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Optimizing application parameters for lactic acid and sodium metasilicate against pathogens on fresh beef, pork and deli meats

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ABSTRACT

Lactic acid (LA) and sodium metasilicate (SM) have been approved for use as antimicrobials on meat. The objectives were to determine optimum concentrations, temperatures and hot-water dips of LA and SM for reduction of *Escherichia coli* O157:H7, non-O157 Shiga-toxin producing *E. coli* (STEC), *Salmonella* spp., and *Listeria monocytogenes* on beef, pork and deli meats. LA was applied at 1, 2, 3, and 4% and SM was applied at 2, 3, 4, and 5%. SM4 and LA4 were the lowest concentrations most effective against all pathogens. LA4 and SM4, the combination of the two (LASM), and distilled water control were applied at 4, 25, and 60 °C. Temperature of application had no effect on pathogens. LA or SM alone were more effective in reduction of pathogens than LASM. Regardless of anti-microbial used in post-packaging lethality treatments, there were no differences in *L. monocytogenes*. Treating deli meats with LA or SM did not reduce *L. monocytogenes*. Both LA and SM can be applied to fresh beef and pork to decrease pathogens.

1. Introduction

Food safety is a constant concern in the meat industry and consideration must be given to methods of ensuring a safe food supply by reduction of pathogens. The CDC (2011) estimates that 48 million (1 in 6) Americans become ill each year due to foodborne illness. Of these, 128,000 are hospitalized and approximately 3000 die of foodborne diseases. *Salmonella* spp., *Escherichia coli*, and *Listeria 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).

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 monitored in the United States. The "big 6" non-O157 Shiga-toxin producing *E. coli* (STEC) 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, 2011). While most *E. coli* infections continue to be linked to ground beef or beef products, there is an increasing amount of fresh produce that also has been implicated. *Salmonella* illnesses have been linked to meats, poultry and poultry products; as well as, peanut butter, cocoa, and fresh produce (FDA, 2012). Across foodborne pathogens, *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 1591 cases of foodborne illness resulting from *L. monocytogenes* with about 255 resulting in death (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. However, *L. monocytogenes* in deli meats continues to be a major concern and ranks 3rd in the top ten pathogen-food combinations in terms of annual disease burden (Batz, Hoffman, & Morris, 2011). The ability of *L. monocytogenes* to grow and thrive at refrigeration temperatures creates a unique problem for the food industry (FDA, 2012).

Lactic acid (LA) is a "Generally Recognized as Safe" (GRAS) food additive commonly used in the meat industry. LA is an organic acid that has been used in abattoirs at 1–2% as a hot carcass rinse to decontaminate red meat carcasses without affecting meat quality (Huffman, 2002; Theron & Lues, 2007). When used at 2–4%, LA has been found to reduce *E. coli* O157:H7 and *S.* Typhimurium (Gill & Badoni, 2004; Yoder et al., 2012). Furthermore, the effectiveness LA on pathogenic organisms, including *L. monocytogenes* was enhanced at high (>60 °C) temperatures Theron & Lues, 2007; Byelashov et al., 2010).

There is very little research on the use of sodium metasilicate (SM) on meat and meat products as an antimicrobial. SM, an alkaline solution that has proven to be effective in reducing Gram-negative bacteria on

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the surface of meat and meat products (Carlson et al., 2008; Pohlman et al., 2009; Weber et al., 2004) has been approved as an antimicrobial for use in ready-to-eat meat and poultry products at 6% (USDA, 2013). SM has been examined as a treatment for fresh beef trimmings before grinding. When used as part of a multi-intervention program, 4% SM 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, but improved or maintained ground beef odor and enhanced beef color (Quilo et al., 2010).

The purpose of this study was to determine optimum concentrations of LSA and SM and application temperatures for the reduction of important food-borne pathogens on beef bottom round muscles and to determine it that optimum concentration and temperature combination provides an effective means of pathogen reduction in fresh beef, pork and deli meats.

2. Materials and methods

2.1. Culture strains

Five strains of *E. coli* O157:H7, 1 strain of each of the big 6 STECs, 5 strains of *Salmonella* spp., and 5 strains of *L. monocytogenes* (Table 1) 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 24 h. The overnight culture produced approximately 9 log CFU/ml culture suspensions which were then used for inoculation. Cultures were centrifuged (5810R Eppendorf, Hauppauge, New York) at 3650 rpm for 20 min at 37 °C. Using the method of Wang and 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 previously determined to result in 8 log CFU/ml cultures. To create the culture cocktails of *E. coli* O157:H7, non-O157 STECs, *Salmonella* spp., and *L. monocytogenes* used for inoculation, equal parts of each strain of microorganism were combined and vortexed. The culture cocktails were then serially diluted using 9 ml peptone (Becton Dickinson and Company, Sparks, Maryland) and plated onto MacConkey Sorbitol Agar (*E. coli*), Xylose Lysine Tergitol 4 Agar (*Salmonella* spp.) or Modified Oxford Medium (*L. monocytogenes*) to determine cell density. All plates were enumerated after incubation at 35 °C for 24 h.

Table 1
Strains and sources of microorganisms used.

Microorganism	ATCC number or ID code	Source
Escherichia coli O157:H7	ATCC 35150	Human — hemorrhagic colitis
Escherichia coli O157:H7	ATCC 43894	Human — hemorrhagic colitis
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 — hemolytic uremic syndrome
Non-O157 STEC (O26)	TWO7814	Human — hemolytic uremic syndrome
Non-O157 STEC (O121)	TWO8039	Human
Non-O157 STEC (O45)	TWO14003	Human
Non-O157 STEC (O111)	TWO7926	Human — hemorrhagic colitis
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 (101 M serotype 4b)
Listeria monocytogenes	AU-5	Laboratory strain (108 M serotype 1/2b)

2.2. Treatment preparation

In phase one, LA and SM antimicrobial treatments were applied various concentrations. LA (analytical grade, Sigma Aldrich, St. Louis, Missouri) concentrations were 1, 2, 3, and 4% (v/v) while SM (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. In phase two, LA at, 4%, and SM at 4%, were applied either alone or in combination (LASAM) at 4, 25, and 60 °C. Antimicrobials were mixed into solution with distilled water (Podolak, Zayas, Kastner, & Fung, 1995a, 1995b). A control treatment of distilled water at 25 °C was also tested. The pH values of all treatments are reported in Table 2. Because of differences in tap water quality, distilled water was used to maintain better control over the process.

2.3. Sample preparation

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

In phase one, fresh meat samples were inoculated with the culture cocktails of *E. coli* O157:H7, non-O157 STECs, *Salmonella* spp., or *L. monocytogenes* (Table 1). In phase two, beef steaks and pork ham steaks were inoculated with the culture cocktails of *E. coli* O157:H7, non-O157 STECs, *Salmonella* spp. while the roast beef, ham and turkey deli meats were inoculated with the *L. monocytogenes* culture cocktail. The surface of the meat was inoculated with 1 ml of a cocktail culture and then evenly spread using a disposable L-shaped culture spreader (VWR International, LLC, Radnor, Pennsylvania). Samples were allowed to sit for 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 milliliters of the assigned treatment was evenly applied over the surface of the meat and a 30 min contact time was allotted to each sample. In the case of deli meat samples, one half of the samples 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 (Muriana, Quimby, Davidson, & Grooms, 2002).

A modified plating method from Podolak et al. (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 milliliters 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

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