COMBINING LACTIC ACID AND SODIUM LAURYL SULFATE AS A POST-LETHALITY DIPPING SOLUTION AGAINST *LISTERIA MONOCYTOGENES* ON FRANKFURTERS FORMULATED WITH OR WITHOUT SODIUM LACTATE AND STORED VACUUM-PACKAGED AT 4 ºC

Aubrey Mendonca*[#1], Aura Daraba[#3], Oleksandr Byelashov[1], Joseph Sebranek[1,2], Joseph Cordray[2]
[1]Department of Food Science and Human Nutrition, Iowa State University, Ames, Iowa 50011, USA
[2]Department of Animal Science, Iowa State University, Ames, Iowa 50011, USA
[3]Department Food Science, Food Engineering, Biotechnology and Aquaculture
University "Dunarea de Jos" of Galati, Romania
[#]Both authors contributed equally to this manuscript
*Corresponding author: Address: Iowa State University, 2312 Food Sciences Bldg., Ames, Iowa, 50010, USA
Phone: 515 294 2950
E-mail: amendon@iastate.edu

ABSTRACT
Frankfurters formulated with or without sodium lactate [SL; 2% (w/w)] and surface-treated by with lactic acid [LA; 0 and 5% (v/v)] and sodium lauryl sulfate [SLS; 0, 0.5 and 1% (w/v)] alone or combined, were evaluated as an USDA-FSIS Alternative 1 post-lethality intervention against Listeria monocytogenes (LM). Post-peeling dipping of frankfurters in 5% LA or 1% SLS alone and in 5% LA and 1% SLS combined, reduced initial numbers of LM by ~ 0.4 to 0.7 and 3.8 log_{10}cfu/frankfurter, respectively. During 90 days at 4 °C, numbers of LM survivors did not increase on frankfurters (with 2% SL) that were dipped for 3 minutes in LA + SLS. For all treatments, Hunter L-, a- and b-values were unaffected and pH of the frankfurters was decreased only by treatment with 5% LA alone ($P<0.05$). The use of LA (5%) + SLS (1%) for surface treatment of frankfurters formulated with 2% SL has good potential to control the growth of L. monocytogenes in those ready-to-eat (RTE) meats.

Key words: Frankfurters, *Listeria monocytogenes*, post-lethality intervention, sodium lactate, lactic acid, sodium lauryl sulfate.

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1. INTRODUCTION

Meat producers, retailers, and consumers continue to be challenged by the occurrence of *L. monocytogenes* (LM) in ready-to-eat (RTE) meats(USDA-FSIS, 2019; European Food Safety Agency (EFSA), 2019; Ricci *et.al*, 2018; New South Wales (NSW) Food Authority,2013). The most recent massive LM outbreak related to the consumption of RTE meats occurred between 2017 and 2018 in South Africa and resulted in 1,053 cases and 212 deaths (Murano, 2018). This outbreak is viewed as the largest-ever *Listeria* outbreak in the world by United Nations (UN; 2018) and by World Health Organization (WHO; 2018).In Australia, in 2018 were reported 20 *Listeria* outbreak cases that resulted in the hospitalization of all cases, seven deaths and one miscarriage associated with the outbreak (WHO, 2018). According to the World Health Organization's and FAO's Technical Report (WHO/FAO, 2004), frankfurters, widely consumed as convenience or traditional food, are declared as a food carrying a risk which varies from very high to low risk depending on the serving temperature. In the U.S., it was estimated that LM caused approximately 1,600 foodborne illnesses, 1,500 hospitalizations, and 260 deaths annually (USDA-FSIS, 2014) and ready-to-eat (RTE) meats pose the greatest risk to public health (Donnelly and Diez-Gonzalez, 2014; USDA-FSIS, 2003).

Listeria monocytogenes, a gram-positive psychrotrophic foodborne pathogen,widely distributed in nature, poses a continuous, major concern for food regulatory agencies, food processors, and consumers (Slutsker and

Schuchat, 1999), and has been extensively studied and frequently reported as a major food safety risk. The disease caused by this pathogen can be life-threatening with a great negative impacton immunocompromised individuals who representa high risk group (i.e.: chemotherapy patients, the elderly, neonates, and pregnant women) (Lomonaco*et al.*, 2015;Swaminathan*et al*., 2007). This pathogen is killed during proper thermal processing but is of major concern as a post-processing contaminant of ready-to-eat meat products, such frankfurters, due to its ability to grow during refrigerated storage of those products (Buchanan *et al*., 2017; Kim *et al*., 2009; Samelis*et al*., 2002). Despite the progress achieved in testing antimicrobials against *L. monocytogenes* on RTE meats in the past few decades, listeriosis outbreaks still occur, and effective approaches are still needed to prevent meat-borne contamination.

Frankfurters are ready-to-eat meats and their microbial safety primarily relies on their formulation and storage conditions (i.e.: salt, curing agents, and proper refrigerated storage). If properly applied the cooking process and parameters, *L. monocytogenes* does not survive in frankfurters (Zaika*et al*., 1989) but further contamination can occur during post-processing/post-lethality steps such as: peeling, before packaging, or during subsequent improper handling either in stores and deli sections, fast-foods, or households etc.Unfortunately, *L. monocytogenes* can overcome these hurdles and grow at low temperatures (Gandhi, 2007; Glass *et al*., 2002). Therefore, it is not surprising that frankfurters have been implicated in multiple listeriosis outbreaks, and non-reheated frankfurters are a high-risk meat product for listeriosis (WHO, 2018; USDA-FSIS, 2010). This type of food safety-related events strongly indicatesthat: 1)there is still a risk of listeriosis related to consumption of RTE meats, and 2) there is a need for applying more effective antimicrobial post-lethality interventions against LM in RTE meats topreventing the growth of this pathogen throughout the whole food chain and during the shelf-life of the product.

To prevent meat-borne contamination and listeriosis outbreaks, US processors of RTE meat products are required to adopt one of three alternatives: *Alternative 1*- a post-lethality inactivation treatment for L. monocytogenes combined with a growth inhibitor for the pathogen; *Alternative 2*- a post-lethality inactivation treatment or a growth inhibitor, or *Alternative 3*- sanitation and environmental tests. The adopted alternative must be stated in processors' hazard analysis critical control point (HACCP) plan or perquisite programs. Also, the effectiveness of the alternative in controlling L. monocytogenes should be validated and the validation data to be submitted to FSIS (USDA-FSIS, 2003). More importantly, when planning for an antimicrobial intervention on a food product, "*one size fits all*" principle does not apply, and subsequent testing and validation of the efficacy of that particular antimicrobial intervention is paramount (Consortium of Food Process Validation Experts (CFPVE), 2013). In this regard, variations in the formulation of the food product, the type and concentration of chemicals used as intervention, stage of intervention application (i.e.: point to be applied in the production line), duration and method of application etc. are important factors to be tested and validated. (Bangel, 2012; Crozier-Dodson *et al.*, 2005).

Sodium lauryl sulfate (SLS), although not yet approved for being used in meat product formulation, is a generally regarded as a safe (GRAS) food additive when used at levels of 10 to 5000 ppm. Usually SLS is used in animal fats, vegetable oils, fruit juices and beverages, gelatin, marshmallows and egg whites (FDA-Code of Federal Regulations, 2017). In Europe, SLS is used in pharmaceutical preparations as an emulsifying agent, modified-release agent, penetration enhancer, solubilizing agent, tablet and capsule lubricant (European Medical Agency (EMA), 2015). Due to its surfactant properties SLS can cause cytoplasmic membrane damage in bacteria (Dychdala, 1983) by facilitating better penetration of lactic

acid into the bacterial cell. Also, SLS has been shown to be a viral inactivator due to its inhibition of enveloped and non-enveloped viruses involved in human or animal diseases (Madeira de Sousa *et al*., 2019) by causing dissociation of the viral envelope and denaturates the capsid proteins involved in the viral replication cycle from adhesion to viral encapsidation (Piret*et al*., 2002).

The growth inhibitory effects of surface treatments with lactic acid or lactates combined with sodium lauryl sulfate against *L. monocytogenes* have been reported for frankfurters formulated with different meat composition (Byelashov, 2010; Byelashov, 2005; Samelis 2002). The ability of lactic acid applied as a dipping or spraying solutions to suppress the growth of LM has been also reported and lactic acid's efficacy was dependent on its concentration, when used singly or combined with other antimicrobials. The addition of sodium lactate (SL) to RTE meat formulations was more effective in inhibiting LM growth compared to only dipping meats in antimicrobial solutions (Camelia Grosulescu*et al*., 2011; Choi and Chin, 2003).

To our knowledge there are no published reports on the application of lactic acid (LA) and sodium lauryl sulfate(SLS) mixtures,as dipping solutions, together with a growth inhibitor such as sodium lactate (SL) to control *L. monocytogenes* growth and survival in frankfurters. Our experiment used some unique formulations, namely frankfurters formulated with or without sodium lactate [SL; 2% (w/w)] and surface-treated by with lactic acid [LA; 0 and 5% (v/v)] and sodium lauryl sulfate [SLS; 0, 0.5 and 1% (w/v)] alone or combined. Accordingly, the main objective of the present challenge study was to evaluate a novel combination of lactic acid and the surfactant sodium lauryl sulfate as a potential *Alternative 1*post-lethality surface-treatment intervention for controlling *L. monocytogenes* on vacuum-packed frankfurters formulated with or without 2% sodium lactate, during storage at 4°C for 90 days.

2. MATERIALS AND METHODS

2.1. Experimental design

Six dipping solutions, including the control(deionized water; DW), two dipping times (1 and 3 min.), seven storage times (1, 14, 28, 42, 56, 70, and 90 days) at 4 °C, and frankfurters formulated with 2% SL or without SL were used in present study. The experimental unit for the dipping solutions and dipping time treatments was two frankfurters. A randomized complete-block full factorial experimental design was used. Three replications of each experiment were performed.

2.2. Preparation of frankfurters

The frankfurters were prepared from pork fat trimmings (40:60 lean:fat ratio) and lean beef trimmings (80:20 lean:fat ratio) at Iowa State University Meat Laboratory, using the formulation previously described by Lu *et al*., 2005. The meat was ground through a 0.79-cm grinder plate, and divided into two batches. In one batch, sodium lactate, as a powder (SL; Purac Inc., Lincolnshire, IL), was added along with salt, sodium erythrobate, sodium nitrite, seasoning and water with ice during emulsification in a vacuum chopper (Kutter Supplies, Inc., Randolph, MA.). The second batch, used as a control, was prepared in the same way as the first batch using the same ingredients but without SL. Subsequently, the meat batters were extruded through a meat stuffer (Risco® Model RS 4003-165; Stoughton, Mass., U.S.A.) into 22-mm peelable cellulose casings (DevroTeepack™ Wiene-Pack® Coastal corrugated Inc., N. Charleston, S.C., U.S.A.). The sausage was linked at 14.0 cm length by 2.2 cm in diameter using a poly-clip system (GmbH and Co., KG, Frankfurt, Germany).

The linked product was hung on racks and cooked for 90 minutes using the conventional cooking-smoking cycle in a humidity-controlled smoke house (Alkar, DEC Intl. Inc., Lodi, Wis., U.S.A.) to an internal temperature of 71.1 ºC. Natural smoke (hardwood sawdust; Frantz Co., Milwaukee, WI) was applied during the cooking cycle. At the end of the

cycle, frankfurters were showered with cold water, and then kept in a walk-in refrigerator at 4 °C for 18-19 hours. The following day, the frankfurters were peeled, (Peeler Townsend 2600, Des Moines, IA), sealed in vacuum bags and stored at – 20 ºC in a walk-in freezer until used in the experiments.

2.3. Treatment of frankfurters

For each replication of the experiment the frozen frankfurters were transferred into a walk-in refrigerator and thawed overnight at 4°C. After thawing, frankfurters were aseptically removed from the bulk package and placed into a sanitized aluminum basket (28 frankfurters per basket). Each basket with frankfurters was immersed in 3 liters of freshly prepared solutions of 5% (vol/vol) lactic acid (LA; Birko Co. Denver, CO.), 0.5% (wt/vol) SLS (Fisher Scientific, Fair Law, NJ), 1% SLS, 0.5% SLS + 5% LA, 1% SLS+ 5% LA, or deionized water (DW; Control) for 1 or 3 min, at room temperature (23 ± 1 ºC). After immersion, the baskets with frankfurters were removed and allowed to drain for about 2 minutes. Treated frankfurters were placed individually in vacuum packaging bags (Cryovac B-2540, Cryovac Sealed Air Corp., Duncan, SC; water vapor transmission = 0.5-0.6g at 100 ºF, 100% RH/100 sq.in./24h; oxygen transmission rate = 36 CC at 40 ºF/m²/24h/0%RH) prior to inoculation with the pathogen.

2.4. Preparation of inoculum

The inoculum consisted of a five-strain *L. monocytogenes* cocktail obtained from: H7962 serotype 4b, H7969 serotype 4b, H7762 serotype 4b, Scott A/NADC 2045 serotype 4b, and H7764 serotype 1/2a. Except for *L. monocytogenes* Scott A, which was obtained from the National Animal Disease Center, Agricultural research Service, U.S. Department of Agriculture, Ames, Iowa, all other strains were isolates from the 1998-1999 Bil Mar Food Outbreak (CDC, Atlanta, GA). The working cultures of *L. monocytogenes* strainswere prepared from frozen stocks[-70 °C, in brain heart infusion broth (Difco Becton Dickinson, Sparks, MD.) with 10% glycerol]. Each *L. monocytogenes* working culture was prepared by transferring a loopful of the appropriate stock culture into 10 ml of tryptic soy broth (Difco) supplemented with 0.6% yeast extract (Difco; TSBYE) followed by 18 hours of incubation at 35 °C. Prior to each replication of the experiment, two consecutive 18-hour transfers of each *L. monocytogenes* strain were prepared in TSBYE at 35°C. The five-strain cocktail was prepared by transferring 6 ml of each culture into a sterilized 30-ml centrifuge tube. Cells were harvested by centrifugation (10,000×g, 10 min, 4°C) and the supernatant was discarded. Pelleted cells were suspended in 30 ml of 0.1% (w/v) peptone and harvested by centrifugation. This procedure was repeated twice to obtain the washed cells. The washed cell suspension was diluted (10-fold) to obtain the inoculum with a viable count of ~ 10^8cfu/ml. The viable cell population was determined by plating the cell suspension on tryptic soy agar (TSA; Difco) supplemented with 0.6 % yeast extract (TSAYE) and counting bacterial colonies on TSAYE after incubation at 35°C for 24 h.

2.5. Inoculation of frankfurters

Frankfurters were placed in Cryovac bags (one frankfurters per bag) and spot-inoculated with 0.1 ml of the five-strain cocktail of *L. monocytogenes* to give an initial population of 10^7cfu/frankfurter. To spread the inoculum evenly over the frankfurters'surface, each frankfurter was manually massaged from outside of the bag for 10 sec. Subsequently, the bags with frankfurters were vacuumed at 95 kPa using a Multivac A 300/51 vacuum packaging machine (Multivac Sepp Haggenmuller, Gmblt& Co., Wolfertschwenden, Germany) and stored at 4 ºC for 90 days in a walk-in refrigerator.

2.6. Measurement of pH

Measurements of pH were performed at 0, 1, 14, 42, 70 and 90 days of storage at 4ºC. Following the procedure described by Sebranek (2001), a 10-gram sample of non-inoculated frankfurters was pummeled with 90 ml of distilled water using a Seward Stomacher 400 Lab-blender (Seward Ltd., London, England) for one minute, at medium speed. The pH of frankfurter slurry was measured at 23 ± 1 ºC,

using an Orion Model 525 pH meter (Orion Research Inc., Boston. MA) fitted with a glass electrode.

2.7. Color measurement

The influence of surface treatments on color of frankfurters was evaluated. The Hunter *L* (lightness), *a* (redness), and *b* (yellowness) values of non-inoculated frankfurters were measured on the day of packaging (day 0) and at 42 and 90 days of storage at 4 ºC using a Hunter Lab Scan Colorimeter (Hunter Associated Labs. Inc., Reston, VA). Before readings, the Hunter Lab Scan Colorimeter was calibrated against black and white reference tiles which were covered with the same material that was used for frankfurters' vacuum packaging. Area view and port size were 0.25 and 0.40 inch, respectively. The results were expressed as the average of three measurements performed on different sites on the frankfurters' surface.

2.8. Microbiological analysis

At 1, 14, 28, 42, 70 and 90 storage days the vacuum packaged frankfurters were aseptically opened and 20 ml of 0.1% (w/v) sterile peptone (Difco) were added to each package. Each frankfurter's surface was vigorously "washed" by manually rubbing the product from outside of the package, followed by shaking for 30 seconds, to release the pathogen into the wash solution. Ten-fold serial dilutions of the wash solution were prepared in 0.1% (w/v) of peptone. Aliquots (0.1 ml) of appropriate dilutions were surface-plated onto modified Oxford agar (MOX; Difco). All inoculated agar plates were incubated at 35 ºC for 48 hours and the typical colonies of *L. monocytogenes* were counted and expressed as $\log_{10}$cfu/ frankfurter.

2.9. Statistical analysis

Three replications of the experiment were conducted each with two samples per treatment. Analysis of variance was used to assess the significant differences among the mean values. Tukey's honestly significant difference test was used to determine whether there was a significant difference in microbial reductions, pH values and Hunter color *L*-, *a*- and *b*-values. Differences were considered statistically significant when the associated *P*-value was less than 0.05 (SAS Institute, 1995).

3. RESULTS AND DISCUSSION

RESULTS

1. Antimicrobial efficacy

Survival and growth of *L. monocytogenes* in vacuum packaged frankfurters (formulated with or without 2.0% SL) that were immersed for 1.0 or 3.0 minutes in deionized water (control) or antimicrobial solutions and stored at 4 ºC for 90 days is presented in Figures 1-4.

1.1. Frankfurters formulated without sodium lactate. For the frankfurters formulated without SL, the *L. monocytogenes* viable counts depended on the dipping time (1 or 3 minutes) and the characteristics of the dipping solution (formulation and concentration) (Figures 1 and 2).

Dipping of frankfurters for 1 minute in the tested solutions did not affect the growth of the pathogen, and for all the samples LM reached levels between 10^9 and 10^{10}, respectively, after 90 days of storage at 4C. In this scenario, the formulation of the dipping solutions affected the lag phase and growth rate but only for up to 28 days of storage. The lower the concentration of the antimicrobials, alone or in combinations, the lower the reduction of the initial viable numbers of LM, the shorter lag phases, and faster recovery and growth. Exceptions were observed when frankfurters were dipped in 5% LA+0.5% SLS or 5% LA+1% SLS when significant biological reductions of the initial numbers of the LM viable cells were noted: 2 logs reduction (for 5% LA+0.5% SLS) and almost 4 logs (for 5% LA+1% SLS) . These results indicate that 5% LA+0.5% SLS or 5% LA+1% SLS exhibit stronger bactericidal action. In control frankfurters, as expected, the *L. monocytogenes* exhibited a 14-day lag phase and grew to approximately 9.0 $\log_{10}$ cfu/frankfurter at day 56. Compared to control, there were no significant differences in viable counts of LM in frankfurters treated with 0.5% SLS alone (P>0.05). The SLS (1.0%) extended the lag phase of the pathogen from 14 to 28 days after which viable counts increased and

also reached about 9.0 log_{10} cfu/frankfurter by day 56. Lactic acid (LA; 5.0%) alone exerted a bacteriostatic effect on LM for 42 days after which viable counts increased to 9.0 log_{10}cfu/frankfurter at day 90.

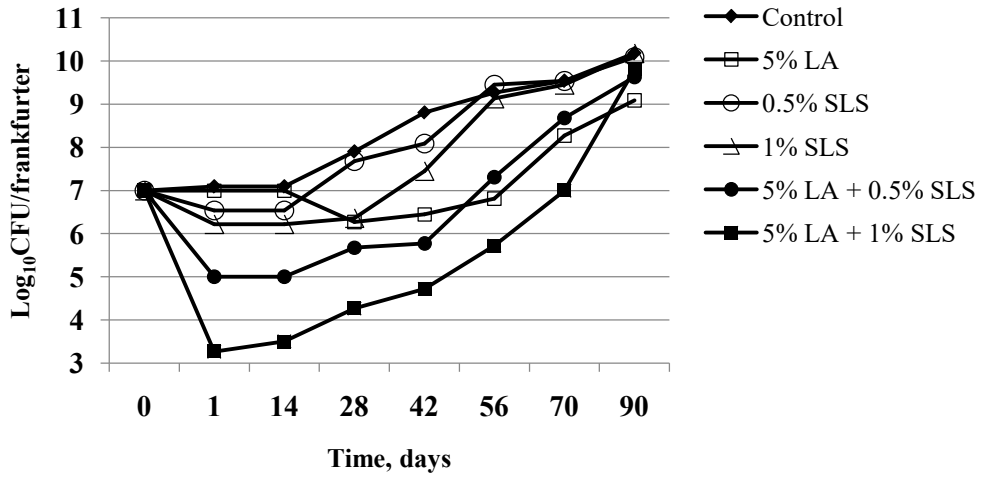

Fig. 1. Survivors of *Listeria monocytogenes* on frankfurters (formulated without sodium lactate) that were immersed for 1 minute in deionized water (control) or solutions of lactic acid (LA; 5%), sodium lauryl sulfate (SLS; 0.5% or 1.0%), or a combination of LA and SLS (LA+SLS) and subsequently inoculated, vacuum packaged, and stored at 4 ºC for 90 days.

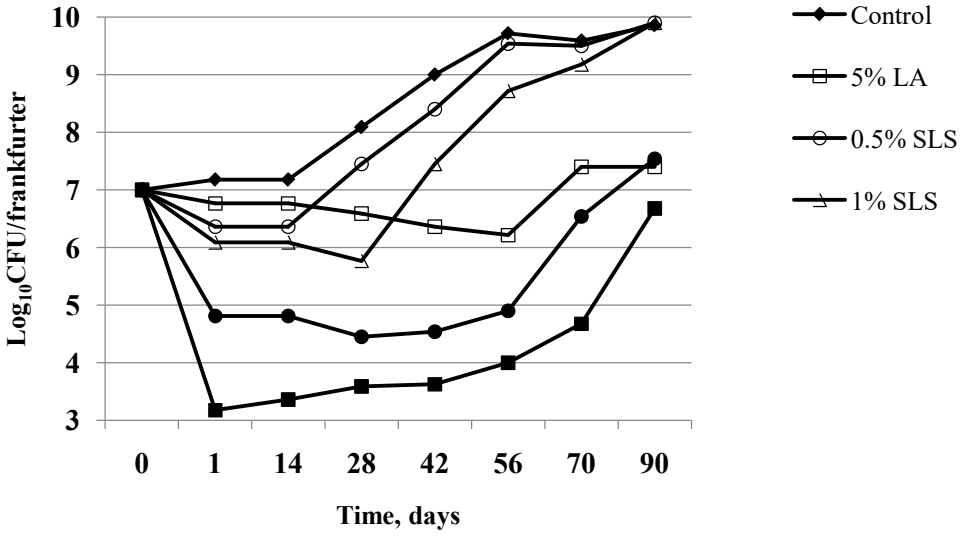

Fig. 2. Survivors of *Listeria monocytogenes* on frankfurters (formulated without sodium lactate) that were immersed for 3 minutes in deionized water (control) or solutions of lactic acid (LA; 5%), sodium lauryl sulfate (SLS; 0.5% or 1.0%), or a combination of LA and SLS (LA+SLS) and subsequently inoculated, vacuum packaged, and stored at 4 ºC for 90 days.

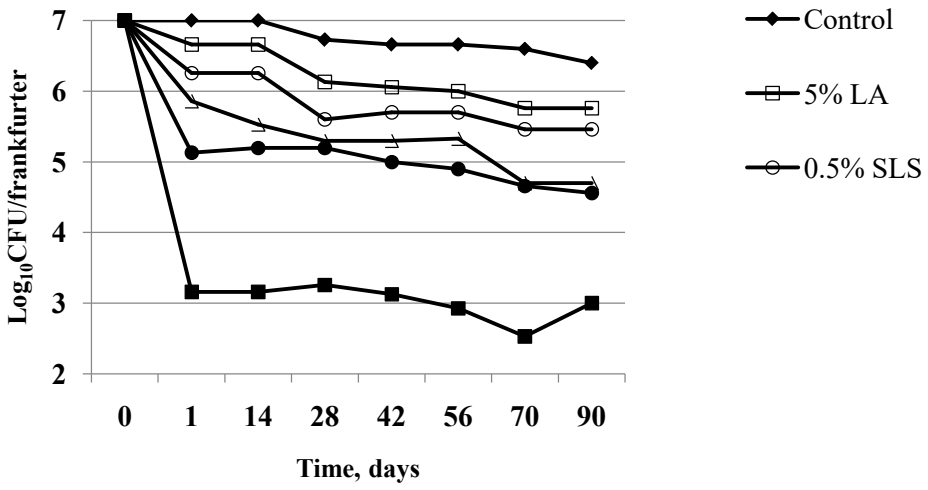

Fig. 3. Survivors of *Listeria monocytogenes* on frankfurters (formulated with 2% sodium lactate) that were immersed for 1 minute in deionized water (control) or solutions of lactic acid (LA; 5%), sodium lauryl sulfate (SLS; 0.5% or 1.0%), or a combination of LA and SLS (LA+SLS) and subsequently inoculated, vacuum packaged, and stored at 4 ºC for 90 days.

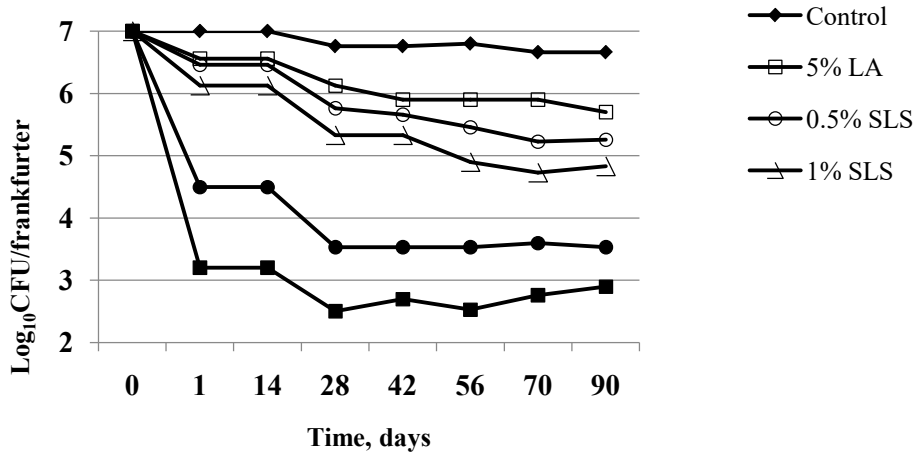

Fig. 4. Survivors of *Listeria monocytogenes* on frankfurters (formulated with 2% sodium lactate) that were immersed for 3 minutes in deionized water (control) or solutions of lactic acid (LA; 5%), sodium lauryl sulfate (SLS; 0.5% or 1.0%), or a combination of LA and SLS (LA+SLS) and subsequently inoculated, vacuum packaged, and stored at 4 ºC for 90 days.

Table 1.The pH valuesof refrigerated (4 ºC) vacuum packaged frankfurters made with or without 2% sodium lactate (SL) and dipped for 3 minutes in deionized water (DW) or lactic acid (LA) in combination with sodium lauryl sulfate (SLS; 1.0%)

SEM is standard error of the means; Mean values with different subscripts (A, B) within a

Surface treatments	Storage time (days)			
	0	28	90	*SEM*
$DW_{0\%\ SL}$	$_{A}6.14^{x}$	$_{A}6.03^{x}$	$_{A}5.75^{y}$	*0.04*
$DW_{2\%SL}$	$_{A}6.13^{x}$	$_{A}6.07^{x}$	$_{A}6.01^{x}$	*0.02*
$5\%LA+\ 1\%SLS_{(0\%\ SL)}$	$_{B}5.85^{x}$	$_{B}5.85^{x}$	$_{A}5.71^{x}$	*0.05*
$5\%LA+1\%SLS_{(2\%SL\ )}$	$_{B}5.87^{x}$	$_{B}5.89^{x}$	$_{A}5.85^{x}$	*0.08*
SEM	*0.05*	*0.02*	*0.07*	

column are significantly different ($P< 0.05$); Mean values with different superscripts (x, y) within a row are significantly different ($P< 0.05$); n=3

Table 2. Hunter color values (*L, a, b*) of refrigerated (4ºC) vacuum packaged frankfurters formulated with or without 2% sodium lactate (SL) and immersed for 3 minutes in deionized water (DW) or lactic acid (LA; 5%) in combination with sodium lauryl sulfate (SLS; 1%)

		Treatments				
Hunter color value	Day of storage	$DW_{0\%SL}$	$DW_{2\%\ SL}$	$5\%LA+\ 1\%SLS_{(0\%\ SL)}$	$5\%LA+1\%SLS_{(2\%SL\ )}$	*SEM*
L	0	56.66	55.96	54.76	55.20	*0.54*
	42	56.80	57.10	56.15	56.81	*0.30*
	90	56.51	57.64	56.92	56.94	*0.30*
	SEM	*0.79*	*0.95*	*0.57*	*0.57*	
a	0	27.42	28.92	28.83	29.47	*0.54*
	42	28.81	28.08	28.69	28.63	*0.60*
	90	26.84	28.04	27.15	27.15	*0.67*
	SEM	*0.86*	*0.39*	*0.34*	*0.89*	
b	0	33.24	33.11	38.63	36.27	*1.23*
	42	35.86	35.43	33.77	35.12	*0.74*
	90	36.22	36.89	35.31	34.94	*1.08*
	SEM	*1.11*	*1.23*	*1.32*	*0.41*	

SEM is standard error of the means; n=3

A major observation regarding the antibacterial activity of SLS and LA was that at day 1, the 0.5% SLS and 1.0% SLS treatments decreased the viable counts by 0.56 and 0.82 log cycles, respectively whereas, LA alone did not alter those counts. Irrespective of the extent of bacteriostatic or bactericidal action of the surface treatments (for 1 min) used in the present study, subsequent growth of *L. monocytogenes* occurred in frankfurters without SL and reached levels of 9.0 to 10.2 log_{10}cfu/frankfurter at day 90 (Figure 1).

A similar trend with regard to bactericidal effects and subsequent growth of LM was observed following immersion of frankfurters in the antimicrobial solutions for 3.0 minutes (Figure 2). Extending the treatment time to 3.0 minutes did not increase the bactericidal effect of the LA/SLS combinations at day 1 ($P>0.05$); however, a major difference was that all treatments involving LA, alone or combined with SLS (0.5 or 1.0%), substantially extended the lag phase of the pathogen. Also, compared to control, the LA-based treatments resulted in significantly lower *L. monocytogenes* populations on frankfurters at day 90 ($P<0.05$). In this regard, viable counts of the pathogen on frankfurters exposed to LA-based treatments ranged from 6.70 to 7.42 log_{10}cfu/frankfurter whereas, counts in control frankfurters and those treated with SLS (0.5 or 1.0%) were as high as 9.73 log_{10}cfu/frankfurter (Figure 2).

1.2. Frankfurters formulated with 2% sodium lactate (2% SL).

Viable populations of *L. monocytogenes* in refrigerated (4ºC) vacuum packaged frankfurters formulated with 2% SL and exposed to water or antimicrobial solution for 1.0 or 3.0 minutes are shown in Figures 3 and 4, respectively. At day 1, reductions in initial viable populations (log_{10}cfu/frankfurter) of LM were 0.34 (for 5% LA), 0.72 (for 0.5% SLS), and 1.23 (for 1.0% SLS). In contrast, initial populations of the pathogen on frankfurters that were treated with 5% LA + SLS (0.5%) and 5% LA + SLS (1.0%) were reduced by 1.9 and 3.92 log cycles, respectively. No growth of LM occurred in control or in treated frankfurters for the entire 90-day storage period (Figure 3).

As observed with frankfurters formulated with 2% SL and surface-treated for 1.0 minute (Figure 3), no increase in growth of LM occurred in control or frankfurters immersed in antimicrobial solutions for 3.0 minutes (Figure 4). Also, the 3.0-minute exposure of 2% SL-containing frankfurters to 5% LA + SLS (0.5%) resulted in an increased killing of LM;

log reductions in viable counts were 2.5 and 3.4 log cycles at day 1 and day 28, respectively. Log reductions in initial populations of the pathogen on frankfurters treated with 5% LA + SLS (1.0%) were 3.80 (day 1) and 4.50 (day 28).

2. Product pH and color

The pH values of dipping solutions were measured to assessing if pH can be considered an additional hurdle for LM. This was not the case in our study since the pH values did not significantly differ among all surface treatments and storage times. (Table 1). The pH values of frankfurters exposed to water or to the most effective antilisterial treatment time (for 3.0 minutes) are presented in Table 2. The pH of LA (5%) + SLS (1.0%) treatment solution was 1.84 and immersion of frankfurters in that solution lowered their pH by 0.26 units (frankfurters formulated with SL) and 0.29 units (frankfurters without SL). There was no significant difference in initial pH (~6.14) of water-dipped frankfurters (control) whether or not SL was present in the formulation ($P > 0.05$). The SL in frankfurters did not significantly change the pH of the product during storage ($P > 0.05$). In contrast, control frankfurters without SL exhibited significant reduction in pH by day 90; the pH decreased from 6.14 to 5.75 ($P < 0.05$). Compared to control frankfurters, those treated with 5% LA + SLS (0.5 or 1%) did not show significant decreases in pH during storage whether or not they were formulated with 2% SL.

The color of the control and treated frankfurters has been assessed for detecting the eventual changes in visual characteristics and for consumer acceptance reasons. No significant differences in Hunter color *L, a* and*b* values were observed for vacuum packaged frankfurters (formulated with or without SL) during storage at 4 °C for 90 days (Table 2).

DISCUSSION

1. Antimicrobial efficacy

In the present study we applied LA (5%) alone or combined with SLS (0.5% or 1.0%) as surface-treatments for frankfurters formulated with or without 2% SL. Treatment of frankfurters with 5% LA alone inhibited *L. monocytogenes* by extending the organism's lag phase of growth. Similar to other short chain lipophilic organic acids, lactic acid is believed to inhibit microbial growth by diffusing the proton motive force (PMF) across the bacterial cytoplasmic membrane (Eklund, 1985). Diffusion of the PMF prevents organisms from generating energy and multiplying, and most of the cellular adenosine triphosphate (ATP) is utilized in pumping protons out of the cell Davidson, 2001). Lactic acid sprays or dips at 0.2 to 2.5% have been shown to inhibit growth of spoilage bacteria in various meat products and extend their shelf life (Dickson and Anderson, 1992; Shrestha and Min, 2006). We observed that this growth inhibition by lactic acid applied to the surface of the frankfurters was transient and the pathogen subsequently grew in frankfurters formulated without 2% SL.

Our observed minimal initial bactericidal effect of SLS (0.5 or 1.0%), used singly, is not surprising considering the pH (ranging from 6.09 to 6.13) of the frankfurters treated with this anionic surfactant (data not shown). The bactericidal action of anionic surfactants such as SLS increases substantially in more acidic conditions (Dychala, 1983). The increased effectiveness of SLS at a lower pH was also observed by Hill and Ivey (1988), Restanio*et al.* (1994), and Tamblyn and Conner (1997). In this respect, the increase in bactericidal activity (~ 1.7 to 3.8 $\log_{10}$ reduction in initial viable counts) of 5% LA combined with SLS (0.5 or 1.0%) against *L. monocytogenes* on frankfurters is expected in view of the lowered pH of frankfurters (pH 5.85) following surface-treatment with this combination. Although LA/SLS treatments tested in the present study exhibited the best bactericidal effect, those treatments, such as 5% LA or SLS (0.5 or 1.0%) used alone, failed to prevent subsequent growth of *L. monocytogenes* on frankfurters (formulated without 2% SL) during the 90-day storage period at 4 ºC. Our findings confirm our earlier observations (Byelashov and Mendonça, 2005,Iowa State University Food

Safety Laboratory) and those reported by Byelashov*et al*. (2008) who demonstrated significant reductions in viable counts of *L. monocytogenes* (2.8 log cycles/cm^2) on frankfurters following a 10-second spray application of LA (5%) + SLS (0.5%) to the surface of the product after inoculation. Those same researchers demonstrated that after 35 days the pathogen initiated growth in frankfurters treated with the LA/SLS treatment and stored under vacuum at 4 ºC.

Results of the present study revealed the potential for *L. monocytogenes* survivors of a bactericidal treatment to subsequently grow and attain substantially high populations in vacuum packaged frankfurters formulated without 2% SL and stored for 90 days at 4 ºC. The efficacy of organic acid salts such as lactate and diacetate, for suppressing growth of *L. monocytogenes* in RTE meats has been widely reported (Barmpalia*et al*., 2004; Bedie*et al*., 2001; Lu, Sebranek, Dickson, Mendonça, & Bailey, 2005; Mbandi&Shelef, 2002; Porto *et al*., 2002; Porto-Fett, Call, Muriana, Freier, &Luchansky, 2010; Samelis*et al*., 2005). Sodium lactate (at concentrations of 1.5% to 3.0%) added alone or in combination with sodium diacetate (0.125% to 0.25%) to RTE meats is used by meat processors to control growth of *L. monocytogenes* inthose food products (Thompson, Carpenter, Martini, & Broadbent, 2008; Tompkin, 2002). While it is well known that lactates may inhibit growth of *L. monocytogenes* in RTE meats during extended refrigeratedstorage, they are not effective in exerting a lethal effect on initial populations of the pathogen.

Under the conditions of this study, our results demonstrated that addition of 2% SL in frankfurter's formulation had a bacteriostatic effect on *L. monocytogenes* and in combination with 5% LA and SLS solutions, applied as surface treatments, resulted in a stronger antilisterial effect (Figures 3 and 4) without altering the desirable color characteristics of the frankfurters (Table 4). While the incorporation of 2% SL in the frankfurter formulation did not increase the initial kill of *L. monocytogenes*, it suppressed growth of the pathogen during the entire storage period on frankfurters. Our results agree with previous research that demonstrated inhibition of *L. monocytogenes* in cured meat products by sodium lactate in combination with other antimicrobials such acetates (Geornaras*et al*., 2006; Mbandi and Shelef, 2002; Porto *et al*., 2002; Samelis*et al*., 2002). Also, Porto *et al*. (2002) reported no growth of *L. monocytogenes* in beef/pork frankfurters prepared with potassium sorbate and stored for 90 days at 4 °C.

2. Product pH and color

Our findings of no significant difference in initial pH (6.14) of water-dipped frankfurters (control) whether or not SL was present in the product formulation (P> 0.05), are consistent with those of Bloukas*et al.* (1996). Those researchers reported no differences in pH of frankfurters prepared with or without 2% of SL. Other studies confirmed that SL does not significantly alter the pH of RTE meats (Brewer *et al.*, 1991; Choi and Chin, 2003; Lin and Lin, 2002; Wang, 2000). Also, our findings that the addition of SL to the formulation of frankfurters did not significantly change the pH of the product during storage are supported by results of previous studies (Deumier and Collignan, 2003; Papadopoulos *et al*., 1991). It was suggested that the relatively stable pH of frankfurters formulated with SL and stored at refrigeration temperatures was attributed to suppression of the growth of lactic acid producing bacteria (Deumier and Collignan, 2003; Papadopoulos *et al.*, 1991). Our observed pH decrease from 6.14 to 5.75 (P< 0.05) in control frankfurters formulated without 2% SL is not surprising considering the rapid growth of *L. monocytogenes* with viable populations reaching about 9.7 $\log_{10}$ cfu/frankfurter at day 90 (Figures 1 and 2). Also, it is likely that the growth of psychrotrophic lactic acid bacteria in the vacuum packaged frankfurters resulted in acid production and concomitant decrease in pH (Bloukas*et al.*, 1996; Samelis*et al.*, 2002).

The relative stability of Hunter *L, a* and *b* color values for treated vacuum packaged frankfurters (formulated with or without SL)

during storage at 4 °C for 90 days confirms reports of earlier published research. Bloukas*et al*. (1996) reported that incorporation of 2% of SL in frankfurters did not affect external and internal color of frankfurters during storage for 6 weeks at 4 °C. However, Choi and Chin (2003) reported an increase in yellowness and decrease in lightness of frankfurters prepared with 3.3% SL during 6 weeks storage at 4 °C. While fresh meats usually become discolored after treatment with organic acids (Pipek*et al*., 2004; Shrestha and Min, 2006) cured meats seem to resist changes in color from treatment with organic acids due to the high stability of nitroso pigments. For example, in the present study, the surface treatment with 5% LA alone did not affect Hunter color *L, a*, and *b* values of the frankfurters (P>0.05) during storage (data not shown).

4. CONCLUSIONS

The growth inhibitory action of SL (2%) and the bactericidal action of 5% LA + 0.5% or 1% SLS could be considered a post-lethality intervention that substantially destroy initial populations of *L. monocytogenes* in frankfurters and prevent growth of survivors (if any) during storage of this RTE product at 4 ºC for 90 days. Regardless the dipping time (1 or 3 minutes), the 2% SL and 5% LA+ 1% SLS could control better the *L. monocytogenes* which has been killed almost 4 to 4.5 $\log_{10}$cfu/frankfurter and could not grow throughout the 90 days of storage at 4 ºC.

Our study indicates that *L. monocytogenes'* behavior and survival in frankfurters could be affected by:i) even relatively small changes in the formulation of the product, namely frankfurters(i.e.: in our study we used 2% SLinstead of 1.8% SL as previously reportedby Byelashov*et al.* (2008) and Samelis*et al.* (2002)); ii) the type of surface treatment, namely dipping versus spraying (i.e.: during spraying the reduction in the numbers of *L. monocytogenes* can be also the effect of mechanical removal), andiii) solution's concentrations and the dipping time.Therefore, whenever the product's or dipping solution's formulation suffer slight modifications further testing and validation is required to be performed to evaluate and validate the efficacy of that particular post-lethality intervention (Consortium of Food Process Validation Experts (CFPVE), 2013).

Our results indicate that the multiple hurdle intervention used in our study has a good potential for application in frankfurters to control *L. monocytogenes* and facilitate meat processors' adoption of the *Alternative 1*as per FSIS final rule (USDA-FSIS, 2003).

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