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Fate of *Listeria monocytogenes* Inoculated onto Turkey Ham and Treated with Sodium Metasilicate[†,‡]

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Abstract: Sodium Metasilicate (SMS) is a USDA approved antimicrobial and has been shown to be inhibitory towards various foodborne pathogens in refrigerated raw poultry and beef trimmings. The objectives of this study were to determine the antimicrobial effects of SMS against *Listeria monocytogenes* in ready-to-eat turkey ham and to ascertain effects of various treatments on pH. Ready-to-eat turkey ham slices were inoculated with *L. monocytogenes*, treated with either 0% SMS (only sterile de-ionized water) and no inoculum (negative control), 0% SMS (only sterile de-ionized water) and inoculum (positive control) and 6% SMS solution with final concentration of 300 ppm and 600 ppm of SMS in turkey ham samples plus inoculum. In each treatment, the turkey ham samples were vacuum-packaged and stored at 4±1°C. All samples were analyzed on day 0, 7, 14, 21 and 28 of storage for presence of *L. monocytogenes* and pH. The use of 6% SMS solution at 300 and 600 ppm concentrations was not effective in reducing *L. monocytogenes* populations ($p>0.05$) in turkey ham and pH values were similar ($p>0.05$) for all treatments from d 0 to d 28. The findings from this study suggested that SMS is ineffective in reducing *L. monocytogenes* in turkey ham, at the currently approved levels and higher concentrations of SMS may be needed to restrict growth of *L. monocytogenes* in ready-to-eat poultry products.

Key words: Sodium metasilicate, *Listeria monocytogenes*, turkey ham, poultry

INTRODUCTION

Listeria monocytogenes is a foodborne pathogen of great public health significance because of its high case fatality rate (CDC, 2010). Outbreaks have been reported with consumption of Ready-to-Eat (RTE) poultry products in the United States (CDC, 2002), which highlights the public health importance of this pathogen in RTE poultry products. Currently USDA Food Safety Inspection Services (USDA-FSIS) maintains a zero-tolerance policy for *L. monocytogenes* in RTE meat and poultry products. Pregnant women, newborns and immunocompromised persons are at the highest risk for contacting listeriosis due to *L. monocytogenes*. It is estimated that every year approximately 2,500 cases of illness and 500 deaths occur due to foodborne listeriosis in the United States (Mead *et al*., 1999). Approximately 13 different serotypes of *L. monocytogenes* have been described and serotypes 1/2a, 1/2b and 4b are the most commonly encountered in illnesses caused by *L. monocytogenes* (Kathariou, 2002; Gorski *et al*., 2006). The presence of *L. monocytogenes* in RTE products usually involves post-processing contamination and is of high significance in terms of food safety. Tremendous research has been conducted to restrict and eliminate *L. monocytogenes* contamination in a variety of RTE food products by using different antimicrobial compounds. Organic acids, bacteriocins, lauric arginate and natural antimicrobials such as nisin and rosemary have been tested in RTE meat and poultry products for their anti-*Listeria* properties and were reported to be effective in restricting growth of *L. monocytogenes* (Burt, 2004; Lungu and Johnson, 2005; Over *et al*., 2009; Ruiz *et al*., 2009). This pathogen has been encountered in RTE meat and poultry products quite frequently, which endangers public health of individuals consuming the contaminated products, particularly the populations at risk.

Sodium Metasilicate (SMS) is an alkaline fast dissolving, fine granular chemical currently approved by USDA FSIS as a processing aid to be used in RTE meat and poultry products up to a 6% solution applied on the surface of the product at levels not exceeding 300 ppm of the finished product (USDA-FSIS, 2012). Sodium metasilicate was effective in reducing gram-negative foodborne pathogens such as *Salmonella* and *Escherichia coli* O157:H7 *in vitro* and in beef trimmings, beef hide and chicken breasts (Weber *et al*., 2004; Carlson *et al*., 2008; Pohlman *et al*., 2009; Sharma *et al*., 2012). However, very little is known about its antimicrobial efficacy against gram-positive pathogens like *Listeria monocytogenes* in RTE meat and poultry

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products. In an earlier study we found that SMS was effective in inactivating *L. monocytogenes* in suspension and using 6% SMS solutions completely inhibited *L. monocytogenes*. It was also observed that there was time and concentration effect on the susceptibility of *L. monocytogenes* to SMS (data not shown). The objectives of this study were to investigate the efficacy of SMS as anti-listerial compound to reduce *L. monocytogenes* populations on RTE turkey ham after four weeks (28 days) of refrigerated storage and to ascertain the effects of various treatments on the pH.

MATERIALS AND METHODS

This study was carried out to determine anti-*Listeria* properties of SMS (Danisco, USA Inc.) as a food antimicrobial when applied to treat turkey ham inoculated with *L. monocytogenes*. The study was carried out at the Meat Microbiology Laboratory, University of Florida, Gainesville, FL.

Bacterial strain and inoculum preparation: Reference strains of *L. monocytogenes* ½ a, ½ b, 4b, Scott A and ATCC 19115 obtained from ABC Research Corporation in Gainesville, FL, were used as the inoculum in this study to evaluate the antimicrobial properties of SMS. Stock cultures were prepared by transferring each reference strain to test tubes containing 10 mL of TSB followed by incubation at 35°C for 24 h. After incubation, the cultures were centrifuged in sterile 15 mL centrifuge tubes (05-539-5, Fisher Scientific, Pittsburgh, PA) at 5,000 g for 10 min at 4°C (Sorvall RC-5, Dupont Instruments, Newton, CT). The supernatant was discarded and the pellets were resuspended in 10 mL of sterile 0.1% peptone water (CM009, Oxoid Ltd., Basingstoke, Hampshire, England), centrifuged again and pellets were suspended in 1 mL of TSB supplemented with 30% glycerol in a 2 mL cryogenic vial (430488, Corning Inc., Corning, NY), stored at -80°C and used as the stock culture for the inoculation studies. The working cultures were prepared by thawing the deep-frozen culture at room temperature for 5 to 10 min and a loopful was streaked onto TSA (MP Biomedicals, LLC, Solon, OH) followed by incubation at 35°C for 24 h. One colony was selected and transferred to 10 mL of TSB and incubated at 35°C for 20 h. Cells were pelleted by centrifugation at 5,000 g for 10 min at 4°C harvested, washed and re-suspended in sterile 0.1% peptone water. A cocktail of all these strains was prepared by mixing equal amount of inoculum for each strain and used as the final inoculum for the study. Preliminary experiments were carried out to determine the final concentration of inoculum between 5 to 6 log cfu/g in turkey ham samples.

Sample preparation, inoculation and treatment: Ready-to-Eat (RTE) turkey ham with expiration date of at least 2 months were purchased from a local supermarket, transported on ice and processed on the same day or kept at 4°C for use within 24 h. The turkey ham meat was sliced into 25 g samples under aseptic conditions and inoculated with approximately 10^8 cfu/mL of *L. monocytogenes* cocktail inoculum to recover 5 to 6 log cfu/g. The samples were left to stand for 20 min at room temperature in order to attach bacteria to the meat surface. Sodium metasilicate solution 6% (pH 13.0 to 13.1) was prepared in sterile de-ionized water and used as treatments for this study. The turkey ham samples were treated with either 0% SMS (only sterile de-ionized water) and no inoculum (negative control), 0% SMS (only sterile de-ionized water) and inoculum (positive control) and 6% SMS solution with final concentration of 300 ppm and 600 ppm of SMS in turkey ham samples plus inoculum. In each treatment, the turkey ham samples were vacuum packaged (one sample per bag) and stored at 4°C for the rest of the study.

Microbiological and pH analyses: Samples were analyzed on d 0, 7, 14, 21 and 28 of storage for presence of *L. monocytogenes* and pH. Turkey ham samples (25 g each) were transferred aseptically from storage bags to 225 mL of sterile 0.1% peptone water in a sterile stomacher bag (01-002-56, Fisher Scientific, Pittsburgh, PA) and manually agitated for approximately 1 min to loosen and suspend bacteria in solution. Serial dilutions were prepared by transferring 1.0 mL of the sample homogenate to 9 mL of 0.1% sterile peptone water. A volume of 0.1 mL from each dilution was pipetted onto duplicate pre-poured modified oxford medium (MOX) agar plates prepared with MOX supplement for *L. monocytogenes* recovery. The plates were incubated aerobically for 48 h at 35°C. After incubation, colony forming unit (cfu) from each plate were counted, averaged and reported as log cfu/g of the sample. The pH for each sample homogenate was measured by placing the pH probe into the sample homogenate immediately after the microbiological analyses were completed. All pH measurements were recorded in duplicate using an Accumet pH meter (AB15 Accumet Basic, Fisher Scientific, Pittsburgh, PA).

Data analyses: A total of 120 samples were analyzed and a complete randomized block design was used. Samples consisting of 4 treatments, duplicate samples for each treatment, 5 storage d and 3 trials were analyzed by GLIMMIX procedures of SAS (SAS Institute, 2009) to analyze differences between trials, among treatments and storage day and treatment x day interaction. The mean separation was performed using Tukey-Kramer and a level of significance of $\alpha = 0.05$ was used to determine any significant differences among means.

Table 1: *Listeria monocytogenes* counts (LOG CFU/G) in Turkey ham inoculated with *L. monocytogenes*, treated with sodium metasilicate and stored at 4±1°C for 28 days

	Day of sampling				
Treatment	0	7	14	21	28
Negative control	ND	ND	ND	ND	ND
Positive control	6.47^{a,x}	6.34^{a,x}	6.40^{a,x}	6.64^{a,x}	7.29^{a,x}
6% SMS 300 ppm	6.22^{a,y}	6.12^{a,y}	6.40^{a,y,x}	6.76^{a,y,x}	7.27^{a,x}
6% SMS 600 ppm	6.11^{a,y}	6.01^{a,y}	6.51^{a,y,x}	6.89^{a,y,x}	7.29^{a,x}

$^{a-b}$Means within a column lacking a common superscript differ ($p<0.05$).
$^{x-z}$Means within a row lacking a common superscript differ ($p<0.05$). ND = Not detected, N = 6 values per mean, SMS = Sodium metasilicate

Table 2: pH measurements for Turkey ham inoculated with *Listeria monocytogenes*, treated with sodium metasilicate and stored at 4±1°C for 28 days

	Day of sampling				
Treatment	0	7	14	21	28
Negative control	6.86^{a,x}	6.88^{a,x}	6.65^{a,x}	6.59^{a,x}	6.59^{a,x}
Positive control	6.80^{a,x}	6.87^{a,x}	6.62^{a,x,y}	6.59^{a,x,y}	6.45^{a,y}
6% SMS 300 ppm	6.92^{a,x}	6.94^{a,x}	6.72^{a,x,y}	6.71^{a,x,y}	6.54^{a,y}
6% SMS 600 ppm	6.99^{a,x}	7.01^{a,x}	6.79^{a,x,y}	6.77^{a,x,y}	6.62^{a,y}

$^{a-b}$Means within a column lacking a common superscript differ ($p<0.05$).
$^{x-z}$Means within a row lacking a common superscript differ ($p<0.05$). ND = Not detected, N = 6 values per mean, SMS = Sodium metasilicate

RESULTS AND DISCUSSION

***L. monocytogenes* recovery and analysis:** No *L. monocytogenes* were isolated in the negative control. In positive control the counts of *L. monocytogenes* ranged from 6.34 to 7.29 log cfu/g day 0 through 28 day of storage (Table 1). No antimicrobial effects of SMS were observed for 300 and 600 ppm concentrations of 6% SMS treatments, as evidenced by similar ($p>0.05$) *L. monocytogenes* counts among the positive control and SMS treatments on all sampling days. The SMS treatments were similar ($p>0.05$) on all sampling days. However, *L. monocytogenes* was able to significantly grow and populate even with SMS treatments as evidenced from almost 1 log unit increase in *L. monocytogenes* population on day 28 as compared to initial inoculum. This increase in growth of *L. monocytogenes* was correspondent to significant decrease in pH during that period (Table 2). The findings from this study suggested that SMS is ineffective in reducing *L. monocytogenes* in turkey ham, at the currently approved levels. In an earlier study we found that SMS was effective in inactivating *L. monocytogenes in vitro* and it was also observed that there was time and concentration effect on the susceptibility of *L. monocytogenes* to SMS (data not shown). The high pH was found to be responsible for inhibitory effect of SMS against *L. monocytogenes* in that study.

pH Analysis: The pH values were similar ($p>0.05$) for all treatments from day 0 to day 28 in this study (Table 2). The reduction in *L. monocytogenes* populations reported in pure culture experiment (data not shown) was as a result of high pH generated by SMS solutions, which was not observed in this study. It can be assumed that the pH of the 6% SMS treatment with final levels of 300 and 600 ppm were insufficient to cause enough elevation in pH so as to have any effect on growth of *L. monocytogenes* in turkey ham.

In summary, SMS was ineffective in reducing *L. monocytogenes* populations in turkey ham. The antimicrobial efficacy of SMS against *L. monocytogenes* had no effect with an increase in concentration from 300 to 600 ppm of 6% SMS in turkey ham. The findings of this study indicate that the use of SMS at the currently approved levels is not sufficient enough to cause any inhibitory effect on *L. monocytogenes* and higher concentrations of SMS may be needed to restrict the growth of *L. monocytogenes* in RTE poultry products.

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REFERENCES

Burt, S., 2004. Essential oils: Their antibacterial properties and potential applications in foods - a review. Int. J. Food Microbiol., 94: 223-253.

Carlson, B.A., J. Ruby, G.C. Smith, J.N. Sofos, G.R. Bellinger, W. Warren-Serna, B. Centrella, R.A. Bowling and K.E. Belk, 2008. Comparison of antimicrobial efficacy of multiple beef hide decontamination strategies to reduce levels of *Escherichia coli* O157:H7 and *Salmonella*. J. Food Prot., 71: 2223-2227.

CDC (Centers for Disease Control and Prevention), 2002. Public health dispatch: Outbreak of Listeriosis - Northeastern United States, 2002. Morb. Mortal. Wkly. Rep. 51: 950-951.

CDC (Centers for Disease Control and Prevention) 2010. Surveillance for foodborne disease outbreaks: United States, 2007. Morb. Mortal. Wkly. Rep. 59: 973-979.

Gorski, L., D. Flaherty and R.E. Mandrell, 2006. Competitive fitness of *Listeria monocytogenes* serotype 1/2a and 4b strains in mixed cultures with and without food in the U.S. Food and Drug Administration enrichment protocol. Appl. Environ. Microbiol., 72: 776-783.

Kathariou, S., 2002. *Listeria monocytogenes* virulence and pathogenicity, a food safety perspective. J. Food Prot., 65: 1811-1829.

Lungu, B. and M.G. Johnson, 2005. Fate of *Listeria monocytogenes* inoculated onto the surface of model turkey frankfurter pieces treated with zein coatings containing nisin, sodium diacetate and sodium lactate at 4°C. J. Food Prot., 68: 855-859.

Mead, P.S., L. Slutsker, V. Dietz, L.F. McCaig, J.S. Bresee, C. Shapiro, P.M. Griffin and R.V. Tauxe, 1999. Food-related illness and death in the United States. Emerg. Infect. Dis., 5: 607-625.

Over, K.F., N. Hettiarachchy, M.G. Johnson and B. Davis, 2009. Effect of organic acids and plant extracts on *Escherichia coli* O157:H7, *Listeria monocytogenes* and *Salmonella typhimurium* in broth culture model and chicken meat systems. J. Food Sci., 74: M515-521.

Pohlman, F.W., P.N. Dias-Morse, S.A. Quilo, A.H. Brown, P.G. Crandall, R.T. Baublitis, R.P. Story, C. Bokina and G. Rajaratnam, 2009. Microbial, instrumental color and sensory characteristics of ground beef processed from beef trimmings treated with potassium lactate, sodium lactate, sodium metasilicate, peroxyacetic acid or acidified sodium chlorite as single antimicrobial interventions. J. Muscle Foods, 20: 54-69.

Ruiz, A., S.K. Williams, N. Djeri, A. Jr. Hinton and G.E. Rodrick, 2009. Nisin, rosemary and ethylenediaminetetraacetic acid affect the growth of *Listeria monocytogenes* on ready-to-eat turkey ham stored at four degrees Celsius for sixty-three days. Poult. Sci., 88: 1765-1772.

SAS Institute, 2009. The SAS system for Windows. Release 9.2. SAS Inst., Cary, NC.

Sharma, C.S., S.K. Williams, K.R. Schneider, R.H. Schmidt and G.E. Rodrick, 2012. Sodium metasilicate affects growth of *Salmonella typhimurium* in fresh, boneless uncooked chicken breast fillets stored at four degrees celsius for seven days. Poult. Sci., 91: 719-723.

USDA-FSIS (United States Department of Agriculture Food Safety Inspection Services), 2012. FSIS directive 7120.1 Revision 10. Safe and suitable ingredients used in the production of meat, poultry and egg products. http://www.fsis.usda.gov/OPPDE/rdad/FSISDirectives/7120.1.pdf.

Weber, G., J.K. O'Brien and F.G. Bender, 2004. Control of *Escherichia coli* O157:H7 with sodium metasilicate. J. Food Prot., 67: 1501-1506.

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