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Viability of *Listeria monocytogenes* on commercially-prepared hams surface treated with acidic calcium sulfate and lauric arginate and stored at 4 °C ☆

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Abstract

We demonstrated the effectiveness of delivering an antimicrobial purge/fluid into shrink-wrap bags immediately prior to introducing the product and vacuum sealing, namely the "Sprayed Lethality In Container" (SLIC™) intervention delivery method. The pathogen was *Listeria monocytogenes*, the antimicrobials were acidic calcium sulfate (ACS; calcium sulfate plus lactic acid; 1:1 or 1:2 in dH_2O) and lauric arginate (LAE; Ethyl-*N*-dodecanoyl-L-arginate hydrochloride; 5% or 10% in dH_2O), and the product was commercially prepared "table brown" ham (ca. 3 pounds each). Hams were surface inoculated with a five-strain cocktail of *L. monocytogenes* (ca. 7.0 $\log_{10}$ CFU per ham), added to shrink-wrap bags that already contained ACS or LAE, vacuum-sealed, and stored at 4 °C for 24 h. Pathogen levels decreased by 1.2, 1.6, 2.4, and 3.1 $\log_{10}$ CFU/ham and 0.7, 1.6, 2.2, and 2.6 $\log_{10}$ CFU/ham in samples treated with 2, 4, 6, and 8 mL of a 1:1 and 1:2 solution of ACS, respectively. In samples treated with 2, 4, 6, and 8 mL of a 5% solution of LAE, pathogen levels decreased by 3.3, 6.5, 5.6, and 6.5 $\log_{10}$ CFU/ham, whereas when treated with a 10% solution of LAE pathogen levels decreased ca. 6.5 $\log_{10}$ CFU/ham for all application volumes tested. The efficacy of ACS and LAE were further evaluated in shelf-life studies wherein hams were surface inoculated with either ca. 3.0 or 7.0 $\log_{10}$ CFU of *L. monocytogenes*, added to shrink-wrap bags that contained 0, 4, 6, or 8 mL of either a 1:2 solution of ACS or a 5% solution of LAE, vacuum-sealed, and stored at 4 °C for 60 days. For hams inoculated with 7.0 $\log_{10}$ CFU, *L. monocytogenes* levels decreased by ca.1.2, 1.5, and 2.0 $\log_{10}$ CFU/ham and 5.1, 5.4, and 5.5 $\log_{10}$ CFU/ham within 24 h at 4 °C in samples treated with 4, 6, and 8 mL of a 1:2 solution of ACS and a 5% solution of LAE, respectively, compared to control hams that were not treated with either antimicrobial. Thereafter, pathogen levels remained relatively unchanged (±1.0 $\log_{10}$ CFU/ham) after 60 days at 4 °C in hams treated with 4, 6, and 8 mL of a 1:2 solution of ACS and increased by ca. 2.0–5.0 $\log_{10}$ CFU/ham in samples treated with 4, 6, and 8 mL of a 5% solution of LAE. For hams inoculated with 3.0 $\log_{10}$ CFU, *L. monocytogenes* levels decreased by 1.3, 1.9, and 1.8 $\log_{10}$ CFU/ham within 24 h at 4 °C in samples treated with 4, 6, and 8 mL of a 1:2 solution of ACS, respectively, compared to control hams that were not treated. Likewise, levels of the pathogen were reduced to below the limit of detection (i.e., 1.48 $\log_{10}$ CFU/ham) in the presence of 4, 6, and 8 mL of a 5% solution of LAE within 24 h at 4 °C. After 60 days at 4 °C, pathogen levels remained relatively unchanged (±0.3 $\log_{10}$ CFU/ham) in hams treated with 4, 6, and 8 mL of a 1:2 solution of ACS. However, levels of *L. monocytogenes* increased by ca. 2.0 $\log_{10}$ CFU/ham in samples treated with 4 and 6 mL of a 5% LAE solution within 60 days but remained below the detection limit on samples treated with 8 mL of this antimicrobial. These data confirmed that application via SLIC™ of both ACS and LAE, at the concentrations and volumes used in this study, appreciably reduced levels of *L. monocyt-*

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ogenes on the surface of hams within 24 h at 4 °C and showed potential for controlling outgrowth of the pathogen over 60 days of refrigerated storage.

Keywords: Ham; *Listeria monocytogenes*; SLIC™; Acidic calcium sulfate; Lauric arginate; Shelf life

1. Introduction

In recent years, there have been at least three large outbreaks of listeriosis in the United States that were associated with ready-to-eat (RTE) frankfurters and/or delicatessen-type meats (CDC, 1998; CDC, 2000; CDC, 2002). During this same time period, there have also been several large recalls due to contamination of RTE meat and poultry products with *Listeria monocytogenes*. The economic loss due to recalls of meat and poultry products contaminated with this pathogen is estimated at $1.2–$2.4 billion dollars per year in the United States (Thomsen & McKenzie, 2001). In addition, food surveys conducted in the United States between 1990 and 2003 involving ~100,000 samples estimated the prevalence of *L. monocytogenes* at 1.6–7.6% in meat, fish, and vegetable products, most of which were RTE foods (Gombas, Chen, Clavero, & Scott, 2003; Levine, Rose, Green, Ransom, & Hill, 2001; Wallace et al., 2003).

In response to the frequency and magnitude of food recalls, as well as the number and severity of infections, the USDA Food Safety and Inspection Service (USDA/FSIS) established rules/guidelines for RTE meat and poultry manufacturers to better control *L. monocytogenes* in their products (Anonymous, 2003). This ruling provides manufacturers with three options for determining the degree to which regulatory testing would be implemented for their plant/product: alternative 1 – use of both a post-process lethality step AND an antimicrobial to control outgrowth (lowest testing frequency); alternative 2 – use of either a post-processing lethality step OR an antimicrobial to control outgrowth (moderate testing frequency); or alternative 3 – use of appropriate sanitation alone (most testing). These guidelines make it imperative to identify and implement post-process interventions for lethality and/or inhibition of *L. monocytogenes* in RTE meat and poultry products.

As summarized by Crozier-Dodson, Carter, and Zheng (2005), various chemicals are antagonistic towards *L. monocytogenes* in foods when used in bath, dip, or spray applications and/or when added as an ingredient. For example, potassium lactate and sodium diacetate used alone or in combination, are effective at controlling *L. monocytogenes* in RTE meats (Barmpalia et al., 2004; Bedie et al., 2001; Buncic, Fitzgerald, Bell, & Hudson, 1995; Mbandi & Shelef, 2001; Porto et al., 2002; Seman, Borger, Meyer, Hall, & Milkowski, 2002; Stekelenburg, 2003). Sodium, potassium, and calcium lactates have been approved for use as flavorants, shelf-life extenders, and/or antimicrobials. Acidifiers such as acidified sodium chlorite (ASC) are effective for controlling *L. monocytogenes* on beef (Castillo, Lucia, Kemp, & Acuff, 1998) and broiler (Kemp, Aldrich, & Waldroup, 2000) carcasses, and somewhat effective on cook-in-bag turkey breast (Luchansky, Cocoma, & Call, 2004). Moreover, ASC has been approved as an antimicrobial on processed, comminuted, or formed meat products. Other acidifiers, notably acidic calcium sulfate (ACS) which is formulated with organic acids and calcium sulfate, are effective in reducing the levels and controlling the outgrowth of *L. monocytogenes* on the surface of frankfurters during prolonged refrigerated storage (Nunez de Gonzalez, Keeton, Acuff, Ringer, & Lucia, 2004; Acuff, Nunez de Gonzalez, Ringer, & Lucia, 2002). Currently, ACS is considered GRAS and is approved for use in meat products. As a final example, in more limited studies, surfactants such as lauric arginate (LAE) were effective at inhibiting growth of *L. monocytogenes* in cooked meats during refrigerated storage (Bakal & Diaz, 2005). Although the ingredients in LAE have been self-affirmed as GRAS, at present it is not approved for use in meats.

Almost all vacuum packaged meats produce some amount/volume of purge after vacuum packaging. This phenomenon is borne out by an ongoing controversy over the wet versus dry tare regulations used to determine net weight. Recognizing this fact, the National Institute of Standards and Technology (NIST) has issued weighing instructions on how to deal with the purge in determining net weight. Moreover, analyses of listeriosis outbreaks suggest that purge is a likely vehicle/reservoir for *L. monocytogenes* for cases where the product was re-heated/boiled by the infected individual. Working on the assumption that product purge may be the primary reservoir for *L. monocytogenes*, in a previous study (Luchansky, Porto, Wallace, & Call, 2002) we developed and optimized the ARS package rinse method to recover the pathogen from vacuum sealed packages of frankfurters and validated it as being ca. six times more effective than the standard USDA/FSIS product composite enrichment method. Another outcome of our previous work was the genesis of the "Sprayed Lethality In Container" (i.e., SLIC™) concept of treating the purge with an antimicrobial rather than the product or package to control *L. monocytogenes* in

RTE meats. In SLIC™, the vacuum produced by the packaging system distributes the antimicrobial across the surface of the product and kills the targeted pathogen and/or spoilage microbe upon contact. The objectives of the present study were to investigate the lethality of various concentrations and application volumes of ACS and LAE applied via the SLIC™ method towards *L. monocytogenes* inoculated onto the surface of hams and the efficacy of these two compounds to control outgrowth during refrigerated storage.

2. Materials and methods

2.1. Bacterial strains

As described previously (Porto et al., 2002), approximately equal numbers of each of the following five strains of *L. monocytogenes* were used as a cocktail in this study: (i) Scott A (serotype 4b, clinical isolate); (ii) H7776 (serotype 4b, frankfurter isolate); (iii) LM-101M (serotype 4b, beef and pork sausage isolate); (iv) F6854 (serotype 1/2a, turkey frankfurter isolate); and (v) MFS-2 (serotype 1/2a, environmental isolate from a pork processing plant). For each experiment, isolates were passed twice in brain heart infusion (BHI; Difco Laboratories, Detroit, MI) broth at 37 °C so that cells would be in the stationary phase for inoculating hams. Stock cultures were maintained by storage in BHI plus 10% (wt/vol) glycerol in 1.5-ml portions in cryovials and held at −80 °C.

2.2. Lethality studies

To evaluate the lethality of acidic calcium sulfate (ACS; $Safe_2O$-RTE 01, Mionix Corp., Naperville, IL) and lauramide arginine ester (LAE; Ethyl-*N*-dodecanoyl-L-arginate hydrochloride; CAS No. 60372-77-2; Mirenat-N, Vedeqsa, Barcelona, Spain; also known as lauric arginate), ''table brown'' hams (water, ground ham trims, brine, dextrose, sugar, sodium phosphate, sodium erythorbate, and sodium nitrite; ca. 3 pounds each ham) were processed and vacuum-sealed by a commercial processor, that being Hatfield Quality Meats (Hatfield, PA). The hams were boxed, transported back to the laboratory, and stored at 4 °C for up to 7 days. Each ham was aseptically removed from its original packaging, spot inoculated with 2 mL of the cocktail using a pipet to achieve a target level of ca. 7.0 $\log_{10}$ CFU per ham and then transferred to a high-performance shrink-wrap bag (B2570T, Cryovac, Duncan, SC). Just prior to introducing the hams, the inside of each shrink-wrap bag was sprayed with 0, 2, 4, 6, or 8 mL of either a 1:1 (1 part ACS:1 part dH_2O) or 1:2 (1 part ACS:2 parts dH_2O) solution of ACS or a 5% (5 parts LAE:95 parts dH_2O) or 10% (10 parts LAE:90 parts dH_2O) solution of LAE. The antimicrobials for these experiments were introduced via a 24-ounce plastic spray bottle (Koch Supplies, Kansas City, MO). Each bag was then vacuum sealed to 950 mBar using a Multivac A300/16 vacuum-packaging unit (Sepp Haggemüller KG, Wolfertschwenden, Germany), submerged in hot (88 °C) water for approximately 5 s to shrink the bag, and transferred to a 4 °C incubator and held for 24 h. In a single trial, three hams were analyzed for each concentration and volume of ACS and LAE tested after 24 h of refrigerated storage.

2.3. Validation studies

To validate the initial post-process lethality of ACS and LAE, a fresh batch of the same formulation of hams was obtained from the same commercial manufacturer as described previously. The hams were spot inoculated with 2 mL of the *L. monocytogenes* cocktail to achieve a target level of ca. 7.0 $\log_{10}$ CFU per ham, transferred to shrink-wrap bags (Cryovac) that were previously sprayed on the inside with 0, 2.5, 4.5, or 6.5 mL of a 1:2 solution of ACS or a 5% solution of LAE, vacuum sealed, submerged in hot (88 °C) water, and placed at 4 °C. In each of three trials, three hams were analyzed at each concentration and volume of ACS and LAE tested after 24 h of refrigerated storage.

2.4. Shelf-life studies

To evaluate the efficacy of ACS and LAE over the expected refrigerated shelf life of the product, a fresh batch of the same formulation of hams was obtained from the same commercial manufacturer as described previously. For these studies, hams were spot inoculated with 2 mL of the *L. monocytogenes* cocktail to achieve a target level of either 3.0 or 7.0 $\log_{10}$ CFU per ham. At each inoculation level one portion of the hams was transferred to shrink-wrap bags that were previously sprayed on the inside with 4, 6, or 8 mL of a 1:2 solution of ACS applied using a commercial spraying (AutoJet Spray System # 45570-22-10-120 V, Spraying Systems Co., Wheaton, IL) and commercial bagging (Taped Bag Loader # BL189, Cryovac) apparatus. An otherwise similar portion of the inoculated hams was transferred to shrink-wrap bags that just prior to introduction of the hams were sprayed with 4, 6, or 8 mL of a 5% LAE solution using the commercial spraying and bagging apparatus. Control hams were also spot inoculated with either 3.0 or 7.0 $\log_{10}$ CFU of *L. monocytogenes* per ham and were transferred to shrink-wrap bags that were not sprayed with either compound. As described previously, hams were vacuum-sealed, submerged in hot (88 °C) water, and stored at 4 °C. Hams were analyzed 1, 7, 14, 21, 28, 40, and, 60 days post-inoculation. For each of two trials, three hams were analyzed at each sampling

point for both inoculation levels and for both chemicals tested.

2.5. Microbiological analyses

Surviving *L. monocytogenes* were enumerated using the USDA/ARS package rinse method (Luchansky et al., 2002) and spread-plating 250 μL of the resulting rinse fluid or dilutions thereof onto duplicate modified Oxford (MOX; Cook, 1999) agar plates using a sterile cell spreader and incubating for 48 h at 37 °C. *Listeria* numbers were expressed as $\log_{10}$ CFU per ham with each package containing a single ham; the detection limit was 1.48 $\log_{10}$ CFU/ham. Periodically, isolates were retained from randomly selected samples and confirmed as *L. monocytogenes* following the recommended/standard USDA/FSIS protocol (Cook, 1999).

2.6. Chemical analyses

The pH of the rinsate obtained from washing the contents of representative packages was determined using a Corning model 3-in-1 combination electrode and model 340 meter (Corning Inc., Corning, NY). The pH was determined for control and experimental samples for the validation and shelf life components of this study. The batch-to-batch variation in formulation was evaluated by testing a randomly selected ham from each of five production batches. The proximate composition of representative hams was determined using methods approved and described by the Association of Official Analytical Chemists (McNeal, 1990) as conducted by a commercial testing laboratory.

2.7. Statistical analyses

Data were analyzed using version 8.0 of the SAS statistical package (SAS Institute, Inc., Cary, NC). Analysis of covariance was performed to evaluate the effect of type, concentration, and volume of antimicrobial on the initial lethality and the subsequent ability of ACS and LAE to control the outgrowth of *L. monocytogenes* during extended storage at 4 °C. Results are reported as statistically significant at the level of $P > 0.05$.

3. Results and discussion

3.1. Proximate composition

Chemical analyses (Table 1) revealed significant ($P > 0.05$) variations among NaCl, fat, carbohydrate, lactic acid, and nitrite levels among the samples representing the five production batches of the same formulation of ham, but did not reveal appreciable differences in levels of the other chemicals assayed. These data reveal considerable batch-to-batch variation for this type of ham, but further experiments will be needed to determine the effect, if any, of these variations on the fate of *L. monocytogenes*.

3.2. Lethality studies

A five-strain cocktail (ca. 7.0 $\log_{10}$ CFU per ham) was used to evaluate the initial lethality of ACS and LAE towards *L. monocytogenes* on hams. Relative to samples that were not treated with ACS, *L. monocytogenes* levels decreased within 24 h at 4 °C by ca.1.2, 1.6, 2.4, and 3.1 $\log_{10}$ CFU/ham in samples treated with 2, 4, 6, and 8 mL of a 1:1 solution of ACS and 0.7, 1.6, 2.2, and 2.6 $\log_{10}$ CFU/ham in samples treated with 2, 4, 6, and 8 mL of a 1:2 solution of ACS (Table 2). In general, the larger the volume and the higher the concentration of ACS applied, the greater the decrease in *L. monocytogenes* levels on hams that were stored at 4 °C for 24 h. Regardless, there was not an appreciable difference ($P < 0.05$) in lethality between a 1:1 and a 1:2 solution of ACS at any of the four volumes applied.

In samples treated with LAE (Table 2), *L. monocytogenes* levels decreased by ca. 3.3, 6.5, 5.6, and 6.5 $\log_{10}$ CFU/ham in hams that received 2, 4, 6, and 8 mL of a 5% solution of LAE. In hams that were treated with a 10% solution of LAE, pathogen levels decreased by ca. 6.5 $\log_{10}$ CFU/ham for all four

Table 1
Proximate composition analyses of a single ham from each of five separate production batches

Sample	Chemical trait								
	NaCl (g/100 g)	pH	Moisture (g/100 g)	Protein (g/100 g)	Fat (g/100 g)	CHOs (g/100 g)	Phenolics (μg/g)	Lactic acid (%)	Nitrite (μg/g)
Batch 1	3.4[a]	6.2[a]	72.3[a]	16.0[a]	2.3[b]	4.0[a]	2305[a]	0.8[b]	1.3[b]
Batch 2	1.8[b]	6.2[a]	68.9[a]	17.5[a]	3.1[b]	6.0[a]	1852[a]	1.6[a]	3.5[a]
Batch 3	2.4[a]	6.2[a]	70.7[a]	17.2[a]	4.3[a]	3.2[b]	1819[a]	1.6[a]	2.7[a]
Batch 4	1.6[c]	6.1[a]	72.1[a]	18.5[a]	1.9[c]	4.0[a]	2044[a]	1.0[a]	1.7[b]
Batch 5	2.2[b]	6.1[a]	74.5[a]	19.0[a]	2.9[b]	0[c]	1647[a]	0.6[b]	1.8[b]
Average of 5	2.28	6.17	71.7	17.64	2.90	3.44	1933	1.12	2.20
Standard Deviation	0.7	0.05	2.07	1.17	0.92	2.18	251	0.46	0.89

[a–c] Values in the same column with the same letter are statistically similar.

Table 2
Use of SLIC™ to evaluate the lethality of varying levels of acidic calcium sulfate (ACS) and lauric arginate (LAE) towards *L. monocytogenes* (ca.7.0 $\log_{10}$ CFU total) after 24 h at 4 °C ($N = 1$ trial, $n = 3$ hams per sampling interval)

Samples	Volume applied (mL)				
	0	2	4	6	8
1:1 ACS	7.1[a,d] (6.0)[e]	5.9[b] (5.6)	5.5[b] (5.4)	4.7[b] (4.6)	4.0[b] (3.1)
1:2 ACS	7.1[a] (6.0)	6.4[b] (5.3)	5.5[b] (3.9)	4.9[b] (4.3)	4.9[b] (4.2)
5% LAE	7.3[a] (6.6)	4.0[b] (3.8)	1.48[c,f] (0)	1.48[c] (0)	1.48[c] (0)
10% LAE	7.3[a] (6.6)	1.48[c] (0)	1.48[c] (0)	1.48[c] (0)	1.48[c] (0)

[a–c] Values with the same letter are statistically similar.
[d] $\log_{10}$ CFU/ham.
[e] Standard deviation ($\log_{10}$).
[f] Limit of detection = 1.48 $\log_{10}$ CFU/ham.

application volumes tested. With the exception of the 2 mL application volume of the 5% LAE solution, there was no statistical difference in lethality between the two concentrations of LAE. However, the lethality achieved with either concentration of LAE was significantly greater ($P > 0.05$) than that which was achieved with either concentration of ACS, regardless of the application volume.

3.3. Validation studies

Based on the results of the prefatory experiments detailed in the previous section, we validated the SLIC™ strategy for delivery of ACS and LAE to control *L. monocytogenes* on hams. In three individual validation experiments, each ham was surface inoculated with ca. 7.0 $\log_{10}$ CFU of *L. monocytogenes* and treated with either a 5% solution of LAE or a 1:2 solution of ACS. These concentrations of ACS and LAE were recommended by the manufacturer. Moreover, when used at a concentration of 1:1, ACS adversely affected product taste (data not shown). Also, use of a 5% solution of LAE was equivalent in cost to use of a 1:2 solution of ACS. After 24 h at 4 °C, on average pathogen levels decreased by ca. 1.0, 1.5, and 2.5 $\log_{10}$ CFU/ham in product treated with 2.5, 4.5, and 6.5 mL of a 1:2 solution of ACS and by ca. 4.6, 5.9, and 6.1 $\log_{10}$ CFU/ham in product treated with 2.5, 4.5, and 6.5 mL of a 5% solution of LAE compared to otherwise similar control hams that were not treated with an antimicrobial (Table 3). These data validate the post-process lethality of both ACS and LAE towards *L. monocytogenes*. At all volumes tested, LAE caused a significantly greater reduction in levels of *L. monocytogenes* than ACS. Although the results were not different statistically at the level of $P > 0.05$, in general, we observed greater reductions in pathogen levels with larger volumes of both ACS and LAE. Lastly, after 24 h at 4 °C, the pH of the rinse fluid recovered from hams treated with a 1:2 solution of ACS (pH 5.25–5.77) was statistically ($P > 0.05$) lower than the pH of the rinse fluid recovered from hams treated with a 5% solution of LAE (pH 6.34–6.36) or from rinse fluid recovered from control hams that were not treated with either compound (pH 6.28; data not shown).

3.4. Shelf-life studies

Another objective of this study was to establish if ACS and/or LAE when delivered via the SLIC™ method would inhibit outgrowth of *L. monocytogenes* during the expected shelf life of the product. In shelf-life studies using an initial inoculum of ca. 7.0 $\log_{10}$ CFU/ham, pathogen levels were reduced after 24 h at 4 °C by ca. 1.2, 1.5, and 2.0 $\log_{10}$ CFU/ham and 5.1, 5.4, and 5.5 $\log_{10}$ CFU/ham in samples treated with 4, 6, and 8 mL of a 1:2 solution of ACS and a 5% solution of LAE, respectively, relative to samples that were not treated with either antimicrobial (Table 4). Thereafter, pathogen levels increased by ca. 4.6, 3.0, and 2.0 $\log_{10}$ CFU/ham within 60 days in samples treated with 4, 6, and 8 mL of a 5% solution of LAE. In contrast, levels of *L. monocytogenes* decreased by ca. 0.5 and 1.0 $\log_{10}$ CFU/ham in product treated with 6 and 8 mL of a 1:2 solution of ACS within 60 days but increased by ca.

Table 3
Validation of SLIC™ using varying levels of acidic calcium sulfate (ACS) and lauric arginate (LAE) to control *L. monocytogenes* (ca.7.0 $\log_{10}$ CFU total) on hams after storage for 24 h at 4 °C ($N = 3$ trials, $n = 3$ hams per sampling interval)

Samples	Volume applied (mL)			
	0	2.5	4.5	6.5
1:2 ACS	7.0[a,e] (7.0)[f]	5.8[a] (5.5)	5.6[a] (5.7)	4.5[b] (4.7)
5% LAE	7.0[a] (7.0)	3.1[c] (3.4)	1.7[d] (1.9)	1.48[d,g] (0)

[a–d] Values with the same letter are statistically similar.
[e] $\log_{10}$ CFU/ham.
[f] Standard deviation ($\log_{10}$).
[g] Limit of detection = 1.48 $\log_{10}$ CFU/ham.

Table 4
Evaluation of SLIC™ using varying levels of acidic calcium sulfate (ACS) and lauric arginate (LAE) to control *L. monocytogenes* (ca.7.0 $\log_{10}$ CFU total) over the expected refrigerated shelf life of hams ($N = 2$ trials, $n = 3$ hams per sampling interval)

Samples	Storage time (days)						
	1	7	14	21	28	40	60
Control hams	6.9[a,e] (6.4)[f]	7.3[a] (7.6)	6.5[a] (6.2)	6.6[a] (6.3)	7.1[a] (7.0)	8.1[a] (8.1)	9.0[a] (8.6)
4 mL ACS	5.7[b] (5.6)	5.2[b] (5.0)	5.0[b] (5.0)	5.0[b] (5.0)	5.8[b] (5.5)	5.5[b] (5.3)	6.2[d] (6.3)
6 mL ACS	5.4[b] (5.3)	4.8[b] (4.9)	5.1[b] (4.8)	5.2[b] (5.2)	5.2[b] (5.0)	5.6[b] (5.5)	4.9[b] (6.0)
8 mL ACS	5.0[b] (5.2)	4.6[b] (4.7)	4.3[b] (4.2)	4.7[b] (4.9)	4.2[b] (3.9)	4.9[b] (4.7)	3.7[b] (3.5)
4 mL LAE	1.8[c] (1.9)	2.5[c] (2.5)	2.6[c] (2.9)	3.7[c] (3.9)	4.5[b] (4.3)	4.9[b] (5.1)	6.5[d] (6.4)
6 mL LAE	1.5[c] (1.2)	1.48[c,g] (0)	1.8[c] (1.9)	2.0[c] (2.3)	1.7[c] (1.9)	4.1[b] (4.3)	4.5[b] (4.3)
8 mL LAE	1.48[c] (0)	1.48[c] (0)	1.48[c] (0)	1.48[c] (0)	1.48[c] (0)	1.8[c] (1.7)	3.5[b] (3.7)

[a–d] Values with the same letter are statistically similar.
[e] $\log_{10}$ CFU/ham.
[f] Standard deviation ($\log_{10}$).
[g] Limit of detection = 1.48 $\log_{10}$ CFU/ham.

0.5 $\log_{10}$ CFU/ham in product treated with 4 mL. In hams that were not treated with either compound, *L. monocytogenes* levels increased by ca. 2.1 $\log_{10}$ CFU/ham within 60 days. Statistical analyses confirmed that from day 1 through day 60 for all volumes of ACS and LAE tested, levels of *L. monocytogenes* were appreciably lower for hams that were treated with these antimicrobials compared to control hams that were not treated. In addition, through about 28 days of refrigerated storage pathogen levels were significantly lower in samples treated with LAE compared to samples treated with ACS for all application volumes tested. However, after 60 days there was no significant difference in levels of *L. monocytogenes* between samples treated with ACS or LAE. Lastly, after 24 h at 4 °C, the pH of the rinse fluid recovered from hams treated with a 1:2 solution of ACS (pH 5.14–5.49) was significantly lower than the pH of the rinse fluid recovered from hams treated with a 5% solution of LAE (pH 6.21–6.33) or from rinse fluid recovered from hams that were not treated with either compound (pH 6.36). However, the pH of the rinse fluid for both the experimental and control hams was ca. pH 6.0 after 60 days of refrigerated storage (data not shown).

In shelf-life studies using an initial inoculum of ca. 3.0 $\log_{10}$ CFU/ham, *L. monocytogenes* levels were reduced by ca. 1.3, 1.9, and 1.8 within 24 h at 4 °C in samples treated with 4, 6, and 8 mL of a 1:2 solution of ACS, respectively, compared to control hams that were not treated (Table 5). Likewise, levels of the pathogen were reduced to below the limit of detection in the presence of 4, 6, and 8 mL of a 5% solution of LAE within 24 h at 4 °C. After 60 days at 4 °C, pathogen levels remained relatively unchanged (±0.3 $\log_{10}$ CFU/ham) in hams treated with 4, 6, and 8 mL of a 1:2 solution of ACS. However, after 60 days at 4 °C levels of *L. monocytogenes* increased by ca. 2.0 $\log_{10}$ CFU/ham in samples treated with 4 and 6 mL of a 5% LAE solution but remained below the detection limit on samples treated with 8 mL of this antimicrobial. Statistical analyses of these data confirmed that from day 1 through day 40 for all volumes of ACS and LAE tested levels of *L. monocytogenes* were appreciably lower for hams that were treated with these antimicrobials compared to control hams that were not treated. Statistical analyses also confirmed that between day 1 and day 40 of refrigerated storage there were no appreciable differences between ACS and LAE at the application volumes tested, nor were there

Table 5
Evaluation of SLIC™ using varying levels of acidic calcium sulfate (ACS) and lauric arginate (LAE) to control *L. monocytogenes* (ca.3.0 $\log_{10}$ CFU total) over the expected refrigerated shelf life of hams ($N = 2$ trials, $n = 3$ hams per sampling interval)

Samples	Storage time (days)						
	1	7	14	21	28	40	60
Control hams	3.4[a,c] (3.0)[d]	3.3[a] (3.3)	3.5[a] (2.8)	3.4[a] (3.1)	3.7[a] (3.7)	3.7[a] (3.8)	4.7[a] (4.8)
4 mL ACS	2.2[b] (2.2)	2.2[b] (2.2)	2.5[b] (2.4)	2.1[b] (1.7)	1.9[b] (2.0)	1.9[b] (2.1)	1.9[b] (2.0)
6 mL ACS	1.6[b] (1.4)	2.0[b] (2.0)	2.2[b] (2.3)	1.8[b] (1.7)	1.7[b] (1.8)	1.6[b] (1.5)	1.8[b] (1.8)
8 mL ACS	1.6[b] (1.6)	1.6[b] (1.4)	1.8[b] (1.9)	1.7[b] (1.7)	1.7[b] (1.7)	1.48[b,e] (0)	1.48[b] (0)
4 mL LAE	1.48[b] (0)	1.6[b] (1.7)	1.48[b] (0)	1.6[b] (1.4)	2.1[b] (2.3)	1.5[b] (1.4)	4.0[a] (4.4)
6 mL LAE	1.48[b] (0)	1.48[b] (0)	1.48[b] (0)	1.6[b] (1.4)	1.48[b] (0)	1.5[b] (4.4)	3.4[a] (3.7)
8 mL LAE	1.48[b] (0)	1.48[b] (0)	1.48[b] (0)	1.48[b] (0)	1.48[b] (0)	1.48[b] (0)	1.48[b] (0)

[a,b] Values with the same letter are statistically similar.
[c] $\log_{10}$ CFU/ham.
[d] Standard deviation ($\log_{10}$).
[e] Limit of detection = 1.48 $\log_{10}$ CFU/ham.

any appreciable differences among any of the volumes tested for either ACS or LAE. Likewise, after 60 days, with the exception of samples treated with 4 or 6 mL of a 5% solution of LAE, all other treatments showed appreciably lower levels of *L. monocytogenes* compared to the untreated (control) samples. Lastly, following 24 h of refrigerated storage, the pH of the rinse fluid recovered from hams treated with a 1:2 solution of ACS (pH 5.49–5.63) was appreciably ($P > 0.05$) lower than the pH of the rinse fluid recovered from hams treated with a 5% solution of LAE (pH 6.28–6.32) or from rinse fluid recovered from hams that were not treated with either compound (pH 6.26). However, as was observed for hams inoculated with ca. 7.0 $\log_{10}$ CFU, there was no statistical difference in the pH of the rinse fluid between the experimental and control hams (both were ca. pH 6.0) after 60 days of refrigerated storage (data not shown).

4. Conclusions

The present study evaluated both the lethality and inhibition of two food grade chemicals, acidic calcium sulfate and lauric arginate, as applied via SLIC™ for control of *L. monocytogenes* on hams during refrigerated storage. Other researchers also reported that these compounds decreased the levels and prevented the outgrowth of *L. monocytogenes* in RTE products. For example, Keeton et al. (2002) demonstrated that dipping inoculated frankfurters into a 1:2 solution of ACS reduced *L. monocytogenes* levels by 5.8 $\log_{10}$ CFU and prevented subsequent outgrowth after 12 weeks of storage at 4.5 °C. Similar results were obtained by Nunez de Gonzalez et al. (2004) who dipped frankfurters formulated with potassium lactate into a 1:2 solution of ACS and observed a 5.4 $\log_{10}$ CFU reduction in levels of *L. monocytogenes*. To our knowledge, there have been no peer-reviewed publications describing the use of LAE to control pathogens directly in foods; however, Bakal and Diaz (2005) summarized the results of studies in synthetic media demonstrating that LAE at levels ranging from 8 to 128 μg/ml is bacteriostatic towards both Gram-positive and Gram-negative food borne pathogens. Herein, we validated the efficacy of the SLIC™ delivery strategy for reducing levels of *L. monocytogenes* on the surface of hams by at least 2.0 $\log_{10}$ CFU/ham using a 1:1 or 1:2 solution of ACS and by at least 5.0 $\log_{10}$ CFU/ham using a 5% solution of LAE within 24 h at 4 °C. In addition, at a relatively low inoculum level (3.0 $\log_{10}$ CFU/ham) both chemicals applied using the SLIC™ approach were effective at controlling the outgrowth of *L. monocytogenes* for at least 40 days of refrigerated storage. In shelf-life studies using an initial inoculum of ca. 7.0 $\log_{10}$ CFU/ham, in general ACS and LAE were successful at controlling the further outgrowth of *L. monocytogenes* for at least 60 and 28 days of refrigerated storage, respectively.

The SLIC™ delivery strategy displayed considerable potential for controlling *L. monocytogenes* in RTE meat and poultry products. The results validated herein will allow manufacturers to meet the requirements of alternative 2 and perhaps alternative 1 depending on formulation and on the antimicrobial selected and the dose delivered via SLIC™. The SLIC™ method should also be directly applicable for other products and other packaging systems. In fact, studies are underway to validate delivery of antimicrobials via SLIC™ for control of *L. monocytogenes* on frankfurters packaged using a "roll stock" apparatus. Additional studies using SLIC™ should be conducted to determine if both lethality and inhibition could be achieved via SLIC™ and whether antimicrobials other than ACS or LAE, alone or in combination, will demonstrate greater control of *L. monocytogenes* and/or be effective against other pathogens. The beneficial economics of using SLIC™ (see below) and its conservative use of antimicrobials make it a very desirable alternative to other more costly means of assuring the safety of RTE meats. In SLIC™, the amount of antimicrobial added to the package is determined by the surface area of the product, as opposed to the random and normally excessive application used in spray and bath systems. Unlike spray and bath systems, SLIC™ affords the antimicrobial almost unlimited time, that being throughout shelf life, to work against undesirable microorganisms, whereas bath and spray applications must be regulated by the time (usually seconds) of exposure. In addition, in SLIC™ the antimicrobial is added and active after any opportunity for post-packaging contamination is eliminated. The novelty/significance of the SLIC™ concept is not that ACS or LAE display antilisterial activity but rather that SLIC™ is a far more facile, effective, and economical delivery method for antimicrobials than current/traditional techniques.

Regarding economic benefits, SLIC™ uses specific and much lower doses of an antimicrobial than direct (internal) addition, bathing, and/or spraying. In SLIC™, the volume applied to the product is determined by the surface area to be treated to achieve sufficient distribution/coverage and, in turn, eliminate any "cold spots" that would be lacking antimicrobial while possibly harboring *L. monocytogenes.* When selecting the volume, consideration must also be given to concerns about flavor and/or texture that may result from the added purge. Regardless, because of the metered dose concept, very small amounts of chemical are used. In general, the cost of applying antimicrobials by bathing, dipping, or spraying can range from $0.02 to $0.03 per pound of product treated, whereas SLIC™ costs are estimated to range from $0.002 to $0.009 per pound. More specifically, we estimate that the savings of using SLIC™ and LAE and/or ACS compared to using potassium lactate and sodium diacetate as an ingredient

would amount to ca. $1,000,000–2,000,000 per year for a "large" (USDA/FSIS definition) processing plant. Other advantages of SLIC™ are a reduced impact on flavor and quality due to its use of comparatively lower volumes of antimicrobials. Also, it is likely that consumers will ingest little or no antimicrobials introduced by SLIC™, since purge is rarely consumed in any significant quantity by the end user. For all of these reasons, and for its ability to address current regulatory guidelines, provide considerable economic benefit to industry, and enhance food safety/quality for consumers, the SLIC™ concept should be adopted for routine use by manufacturers of RTE meat and poultry products.

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