

COMPARISON OF LAURIC ARGINATE ESTER AND ACIDIFIED SODIUM  
CHLORITE AS POST-LETHALITY INTERVENTIONS ON PROCESSED MEATS

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## ABSTRACT

Acidified sodium chlorite and lauric arginate ester were evaluated to 1) confirm an application formula using a Sprayed Lethality In Container (SLIC) method based on the product size, 2) evaluate microbial effectiveness on *Listeria innocua*, and 3) analyze the impact on quality and sensory characteristics when applied to pork German sausage. The amount of ASC and LAE required to provide full coverage was determined to be 4 ml per 16 oz. While there was no significance in *Listeria innocua* concentrations on ASC and LAE treated sausage on d 14, they were both significantly lower ( $P < 0.05$ ) than the control. While  $L^*$  and  $a^*$  values were similar between treatments ( $P > 0.05$ ), sausage links treated with LAE had greater  $b^*$  values ( $P < 0.05$ ) than those treated with ASC. Sensory analysis panelists were unable to identify any differences ( $P > 0.05$ ) between ASC and LAE treated sausages. It can be concluded that ASC and LAE have no significant sensory effect when applied to processed meats as post-lethality interventions.

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## INTRODUCTION

According to the Center for Disease Control and Prevention, approximately 48 million people are affected by a foodborne illness annually (CDC, 2012). Those at high risk for contracting a foodborne illness include infants, toddlers, elderly, pregnant women, and the immunocompromised (FSIS-USDA, 2016). These populations are more likely to experience adverse effects to a foodborne illness than healthy, young adults. As a result, an increase use in post-lethality interventions are seen within the food industry to reduce the possible contamination of pathogenic bacteria such as *Listeria monocytogenes* on ready-to-eat (RTE) and processed meat products. Ready-to-eat foods, such as deli meat and frankfurters, have been fully cooked to reach lethality prior to packaging, and require no further heat treatment before consumption. Post-lethality antimicrobial treatments may be applied to RTE products in various ways to increase the safety of these products even further. One method utilized in the food industry is the Sprayed Lethality In Container (SLIC) method.

Acidified sodium chlorite (ASC) and lauric arginate ester (LAE) are two GRAS (generally recognized as safe) approved antimicrobials that may be applied as post-lethality interventions. Ideally, post-lethality interventions should not cause any significant quality issues, such as color fading, to the final product.

The objectives of this study included: 1) creating a formulation for the application of ASC and LAE using a Sprayed Lethality In Container (SLIC) method based on product surface area, 2) evaluating the effectiveness of both antimicrobials on *Listeria innocua*, and 3) measuring quality characteristics of pork German sausage treated with ASC and LAE.



## LITERATURE REVIEW

### **Acidified Sodium Chlorite: Antimicrobial Characteristics**

Acidified sodium chlorite (ASC) is a combination of sodium chlorite and any GRAS approved acid (Lim and Mustapha, 2004). ASC is a food-grade antimicrobial approved by both the U.S. FDA and FSIS-USDA for use in red meat and poultry at levels resulting in sodium chlorite concentrations from 500 ppm to 1200 ppm (Code of Federal Regulations, 2018). Acidified sodium chlorite functions by disrupting the bacterial cellular membrane, resulting in cellular oxidation (Rao, 2007). While acidified sodium chlorite has been proven effective at eliminating *Escherichia coli* O157:H7, *Listeria monocytogenes*, and *Salmonella* spp. by numerous studies, some studies suggest otherwise.

Lim and Mustapha (2004) found that when ASC was sprayed on the surface of fresh beef, it reduced the number of *E. coli* O157:H7 by 2.50 log<sub>10</sub>CFU/cm<sup>2</sup> immediately following application (day 0). Bosilevac et al. (2004) reported that ASC significantly ( $P < 0.05$ ) reduced the total number of gram-negative bacteria and aerobic plate counts present on beef trim when applied at 600 ppm and 300 ppm. Controversially, Gill and Badoni (2004) reported ASC having little to no effect on total aerobic organisms, generic *E. coli*, and coliforms when applied to beef carcasses.

### **Lauric Arginate Ester: Antimicrobial Characteristics**

Lauric arginate ester (LAE) is a food-grade antimicrobial derived from lauric acid, L-arginine, and ethanol (Bonnaud et al., 2010). Lauric arginate is accepted for food use by the European Food Safety Authority (EFSA, 2013) and categorized as GRAS by the FDA at 200 ppm by weight of the finished food product (U.S. FDA, 2005). Lauric arginate ester may be sprayed on the surface of ready-to-eat meats to inhibit the growth of pathogenic bacteria such as *Listeria monocytogenes*. As an antimicrobial, LAE targets the metabolic processes of

the cytoplasmic membrane, resulting in the disruption of the plasma membrane lipid bilayer (Bakal and Diaz, 2005, Rodriguez et al., 2004). Without a properly functioning membrane, the cell is unable to consume nutrients and excrete waste, ultimately resulting in cell death.

In a study where a 5% LAE solution was applied to boneless hams, a reduction up to 3 log<sub>10</sub>CFU of *Listeria monocytogenes* was recorded within 24 h of application (Luchansky et al., 2005). Similarly, LAE reportedly reduced up to 2 log<sub>10</sub>CFU/package within 48 h of application on frankfurters previously inoculated with *L. monocytogenes* (Taormina and Dorsa, 2009). When applied to food-contact surfaces, Sadekuzzaman et al. (2017) reported that LAE effectively reduced *Escherichia coli* O157:H7, *Listeria monocytogenes*, and *Salmonella typhimurium* biofilms on stainless steel and rubber surfaces. Results showed a bacterial reduction up to 7 log<sub>10</sub>CFU/cm<sup>2</sup> on stainless steel surfaces and a 3.5 log<sub>10</sub>CFU/cm<sup>2</sup> on rubber surfaces.

### **Meat Color**

The color processed meat products exhibit in a retail setting is an important determining factor for the consumer's willingness to purchase (Dunsing, 1959). If the product expresses negative traits such as color fading, the consumer will likely be deterred from purchasing the product. Therefore, investigating any surface discoloration possibly associated with the application of ASC and LAE will help determine consumer acceptability.

Studies report surface discoloration of fresh meat products when treated with ASC. Visvalingam and Holley (2018) reported that when  $\geq 30$  ppm of ASC was applied to raw ground beef, redness values decreased to  $\leq 16$  on a 20-point scale. Similarly, Lim and Mustapha (2004) reported a loss of both light color and redness in fresh beef when treated with ASC. However, because acidified sodium chlorite is generally applied to fresh meat, there is limited research on its application to processed meats.

## **Sprayed Lethality in Container**

A Sprayed Lethality In Container or SLIC system is a method used to apply liquid antimicrobials to RTE and processed meats before or after they are placed into a vacuum package. The SLIC system requires only a small amount of antimicrobial when compared to other methods such as bathing, dipping, spraying, or adding as an ingredient (A & B Ingredients, 2019). Luchansky et al. (2005) reported a minimum reduction of 5 logs in *L. monocytogenes* when LAE was applied using the SLIC system. Similarly, Stella et al. (2017) reported that LAE applied via SLIC was effective in reducing *E. coli* O157:H7 up to 1.6 log<sub>10</sub>CFU. While the SLIC system may be used for the application of LAE, few studies have utilized this method for the application of ASC.

## **Sensory Analysis: Triangle Test**

A triangle test is used to determine if there is a significant difference between two products with a difference, such as a different ingredient added, and is used in the industry for sensory analysis (Ennis, 1990). Panelists are presented with 3 samples at once and asked to identify the odd sample. Because the nature of the difference is unknown to the panelists, the triangle test has the ability to reveal any discriminable sensory difference (MacRae, 1995). In a study observing three different residual oxygen levels on color stability in pasteurized hams, sensory panelists were unable to distinguish a difference between the three different oxygen levels after 18 days of storage, but were able to distinguish between hams packaged with 0.5% and 0.02% oxygen after 27 days of storage (Moller et al., 2000). By utilizing a triangle test, it is possible to find a significant difference in processed meat products.

## MATERIALS AND METHODS

### **Product Production**

Pork German sausage (n = 60) (NAMP# 811 Smoked Sausage) was utilized for all trials. The sausage was portioned into 16, 8, 4, and 2 oz links for trial one and 8 oz links for trial two, which consisted of pork, water, salt, ground black pepper, ground red pepper, garlic powder, onion powder, sodium nitrite, and stuffed into natural pork casings. The sausage was cooked according to Appendix A (FSIS-USDA, 1999a), where it reached lethality (66.11°C) for one minute to eliminate pathogenic bacteria. The sausage was then cooled according to Appendix B (FSIS-USDA, 1999b), where temperatures were reduced to 26.67°C within 5 h and below 7.22°C in 10 h, to ensure safety of the product. This process took place at the Angelo State University Food Safety and Product Development Laboratory. All procedures involving human subjects were approved by the ASU IRB committee before the start of research (#BRA-073119).

### **Antimicrobial Solutions**

The antimicrobial treatments used in this study were acidified sodium chlorite (Sodium Chlorite and Citric Acid, Crimson Chemicals, Fort Worth, TX) and lauric arginate ester (CytoGuard LA 20, A&B Ingredients, Fairfield, NJ). Acidified sodium chlorite was mixed by adding 1.751 ml of sodium chlorite to 150 ml of deionized water and mixed for 20 sec, then 1.1 ml of citric acid was added and mixed for an additional 20 sec. The ASC mixture was then titrated to measure the available chlorine in ppm, which was determined to be 1200 ppm. The lauric arginate ester was created by adding 0.125 ml of CytoGuard LA 20 to 142.5 ml of deionized water and mixed for 20 sec., following the industry instructions provided, creating a 200 ppm concentration of LAE. A new solution of ASC and LAE was created prior to each replication within each trial.

### **Trial One: Confirmation of Formulation**

A fluorescent dye (1 ml) and a visible blue dye (2 drops) was added to both antimicrobial treatment mixtures of ASC and LAE for visual detection once the sausage link had been placed into the vacuum package bag and sealed. Four different sausage link sizes were used to create a formulation: 16, 8, 4, and 2 oz. Following the measurement of the length and width of each individual sausage, links were individually placed into a vacuum package, where an antimicrobial treatment was added. The amount of antimicrobial treatment added to each link size was as follows: 4 ml/16 oz, 2 ml/8 oz, 1 ml/4 oz, and 0.5 ml/2 oz. Five links were used for both ASC and LAE with a total of 10 per weight category (5 sample/treatment; n = 80). Once vacuum sealed, packages were observed under both white light and black light to determine if the entire surface area of the sausage link was covered.

### **Trial Two: Analysis**

For trial two, a total of 300 sausage links were created. Links were chosen for use based on uniformity where 135 links were used for microbial inoculation and 140 links were used for quality analysis. For quality analysis, 70 – 8 oz sausage links were randomly assigned to one of two treatments (n = 140); ASC (acidified sodium chlorite) and LAE (lauric arginate ester). Two ml of each antimicrobial treatment was pipetted into each vacuum package following the insertion of the sausage to mimic the SLIC system. The bags were then vacuum sealed and stored in the dark for two weeks at 4°C to simulate retail storage conditions. The sausage from this portion was utilized in the sensory panels.

### **Microbial Inoculation**

This portion of trial two was performed in the Angelo State University Food Microbiology Laboratory. The exterior portion of 135 – 8 oz pork German sausage links with

the ends removed resulting in a 15.24 cm (approximately 4 oz) link, were inoculated with *Listeria innocua*, a non-pathogenic surrogate for *Listeria monocytogenes* (n = 135).

#### *Listeria innocua*

The inoculum culture containing two strains of BSL 1 (ATTC #51742 and #33090) *Listeria innocua* was utilized for this project. The original inoculum contained 8.61 log<sub>10</sub>CFU/ml.

#### *Inoculation*

Inoculation was performed based on the procedures of Taormina and Dorsa (2009). For dip inoculation, links were portioned into six-inch pieces and fully immersed in the *Listeria innocua* bath for 5 min under a BSL 2 certified hood. Following the dip, the sausages were placed on sanitized racks to dry at room temperature, approximately 23°C for 30 min to allow for bacterial attachment to the surface. Following the drying period, each individual sausage link was removed from under the hood and aseptically placed into a vacuum package where it was then randomly assigned to one of the three treatments. Forty five sausages (n = 45/treatment, 135 total) were treated with 1 ml of either ASC, LAE, or no treatment (CON). Sausages were then vacuum sealed and stored in the dark at 4°C.

Five links were randomly selected and tested from each treatment (n = 15) following two hours of storage (Day 0) and plated to establish an initial bacterial count. Twenty links from each treatment were randomly assigned to d 1 and d 14 analysis (n = 60). Packages were opened using sterile scissors and then 25 ml of buffered peptone water (BPW) was added to the package via pipette. The bag opening was then closed, inverted, and massaged/rinsed for 1 min to insure even distribution of BPW across the entire link. Following the rinse, serial dilutions were performed and plated on duplicate 3M Aerobic Plate Count (APC) Petrifilm. The plates were incubated for 48 h at 37°C. Plates were

enumerated, with the *Listeria innocua* having a colony morphology of violet red. Data was entered into Excel for further analysis. All inoculated sausage links from this section were autoclaved and properly disposed.

### **pH Measurement**

Following 14 days of storage, the pH of the sausage surface was measured using pH litmus paper strips (Hydrion pH paper; Brooklyn, New York). Measurements were collected from the geometric center of the sausage link immediately following the removal from the vacuum package (n = 96). The pH was determined by comparing color results to a color scale provided by the Hydrion Company.

### **Color Evaluation**

#### *Objective*

Color of the pork German sausage was measured using a Minolta Colorimeter (Model CR-410, Minolta Corp., Ramsey, NJ) after 14 days of refrigerated storage (n = 140). The colorimeter was calibrated by using a white tile, then used to measure the sausage color through the vacuum packaged bags. Each sausage was measured at three different set locations. The color score included lightness (L\*), redness (a\*), and yellowness (a\*) values. These values were used to determine if any color fading occurred to the exterior portion of the sausage due to the antimicrobial applications.

#### *Subjective*

A trained color panel was utilized to perform a visual color test on the sausage links according to the American Meat Science Association Guidelines (AMSA, 1995). Eight panelists were presented with 12 samples (10.16 cm long) and asked to visually evaluate characteristics including degree of fading on a hedonic scale of 1-5 (1= no fading, 5= extreme fading), and percent in which fading was present on each individual link (1= none,

5= 76-100%). A total of five color panels were conducted, resulting in a total of 30 samples per treatment evaluated (n = 60).

### **Sensory Evaluation**

Panelists performed a triangle test based on the work of Meilgaard et al. (2007) to evaluate sensory characteristics. Sausage links were portioned into 1.27 cm bite-sized pieces. Panelists were presented with three coded samples at one time for tasting. Each plate presented had two identical treatment samples and one odd/different sample, with a total of 12 plates per panel (18 samples/treatment; 12 plates/panel). Panelists ingested each sample in alphabetical order as coded, and were asked to identify which they believed to be the odd/different sample, as well as note any off-flavor present. If unable to confidently identify the odd sample, panelists were instructed to make an informed guess. A minimum of six correct responses out of eight panelists (75%) was required to declare a distinct sensory difference at a significant of 0.05. A total of six panels occurred resulting in a total of 864 samples per treatment evaluated (n = 1728).

### **Statistical Analysis**

Data was entered into Excel and imported into SAS (Cary, NC; Version 9.4) for analysis. Bacterial populations were converted into log<sub>10</sub> for analysis. Response variables including bacterial populations, pH, colorimeter scores, and color scores from the trained panel were analyzed using the Mixed Procedure of SAS. Results from the triangle test were analyzed using Chi-square analysis in the Frequency Procedure of SAS. A minimum of six correct responses out of eight panelists (75%) was required to declare a distinct sensory difference at a significant level of 0.05. All differences were evaluated at a predetermined  $\alpha \leq 0.05$ .



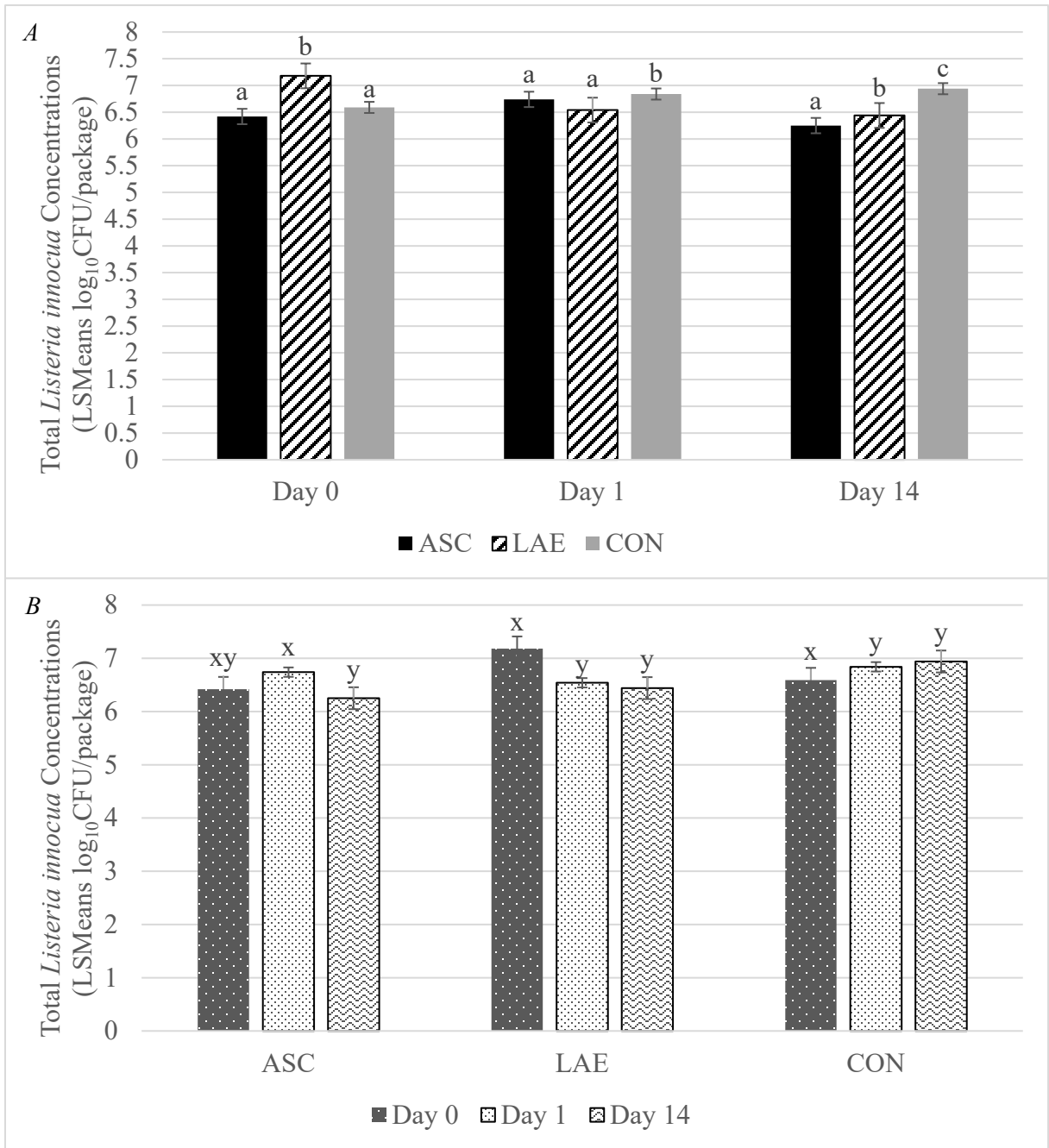
## RESULTS AND DISCUSSION

### **Trial One: Confirmation of Formulation**

The objective of trial one included creating a formulation for the application of acidified sodium chlorite (ASC) and lauric arginate ester (LAE) on pork German sausage links utilizing a SLIC system. A formulation was calculated for the surface area of 16, 8, 4, and 2 oz sausage links by using a fluorescent food dye to determine the volume needed for full coverage on the products surface. Five links were used for both ASC and LAE with a total of 10 per weight category (5 sample/treatment; n = 80). The amount of antimicrobial treatment added to each link size was as follows: 4 ml/16 oz, 2 ml/8 oz, 1 ml/4 oz, and 0.5 ml/2 oz. It was determined that 4 ml of antimicrobial was required for every 16 oz of sausage to provide full coverage.

### **Trial Two: Microbial and Quality Analysis**

The objective of this portion of trial two was to evaluate the effectiveness of ASC and LAE on *Listeria innocua*. Figure 1a shows the least squares means (LSMeans) for the comparison of *Listeria innocua* concentrations present on sausage links treated with ASC, LAE, and CON between d 0, 1, and 14. There was an interaction noted between day and treatment effects ( $P < 0.0001$ ). On d 0, sausage treated with LAE (7.18 log<sub>10</sub>CFU/package) had a significantly higher concentration ( $P < 0.05$ ) than both ASC (6.42 log<sub>10</sub>CFU/package) and CON (6.59 log<sub>10</sub>CFU/package). On d 1, the control (6.84 log<sub>10</sub>CFU/package) had a significantly higher concentration ( $P < 0.05$ ) when compared to the ASC (6.74 log<sub>10</sub>CFU/package) and LAE (6.54 log<sub>10</sub>CFU/package) treated sausage. All three treatments differed on d 14, with ASC having the lowest *Listeria innocua* concentration and the control having the highest ( $P < 0.05$ ).



**Figure 1.** Least-squares means (LSMeans) of total *Listeria innocua* concentrations from inoculated sausage links treated with acidified sodium chlorite, lauric arginate ester, and no treatment between on days 0, 1, and 14 under vacuum packaged refrigerated storage ( $n = 90$ ). Error bars represent the SE of the LSMeans.

A) <sup>a,b,c</sup> Treatment values within a sampling period with the same superscript do not differ ( $P > 0.05$ ).

B) <sup>x,y</sup> Period values within a treatment type with the same superscript do not differ ( $P > 0.05$ )

Figure 1b shows the least squares means (LSMeans) of *Listeria innocua* concentrations present on sausage links treated with ASC, LAE, and CON and how each day (0, 1, and 14) compared within treatments. Sausage treated with ASC were statistically the same from d 0 to d 1 (6.42 log<sub>10</sub>CFU/package; 6.74 log<sub>10</sub>CFU/package, respectively) and showed a decrease ( $P < 0.05$ ) between d 1 and 14 (6.42 log<sub>10</sub>CFU/package; 6.25 log<sub>10</sub>CFU/package, respectively). Although sausage treated with ASC had the lowest *L. innocua* concentration between all three treatments on d 14, it did not have the greatest log reduction. Lim and Mustapha (2004) stated acidified sodium chlorite had the greatest log reduction on *E. coli* O157:H7 (4.62 log reduction) when compared to cetylpyridinium chloride and potassium sorbate when applied to fresh beef. However, when applied to fresh beef inoculated with *L. monocytogenes*, ASC did not have as great of a log reduction (1.81 log reduction) when compared to *E. coli* O157:H7. The mentioned study suggests ASC is more effective on gram negative bacteria such as *E. coli* O157:H7 than gram positive bacteria such as *Listeria monocytogenes*. The results of the current study agree with this finding since ASC did not have the greatest log reduction on the *L. innocua*. The concentration of *L. innocua* decreased the most from d 0 to 1 when treated with LAE, with a 0.74 log reduction. A minimum of 1 log reduction is required to be considered significant because it is a 90% reduction in bacteria, therefore, LAE was not significant in reducing *L. innocua* (Endurocide Limited, 2018). Taormina and Dorsa (2009) reported a greater log reduction (1.5 log<sub>10</sub>CFU/g) within the first 48 hours when LAE was applied to frankfurters compared to d 0 and 8. Similarly, Martin et al. (2010) reported a minimum of 1 log reduction in frankfurters treated with LAE within the first 12 h of application. This agrees with the current study that LAE is more effective within the first 12 to 48 h of application. While ASC

and LAE treated links had no significant difference ( $P > 0.05$ ) in *Listeria innocua* when compared to each other, they were both significantly lower than the control ( $P < 0.05$ ).

Limitations to this trial existed due to the fact that only a ‘worst case scenario’ microbial concentration was utilized. As an implication for further research, it may be recommended to also use a low concentration of the *Listeria innocua* in order to create results more applicable to real world scenarios

## **pH**

The pH was collected from each sausage link immediately following the removal from the vacuum packaging after 14 d of refrigerated storage. Litmus paper was placed on the geometric center of the sausage link surface, and measured on a scale of 0 to 13. A total of 48 samples were collected for each treatment ( $n = 96$ ; 48 sample/treatment). Results for both ASC and LAE treated sausages yielded a consistent pH of 5 (data not shown in table format). These results are similar to Lim and Mustapha (2004) where the surface of fresh beef treated with a 1000 ppm solution of ASC had a pH of 4.99, as well as Quilo et al. (2009) where the pH of ground beef treated with 1000 ppm ASC ranged from 5.25 on d 0 to 5.56 on day 7. Results for the LAE treatment, however, disagree with Yang et al., (2017) where LAE treated chicken breasts reported a pH range from 6.02 to 6.63.

Some limitations may have occurred from using litmus paper because it was only able to show whole pH numbers, making it less accurate. Another limitation that occurred was the fact that the pH was only tested after 14 days of storage instead of being tested at different periods of refrigerated storage. Therefore, it may be recommended to use some other method for testing the surface pH, as well as testing the pH during different periods of storage.

## **Color Evaluation**

### *Objective*

Color scores were collected from the two treatments (ASC, LAE) following a 14 d refrigerated storage period, with a total of three replications (n = 140). Measurements were recorded as L\*, a\*, and b\* values. L\* values measure the degree of lightness (0= black; 100= white), a\* values measure the degree of redness (- = green; + = red), and b\* values measure the yellowness of the product (- = blue; + = yellow). Table 1 shows the least squares means on objective color with Minolta L\*, a\*, and b\* values on pork German sausage treated with ASC and LAE. There was no significant interaction found between replication and treatment for L\* values ( $P > 0.05$ ), a\* values ( $P = 0.5678$ ), and b\* values ( $P = 0.3841$ ) of sausage treated with ASC and LAE. A statistical difference ( $P < 0.05$ ) was found in the b\* value of LAE treated sausage which was more yellow in color than those treated with ASC (37.84; 36.48, respectively). L\* and a\* values for both ASC and LAE were similar between treatments ( $P > 0.05$ ). When evaluating differences between replications, there was no statistical difference noted between a\* and b\* values, there was however, a statistical difference ( $P < 0.05$ ) in the L\* values between replication 1 (35.71) and replications 2 and 3 (34.81; 34.44, respectively).

Results from the current study agree with Quilo et al., (2009) who reported a maintained redness in ground beef trim treated with ASC when compared to trim treated with potassium lactate, sodium metasilicate, and peroxyacetic acid. Results of the LAE treatment agree with Yang et al., (2017) where chicken breast treated with LAE exhibited greater b\* values when compared to that of a control.

**Table 1.** Least-squares means (LSMeans) of objective color scores on Minolta lightness (L\*), redness (a\*), and yellowness (b\*) on pork german sausage (n = 140) treated with acidified sodium chlorite and lauric arginate ester as post-lethality interventions.

	L*	SEM	a*	SEM	b*	SEM
Treatment						
ASC	34.97	0.23	47.93	1.45	36.48 <sup>x</sup>	0.22
LAE	35.01	0.23	47.91	1.44	37.84 <sup>y</sup>	0.22
Replication						
1	35.71 <sup>x</sup>	0.29	46.75	1.84	37.05	0.28
2	34.81 <sup>y</sup>	0.27	50.69	1.74	37.53	0.26
3	34.44 <sup>y</sup>	0.27	46.32	1.74	36.90	0.26

<sup>x,y</sup> Least squares means within a main effect and attribution type lacking common superscript differ ( $P < 0.05$ ).

L\*: 0= black; 100= white

a\*: + red; - green

b\*: + yellow; - blue

Because differences between treatments were minimal, it can be determined that there was no biological difference between the treatments (ASC and LAE). From this observation, it can be concluded that using ASC and LAE as a post-lethality intervention will have little to no negative visible impacts when applied to RTE and processed meats.

### *Subjective*

Panelists were asked to evaluate 12 sausage links per panel, which had been portioned into 10.16 cm pieces. Panelists were asked to evaluate the degree of fading on a hedonic scale of 1 – 5 (1= no fading, 5= extreme fading), and percent in which fading was present on each individual link (1= none, 5= 76 – 100%). A total of five color panels were conducted with six samples of each treatment per panel (6 samples/treatment; 12 samples/panel).

Table 2 reports the least squares means on subjective color scores of pork German sausage treated with ASC and LAE. There was a statistical interaction ( $P = 0.0240$ ) found between replications and treatment on degree of fading. For ASC, there were no differences ( $P > 0.05$ ) found in degree of fading within replications 1, 2, and 3 (2.02; 2.14; 2.00, respectively) and no differences ( $P > 0.05$ ) found in percent of fading within replications 1, 2, and 3 (2.02; 2.24; 2.02, respectively). For LAE degree of fading, while replication 2 and 3 differed ( $P = 0.0034$ ) from each other (1.76; 2.13, respectively), they did not differ from replication 1 (1.90). There was also a statistical difference ( $P < 0.0001$ ) found between replications and treatment on percent of fading for LAE treated links where replication 3 (2.51) differed from 1 and 2 (1.90; 1.65, respectively).

In a similar study where poultry was dipped treated with ASC, untrained sensory panelist were unable to detect differences in color between treatments, and considered it acceptable (Rio et al., 2007). In another study, panelists were unable to identify frankfurters

**Table 2.** Least-squares means ( $\pm$ SEM) on subjective color scores of pork german sausage (n= 60) treated with acidified sodium chlorite and lauric arginate ester as post-lethality interventions.

Replication	ASC		LAE	
	Degree <sup>1</sup>	Percent <sup>2</sup>	Degree <sup>1</sup>	Percent <sup>2</sup>
1	2.02 $\pm$ 0.16	2.02 $\pm$ 0.18	1.90 $\pm$ 0.16 <sup>a xy</sup>	1.90 $\pm$ 0.18 <sup>a x</sup>
2	2.14 $\pm$ 0.11	2.24 $\pm$ 0.13	1.76 $\pm$ 0.11 <sup>b x</sup>	1.65 $\pm$ 0.13 <sup>b x</sup>
3	2.00 $\pm$ 0.12	2.02 $\pm$ 0.12	2.13 $\pm$ 0.12 <sup>a y</sup>	2.51 $\pm$ 0.12 <sup>b y</sup>

<sup>1</sup>Degree of fading (1= no fading, 5= extreme fading)

<sup>2</sup>Percent of fading (1= none, 5= 76 – 100%)

<sup>a,b</sup> Values within a replication lacking a common superscript differ ( $P < 0.05$ ).

<sup>x,y</sup> Values within a treatment type lacking a common superscript differ ( $P < 0.05$ ).



treated with LAE and those with no treatment (Martin et al., 2009).

Results may be due to the fact that some sausages did not have an even smoke color to begin with, making it difficult to determine if smoke fading had occurred as a result of application of the antimicrobial treatments. The lack of even smoke color on the original sample product was a direct result of sausage links touching while being smoked. A suggestion for future studies may be to do smaller batches to ensure even smoke distribution, or even attempt to run a smokeless cycle.

### **Sensory Analysis**

Sensory data was collected utilizing a triangle panel. Panelists were presented with three coded samples (two identical and one different) on one plate at a time and asked to indicate which they believed to be the odd sample. A total of 12 plates were presented per panel with a total of 18 samples of each treatment per panel (18 samples/treatment; 12 plates/panel). Samples were presented under a red light to eliminate any visual color differences. A minimum of six correct responses (75%) were required out of eight panelists for there to be a distinct flavor difference identified at a significance level of  $P < 0.05$ .

Table 3 shows the percent of correctly identified samples within each panel. The average correctly identified samples for ASC treated sausage was 32.99% and 33.68% for LAE treated sausage. These low success rates indicate there was little sensory impact on the product. Bonnaud et al. (2010) stated that high amounts of antimicrobials, such as LAE, contribute to a bitter taste when applied to food products; the current study, however, did not find a discernible difference between treatments ( $P > 0.05$ ). In a similar study, panelists were unable to determine the difference between queso fresco treated with LAE and the control when presented in a triangle test (Soni et al., 2010). Rio et al., (2007) reported that ASC

**Table 3.** Percent of correctly identified samples in a triangle sensory panel of pork german sausage (n = 1728) treated with acidified sodium chlorite and lauric arginate ester as post-lethality interventions.

Treatment	Panel						Avg
	1	2	3	4	5	6	
ASC	27.08	43.75	33.33	35.42	25.00	33.33	32.99
LAE	25.00	33.33	39.58	35.42	35.42	33.33	33.68

Each panelist was presented with 3 coded samples, two from the same treatment and one from a different treatment and asked to identify the odd sample. For a significant level of confidence for the products to be identifiably different from each other, a minimum of 75% correctly identified samples were required to be determined significant ( $P < 0.05$ ).

treated chicken legs were considered acceptable by an untrained sensory panel when evaluating qualities such as color, smell, and product acceptability. In agreement with these studies, there was no significant difference noted in taste with the current study; therefore, it can be concluded that ASC and LAE have no significant sensory effect when applied to RTE products as post-lethality interventions.

## CONCLUSION

The amount of both antimicrobials required to provide full coverage is 4 ml for every 16 oz. When applied to inoculated links, sausage treated with LAE reported a higher reduction (0.74 log reduction) of *Listeria innocua* on d 14 than those treated with ASC (0.17 log reduction). Although LAE had the lowest APC count, the log reduction was not considered significant because it was not greater than a 1 log (90%) reduction. While results from ASC and LAE had no significant difference ( $P > 0.05$ ) when compared to each other, they were both significantly lower than the control ( $P < 0.05$ ). After 14 days of refrigerated storage, sausage treated with ASC and LAE both reported a pH of 5. Sausage treated with LAE reported higher  $b^*$  values (more yellow) than those treated with ASC ( $P < 0.05$ ). Panelists from the sensory analysis panel were unable to consistently identify any differences ( $P > 0.05$ ) between ASC and LAE treated sausages.

From the data collected, it can be determined that acidified sodium chlorite and lauric arginate ester both performed similarly across all trials within the current study. It can be concluded that ASC and LAE may be used to achieve similar results in eliminating pathogenic bacteria without negatively impacting sensory characteristic of RTE foods and processed meats in the meat industry.

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APPENDIX A



7/31/2019

Dr. Loree Branham  
Dept. of Agriculture  
Angelo State University  
San Angelo, TX 76909

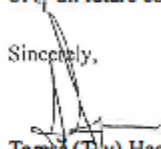
Dear Loree:

The proposed project submitted by your student Alicia Curtis titled, "*Comparison of Lauric Arginate and Acidified Sodium Chlorite as Post-Lethality Interventions on Processed Meats*" has been approved in accordance with federal regulations 45 CFR 46.

The approval is effective beginning July 31, 2019. Please be aware that the protocol will expire one year from its original approval date. If the study will continue beyond that date, you must submit a request for continuation before the current protocol expires.

The approved addendum is for protocol #BRA-073119. Please include this number in the subject line of all future communications with the IRB regarding the protocol.

Sincerely,

  
Teresa (Tay) Hack, Ph.D.  
Chair, Institutional Review Board

Dr. Teresa Hack, IRB Chair | ASU Station #11025 | San Angelo, Texas 76909  
Phone: (325) 439-6121 | Fax: (325) 432-2194

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## BIOGRAPHY

Alicia Rachael Curtis was born in Newport, RI to a military family. She spent the majority of her life moving, and eventually resided with her grandparents, Michael and Peggy Nester, in 2011. She graduated from Hondo High School in 2014, then attended Angelo State University. She received her Bachelor of Science in Animal Science with a split minor in Food Science and Range and Wildlife Management December of 2017. During her time as an undergraduate, she was involved in Block and Bridle as an officer, Young Life Ministries as a college leader, and received Dean's List status in fall and spring of 2017. She spent the summer of 2017 as a farm intern with Seaboard Foods and received multiple certifications.

Upon graduation, she attended Graduate School at Angelo State University and received her Master of Science in Animal Science with an emphasis in Meat and Food Science in December of 2019. During her time as a graduate student, she was inducted into the National Honor Society of Phi Kappa Phi in the spring of 2019. She was also a graduate assistant, where she held multiple responsibilities involving multiple undergraduate classes, helping other graduate students conduct research, and was actively involved at Angelo State University's Food Safety and Product Development Laboratory.