply LA or SM at refrigeration temperatures with the same benefits as applying them at a higher temperature. Both lactic acid and sodium metasilicate can be applied to fresh beef and pork as an effective hurdle technology in the fight for food safety. Treating deli meats with lactic acid or sodium metasilicate did not reduce *L. monocytogenes* loads. However, adding a post-packaging lethality treatment was able to minimize overall microbial contamination.

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It is bittersweet to be ready to start the next chapter of my life. Thank you to everyone for making Auburn home.

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Chapter I

Review of Literature

Food Safety

Food safety is a constant concern in the meat industry and consideration is given to methods of ensuring a safe food supply by reduction of pathogens. The Centers for Disease Control and Prevention (CDC) estimates that 1 in 6 Americans become ill each year due to foodborne illness. That is equivalent to approximately 48 million people. Of these, 128,000 are hospitalized and about 3,000 die of foodborne diseases (Weber, O'Brien, & Bender, 2004). Regulatory agencies, researchers, and employees in the meat industry mainly focus on *Escherichia coli* O157:H7 and non-O157 shiga-toxin producing *E. coli* (STEC) serotypes and *Salmonella* spp. in fresh meat. In ready to eat meats *Listeria monocytogenes* is the common pathogen of concern. While *Salmonella* spp., *E. coli*, and *L. monocytogenes* are common pathogens on the "top five foodborne pathogens" lists compiled by the CDC (Weber, O'Brien, & Bender, 2004). The United States Department of Agriculture Food Safety and Inspection Service (USDA FSIS) has a zero tolerance policy for *L. monocytogenes* in ready-to-eat meat and poultry products. This rule requires meat processors to control *L. monocytogenes* by using one of three alternatives. Alternative 1 includes both a post-lethality treatment and a process or antimicrobial agent, Alternative 2 requires either a post-lethality treatment or a process or antimicrobial agent, and Alternative 3 requires the implementation of sanitation procedures and frequent USDA FSIS environmental testing (USDA, 2003). More recently, the USDA

FSIS expanded its ruling on *E. coli* O157 in raw, non-intact beef to include six non-O157 serotypes including: O26, O45, O103, O111, O121, and O145 (USDA 2011).

Intrinsic Factors Affecting Microorganisms

Water Activity

Microorganisms need water to grow and thrive in food products. The water activity (a_w) of foods is normally how this water described (FDA, 2013). The ratio of water vapor pressure of the food substrate to the vapor pressure of pure water at the same temperature is defined as water activity (Jay, Loessner, & Golden, 2006). Thus, a_w describes how much water is "unbound" and available for chemical/biochemical reactions and microbial growth facilitation (FDA, 2013). Pure water has an a_w of 1.0 and most fresh foods have an a_w of > 0.98 (Nester, Anderson, Roberts, Pearsall, & Nester, 2001). The a_w in foods can be lowered with the addition of salts and sugars, binding the unbound water, or by physically removing the unbound water through drying, baking, or cooking the food (FDA, 2013). Most microorganisms require an $a_w > 0.90$ (Nester, Anderson, Roberts, Pearsall, & Nester, 2001); however, the taxonomic classification of the microorganism can indicate how sensitive the bacteria will be to a_w changes (FDA, 2013). Gram negative bacteria are usually more sensitive to low a_w than Gram positive microorganisms (FDA, 2013). Selected pathogen a_w requirements are listed in Table 1. Small changes in a_w can have differential effects on bacterial growth (Gill & Newton, 1976). Most fresh meats have an a_w of 0.99 – 1.00, while cured meats generally have an a_w of 0.87 – 0.95 (FDA, 2013).

pH

The pH of food can affect what microorganisms can survive on the food surface or within the food matrix (Nester, Anderson, Roberts, Pearsall, & Nester, 2001). Pathogens generally do not grow at pH levels below 4.6; however, there are some exceptions (FDA, 2013). Ground beef has a typical pH of 5.1 – 6.1 and ham generally has a pH of 5.9 – 6.1 (FDA, 2013). Normal meat pH is between 5.5 and 5.7; however, there can be differences in pH between carcasses and between different muscles from the same carcass (Gill & Newton, 1978). Table 1 includes approximate pH values allowing the growth of selected pathogens in food. Increasing the acidity of foods either through fermentation or the use of weak acids has been used as a food preservation method since ancient times (FDA, 2013). Meats can resist pH changes better than other foods such as vegetables because of the buffering ability of meat (Jay, Loessner, & Golden, 2006). The protein in meat contributes to the buffering capacity of the meat (Jay, Loessner, & Golden, 2006).

Nutrient Availability

Bacteria need five nutrients in order to grown and function normally: water, a source of energy, a source of nitrogen, vitamins, and minerals (Jay, Loessner, & Golden, 2006). An organism that requires a vitamin that the organism cannot synthesize will not grow (Nester, Anderson, Roberts, Pearsall, & Nester, 2001). Nutrients vary among foods. For example, meats have high levels of proteins, lipids, minerals, and vitamins with low levels of carbohydrates; most vegetables are high in carbohydrates, but have varying levels of proteins, vitamins, and minerals (FDA, 2013). In general, Gram positive

microorganism have more stringent nutrient requirements than Gram negative microorganism as Gram positive microbes are less likely to be able to synthesize required nutrients not in the environment (Jay, Loessner, & Golden, 2006). Gram negative bacteria are more likely to receive the nutrients they need from the food environment they are in (Jay, Loessner, & Golden, 2006). The most predominate microorganisms found in food are those that can use the nutrients available in the food source (FDA, 2013).

Biological Structures

Some foods have biological structures that may prevent the entry and growth of pathogens (FDA, 2013). Rinds, shells, and other coverings provide protection from some bacteria (Nester, Anderson, Roberts, Pearsall, & Nester, 2001). However, if the protective covering becomes damaged the covering will no longer be protective (Jay, Loessner, & Golden, 2006). In the case of meats, the hide of the animal protects the muscle and then the outside surface of an intact piece of meat protects the inner meat (Jay, Loessner, & Golden, 2006). These barriers will be destroyed once the meat is cut, chopped or ground; thus, allowing for bacteria to gain access to the interior of the meat (FDA, 2013).

Oxidation-Reduction Potential

Oxidation-reduction potential (Eh) is defined as the ratio of the total oxidizing (electron accepting) power to the total reducing (electron donating) power of the substance (FDA, 2013). Aerobic microorganisms require positive (oxidized) Eh values, anaerobic microorganisms require negative (reduced) Eh values, and facultative anaerobic bacteria can survive and grow in either condition (Jay, Loessner, & Golden,

2006). Raw, post-rigor muscle has an Eh of -60 to -150 mV and cooked sausages and canned meat have an Eh of -20 to -150 mV (FDA, 2013).

Naturally Occurring Antimicrobials

Some foods naturally contain antimicrobials such as lysozyme in egg whites (Nester, Anderson, Roberts, Pearsall, & Nester, 2001). Food processing techniques, such as smoking, can form antimicrobial compounds on the surface of the meat (Mossel, Corry, Struijk, & Baird, 1996). Phenol is found in smoke condensate and is not only an antimicrobial, but also lowers the pH (FDA, 2013).

Extrinsic Factors Affecting Microorganisms

Storage Temperatures

All microorganisms have a defined temperature range in which they grow (FDA, 2013). The range of select pathogens can be found in Table 1. Microbes can be divided into four groups depending on the optimum temperature in which they grow and thrive: psychrotrophs, psychrophiles, mesophiles, and thermophiles (FDA, 2013). Almost all human pathogens are included in the mesophile group (FDA, 2013). When determining storage temperatures, the quality of the food must be kept in mind (Jay, Loessner, & Golden, 2006). Mesophilic bacteria can be inhibited by the use of cold storage; however, those same cold temperatures will facilitate the growth of psychrotrophic organisms. Small temperature changes can change the microbial profile of meat (Sun & Holley, 2012).

Atmosphere Composition

Some gasses, such as carbon dioxide (CO_2), ozone (O_3), and oxygen (O_2), are toxic to certain pathogens (FDA, 2013). Incorporating these gases into the packaging of the food can provide an antimicrobial affect (Nester, Anderson, Roberts, Pearsall, & Nester, 2001; Gill & Newton, 1978). Technologies commonly used to control the atmosphere of the food storage environment include modified atmosphere packaging (MAP), controlled atmosphere packaging (CAP), controlled atmosphere storage (CAS), direct addition of carbon dioxide (DAC), and hypobaric storage (Loss & Hotchkiss, 2001). MAP is most commonly used in the meat processing industry (FDA, 2013). Common compositions of gasses used in MAP can be found in Table 2 for select food types. Another common packaging type is vacuum packaging which restricts the O_2 levels and allows for CO_2 levels of about 20%, largely inhibiting the growth of Gram negative aerobes (Gill & Newton, 1978).

Microorganisms

Escherichia coli

Escherichia coli is a Gram-negative non-spore forming short rod. There are both non-pathogenic and pathogenic *E. coli* groups. There are six pathogenic groups enterotoxigenic (ETEC), enteropathogenic (EPEC), enterohemorrhagic (EHEC), enteroinvasive (EIEC), enteroaggregative (EAEC), and diffusely adherent (DAEC). While all six strains are pathogenic, EHEC organisms are most commonly associated with foodborne outbreaks (FDA, 2012).

While about 75% of all *E. coli* foodborne infections world-wide are caused by O157:H7 there is another group of non-O157 *E. coli* serotypes that is currently being tested for in the United States. The "big 6" non-O157 are O26, O45, O103, O111, O121, and O145. The "big 6" account for 71% of the non-O157:H7 infections; however, there are other infectious serotypes that can result in illness (Brooks, et al., 2005).

EHEC has a very low infectious dose of 10 to 100 cells (FDA, 2012). Symptoms of EHEC infection, developing after an incubation period of 2 hours to 6 days, can include nausea, abdominal cramps, vomiting, and watery or bloody diarrhea (Nester, Anderson, Roberts, Pearsall, & Nester, 2001). In some extreme cases, hemorrhagic colitis can progress to hemolytic uremic syndrome (HUS) 3%-7% of the time. Patients with HUS have a mortality rate of 4%-5% (FDA, 2012).

E. coli O157:H7 results in 4% of domestically acquired foodborne illnesses that result in hospitalization (CDC, 2011). While most infections continue to be a result of ground beef or beef products, there is an increasing amount of produce products (FDA, 2012). There are about 63,153 cases of O157:H7 EHEC and about 112,752 cases of non-O157 EHEC infections annually (Scallan, et al., 2011). The largest meat recall associated with EHEC to date was in 1997 when Hudson Foods recalled six lots of frozen ground beef patties and burgers (CDC, 1997). Fifteen individual patients were determined to be affected by contaminate meat from Hudson Foods; five were hospitalized, but none developed HUS (CDC, 1997). The most recent meat related EHEC outbreak was attributed to Lebanon bologna in 2011 (CDC, 2011b). Fourteen persons from five states

were infected with the outbreak strain; none developed HUS, but three were hospitalized (CDC, 2011b). The last reported case of HUS associated with a meat product was in 2010 when beef non-intact steaks were recalled (CDC, 2010).

***Salmonella* spp.**

Salmonella is a Gram-negative, non-spore forming, motile rod. *S. enterica* and *S. bongori* are the two species that cause illness in humans. There are six subspecies of *S. enterica*. The most common subspecies is *S. enterica* subsp. *enterica*. Serotypes within the subspecies include Enteritidis and Typhimurium – two of the most common serotypes in the United States out of the 2,579 identified (FDA, 2012).

Nontyphoidal salmonellosis and typhoid fever (only caused by *S.* Typhi or *S.* Paratyphi A.) are the two types of illnesses that can be caused by *Salmonella* infection. Nontyphoidal salmonellosis can have an infectious dose of one cell while typhoid fever's infectious dose is fewer than 1,000 cells. Mortality rates of nontyphoidal salmonellosis range from less than 1% to about 3.6%; however, untreated typhoid fever can have a mortality rate of up to 10% (FDA, 2012).

Salmonella is found widely in the environment and in the intestines of many animals. However, *S.* Typhi and *S.* Paratyphi A. are only found in human hosts. *Salmonella* illnesses have been linked to meats, poultry, and poultry products; as well as, peanut butter, cocoa, and produce (FDA, 2012). There are an estimated 1,027,561

nontyphoidal (foodborne) salmonellosis cases and 1,821 typhoid fever cases annually (Scallan, et al., 2011).

In foodborne cases, the incubation period before symptoms begin is 6 to 72 hours. Symptoms of salmonellosis most often include diarrhea and vomiting. Less common symptoms are a prolonged fever, headache, abdominal pain, abscesses, and shock (Nester, Anderson, Roberts, Pearsall, & Nester, 2001). In 2007, 401 reported cases of salmonellosis with 338 hospitalizations were linked to frozen pot pies (CDC, 2008). ConAgra Foods, Inc. recalled 9 brands of pot pies and labeling issues were addressed highlighting the need to thoroughly cook not-ready-to-eat frozen foods (CDC, 2008). *Salmonella* spp. contamination in beef products is becoming an important issue. Cargill Meat Solutions recalled about 29,000 pounds of fresh ground beef in 2012 due to *Salmonella* Enteritidis infections (CDC, 2012). In total, 46 cases were reported in 9 states resulting in 12 hospitalizations (CDC, 2012). The most recent salmonellosis outbreak associated with poultry was in January 2014 when 9 reported cases in Tennessee were linked to Tyson brand mechanically separated chicken (CDC, 2014).

Listeria monocytogenes

Listeria monocytogenes is a Gram-positive, motile rod. There are thirteen serotypes, of which, three have been associated with the largest amount of foodborne illnesses. *L. monocytogenes* is both salt and cold tolerant and is widely found within the environment (FDA, 2012). Across organisms, *L. monocytogenes* is not a leading cause of illness, but it is a leading cause of death from foodborne illness (Scallan, et al., 2011).

The CDC estimates 1,591 cases of foodborne illness resulting from *L. monocytogenes* with about 255 resulting in death (Scallan, et al., 2011). The infectious dose is unknown, but estimated to be less than 1,000 cells. *L. monocytogenes* infection occurs more often in pregnant women than other populations. While the women generally have mild flu-like symptoms the infection results in spontaneous abortions and stillbirths one third of the time (FDA, 2012).

Many foods have been associated with *L. monocytogenes* outbreaks including raw and ready-to-eat meats, dairy, dairy products, and produce. The ability to grow and thrive at refrigeration temperatures creates a unique problem for the food industry (FDA, 2012). The most recent listeriosis outbreak associated with meat products was in 2002 when Pilgram's Pride recalled 27.4 million pounds of fresh and frozen ready-to-eat turkey and chicken products. Forty-six confirmed cases (including 7 deaths and 3 stillbirths or miscarriages) were linked to the contaminated products (CDC, 2002). Preventing listeriosis requires interventions in all stages of the food chain from the processing facility to the home (Lianou, et al., 2007).

Topical Treatments

Lactic Acid

Lactic acid is a "Generally Recognized As Safe" (GRAS) food additive commonly used in the meat industry. Lactic acid is an organic acid that has been used on abattoirs as a hot carcass rinse (Huffman, 2002). Lactic acid has been used at 1 to 2% to

decontaminate red meat carcasses without affecting meat quality (Theron & Lues, 2007). When used at high temperatures (>60 °C) lactic acid has been proven to control pathogenic bacteria on carcasses (Theron & Lues, 2007). Organic acids, such as lactic acid, cause bactericidal and bacteriostatic results by reducing the pH of the substrate to lower the internal cellular pH that then disrupts the cell membrane (Chung & Murdock, 1991).

Spraying or dipping cured meats post-processing in organic acids has been proven to reduce *L. monocytogenes* (Theron & Lues, 2007). Lactic acid at 2% has been found to reduce *E. coli* O157:H7 and *Salmonella* Typhimurium 3.54 and 4.68 CFU/cm^2, respectively (Yoder, et al., 2012). Gill and Badoni (2004) found that 4% lactic acid reduced bacteria by >1 log unit compared to a water treatment. Lactic acid's acceptable limit for use in products is the highest level that will not negatively impact sensory characteristics; however, higher acid concentrations can affect the buffering ability of the meat (Smulders, Barendsen, Vanlogtestijn, Mossel, & Vanermarel, 1986). The use of lactic acid use in other areas of meat processing has not been widely explored. Lactic acid (2%) has been showed to reduce the amount of surface bacteria on turkey rolls (*L. monocytogenes*), pork bellies (*Salmonella*), and chicken skins (*Salmonella*), but on not beef plates (*E. coli* O157:H7) (Carpenter, Smith, & Broadbent, 2011). Relatively high levels of *E. coli* can be reduced on cuts or trimmings with 5% lactic acid at ≥ 0.1 ml/cm^2 by between 0.5 and 1 log units (Youssef, Yang, Badoni, & Gill, 2012). Lactic acid (4%) applied as a spray effectively reduced both non-O157 and O157:H7 STEC when beef flanks were inoculated at approximately 10^4 CFU/cm^2 (Kalchayanand, et al., 2012).

When utilizing meat brines, adding 3.3% lactic acid has been shown to decrease *E. coli* O157:H7 during storage (Adler, et al., 2011). Stivarius et al. (2002) found that 5% lactic acid applied to beef trimmings before grinding reduced *E. coli*, coliforms, and aerobic plate count, but not *Salmonella* Typhimurium. Concentration and temperature of lactic acid have an affect on *L. monocytogenes* when applied to frankfurters (Byelashov, et al., 2010).

Other Organic Acids

Aside from lactic acid, there are many other organic acids currently being used in the food industry. Acetic acid, acetates, diacetates, and dehyroacetic acid are primarily used in dairy products and meats to target yeasts and bacteria; sodium propionate is commonly used in meat products to target molds (Mani-Lopez, Garcia, & Lopez-Malo, 2011). Other products utilized in meat and meat products as food preservatives are propionic, citric, and benzoic acids (Theron & Lues, 2007). Acetic acid, the principal ingredient of vinegar, has a pungent odor and flavor that limits its use in foods; many pickled products include acetic acid, acetates, or diacetates (Mani-Lopez, Garcia, & Lopez-Malo, 2011). As a beef carcass wash, fumaric acid has been shown to be effective against *L. monocytogenes* alone and in combination with lactic and acetic acids (Podolak, Zayas, Kastner, & Fung, 1995a). Citric acid and citrates are commonly used in the poultry industry in chill tanks to control *Salmonella* spp. (Mani-Lopez, Garcia, & Lopez-Malo, 2011). Other organic acids with similar uses that are not as commonly utilized include malic, propionic, and tartaric acids (Mani-Lopez, Garcia, & Lopez-Malo, 2011). Fumaric, acetic, and lactic acids have all proved to reduce *E. coli* O157:H7, *Salmonella*

Typhimurium, and *L. monocytogenes* counts on beef lean muscle over time (Podolak, Zayas, Kastner, & Fung, 1995a; Podolak, Zayas, Kastner, & Fung, 1995b). Spray application of peroxyacetic acid did not effectively reduce *E. coli* O111, but it did reduce other STEC strains when inoculated at about 10^4 CFU/cm^2 (Kalchayanand, et al., 2012). The same study found that under the same inoculation conditions acidified sodium chlorite did not reduce *E. coli* O26, O111, or O145 serogroups (Kalchayanand, et al., 2012). In general, organic acids are more effective at reducing bacteria than hot water, but the discoloration and off-odor properties that organic acid use can lead to are main concerns when determining which acids and concentrations should be utilized (Sun & Holley, 2012)

Sodium Metasilicate

Very little research has been performed on the use of sodium metasilicate on meat and meat products. This area has previously been unexplored especially in regards to pork and processed meats. Sodium metasilicate is approved for antimicrobial use in ready-to-eat meat and poultry products up to 6% (USDA, 2013). It is an alkaline solution that has proven to be effective in reducing Gram-negative bacteria from the surface of meat and meat products (Carlson, et al., 2008; Pohlman, Dias-Morse, & Rajaratnam, 2005; Pohlman, et al., 2009; Weber, et al., 2004). Sodium metasilicate acts on the cytoplasmic membrane and causes the cells to lyse (Sharma, Williams, Schneider, Scmidt, & Rodrick, 2013b). Little research has been conducted on the effectiveness of sodium metasilicate on Gram-positive bacteria, but one *in vitro* study found that sodium metasilicate at 1, 2, or 3% reduced *L. monocytogenes* by >5 logs after a 30 min exposure

time (Sharma, Williams, Schneider, Schmidt, & Rodrick, 2012a). Sharma et al. (2012a) also discovered that applying 4% sodium metasilicate to a culture of *L. monocytogenes* with a 30 min exposure time resulted in an undetectable amount of *L. monocytogenes.* Sodium metasilicate has been explored as a treatment for fresh beef trimmings before grinding. Geornaras et al. (2012b) found that 4% sodium metasilicate reduced *E. coli* O157:H7 and multidrug-resistant and antibiotic susceptible *Salmonella* by 1.3-1.5 log CFU/cm^2 when applied as a dip to beef trimmings. Sodium metasilicate (4%) applied to beef trimmings before grind has been shown to reduce coliforms, *E. coli*, aerobic plate counts, and *Salmonella* over 7 days of storage (Pohlman, et al., 2009). One study explored the ability of sodium metasilicate to reduce *E. coli* and *Salmonella* typhimurium when used as a dip on beef trimmings before grinding and discovered that both species were decreased; however, the bacteria were inoculated in the same solution and some reduction could be the result of organisms outcompeting each other (Pohlman, F. W., Dias-Morse, P. N., & Rajaratnam, G., 2005). When used as part of a multi-hurdle program, 4% sodium metasilicate in combination with 3% potassium lactate or 200-ppm peroxyacetic acid did not reduce *E. coli* or *Salmonella typhimurium* when applied to beef trimming before grinding (Quilo, et al., 2010). However, 4% sodium metasilicate in combination with 3% potassium lactate or 200-ppm peroxyacetic acid improved or maintained ground beef odor when applied to beef trimmings before grinding, and 4% sodium metasilicate in combination with 200-ppm peroxyacetic acid enhanced beef color when applied to beef trimming before grinding (Quilo, et al., 2010). Sodium metasilicate at 2.2% has shown the ability to reduce *E. coli* O157:H7 in brines both immediately and throughout storage (Adler, et al., 2011). While being effective against microorganisms

sodium metasilicate doesn't affect meat quality (Quilo, et al., 2009).

Other Alkaline Solutions

Several alkaline solutions are used to reduce the risk of pathogens in the meat industry. Potassium hydroxide, sodium hypochlorite, and sodium hydroxide have been used as food surface cleaners (Sharma & Beuchat, 2004). Sharma & Beuchat (2004) discovered that high pH and chlorine combined with sodium hydroxide or potassium hydroxide add to the bactericidal effectiveness of alkaline cleaners. Sodium hypochlorite has been used to reduce *E. coli*, *Campylobacter*, and *Salmonella* from broiler carcasses after spray washing (Northcutt, Smith, Ingram, Hinton, & Musgrove, 2006). Using 0.1% ammonium hydroxide in a brine solution was less effective on total aerobic and anaerobic bacteria than 4.5% sodium tripolyphosphate (Parsons, VanOverbeke, Goad, & Mireles DeWitt, 2010); however, in a similar study the opposite was reported (Cerruto-Noya, VanOverbeke, & Mireles DeWitt, 2009).

Research Objectives

There are many different antimicrobial solutions that are currently being used in food applications. Expanding the use of these solutions may decrease foodborne pathogenic bacteria. Identifying cross-functional solutions that can be used in the meat industry may increase the safety of the meat supply. The effectiveness of lactic acid and sodium metasilicate will depend on the concentration, temperature, and whether the solutions are applied separately or in combination. While other researchers have investigated similar topics, those studies did not investigate individual inoculation of

microorganisms, fresh beef or pork steaks, or deli meats. Therefore, the objectives of this research addressed those concerns.

The objective of the first study was to determine the optimal application concentration and temperature of lactic acid and sodium metasilicate for bactericidal reduction of inoculated pathogens. Each of these antimicrobials has been used at a variety of concentrations. Lactic acid was analyzed at 1, 2, 3, and 4% (LA1, LA2, LA3, and LA4) and sodium metasilicate at 2, 3, 4, and 5% (SM2, SM3, SM4, and SM5). Concentrations were determined based on current industry use and United States Department of Agriculture Food Safety and Inspection Service Directive 7120.1 revision 15 (USDA, 2013). Temperature of application may change the effectiveness. Temperatures of 4, 25, and 60 °C were utilized. Temperatures were selected to represent meat processing (4 °C), room (25 °C), and warm (60 °C) temperatures. The optimal concentration of LA, SM, and their combination (LASM) were utilized.

The objective of the third study was to determine the bactericidal reduction of inoculated pathogens of the optimal concentration and temperature of LA, SM, and LASM on fresh beef, pork, and deli meats (roast beef, ham, and turkey).

Table 1. Selected intrinsic factors affecting chosen pathogen growth.

Microorganism	Approximate a_w values for growth of selected pathogens in food			Approximate pH values allowing the growth of selected pathogens in food			Approximate temperatures allowing growth of selected pathogens (°C)		
	Minimum	Optimum	Maximum	Minimum	Optimum	Maximum	Minimum	Optimum	Maximum
Enterohemorrhagic *Escherichia* coli	0.95	0.99		4.4	6.0 – 7.0	9.0	7	35 – 40	46
Salmonella spp.	0.94	0.99	> 0.99	4.2[a]	7.0 – 7.5	9.5	5	35 – 37	45 – 47
Listeria monocytogenes	0.92			4.39	7.0	9.4	0	30 – 37	45

Sources: Tables 3-2, 3-5, 3-10; FDA 2013

[a] pH minimum as low as 3.8 has been reported when acidulants other than acetic acid or equivalent are used.

Table 2. Examples of gas mixtures used for selected MAP products.

Product	% Carbon Dioxide	% Oxygen	% Nitrogen
Fresh Meat	30	30	30
	15 – 40	60 – 85	0
Cured Meat	20 – 50	0	50 – 80
Sliced Cooked Roast Beef	75	10	15
Processed Meats	0	0	100

Source: Table 3-8; FDA, 2013

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Chapter II

Evaluation of Multiple Concentrations and Temperatures of Lactic Acid and Sodium Metasilicate against Pathogens of Concern on Fresh Beef

Abstract

Lactic acid (LA) has been widely used in abattoirs as an antimicrobial spray for carcass intervention. Sodium metasilicate (SM) has been approved for use on carcasses, trimmings, and ready to eat products. Each of these antimicrobials has been used at a variety of concentrations and temperatures. Utilizing these antimicrobials in different stages during meat processing may assist in the reduction of the risk of pathogenic microorganisms. The first purpose of this study was to determine optimum concentrations of usage of lactic acid and sodium metasilicate for pathogen reduction on beef bottom round muscles. Lactic acid was applied at 1, 2, 3, and 4% (LA1, LA2, LA3, and LA4) and sodium metasilicate was applied at 2, 3, 4, and 5% (SM2, SM3, SM4, and SM5). The second purpose of this study was to determine optimum temperatures of usage of lactic acid and sodium metasilicate for pathogen reduction on beef bottom round muscles. Lactic acid 4% (LA, v/v), sodium metasilicate 4% (SM, w/v), the combination of the two solutions (LASM), and a distilled water control were applied at 4, 25, and 60 °C. Antimicrobials were mixed into solution with distilled water. Beef bottom round was cut into 100 cm^2 pieces. Pieces were then inoculated with *Escherichia coli* O157:H7 (5 strains), non-O157 shiga-toxin producing *Escherichia coli* (STEC, 1 strain each of the

"Big 6"), *Salmonella* spp. (5 strains), or *Listeria monocytogenes* (5 strains). After 30 min of contact time, samples were treated with the antimicrobial solution or control and then allowed 30 min of contact time. Samples were serially diluted and plated on MacConkey Agar with Sorbitol (*E. coli*), XLT4 (*Salmonella* spp.), or Modified Oxford Medium (*L. monocytogenes*). Data were analyzed using the PROC MIXED procedure of SAS and Tukey pairwise comparisons. For all microorganisms, increasing the concentration of lactic acid or sodium metasilicate increased the effectiveness of the treatment. SM4 and LA4 were determined to be the lowest concentrations most effective against all microorganisms tested in this study. By utilizing the lowest concentration of antimicrobial solution necessary to achieve effective pathogen reduction, meat processors can provide a safe and wholesome meat supply. LA and SM reduced ($P<0.05$) the microbial contamination of the meat samples. Temperature of application had no effect ($P>0.05$) on bacterial counts in any of the treatments. LA or SM alone were more effective ($P<0.05$) in reduction of microbes than when used together (LASM). Meat processors can apply LA or SM at refrigeration temperatures and gain the same benefits of applying them at a higher temperature. Both solutions can serve as a hurdle technology in meat processing facilities.

Introduction

Food safety is a constant concern in the meat industry and consideration is given to methods of ensuring a safe food supply by reduction of pathogens. The Centers for Disease Control and Prevention (CDC) estimates that 48 million (1 in 6) Americans become ill each year due to foodborne illness. Of these, 128,000 are hospitalized and

about 3,000 die of foodborne diseases (Weber, O'Brien, & Bender, 2004). *Salmonella* spp., *E. coli*, and *L. monocytogenes* are common pathogens of concern in fresh and processed meats on the "top five pathogens" lists compiled by the CDC (Weber, O'Brien, & Bender, 2004).

Escherichia coli is a Gram-negative non-spore forming short rod. While about 75% of all *E. coli* foodborne infections world-wide are caused by O157:H7 there is another group of non-O157 *E. coli* serotypes that is currently being tested for in the United States. The "big 6" non-O157 are O26, O45, O103, O111, O121, and O145. The "big 6" account for most of the non-O157:H7 foodborne infections (FDA, 2012). *E. coli* O157:H7 results in 4% of domestically acquired foodborne illnesses that result in hospitalization (CDC, 2011a). While most infections continue to be a result of ground beef or beef products, there is an increasing amount of produce that has been implicated sporeforming, motile rod. *Salmonella* illnesses have been linked to meats, poultry, and poultry products; as well as, peanut butter, cocoa, and produce (FDA, 2012). There are an estimated 1,027,561 nontyphoidal salmonellosis cases and 1,821 typhoid fever cases annually (Scallan, et al., 2011). Nontyphoidal salmonellosis and typhoid fever (only caused by *S.* Typhi or *S.* Paratyphi A.) are the two types of illnesses that can be caused by *Salmonella* infection. *Salmonella* illnesses have been linked to meats, poultry, and poultry products; as well as, peanut butter, cocoa, and produce (FDA, 2012). *Listeria monocytogenes* is a Gram-positive, motile rod. Across organisms, *L. monocytogenes* is not a leading cause of illness, but it is a leading cause of death from foodborne illness (Scallan, et al., 2011). *L. monocytogenes* is both salt and cold tolerant and is widely found

within the environment (FDA, 2012). Many foods have been associated with *L. monocytogenes* outbreaks including raw and ready-to-eat meats, dairy, and dairy products. The ability of *L. monocytogenes* to grow and thrive at refrigeration temperatures creates a unique problem for the food industry (FDA, 2012).

Lactic acid is a "Generally Recognized As Safe" (GRAS) food additive commonly used in the meat industry. Lactic acid is an organic acid that has been used in abattoirs as a hot carcass rinse (Huffman, 2002). Lactic acid has been used at 1 to 2% to decontaminate red meat carcasses without affecting meat quality (Theron & Lues, 2007). Lactic acid at 2% has been found to reduce *E. coli* O157:H7 and *Salmonella* Typhimurium by 3.54 and 4.68 CFU/cm^2, respectively (Yoder, et al., 2012). When used at high temperatures (>60 °C) lactic acid has controlled pathogenic bacteria on carcasses (Theron & Lues, 2007). Gill and Badoni (2004) found that 4% lactic acid reduced bacteria by >1 log unit compared to a water treatment. Lactic acid effectiveness is impacted by concentration and lactic acid temperature on *L. monocytogenes* when applied to frankfurters (Byelashov, et al., 2010).

There is very little research on the use of sodium metasilicate on meat and meat products as an antimicrobial. This is an area that has previously been unexplored especially in regards to pork and processed meats. Sodium metasilicate is approved for antimicrobial use in ready-to-eat meat and poultry products at 6% (USDA, 2013). It is an alkaline solution that has proven to be effective in reducing Gram-negative bacteria from the surface of meat and meat products (Carlson, et al., 2008; Pohlman, et al., 2009;

Weber, O'Brien, & Bender, 2004). Sodium metasilicate has been explored as a treatment for fresh beef trimmings before grinding. When used as part of a multi-intervention program, 4% sodium metasilicate in combination with 3% potassium lactate or 200-ppm peroxyacetic acid does not reduce *E. coli* or *Salmonella* Typhimurium when applied to beef trimming before grinding (Quilo, et al., 2010). However, 4% sodium metasilicate in combination with 3% potassium lactate or 200-ppm peroxyacetic acid does improve or maintain ground beef odor when applied to beef trimmings before grinding, and 4% sodium metasilicate in combination with 200-ppm peroxyacetic acid enhances beef color when applied to beef trimming before grinding (Quilo, et al., 2010).

Each of these antimicrobials has been used at a variety of concentrations. Utilizing these antimicrobials in different stages during meat processing may assist in the reduction of the risk of pathogenic microorganisms. The purpose of this study was to determine optimum concentrations of usage of lactic acid and sodium metasilicate for pathogen reduction on beef bottom round muscles.

Materials and Methods

Culture Strains

Five strains of *Escherichia coli* O157:H7, 1 strain of each of the big 6 STECs, 5 strains of *Salmonella* spp., and 5 strains of *Listeria monocytogenes* (Table 3) were used for this study. All media was purchased from Neogen Corporation, Lansing, Michigan unless otherwise stated. Cultured microorganisms were individually transferred to 9 ml sterile tryptic soy broth, vortexed (Labnet International, Inc., Edison, New Jersey), and

incubated at 35 °C for 24h (Jeio Tech, Inc., Des Plaines, Illinois). The overnight culture produced approximately 9 log CFU/ml culture suspensions which were used for inoculation. Cultures were centrifuged at 3650 rpm for 20 min at 37 °C (5810R Eppendorf, Hauppauge, New York). Using the same method as Wang & Harris (2011), the supernatant was discarded and the precipitate was re-suspended in 0.85% sodium chloride (Fisher Scientific, Fair Lawn, New Jersey) solution until a spectrometer (Amersham Biosciences Corporation, Piscataway, New Jersey) absorbance reading of 0.60 was determined yielding about 8 log CFU/ml cultures. To create the culture cocktail used for inoculation, equal parts of each strain of microorganism were combined and vortexed to result in cocktails of *E. coli* O157:H7, non-O157 STECs, *Salmonella* spp., and *L. monocytogenes*. The culture cocktails were serially diluted using 9ml peptone (Becton Dickinson and Company, Sparks, Maryland) and plated on MacConkey Sorbitol Agar (*E. coli*), XLT4 (*Salmonella* spp.) or Modified Oxford Medium (*L. monocytogenes*) to determine cell density. All plates were enumerated after incubation at 35 °C for 24h.

Treatment Preparation

Lactic acid and sodium metasilicate antimicrobial treatments were utilized at various concentrations and temperatures of application. Lactic acid (analytical grade, Sigma Aldrich, St. Louis, Missouri) concentrations were 1, 2, 3, and 4% (v/v) while sodium metasilicate (analytical grade, Sigma Aldrich, St. Louis, Missouri) concentrations were 2, 3, 4, and 5% (w/v). A control treatment of distilled water was also tested. Lactic acid (analytical grade, Sigma Aldrich, St. Louis, Missouri), 4% (v/v), and sodium metasilicate (analytical grade, Sigma Aldrich, St. Louis, Missouri) 4% (w/v), were

applied at 4, 25, and 60 °C. Antimicrobials were mixed into solution with distilled water (Podolak, Zayas, Kastner, & Fung, 1995a; Podolak, Zayas, Kastner, & Fung, 1995b). A control treatment of distilled water at 25 °C was also tested. Table 4 contains the pH values of all treatments. Tap water in the research facility is of poor quality and consistency; thus, distilled water was used to maintain better control over the process.

Sample Preparation

Fresh beef bottom round steaks were cut at the Lambert Powell Meat Laboratory without the use of antimicrobial solutions. Lean meat samples were cut to 100 cm^2 pieces. Each piece was individually inoculated and treated with the antimicrobial treatment assigned.

Fresh beef steaks were inoculated with the culture cocktails of *E. coli* O157:H7, non-O157 STECs, *Salmonella* spp., or *L. monocytogenes*. The surface of the meat was inoculated with 1mL of a cocktail culture and then evenly spread using a disposable L-shaped culture spreader (VWR International, LLC, Radnor, Pennsylvania). Samples were allowed 30 min to allow the bacteria to adhere to the surface of the meat before antimicrobial solutions were applied. Antimicrobial treatments were randomly assigned. Ten ml of the assigned treatment were evenly applied over the surface of the meat. After treatment application, the samples were allowed an additional 30 min contact time.

A modified plating method from Podolak, Zayas, Kastner, & Fung (1995a) was utilized. Since samples were not stored after dilution, a buffered solution was not utilized

and a simple diluent of 0.1% peptone was used instead. One hundred ml of 0.1% peptone was added to each of the meat samples in sterile stomacher bags (Nasco Whirl-Pak, Fort Atkinson, Wisconsin) and then samples were stomached for 2 min at 300 rpm (400 Circular Seward Medical, London, England). Serial dilutions with 9ml 0.1% peptone were created and dilutions were plated on MacConkey Sorbitol Agar (*E. coli*), XLT4 (*Salmonella* spp.), or Modified Oxford Medium (*L. monocytogenes*) to determine cell density. All plates were enumerated after incubation at 35 °C for 24h. Results are reported in CFU/cm^2.

Statistical Analysis

A completely random design was used to conduct these experiments. Each experiment was conducted in triplicate with 3 replications (on separate days) resulting in 9 samples per treatment. No more than 1 data outlier was removed as a sample possibly contaminated with pathogens before inoculation in each treatment group. All data were converted to $\log_{10}$ CFU/cm^2 before statistical analysis. Statistics were completed using PROC MIXED in SAS 9.2 (SAS Institute, Inc., Cary, North Carolina). The fixed effect was the treatment. There were no differences in replications, and no treatment by replication interactions were included as no practical differences were observed. Tukey pairwise comparisons were utilized due to the potential unequal sample sizes that resulted when data points were removed.

Results and Discussion

Concentration

All LA treatments except LA1 reduced ($P < 0.01$) microbial counts of *E. coli* O157:H7 compared to the control sample. There were no differences in non-O157 STEC counts among the control, LA1, and LA2 ($P > 0.37$, Table 5). All LA treatments reduced *L. monocytogenes* counts compared to the control treatment ($P < 0.01$, Table 5). LA4 was the only LA treatment that reduced *Salmonella* spp. counts in comparison to the control sample ($P < 0.01$, Table 5). Lactic acid at a 2% concentration has been shown to reduce both *E. coli* O157:H7 and *Salmonella* as a beef carcass wash (Hardin, Acuff, Lucia, Oman, & Savell, 1995). In contrast, a study conducted using 5% lactic acid did not show a decrease in *Salmonella* Typhimurium in ground beef samples (Stivarius, Pohlman, McElyea, & Waldroup, 2002). It is possible that the increased fat in the Stivarius et al. (2002) study (15%) protected the bacteria more than the current study's surface inoculation and treatment method allowed. However, the same study found that 5% lactic acid reduced *E. coli* and coliform counts. Lactic acid (2%) has also been shown to reduce the amount of surface bacteria on turkey rolls (*L. monocytogenes*), pork bellies (*Salmonella*), and chicken skins (*Salmonella*), but not beef plates (*E. coli* O157:H7) (Carpenter, Smith, & Broadbent, 2011). LA4 was most effective in combating *L. monocytogenes* ($P < 0.03$, Table 4). Gill and Badoni (2004) found that 4% lactic acid reduced total aerobic bacteria by >2 log units compared to a water treatment on chilled, raw beef. In the present study, *Salmonella* spp. and *L. monocytogenes* decreased; however, *E. coli* O157:H7 and non-O157 STEC did not. One study reported that treating cut muscle surfaces with 5% lactic acid resulted in greater effectiveness when the cut muscle surface was inoculated at a higher rather than lower level of *E. coli* (Youssef, Yang, Badoni, & Gill, 2012). While lower inoculation levels of *E. coli* were reduced

there was a greater overall reduction when inoculated at a higher level. This outcome is logical as there is overall less bacteria on the surface of the meat. Yoder et al. (2012) reported that 2% lactic acid reduced *Salmonella* Typhimurium and *E. coli* O157:H7 by 4.68 and 3.54 log CFU/cm^2, respectively on inoculated beef plates. It is possible that these results differ from the current study because of lactic acid application methods. Yoder et. al (2012) utilized a pressurized spray system which could account for the increased bacterial reduction. Lactic acid is one of the most common organic acids used as a whole carcass decontamination step (Huffman, 2002). This data suggests that its effectiveness can be expanded past the abattoir and into fabrication.

As the concentration of sodium metasilicate increased, the pathogen counts decreased. This was also reported by Huang, Williams, Sims, & Simmone (2011) when testing total psychotropic bacteria of fresh chicken breasts marinated in sodium metasilicate at 1, 2, 3, and 4% during cold storage. All SM treatments reduced *E. coli* O157:H7 counts compared to the control sample ($P < 0.04$, Table 6). SM5 was more effective than SM2 ($P < 0.01$) in reducing *E. coli* O157:H7 counts. SM3 and SM4 were equally as effective compared to SM2 and SM5 ($P > 0.16$, Table 6) on *E. coli* O157:H7. No differences were discovered in non-O157 STEC counts among the control, SM2, and SM3 ($P > 0.15$, Table 6). SM4 and SM5 were both more effective than the control sample ($P < 0.01$, Table 6) in controlling non-O157 STEC contamination. Compared to the control treatment, SM4 and SM5 reduced the *Salmonella* spp. counts of samples ($P = 0.02$ and $P < 0.01$, respectively, Table 6). Several studies have utilized 4% sodium metasilicate that have shown favorable bacteria reduction of *E. coli* O157:H7 or

Salmonella in ground beef (Geornaras, et al., 2012a; Geornaras, et al., 2012b; Pohlman, et al., 2009; Pohlman, Dias Morse, & Rajaratnam, 2005; Quilo, et al., 2009; Quilo, et al., 2010). Each of these studies utilized a shorter contact time (30s or 3 min) than the current procedure, but the results were still similar. There were no differences in *L. monocytogenes* counts among the control, SM2, and SM3 ($P > 0.94$, Table 6). SM4 and SM5 were most effective in reducing *L. monocytogenes* compared to the control ($P < 0.03$, Table 6). *Listeria monocytogenes* decreased as sodium metasilicate concentration increased as was observed in an *in vitro* study with a 30 min contact time conducted by Sharma et. al (2012a). It is possible that more favorable results were shown in the *in vitro* study than in the current study due to the ability of the meat to buffer the antimicrobial solutions and *L. monocytogenes* ability to form biofilms on the meat.

Temperature

E. coli O157:H7 and non-O157 STEC counts were reduced by all treatments compared to the inoculated control ($P < 0.01$, Tables 5 and 6, respectively). The control treatment was ineffective in reducing microbial *Salmonella* spp. contamination compared to all other treatments except LASM at 25 and 60 °C ($P < 0.01$ and $P > 0.11$, respectively, Table 9). Compared to the control treatment, the SM and LA treatments effectively lessened *L. monocytogenes* contamination ($P < 0.01$ and $P > 0.85$, respectively); however, the combination treatment (LASM) did not ($P > 0.05$, Table 10).

There were no differences in bacteria prevalence among the application temperatures within a treatment group ($P > 0.33$, Tables 5-8) for any microorganisms

tested. Lactic acid and sodium metasilicate have been used with positive results at a variety of temperatures. In a brine solution, sodium metasilicate has reduced *E. coli* O157:H7 at both 4 and 15 °C (Adler, et al., 2011). At 25 °C sodium metasilicate has been shown to reduce *E. coli* O157:H7, non-O157 STECs, and *Salmonella* Typhimurium on beef trimmings (Geornaras, et al., 2012a; Geornaras, et al., 2012b). Lactic acid (4%) at 55 °C and > 60 °C reduced microbial counts on beef carcasses (Castillo, Lucia, Mercado, & Acuff, 2001).

While there was no difference in effectiveness of reducing *E. coli* O157:H7 between LA and LASM ($P > 0.15$, Table 7), there was an improvement between SM and LASM ($P < 0.01$, Table 7). All LA and SM treatments were equally effective ($P > 0.57$) against non-O157 STEC, but LA and LASM were only equally effective at 4 °C ($P = 0.12$, Table 8). In all other comparisons, LA and SM provided a greater advantage in reducing non-O157 STEC than the combination, LASM ($P < 0.02$, Table 8). The greatest effect on *Salmonella* spp. reduction was observed by the LA treatment ($P < 0.01$, Table 9). The combination treatment (LASM) did not improve effectiveness when compared with LA or SM used alone in *Salmonella* spp. contamination. The only difference among LASM and SM treatments was SM at 25 °C improving *Salmonella* spp. counts compared to LASM at 60 °C ($P = 0.05$, Table 9). The LA treatment was the most effective in reducing the *L. monocytogenes* load ($P < 0.01$, Table 10). While utilizing multiple solutions as part of a multistep intervention program has proven positive (Quilo, et al., 2010), the lactic acid and sodium metasilicate combination did not enhance the individual antimicrobial effectiveness of either solution. Quilo, et al. (2010) combined 4% sodium

metasilicate with 3% potassium lactate or 200-ppm peroxyacetic acid and dipped inoculated beef trimmings in the treatment solution and then ground the meat before sampling. This research found that neither combination reduced *E. coli* or *Salmonella* Typhimurium contamination (Quilo, et al., 2010).

Conclusion

Sodium metasilicate, 4% and lactic acid, 4% were determined to be the lowest concentrations most effective against all microorganisms. While lower concentrations of lactic acid were equally effective against non-O157 STEC (LA3) and *Salmonella* spp. (LA2 and LA3); LA4 was more effective against *E. coli* O157:H7 and *L. monocytogenes*. Similarly, SM3 was consistently as effective as SM4 against *E. coli* O157:H7, non-O157 STEC, and *L. monocytogenes*, but not against *Salmonella* spp. Therefore, to have the greatest impact on all bacteria the greater concentration of 4% is recommended for both lactic acid and sodium metasilicate. By utilizing the lowest concentration of antimicrobial solution necessary to achieve effective pathogen reduction, meat processors can provide a safe and wholesome meat supply. Meat processors can apply 4% lactic acid or 4% sodium metasilicate at refrigeration temperatures (4 °C) and obtain the same microbial benefits of decreased counts of *E. coli* O157:H7, non-O157 STEC, *Salmonella* spp., and *L. monocytogenes* of applying them at a greater temperatures (25 or 60°C). Combining the two antimicrobial solutions (LASM) is not recommended. The high pH of sodium metasilicate (12.82) combined with the low pH of lactic acid (1.84) creates a solution with a pH of 12.53 which is ineffective against pathogenic bacteria. However, individually both solutions can serve as a hurdle technology in meat processing facilities.

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Table 3. Strains of microorganisms used

Microorganism	**ATCC number or ID Code**	**Source**
Escherichia coli O157:H7	ATCC 35150	Human – HC
Escherichia coli O157:H7	ATCC 43894	Human – HC
Escherichia coli O157:H7	AU – 1	Laboratory strain (301)
Escherichia coli O157:H7	AU – 2	Laboratory strain (505B)
Escherichia coli O157:H7	AU – 3	Laboratory strain
Non-O157 STEC (O145)	TWO9356	Human – HUS
Non-O157 STEC (O26)	TWO7814	Human – HUS
Non-O157 STEC (O121)	TWO8039	Human
Non-O157 STEC (O45)	TWO14003	Human
Non-O157 STEC (O111)	TWO7926	Human – HC
Non-O157 STEC (O103)	TWO8101	Human
Salmonella	AU – Enteritidis	Laboratory strain
Salmonella	AU – Kentucky	Laboratory strain
Salmonella	AU – Montevideo	Laboratory strain
Salmonella	AU – Thompson	Laboratory strain
Salmonella	AU – Stanley	Laboratory strain
Listeria monocytogenes	ATCC 49594	Petite Scott A
Listeria monocytogenes	ATCC 19115	Human – Serotype 4b
Listeria monocytogenes	ATCC 7644	Human
Listeria monocytogenes	AU – 4	Laboratory strain (101M serotype 4b)
Listeria monocytogenes	AU – 5	Laboratory strain (108M serotype 1/2b)

Table 4. pH values of lactic acid at 1, 2, 3, 4% (LA1, LA2, LA3, LA4), sodium metasilicate at 2, 3, 4, 5% (SM2, SM3, SM4, SM5), and distilled water.

Solution	pH
LA1	1.92
LA2	1.89
LA3	1.89
LA4	1.84
SM2	12.82
SM3	12.83
SM4	12.82
SM5	12.82
Distilled Water	4.90
LA4 + SM4	12.53

Table 5. Concentration effects of lactic acid at 1, 2, 3, 4% (LA1, LA2, LA3, LA4) on fresh beef bottom round steaks after pathogen inoculation and a 30min contact time.

Microorganism	Treatment	Mean $\log_{10}$ CFU/cm^2	SEM[z]
E. coli O157:H7	Control	7.04[a]	0.19
	LA1	6.74[a]	0.11
	LA2	6.20[b]	0.12
	LA3	5.90[b]	0.11
	LA4	5.76[b]	0.11
Non-O157 STEC	Control	7.00[a]	0.25
	LA1	6.71[a]	0.15
	LA2	6.33[ab]	0.15
	LA3	6.04[bc]	0.15
	LA4	5.43[c]	0.15
Salmonella spp.	Control	6.71[a]	0.43
	LA1	6.41[a]	0.25
	LA2	5.20[ab]	0.25
	LA3	5.26[ab]	0.25
	LA4	4.76[b]	0.25
L. monocytogenes	Control	7.24[a]	0.15
	LA1	6.45[b]	0.09
	LA2	6.37[b]	0.09
	LA3	6.36[b]	0.09
	LA4	5.93[c]	0.09

[a, b, c] Means within a bacteria group lacking a common superscript differ ($P < 0.05$).
[z] Standard Error of the Mean

Table 6. Concentration effects of sodium metasilicate at 2, 3, 4, 5% (SM2, SM3, SM4, SM5) on fresh beef bottom round steaks after pathogen inoculation and a 30min contact time.

Microorganism	Treatment	Mean $\log_{10}$ CFU/cm^2	SEM[z]
E. coli O157:H7	Control	7.04[a]	0.19
	SM2	6.32[b]	0.11
	SM3	6.12[bc]	0.11
	SM4	5.87[bc]	0.11
	SM5	5.70[c]	0.11
Non-O157 STEC	Control	7.00[a]	0.25
	SM2	6.20[ab]	0.15
	SM3	6.20[ab]	0.15
	SM4	5.88[bc]	0.15
	SM5	5.52[c]	0.15
Salmonella spp.	Control	6.71[a]	0.43
	SM2	5.92[ab]	0.25
	SM3	6.16[a]	0.25
	SM4	4.93[bc]	0.25
	SM5	4.70[c]	0.25
L. monocytogenes	Control	7.24[a]	0.15
	SM2	7.20[a]	0.09
	SM3	7.03[ab]	0.09
	SM4	6.64[b]	0.09
	SM5	6.65[b]	0.09

[a, b, c] Means within a bacteria group lacking a common superscript differ ($P < 0.05$).
[z] Standard Error of the Mean

Table 7. Temperature effects of 4% lactic acid (LA), 4% sodium metasilicate (SM), and 4% lactic acid + 4% sodium metasilicate (LASM) on *Escherichia coli* O157:H7 at 4, 25, and 60 °C on fresh beef bottom round steaks after pathogen inoculation and a 30min contact time.

Treatment	Temperature (°C)	Mean $\log_{10}$ CFU/cm^2	SEM[z]
Control	25	7.15[a]	0.10
LA	4	6.15[bc]	0.09
	25	6.19[bc]	0.09
	60	6.00[bcd]	0.09
SM	4	5.71[d]	0.09
	25	5.59[d]	0.09
	60	5.83[cd]	0.09
LASM	4	6.39[b]	0.09
	25	6.47[b]	0.09
	60	6.37[b]	0.09

[a, b, c, d] Means lacking a common superscript differ ($P < 0.05$).

[z] Standard Error of the Mean

Table 8. Temperature effects of 4% lactic acid (LA), 4% sodium metasilicate (SM), and 4% lactic acid + 4% sodium metasilicate (LASM) on non-O157 STEC at 4, 25, and 60 °C on fresh beef bottom round steaks after pathogen inoculation and a 30min contact time.

Treatment	Temperature (°C)	Mean $\log_{10}$ CFU/cm^2	SEM[z]
Control	25	6.97[a]	0.08
LA	4	5.77[cd]	0.08
	25	5.80[cd]	0.08
	60	5.87[cd]	0.08
SM	4	5.70[d]	0.08
	25	5.65[d]	0.08
	60	5.64[d]	0.08
LASM	4	6.21[bc]	0.08
	25	6.38[b]	0.08
	60	6.32[b]	0.08

[a, b, c, d] Means lacking a common superscript differ ($P < 0.05$).

[z] Standard Error of the Mean

Table 9. Temperature effects of 4% lactic acid (LA), 4% sodium metasilicate (SM), and 4% lactic acid + 4% sodium metasilicate (LASM) on *Salmonella* spp. at 4, 25, and 60 °C on fresh beef bottom round steaks after pathogen inoculation and a 30min contact time.

Treatment	Temperature (°C)	Mean $\log_{10}$ CFU/cm^2	SEM[z]
Control	25	6.60[a]	0.18
LA	4	3.87[d]	0.18
	25	3.62[d]	0.18
	60	3.57[d]	0.18
SM	4	5.28[bc]	0.18
	25	5.13[c]	0.18
	60	5.44[bc]	0.18
LASM	4	5.63[bc]	0.18
	25	5.85[abc]	0.18
	60	5.96[ab]	0.18

[a, b, c, d] Means lacking a common superscript differ ($P < 0.05$).

[z] Standard Error of the Mean

Table 10. Temperature effects of 4% lactic acid (LA), 4% sodium metasilicate (SM), and 4% lactic acid + 4% sodium metasilicate (LASM) on *Listeria monocytogenes* at 4, 25, and 60 °C on fresh beef bottom round steaks after pathogen inoculation and a 30min contact time.

Treatment	Temperature (°C)	Mean $\log_{10}$ CFU/cm^2	SEM[z]
Control	25	7.19[a]	0.06
LA	4	6.24[e]	0.06
	25	6.16[e]	0.06
	60	6.03[e]	0.06
SM	4	7.10[d]	0.06
	25	7.05[bcd]	0.06
	60	7.06[cd]	0.06
LASM	4	6.72[ab]	0.06
	25	6.85[abc]	0.06
	60	6.82[abc]	0.06

[a, b, c, d, e] Means lacking a common superscript differ ($P < 0.05$).

[z] Standard Error of the Mean

Chapter III

Evaluation of Lactic Acid and Sodium Metasilicate against Pathogens of Concern on Fresh Beef, Pork, and Deli Meats

Abstract

An important aspect of the meat industry is food safety. Lactic acid is a "Generally Recognized As Safe" (GRAS) food additive commonly used in the meat industry. Lactic acid is most commonly used as a hot carcass rinse. Sodium metasilicate is approved for antimicrobial use in ready-to-eat meat and poultry products. There is very little research about sodium metasilicate use on meat and meat products especially in regards to pork. The United States Department of Agriculture Food Safety and Inspection Service (USDA FSIS) has a zero tolerance policy for *L. monocytogenes* in ready-to-eat meat and poultry products. Spraying or dipping cured meats post-processing in organic acids has been proven to reduce *L. monocytogenes*. Lactic acid concentration and temperature impacts the effectiveness as an antimicrobial agent on *L. monocytogenes* when applied to frankfurters. There is very little research concerning sodium metasilicate as an antimicrobial agent on meat and meat products. This is an area that has previously been unexplored especially in regards to processed meats. The purpose of this study was to determine if lactic acid and sodium metasilicate could effectively lower pathogenic bacteria on fresh beef and pork and deli meats. Lactic acid 4% (LA, v/v), sodium metasilicate 4% (SM, w/v), and a distilled water control were applied to fresh beef and

pork lean muscle and deli meats. Antimicrobials were mixed in solution with distilled water. Fresh meat of beef bottom round and pork ham steaks were cut into 100 cm^2 pieces. Roast beef, ham, and turkey deli meats were manufactured at the Lambert Powell Meat Laboratory without the use of antimicrobial solutions. Meat samples were cut to 100 cm^2 pieces. Each piece was individually inoculated and treated with the antimicrobial treatment assigned. Fresh meat pieces were inoculated with *Escherichia coli* O157:H7 (5 strains), non-O157 shiga-toxin producing *Escherichia coli* (STEC, 1 strain each of the "Big 6"), or *Salmonella* spp. (5 strains). Deli meats were inoculated with *Listeria monocytogenes* (5 strains). After 30 min of contact time samples were treated with the antimicrobial solution or control and then allowed 30 min of contact time. Half of the deli meat samples were vacuum packaged and treated in a hot water bath for 2 min at 90.6 °C. Samples were serially diluted and plated on MacConkey Agar with Sorbitol (*E. coli*), XLT4 (*Salmonella* spp.), or Modified Oxford Medium (MOX, *L. monocytogenes*). Data were analyzed using the PROC MIXED procedure of SAS and Tukey pairwise comparisons. In fresh meat samples, the control treatment resulted in greater microbial counts regardless of inoculum or species than either the LA or SM treatments ($P < 0.01$). Within species, the SM treatment was more effective at reducing *E. coli* O157:H7 contamination than the LA treatment ($P < 0.01$). Beef treated with LA had less *Salmonella* spp. than pork treated with SM ($P = 0.03$). Both lactic acid and sodium metasilicate can be applied to fresh beef and pork as an effective hurdle technology in the fight for food safety. The deli meat treatments including post-packaging lethality decreased the bacterial load of samples in comparison to those that did not receive the post packaging lethality treatment ($P < 0.01$). Regardless of post-packaging lethality

treatments, there were no differences in microbial counts among treatment groups ($P > 0.73$). Treating deli meats with lactic acid or sodium metasilicate did not reduce *L. monocytogenes* loads. However, adding a post-packaging lethality treatment was able to minimize microbial contamination.

Introduction

Food safety is a major concern in the meat industry. The Centers for Disease Control and Prevention (CDC) estimates that 1 in 6 Americans become ill each year due to foodborne illness. Of these 48 million, 128,000 are hospitalized and about 3,000 die of foodborne diseases (Weber, O'Brien, & Bender, 2004). Fresh meat pathogens of concern include: *Escherichia coli* O157:H7, non-O157 shiga toxin producing *E. coli* (STEC) serotypes, and *Salmonella* spp. These pathogens, *Salmonella* spp. and *E. coli* are pathogens on the "top five pathogens" lists compiled by the CDC (Weber, et al., 2004). The USDA FSIS has expanded its ruling on *E. coli* O157 in raw, non-intact beef to include six non-O157 serotypes including: O26, O45, O103, O111, O121, and O145 – the "big 6" (USDA, 2011). In ready to eat meats *Listeria monocytogenes* is the pathogen of concern. *L. monocytogenes* is on the "top five pathogens" lists compiled by the CDC (Weber, O'Brien, & Bender, 2004). The United States Department of Agriculture Food Safety and Inspection Service (USDA FSIS) has a zero tolerance policy for *L. monocytogenes* in ready-to-eat meat and poultry products. This rule requires meat processors to control *L. monocytogenes* by using one of three alternatives. Alternative 1 includes both a post-lethality treatment and a process or antimicrobial agent, Alternative 2 requires either a post-lethality treatment or a process or antimicrobial agent, and

Alternative 3 requires the implantation of sanitation procedures and frequent USDA FSIS environmental testing (USDA, 2003).

Lactic acid is a "Generally Recognized As Safe" (GRAS) food additive commonly used in the meat industry. Lactic acid is an organic acid that has been used in abattoirs as a hot carcass rinse (Huffman, 2002). Gill and Badoni (2004) found that 4% lactic acid on chilled beef carcasses reduced bacteria by > 1 log unit compared to a water treatment. Similarly, lactic acid at 2% has been found to reduce *E. coli* O157:H7 and *Salmonella* Typhimurium by 3.54 and 4.68 CFU/cm^2, respectively on beef surfaces (Yoder, et al., 2012). Lactic acid (2%) has been showed to reduce the amount of surface bacteria on pork bellies (*Salmonella*), and chicken skins (*Salmonella*), but not beef plates (*E. coli* O157:H7) (Carpenter, Smith, & Broadbent, 2011). Its use in other areas of meat processing has not been widely explored. Spraying or dipping cured meats post-processing in organic acids has proven to reduce *L. monocytogenes* (Theron & Lues, 2007). Lactic acid (2%) has been showed to reduce the amount of surface bacteria on turkey rolls (*L. monocytogenes*) (Carpenter, Smith, & Broadbent, 2011). Lactic acid concentration and temperature impacts the effectiveness as an antimicrobial agent on *L. monocytogenes* when applied to frankfurters (Byelashov, et al., 2010).

Very little research has been performed on the use of sodium metasilicate on meat and meat products. This is an area that has previously been unexplored especially in regards to pork and processed meats. Sodium metasilicate is approved for antimicrobial use in ready-to-eat meat and poultry products at 6% (USDA, 2013). It is an alkaline

solution that has proven to be effective in reducing Gram-negative bacteria from the surface of meat and meat products (Carlson, et al., 2008; Pohlman, et al., 2009; Weber, O'Brien, & Bender, 2004). Sodium metasilicate has been explored as a treatment for fresh beef trimmings before grinding. Geornaras, et al. (2012b) found that 4% sodium metasilicate reduced *E. coli* O157:H7 and multidrug-resistant and antibiotic susceptible *Salmonella* by 1.3-1.5 log CFU/cm^2 when applied as a treatment to beef trimmings. Sodium metasilicate (4%) applied to beef trimmings before grind has been shown to reduce coliforms, *E. coli*, aerobic plate counts, and *Salmonella* over 7 days of storage (Pohlman, et al., 2009). Little research has been conducted on the effectiveness of sodium metasilicate on Gram-positive bacteria, but one *in vitro* study found that sodium metasilicate at 1, 2, or 3% reduced *L. monocytogenes* by >5 logs after a 30 min exposure time (Sharma, Williams, Schneider, Schmidt, & Rodrick, 2012a). Sharma et al. (2012a) also discovered that applying 4% sodium metasilicate with a 30 min exposure time resulted in an undetectable amount of *L. monocytogenes.*

Escherichia coli is a Gram-negative non-spore forming short rod. About 75% of all *E. coli* foodborne infections world-wide are caused by O157:H7. The "big 6" account for 71% of the non-O157:H7 infections; however, there are other infectious serotypes that can result in illness (Brooks, et al., 2005). There are some other infectious serotypes that can result in illness (FDA, 2012). While most infections continue to be a result of ground beef or beef products, there is an increasing amount of produce products (FDA, 2012). There are about 63,153 cases of O157:H7 EHEC and about 112,752 cases of non-O157:H7 EHEC infections annually (Scallan, et al., 2011).

Salmonella is a Gram-negative, non-sporeforming, motile rod. *Salmonella* is found widely in the environment and in the intestines of many animals. *Salmonella* illnesses have been linked to meats, poultry, and poultry products; as well as, peanut butter, cocoa, and produce (FDA, 2012). There are an estimated 1,027,561 nontyphoidal salmonellosis cases annually (Scallan, et al., 2011).

Listeria monocytogenes is a Gram-positive, motile rod. It is not a leading cause of illness, but it is a leading cause of death from foodborne illness (Scallan, et al., 2011). The CDC estimates 1,591 cases of foodborne illness resulting from *L. monocytogenes* with about 255 resulting in death (Scallan, et al., 2011). Many foods have been associated with *L. monocytogenes* outbreaks including raw and ready-to-eat meats, dairy, and dairy products. The ability of *L. monocytogenes* to grow and thrive at refrigeration temperatures creates a unique problem for the food industry (FDA, 2012).

Materials and Methods

Culture Strains

Five strains of *Escherichia coli* O157:H7, 1 strain of each of the big 6 STECs, 5 strains of *Salmonella* spp., and 5 strains of *Listeria monocytogenes* (Table 11) were used for this study. All media was purchased from Neogen Corporation, Lansing, Michigan unless otherwise stated. Cultured microorganisms were individually transferred to 9 ml sterile tryptic soy broth, vortexed (Labnet International, Inc., Edison, New Jersey), and incubated at 35 °C for 24h (Jeio Tech, Inc., Des Plaines, Illinois). These approximately 9

log CFU/ml culture suspensions were used for inoculation. Cultures were centrifuged at 3650 rpm for 20 min at 37 °C (5810R Eppendorf, Hauppauge, New York). Using the same method as Wang & Harris (2011), the supernatant was discarded and the precipitate was re-suspended in 0.85% sodium chloride (Fisher Scientific, Fair Lawn, New Jersey) solution until a spectrometer (Amersham Biosciences Corporation, Piscataway, New Jersey) absorbance reading of 0.60 was determined yielding about 8 log CFU/ml cultures. To create the culture cocktail used for inoculation, equal parts of each strain of microorganism were combined and vortexed to result in cocktails of *E. coli* O157:H7, non-O157 STECs, *Salmonella* spp., and *L. monocytogenes*. The culture cocktails were serially diluted using 9ml peptone (Becton Dickinson and Company, Sparks, Maryland) and plated on MacConkey Sorbitol Agar (*E. coli*), XLT4 (*Salmonella* spp.) or Modified Oxford Medium (*L. monocytogenes*) to determine cell density. All plates were enumerated after incubation at 35 °C for 24h.

Treatment Preparation

Lactic acid (analytical grade, Sigma Aldrich, St. Louis, Missouri), 4% (v/v), and sodium metasilicate (analytical grade, Sigma Aldrich, St. Louis, Missouri) 4% (w/v), were applied at 4 °C. Antimicrobials were mixed in solution with distilled water (Podolak, Zayas, Kastner, & Fung, 1995a; Podolak, Zayas, Kastner, & Fung, 1995b). A control treatment of distilled water at 4 °C was also tested. Table 12 contains the pH of all treatments. Tap water in the research facility is of poor quality and consistency; thus, distilled water was used to maintain better control over the process.

Sample Preparation

Fresh beef bottom round steaks, fresh pork ham steaks, roast beef, ham, and turkey deli meats were manufactured at the Lambert Powell Meat Laboratory without the use of antimicrobial solutions. Lean meat samples were cut to 100 cm^2 pieces. Each piece was individually inoculated and treated with the antimicrobial treatment assigned.

Fresh beef steaks were inoculated with the culture cocktails of *E. coli* O157:H7, non-O157 STECs, or *Salmonella* spp.. The surface of the deli meats was inoculated with *L. monocytogenes* cocktail culture. The surface of the meat was inoculated with 1mL of a cocktail culture and then evenly spread using a disposable L-shaped culture spreader (VWR International, LLC, Radnor, Pennsylvania). Samples were allowed 30 min to allow the bacteria to adhere to the surface of the meat before antimicrobial solutions were applied. Antimicrobial treatments were randomly assigned. Ten ml of the assigned treatment were evenly applied over the surface of the meat. After treatment application, the samples were allowed an additional 30 min contact time. Half of the deli meat sample were then vacuum packaged (Promax Packaging Solutions, Claremont, California) and treated in a hot water bath (Thermo Scientific, Marietta, Ohio) for 2 min at 90.6 °C (Murinana, Qumby, Davidson, & Grooms, 2002).

A modified plating method from Podolak, Zayas, Kastner, & Fung (1995a) was utilized. Since samples were not stored after dilution, a buffered solution was not utilized and a simple diluent of 0.1% peptone was used instead. One hundred ml of 0.1% peptone was added to each of the meat samples in sterile stomacher bags (Nasco Whirl-Pak, Fort

Atkinson, Wisconsin) and then samples were stomached for 2 min at 300 rpm (400 Circular Seward Medical, London, England). Serial dilutions with 9ml 0.1% peptone were created and dilutions were plated on MacConkey Sorbitol Agar (*E. coli*), XLT4 (*Salmonella* spp.), or Modified Oxford Medium (*L. monocytogenes*) to determine cell density. All plates were enumerated after incubation at 35 °C for 24h. Results are reported in CFU/cm^2.

Statistical Analysis

A completely random design was used to conduct these experiments. Each experiment was conducted in triplicate with 3 replications (on separate days) resulting in 9 samples per treatment. No more than 1 data outlier was removed as a sample possibly contaminated with pathogens before inoculation in each treatment group. All data were converted to $\log_{10}$ CFU/cm^2 before statistical analysis. Statistics were completed using PROC MIXED in SAS 9.2 (SAS Institute, Inc., Cary, North Carolina). The fixed effect was the treatment. There were no differences in replications, and no treatment by replication interactions were included as no practical differences were observed. Tukey pairwise comparisons were utilized due to the potential unequal sample sizes that resulted when data points were removed.

Results and Discussion

Both LA and SM treatments decreased *E. coli* O157:H7 and non-O157 STEC counts regardless of species than the control treatment ($P < 0.01$, Tables 11 and 12, respectively). Previous studies showed that lactic acid at a lower concentration (2%) does

not improve *E. coli* O157:H7 on beef plates (Carpenter, Smith, & Broadbent, 2011; Yoder, et al., 2012). The increased concentration of lactic acid in study 1 showed that 4% lactic acid was better at controlling *E.* coli O157:H7 than 2% lactic acid. In contrast, lactic acid at a greater concentration (5%) was more effective at reducing *E. coli* contamination when beef products were heavily contaminated than when they were lightly contaminated (Youssef, Yang, Badoni, & Gill, 2012). Similar to the current research, pork loins inoculated with *E. coli* O157:H7 showed decreased bacterial counts after exposure to 3% lactic acid (Choi, Kim, Kim, Kim, & Rhee, 2009). Within species, the SM treatment was more effective at reducing *E. coli* O157:H7 contamination than the LA treatment ($P < 0.01$, Table 13). There were no differences in *E. coli* O157:H7 between the LA treatment when applied to beef and pork or the SM treatment when applied to both species ($P = 0.56$ and $P = 0.41$, respectively, Table 13). The LA and SM treatments were equal in non-O157 STEC counts regardless of species ($P > 0.31$, Table 14). Like the current research, Geornaras, et al. (2012a, 2012b), found that beef trimmings treated with 4% sodium metasilicate showed a reduction of pathogens when inoculated with both *E. coli* O157:H7 and non-O157 STEC or *Salmonella*. The control treatment resulted in higher *Salmonella* spp. counts than that of either the LA or SM treatments ($P < 0.01$, Table 15). At a lower concentration (2%), lactic acid reduced *Salmonella* on pork bellies and beef plates (Carpenter, Smith, & Broadbent, 2011; Yoder, et al., 2013); results that coincide with the current research. Within a treatment group, *Salmonella* spp. counts remained equal regardless of the species ($P > 0.81$, Table 15). Beef treated with LA had less *Salmonella* spp. than pork treated with SM ($P = 0.03$,

Table 15). Similarly, *Salmonella* Typhimurium was reduced with 3% lactic acid on pork loins (Choi, Kim, Kim, Kim, & Rhee, 2009).

Treatments including the hot water dip decreased the bacterial load of samples in comparison to those that did not receive the hot water dip ($P < 0.01$, Table 16). Similarly, *L. monocytogenes* counts were reduced in inoculated ready-to-eat meat products utilizing a post-package pasteurization process defined as 90.6 °C for 2 min (Muriana, Quimby, Davidson, & Grooms, 2002). Regardless of post-packaging hot water dips, there were no differences among treatment groups in regards to *L. monocytogenes* counts ($P > 0.73$, Table 16). In a previous study, lactic acid at a lower concentration (2%) effectively reduced *L. monocytogenes* on turkey rolls when compared with a no wash control; however, it did not reduce microbial contamination any more than a water wash (Carpenter, Smith, & Broadbent, 2011). The current study also found no differences in pathogenic contamination among the treatments and the water control ($P > 0.73$, Table 16). In contrast, when applied as a dip to frankfurters, 3% lactic acid at 4 °C reduced *L. monocytogenes* contamination compared to a distilled water control (Byelashov, et al., 2010). An *in vitro* study found 4% sodium metasilicate reduced a high load of *L. monocytogenes* to an undetectable level after 30 min of exposure (Sharma, Williams, Schneider, Schmidt, & Rodrick, 2012a). This may suggest that *L. monocytogenes* has the ability to form biofilms on meat surfaces to protect it from the sodium metasilicate.

Conclusion

Both 4% lactic acid and 4% sodium metasilicate are effective against *E. coli* O157H7, non-O157 STEC, and *Salmonella* spp. on beef bottom rounds and pork ham steaks. Overall, neither antimicrobial solution outperformed the other within species. Sodium metasilicate (4%) on beef was more effective against *E. coli* O157:H7 than 4% lactic acid. In regards to *Salmonella* spp., 4% lactic acid was more effective on beef than 4% sodium metasilicate was on pork. However, both lactic acid and sodium metasilicate can be applied to fresh beef and pork as an effective hurdle technology in the fight for food safety. Treating deli meats with 4% lactic acid or 4% sodium metasilicate did not reduce *L. monocytogenes* loads compared to the control. However, adding a hot water dip was able to minimize microbial contamination. Therefore, by using either sodium metasilicate or lactic acid at 4% in combination with a hot water post-packaging dip could be an effective hurdle technology for deli meat processors.

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Table 11. Strains of microorganisms used

Microorganism	**ATCC number or ID Code**	**Source**
Escherichia coli O157:H7	ATCC 35150	Human – HC
Escherichia coli O157:H7	ATCC 43894	Human – HC
Escherichia coli O157:H7	AU – 1	Laboratory strain (301)
Escherichia coli O157:H7	AU – 2	Laboratory strain (505B)
Escherichia coli O157:H7	AU – 3	Laboratory strain
Non-O157 STEC (O145)	TWO9356	Human – HUS
Non-O157 STEC (O26)	TWO7814	Human – HUS
Non-O157 STEC (O121)	TWO8039	Human
Non-O157 STEC (O45)	TWO14003	Human
Non-O157 STEC (O111)	TWO7926	Human – HC
Non-O157 STEC (O103)	TWO8101	Human
Salmonella	AU – Enteritidis	Laboratory strain
Salmonella	AU – Kentucky	Laboratory strain
Salmonella	AU – Montevideo	Laboratory strain
Salmonella	AU – Thompson	Laboratory strain
Salmonella	AU – Stanley	Laboratory strain
Listeria monocytogenes	ATCC 49594	Petite Scott A
Listeria monocytogenes	ATCC 19115	Human – Serotype 4b
Listeria monocytogenes	ATCC 7644	Human
Listeria monocytogenes	AU – 4	Laboratory strain (101M serotype 4b)
Listeria monocytogenes	AU – 5	Laboratory strain (108M serotype 1/2b)

Table 12. pH values of lactic acid at 4% (LA4), sodium metasilicate at 4% (SM4), and distilled water.

Solution	pH
LA4	1.84
SM4	12.82
Distilled Water	4.90

Table 13. Microbial effects of 4% lactic acid (LA) and 4% sodium metasilicate (SM) on *Escherichia coli* O157:H7 at 4°C on fresh beef bottom round steaks and fresh pork ham steaks after pathogen inoculation and a 30min contact time.

Treatment	Meat	Mean log_{10} CFU/cm^2	SEM[z]
Control	Beef	6.98[a]	0.08
	Pork	6.91[a]	0.08
LA	Beef	5.93[bc]	0.08
	Pork	6.12[b]	0.08
SM	Beef	5.51[d]	0.08
	Pork	5.71[cd]	0.08

[a, b, c, d] Means lacking a common superscript differ ($P < 0.05$).

[z] Standard Error of the Mean

Table 14. Microbial effects of 4% lactic acid (LA) and 4% sodium metasilicate (SM) on non-O157 STEC at 4°C on fresh beef bottom round steaks and fresh pork ham steaks after pathogen inoculation and a 30min contact time.

Treatment	Meat	Mean log_{10} CFU/cm^2	SEM[z]
Control	Beef	6.97[a]	0.08
	Pork	6.93[a]	0.08
LA	Beef	5.91[b]	0.08
	Pork	5.86[b]	0.08
SM	Beef	5.66[b]	0.08
	Pork	5.86[b]	0.08

[a, b] Means lacking a common superscript differ ($P < 0.05$).
[z] Standard Error of the Mean

Table 15. Microbial effects of 4% lactic acid (LA) and 4% sodium metasilicate (SM) on *Salmonella* spp. at 4°C on fresh beef bottom round steaks and fresh pork ham steaks after pathogen inoculation and a 30min contact time.

Treatment	Meat	Mean log_{10} CFU/cm^2	SEM[z]
Control	Beef	6.67[a]	0.16
	Pork	6.39[a]	0.16
LA	Beef	4.48[c]	0.16
	Pork	4.52[bc]	0.16
SM	Beef	5.13[bc]	0.16
	Pork	5.17[b]	0.16

[a, b, c] Means lacking a common superscript differ ($P < 0.05$).

[z] Standard Error of the Mean

Table 16. Microbial effects of 4% lactic acid (LA) and 4% sodium metasilicate (SM) on *Listeria monocytogenes* at 4°C on deli roast beef, ham, and turkey with and without a post packaging lethality treatment.

Treatment	Meat	Mean $\log_{10}$ CFU/cm^2	SEM[z]
Control Without Hot Water Dip	Roast Beef	7.41[a]	0.48
	Ham	7.33[a]	0.48
	Turkey	7.26[a]	0.48
LA Without Hot Water Dip	Roast Beef	6.84[a]	0.48
	Ham	6.86[a]	0.48
	Turkey	7.03[a]	0.48
SM Without Hot Water Dip	Roast Beef	7.01[a]	0.48
	Ham	7.05[a]	0.48
	Turkey	6.91[a]	0.48
Control With Hot Water Dip	Roast Beef	1.50[b]	0.48
	Ham	1.89[b]	0.48
	Turkey	2.01[b]	0.48
LA With Hot Water Dip	Roast Beef	0.91[b]	0.48
	Ham	1.29[b]	0.48
	Turkey	2.46[b]	0.51
SM With Hot Water Dip	Roast Beef	1.16[b]	0.48
	Ham	1.76[b]	0.48
	Turkey	1.65[b]	0.48

[a, b] Means lacking a common superscript differ ($P < 0.05$).

[z] Standard Error of the Mean

Chapter VII

Implications and Conclusions

Sodium metasilicate, 4% and lactic acid, 4% were determined to be the lowest concentrations most effective against all the tested microorganisms. While in some cases such as *Salmonella* spp. lower concentrations were equally effective; in order to have the greatest impact on all tested bacteria the higher concentration of 4% was used. By utilizing the lowest concentration of antimicrobial solution necessary to achieve effective pathogen reduction, meat processors can provide a safe and wholesome meat supply. Meat processors can apply lactic acid or sodium metasilicate at refrigeration temperatures and obtain the same benefits of applying them at a higher temperature. Combining the two antimicrobial solutions is not recommended as it is ineffective against pathogens. However, individually both solutions can serve as a hurdle technology in meat processing facilities. Both lactic acid and sodium metasilicate can be applied to fresh beef and pork as an effective hurdle technology in the fight for food safety. Treating deli meats with lactic acid or sodium metasilicate did not reduce *L. monocytogenes* loads. However, adding a hot water treatment was able to minimize microbial contamination. Results of the first three studies indicate that lactic acid and sodium metasilicate are viable options for hurdle technologies beyond the abattoirs. The final study shows that neither antimicrobial is a good option for controlling *L. monocytogenes* in processed meats;

however, by applying a post-packaging hot water dip in combination with either treatment they could provide an effective hurdle technology for deli meat processors.