

# Control of *Listeria monocytogenes* on Alternatively Cured Ready-to-Eat Ham Using Natural Antimicrobial Ingredients in Combination with Post-Lethality Interventions

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## Abstract

Ready-to-Eat (RTE) meat and poultry products manufactured with natural or organic methods may be at greater risk for *Listeria monocytogenes* growth, if contaminated, than their conventional counterparts due to the required absence of preservatives and antimicrobials. Thus, the objective of this study was to investigate the use of commercially available natural antimicrobials in combination with post-lethality interventions for the control of *L. monocytogenes* growth and recovery on alternatively-cured RTE ham. Antimicrobials evaluated were cranberry powder (90 MX), vinegar (DV), and vinegar and lemon juice concentrate (LV1 X). Post-lethality interventions studied included high hydrostatic pressure at 400 MPa (HHP), lauricarginate (LAE), octanoic acid (OA), and post-packaging thermal treatment (PPTT). Viable *L. monocytogenes* on modified Oxford (MOX) and thin agar layer (TAL) media were monitored through 98 days of product storage at 4 ± 1°C. The post-lethality treatments of HHP, OA, and LAE significantly reduced initial viable *L. monocytogenes* numbers compared to the control, regardless of the antimicrobial ingredient used in the formulation while PPTT did not. Only when used in combination with DV and LV1 X did HHP, OA, and LAE exhibit sustained suppression, of *L. monocytogenes* recovery and growth throughout refrigerated storage. As a result, the use of natural antimicrobial ingredients such as DV and LV1 X in combination with post-lethality interventions such as HHP, LAE, and OA represents an effective multi-hurdle approach that could be instituted by manufacturers of organic and natural processed meat and poultry products for *L. monocytogenes* control.

**Keywords:** Ham; High-pressure; *Listeria monocytogenes*; Natural; Organic

## Introduction

The popularity of natural and organic foods has been increasing for several years, and has led to noticeable market growth of these food categories [1,2]. In 2013, for example, organic foods in the United States experienced a 13% increase in sales compared to the previous year [3]. Similar increases are expected to continue in the future in spite of the price premiums typically associated with these products [4]. Natural and organic meat products, in particular, have accounted for a significant part of that growth. Stringent regulations that govern the production of natural and organic foods have prevented the use of certain traditional ingredients. For instance, in the manufacture of natural and organic processed meat products, such as boneless ham and frankfurters, the direct addition of nitrite or nitrate, curing ingredients used in the manufacture of such products, and that have strong antimicrobial properties, are not permitted. Additionally, lactate and diacetate, antimicrobials commonly found in ready-to-eat (RTE) meat and poultry products, and that is effective inhibitors of pathogens such as *Listeria monocytogenes*, are not permitted in the manufacture of natural or organic meat products. Thus, RTE meat and poultry products manufactured under uncured, natural, or organic methods are sometimes termed “alternatively cured” or “naturally cured”. The requirements for these products suggest that they are likely to be at a greater risk than their conventional counterparts for growth of *L. monocytogenes* if contamination occurs, and previous reports have supported this concern as well [5-7].

The use of natural antimicrobials or post-lethality interventions in the manufacture of natural and organic meat products has been studied by several researchers and meat processors alike [8-11]. The United States Department of Agriculture Food Safety Inspection Services (USDA-FSIS) defines a post-lethality treatment as “...a lethality

treatment that is applied or is effective after post-lethality exposure. It is applied to the final product or sealed package of product in order to reduce or eliminate the level of pathogens resulting from contamination from post-lethality exposure” [12]. High hydrostatic pressure processing (HHP), for example, is one such post-lethality intervention that takes place after the product has gone through the lethality or cooking step [12,13]. Other examples of post-lethality interventions include sprays or solutions such as lauric arginate (lauramide arginine ethyl ester or LAE) and octanoic acid (sometimes referred to as caprylic acid or OA) as well as post-packaging thermal treatment or pasteurization, all of which can be applied to the finished product. The USDA-FSIS lists lauric arginate as a safe and suitable ingredient for the production of meat and poultry products, and allows up to 44 mg/kg (ppm) (± a 20% tolerance) by weight of the product to be applied to the inside of a package as a processing aid [14]. When used at this level, lauric arginate is considered a processing aid, would not have to be declared on the label of the product, and could be used in the manufacture of uncured, no-nitrate-or-nitrite-added (alternatively-cured), RTE natural or organic meat and poultry products. Similarly, the USDA-FSIS also allows for octanoic acid to be used as a processing aid if applied to the surface of an RTE meat and poultry product at

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a rate not to exceed 400 mg/kg octanoic acid by weight of the final product [14]. Octanoic acid is a saturated ( $C_{8,0}$ ) fatty acid ( $pK_a$  4.89) naturally found in coconut oil and bovine milk [15].

While natural sources of antimicrobials could potentially replace chemical preservatives as a means to address *L. monocytogenes* [10,16,17], it has also been shown that the anti listerial properties of antimicrobials can vary as a result of the fat content of the food [18] and other variables including protein content, pH,  $a_w$ , and other ingredients added.

Thus, there is significant concern for the potential recovery and growth of sub lethally injured and uninjured *L. monocytogenes* during the storage life of alternatively-cured RTE ham and frankfurters that do not include the antimicrobial agents normally used in conventional cured meats. Such concerns highlight the need for a combination of antimicrobial hurdles to be investigated and, eventually, implemented in order to fully address *L. monocytogenes* control in natural and organic RTE meat and poultry products.

Previous work in our laboratory [19] demonstrated that post-lethality interventions such as HHP, OA, and LAE can deliver an initial lethality for *L. monocytogenes*, but survivors will grow in processed meats following the treatment. Secondly, we have also observed that natural antimicrobials such as vinegar and vinegar and lemon juice concentrate can impart a bacteriostatic effect on this pathogen, thus suppressing subsequent growth, but without reducing the initial population.

Consequently, the objective of this study was to assess the commercially available natural antimicrobial ingredients that are currently allowed for natural and organic meat and poultry products when used in combination with post-lethality interventions to both reduce the initial contaminating population, and subsequently inhibit the recovery and growth of any *L. monocytogenes* survivors. We hypothesized that a combination of treatments that achieves both initial lethality and sustained suppression of growth of survivors would effectively improve the overall control of *L. monocytogenes* on alternatively-cured processed meat products.

## Materials and Methods

### Manufacture of hams

Thirteen ham formulations (twelve experimental and one control formulation) were manufactured at the Iowa State University Meat Laboratory using inside (*gracilis* and *semimembranosus*) ham muscles. The formulations consisted of 18.14 kg of ham insides, 3.66 kg water, 0.50 kg salt, 0.30 kg sugar and 74.84 g celery powder plus the selected antimicrobials. The ham muscles were obtained from a local processor and frozen prior to use to ensure uniformity of raw materials. The ham muscles were tempered to  $-2^\circ\text{C}$ , then coarse ground through a grinder plate with 9.53-mm-diameter holes (Biro MFG Co., Marblehead, OH). Nonmeat ingredients (water, salt, sugar) were added and mixed with ground ham muscles at 26 rpm for 2 min using a double action, paddle-and-ribbon mixer (Leland Southwest, Fort Worth, TX). Pre-converted (nitrate converted to nitrite) celery powder (Veg Stable 504, Florida Food Products, Inc., Eustis, FL) containing 1.5% (wt/wt) nitrite was used as the natural, alternative source of nitrite. All products were formulated to contain 50 mg/kg (ppm) ingoing natural nitrite to represent the reduced ingoing nitrite concentration that is typical of many natural and organic processed meat products. Control hams were formulated without antimicrobials or post-lethality interventions to best represent the natural and organic hams currently produced. Three commercially

available natural antimicrobial ingredients were evaluated in this study; cranberry powder (90 MX; Ocean Spray International, Middleboro, MA), buffered vinegar (DV; WTI Ingredients, Inc., Jefferson, GA), and buffered vinegar and lemon juice concentrate (LV1 X; WTI Ingredients, Inc.) (wt/wt). Each ingredient was added at a concentration (1.0%, 1.0%, 2.5%, respectively) recommended by the respective supplier. The pH of 10% solutions (w/v) of the 90 MX, DV, and LV1 X ingredients were 3.89, 5.87 and 5.57 respectively.

The hams and appropriate ingredients were mixed, then reground using a grinder plate with 6.35 mm diameter holes and stuffed into a 50 mm diameter impermeable plastic casing (Nalobar APM 45, Kalle USA, Gurnee, IL) using a rotary vane vacuum stuffer (RS 1040 C, Risco USA Corp., South Eaton, MA). All samples were then placed in a single-truck smokehouse (Maurer, AG, Reichenau, Germany) and heated to an internal temperature of  $71.1^\circ\text{C}$ . The hams were then placed in a  $0^\circ\text{C}$  cooler overnight to stabilize. The next day (day 0 of the experiment), the hams were sliced into approximately 12.0 mm thick slices using a hand slicer (SE 12 D, Bizerba, Piscataway, NJ), placed into barrier bags (B2470, Cryovac Sealed Air Corporation, Duncan, SC; oxygen transmission rate of 3-6 cc/m<sup>2</sup>, 24 h at  $4^\circ\text{C}$ , 0% RH; water vapor transmission rate of 0.5-0.6 g/0.6 m<sup>2</sup> at  $38^\circ\text{C}$  (100% RH, 24 h), and vacuum-sealed (UV 2100, Multivac, Inc., Kansas City, MO). Hams for physicochemical analyses were placed in boxes, transferred to a holding cooler in the Iowa State University Meat Laboratory and stored at  $4 \pm 1^\circ\text{C}$  until analyses were conducted. Hams for microbial analyses were placed in boxes with vacuum packaged ice, transferred to the Iowa State University Microbial Food Safety Laboratory in the Food Science and Human Nutrition Department for subsequent inoculation, and stored at  $4 \pm 1^\circ\text{C}$  for the duration of the experiment. Two complete independent replications of the entire experiment were performed.

### Product analyses

Proximate analysis was conducted for moisture, fat, and protein of homogenized control and treatment formulations on day 0 using AOAC methods 950.46, 960.63, and 992.15, respectively [20-22]. Samples were prepared in duplicate for each ham formulation.

Product pH was measured by placing a pH probe (FC20, Hanna Instruments, Woonsocket, RI) into homogenized (KFP715 food processor, Kitchenaid, St. Joseph, MI) samples from the control and treatments that were prepared by first blending the ground ham with distilled, de-ionized water in a 1:9 ratio, and then measuring the pH with a pH/ion meter (Accumet 925 pH/ion meter, Fisher Scientific, Waltham, MA). Calibration was conducted using phosphate buffers of pH 4.0, 7.0, and 10.0. Duplicate readings were taken for each product formulation on day 0.

Available moisture was determined using a water activity meter (AquaLab 4 TE, Decagon Devices Inc., Pullman, WA). Samples were cut into small pieces, placed in disposable sample cups, covered, and allowed to equilibrate to room temperature (5-10 min). Measurements were obtained on day 0 and were performed in duplicate for the control and all treatments. Calibration was performed using 1.00 and 0.76 sodium chloride water activity standards.

Residual nitrite concentration was determined utilizing AOAC method 973.31 [23]. Samples from each treatment were frozen at  $-20 \pm 1^\circ\text{C}$  on day 0 and evaluated in duplicate at a later date.

### Inoculation of samples

*L. monocytogenes* strains Scott A NADC 2045 serotype 4b, H7969

serotype 4b, H7962 serotype 4b, H7596 serotype 4b, and H7762 serotype 4b were obtained from the Iowa State University Microbial Food Safety Laboratory in the Food Science and Human Nutrition Department. These strains were selected because each has been isolated from cases of food-borne disease outbreaks. Each strain was cultured separately in tryptic soy broth supplemented with 0.6% yeast extract (TSBYE) (Difco, Becton Dickinson, Sparks, MD) for 24 h at 35°C. A minimum of two consecutive 24 h transfers of each strain to fresh TSBYE (35°C) were performed prior to each experiment. The cells were harvested by centrifugation (10 min at 10,000 × g and 4°C) in a Sorvall Super T21 centrifuge (American Laboratory Trading, Inc., East Lyme, CT). The supernatant was discarded and the pelleted cells were re-suspended in 30.0 ml of sterile buffered peptone water (BPW) (Difco, Becton Dickinson). The total concentration of the five-strain mixed culture was approximately 10<sup>9</sup> CFU per ml based on the washed cell suspension. Two serial dilutions (100-fold each) of the cell suspension were prepared in BPW to give a final inoculum concentration of approximately 10<sup>5</sup> CFU per ml. This diluted five-strain mixed culture was used to inoculate the ham samples.

While in the Microbial Food Safety Laboratory, each packaged sample was reopened and the surface of the product was aseptically inoculated with 0.2 ml per package, using the diluted five-strain mixed culture of the pathogen. The viable cell concentration at inoculation was approximately 10<sup>3</sup> CFU per g of ham slice. The bags were then vacuum-sealed using a model A300/52 vacuum packaging machine (Multivac, Inc.) and stored at 4 ± 1°C for the duration of the experiment.

### Post-lethality interventions

Four post-lethality interventions were evaluated in this study; high hydrostatic pressure (HHP), octanoic acid (OA), lauricarginate (LAE), and post-packaging thermal treatment (PPTT). Ham slices from each formulation were randomly assigned to these post-lethality interventions. All post-lethality interventions were applied to the product within two hours following inoculation on day 0 of the study.

The HHP parameters were 400 MPa, 4 min dwell time at 12 ± 2°C initial fluid temperature of the pressurization fluid. The 400 MPa HHP treatment was utilized for this study rather than the more common 600 MPa that is used for commercial products to allow a measurable number of the organisms to survive so that the effects of the antimicrobials in combination with HHP could be assessed. Inoculated samples were transported to the High Pressure Processing Laboratory at the Iowa State University Food Science and Human Nutrition Department and subjected to HHP treatment using a FOOD-LAB 900 Plunger Press system (Standsted Fluid Power Ltd., Standsted, UK). The pressurization fluid was a 50.0% propylene glycol (GWT Koilguard; GWT Global Water Technology, Inc., Indianapolis, IN) and 50.0% water solution (v/v). The average rate of pressurization was 350 MPa per min and depressurization occurred within 7 s. Adiabatic heating of the pressurization fluid was 4.6°C ± 0.8°C/100 MPa.

Octanoic acid (Octa-Gone; Eco Lab, Inc., Eagan, MN) was applied according to the manufacturer's recommendations. Octa-Gone contains approximately 3.6% octanoic acid (v/v). A 23.4% Octa-Gone solution (v/v) was prepared by mixing Octa-Gone with sterile de-ionized water at 4 ± 1°C. Based on average surface area measurements obtained as previously described, the OA solution was aseptically dispensed into the bag containing the ham slice (0.0186 ml per cm<sup>2</sup>) and vacuum-sealed.

Lauricarginate (Protect-M; Purac America, Lincolnshire, IL) was also applied according to the manufacturer's recommendations.

Protect-M contains approximately 10.0% lauricarginate (v/v). A 2.5% Protect-M solution (v/v) was prepared by mixing Protect-M with sterile de-ionized water at 4 ± 1°C. Based on the ham slice surface area measurements, the LAE solution was aseptically dispensed into the bag containing the ham slice (7.19 × 10<sup>-3</sup> ml per cm<sup>2</sup>) and vacuum-sealed.

PPTT was conducted by immersing packages of ham in water at 71.0 ± 1.0°C water for 30 s using a water bath (Isotemp-228, Fisher Scientific). Seven packages were immersed as a group so that water temperature would not change by more than 1.0°C. Water temperature was monitored throughout the process. Packages were held in heated water for the prescribed length of time and then placed on ice immediately to chill before placement in refrigerated storage.

### Microbial analysis

Microbial analysis of ham samples for viable *L. monocytogenes* was conducted on days 1, 14, 28, 42, 56, 70, 84, and 98 of storage. On the appropriate day, two packages for each treatment were removed from the holding cooler, opened aseptically, and their contents placed inside a sterile Whirl-Pak stomacher bag (Nasco, Ft. Atkinson, WI). Fifty (50.0) ml of sterile BPW was added to each bag, and the bags shaken by hand for approximately 30 s. The rinse solution from each ham sample was then serially diluted (10-fold) in BPW to obtain pre-determined dilutions of the samples according to the sampling day. One ml (for undiluted rinsate, divided into three ~0.33-ml aliquots plated on three separate plates) or 0.1 ml of the appropriate dilution was surfaced plated on modified listeria selective agar (Oxford, MOX)(Difco, Becton Dickinson). The dry ingredients used to manufacture the MOX were 42.5 g of Columbia agar base (Difco, Becton Dickinson), 15.0 g of lithium chloride (Difco, Becton Dickinson), 1.0 g of esculin hydrate (Sigma-Aldrich, St. Louis, MO), and 0.5 g of ferric ammonium citrate (Difco, Becton Dickinson) per liter of de-ionized water. Additionally, an aliquot of 1.0 ml (for undiluted rinsate, divided into three ~0.33-ml aliquots plated on three separate plates) or 0.1 ml of the appropriate dilution was surface-plated on thin agar layer medium base (TAL) that was made according to Kang and Fung [24]. Within 48 h before use, MOX plates to be made into TAL were aseptically overlaid with 7.0 ml of sterile tryptic soy agar (Difco, Becton Dickinson) held at 55°C to facilitate the even distribution of the molten agar. Each sample was plated in duplicate. All inoculated plates were incubated in an inverted position at 35°C for 48 h, after which time they were removed from the incubator, and colonies typical of *L. monocytogenes* were enumerated. The populations (CFU per ml) were averaged and then converted to log<sub>10</sub> CFU per g using the average weight of the sliced ham from the two replications of the experiment (n=40). The detection limit of our sampling protocols was ≥0.30 log<sub>10</sub> CFU per g based on a sample weight of 25.0 g.

### Statistical analysis

The overall design of the experiment was a factorial design. The generalized linear mixed models (GLIMMIX) procedure of Statistical Analysis System (version 9.3, SAS Institute Inc., Cary, NC) was used for statistical analysis. *L. monocytogenes* growth data were analyzed for treatment effects within day. Day and treatment x day interactions were also analyzed. The effects of each post-lethality intervention were analyzed separately for each natural antimicrobial ingredient studied. Likewise, the effects of each natural antimicrobial ingredient were analyzed separately for each post-lethality intervention studied. Where significant effects (P<0.05) were found, pair-wise comparisons between the least squares means were computed for each day using Tukey's honestly significant difference adjustment.

## Results and Discussion

The mean weight of the ham slices was  $24.57 \pm 0.64$  g, while the mean diameter, height, and surface area were  $4.72 \pm 0.06$  cm,  $1.31 \pm 0.01$  cm, and  $54.51 \pm 1.13$  cm<sup>2</sup>, respectively (data not shown;  $n=40$  for all measurements). These ham slice dimensions were used to calculate ham slice surface area for LAE and OA treatment volumes of 0.39 and 1.01 ml per package, respectively. The dosages of each compound were calculated according to the respective manufacturer's recommendations as previously described. These dosages resulted in LAE and OA treatment concentrations of 39.82 and 343.03 mg/kg (ppm), respectively.

### Physicochemical traits

Physicochemical characteristics of the hams can be found in Table 1. All treatments exhibited significantly lower  $a_w$  values than the control treatment ( $P<0.05$ ). The DV and LV1 X treatments, in turn, resulted in significantly lower  $a_w$  values when compared to the 90 MX treatment ( $P<0.05$ ). Final product pH was also affected by natural antimicrobial compound added. The pH of the control treatment was not significantly different from that of the DV treatment ( $P>0.05$ ), but did significantly differ from both the LV1 X and the 90 MX treatments ( $P<0.05$ ). These differences in pH most likely resulted from the presence of acidic compounds in the natural antimicrobial compounds utilized. Cranberry has been reported to contain phenolic acids and exhibit a high titratable acidity [25]. Xi et al. [16] obtained similar pH results when using different ingoing levels of cranberry powder in a cooked meat model system and in frankfurters [17]. Similarly, the vinegar and vinegar and lemon juice concentrates used in this study also contain acidic compounds, such as acetic and citric acid, and can be expected to result in the observed lower pH in products made with those ingredients. No significant differences in protein % and moisture % were found between the treatments ( $P>0.05$ ). Fat %, however, was significantly lower in the 90 MX treatment compared to both the DV and LV1 X treatments ( $P<0.05$ ). Although some of these differences were statistically significant, the differences were very small and were not expected to affect the results of this study.

The residual nitrite concentration found in the 90 MX treatment was lower ( $P<0.05$ ) than that of the control and DV treatments. No significant differences between all other treatments were detected ( $P>0.05$ ). Although all ham formulations were manufactured with 50 mg/kg (ppm) natural nitrite on an ingoing basis, the highest residual nitrite concentration observed in all of the treatments on day 0 of the study was 36.01 mg/kg (ppm) (control treatment). This indicates that part of the ingoing nitrite was depleted in curing and other reactions that took place, as expected, during product manufacture.

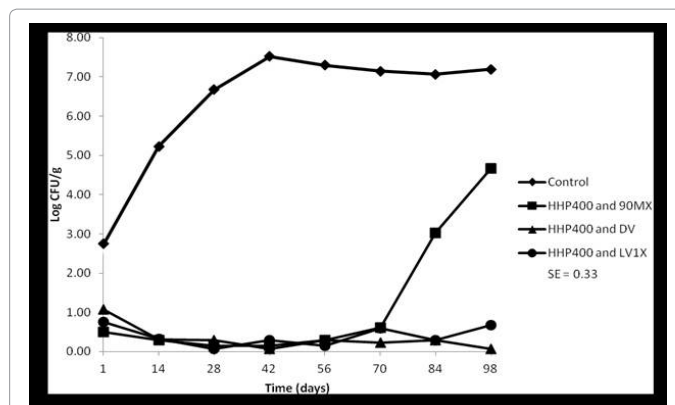
Treatment <sup>b</sup>	$a_w$	pH	Fat %	Moisture %	Protein %	Residual Nitrite (mg/kg)
Control	0.9819 <sup>a</sup>	6.35 <sup>b</sup>	1.96 <sup>ab</sup>	75.84	18.09	36.01 <sup>bc</sup>
90MX	0.9793 <sup>b</sup>	6.05 <sup>a</sup>	1.58 <sup>a</sup>	75.82	17.95	31.32 <sup>a</sup>
DV	0.9759 <sup>c</sup>	6.24 <sup>bc</sup>	2.26 <sup>b</sup>	75.30	17.88	35.36 <sup>bc</sup>
LV1X	0.9772 <sup>c</sup>	6.18 <sup>bc</sup>	2.32 <sup>b</sup>	74.93	18.02	33.56 <sup>bc</sup>
SE <sup>c</sup>	0.0005	0.04	0.19	0.29	0.20	1.11

<sup>a</sup>Values are least squares means. Within a column, means with different superscripts (a through c) are significantly different ( $P<0.05$ )

<sup>b</sup>Control, naturally-cured control; 90MX, cranberry powder; DV, vinegar; LV1X, vinegar and lemon juice concentrate

<sup>c</sup>Standard error of the differences of least squares means

**Table 1:** Effect of natural antimicrobial ingredients on physicochemical properties of naturally-cured RTE ham<sup>a</sup>.



**Figure 1:** Effect of high hydrostatic pressure treatment in combination with natural 535 antimicrobials on viable *Listeria monocytogenes* (log<sub>10</sub> CFU per gram) on alternatively-cured RTE ham stored at  $4 \pm 1$  °C, using modified Oxford medium.

Honikel [26] reported that as much as 65% of the ingoing nitrite can be depleted during product manufacture. Similarly, Xi et al. [17] reported that as much as 75% of the ingoing nitrite can be depleted during the manufacture of frankfurters. Factors such as product pH, cooking temperature, and addition of reducing agents have been long recognized as important factors affecting residual nitrite concentrations in meat systems [27]. Thus, the significant ( $P<0.05$ ) decrease in pH brought about by the natural antimicrobial ingredients used in this study, especially cranberry powder, was expected to influence residual nitrite concentrations.

### Viable *Listeria monocytogenes* populations

The growth mediums used, MOX and TAL, did not significantly differ ( $P>0.05$ ) within treatment on any given day, indicating that, under the conditions of this study, the use of the TAL technique offered no significant advantage compared to using a traditional medium such as MOX. Thus, the discussion about viable *L. monocytogenes* populations as affected by treatment is limited to the results obtained using MOX.

The ham formulations included controls that were manufactured without antimicrobials or post-lethality treatments to provide comparison to the treatment combinations. The 400 MPa HHP treatment used in combination with all of the natural antimicrobial ingredients studied resulted in a significant ( $P<0.05$ ) reduction in viable *L. monocytogenes* populations on day 1 when compared to the control treatment (Figure 1). More specifically, the HHP treatment resulted in populations that were 2.25, 1.99, and 1.67 log<sub>10</sub> CFU per g lower ( $P<0.05$ ) on day 1 when combined with 90 MX, LV1 X, and DV, respectively, and relative to the control treatment. The differences in log<sub>10</sub> CFU per g reductions observed on day 1 in the different treatments subjected to HHP, however, were not significant ( $P>0.05$ ) compared to each other, indicating that the three antimicrobial ingredients used did not influence the bactericidal properties of the HHP treatment applied. These results confirm the bactericidal properties of HHP at 400 MPa against *L. monocytogenes*. However, only when combined with DV or LV1 X was the initial reduction in viable *L. monocytogenes* achieved by 400 MPa HHP sustained throughout the duration of the study. The combination of 400 MPa and 90 MX resulted in an increase in the *L. monocytogenes* populations after day 70 that reached about 5 log<sub>10</sub> CFU per g by the end of the study.

Damage to the cell membrane seems to be the likely mode of action for HHP, and it has been reported that damage to bacterial

cell membranes can be extensive, often resulting in cell death [28,29]. Changes in membrane permeability, scarring around the cell wall, separation of the cell wall from the membrane and protein denaturation, as well as damage to transport systems have also been reported in HHP-treated microbial populations [30,31]. Thus, it is likely that the bacteriostatic effect observed in the HHP treatments combined with ingredients such as vinegar or vinegar and lemon juice concentrate was a result of the migration of growth inhibitory compounds present in these ingredients into the bacterial cells. As a result, the use of HHP at 400 MPa in combination with DV or LV1 X represents a promising multiple-hurdle approach for addressing the potential presence of *L. monocytogenes* in processed meats, and for inhibiting the potential recovery and growth of those cells that remain viable over the refrigerated storage of the products. Further, it appears that the use of these antimicrobials may permit reduced HHP pressure of 400 MPa as an alternative to the higher 600 MPa that is currently used in commercial applications where HHP is used alone. Reduction of pressure used in the HHP process would increase product throughput for the process and result in lower maintenance cost, both of which are important in determining total cost of the treatment [32,33].

Combining OA with the natural antimicrobial ingredients evaluated in this study (Figure 2) yielded similar patterns to those obtained when combining HHP with the same ingredients in terms of viable *L. monocytogenes* populations observed. Significant ( $P < 0.05$ ) reductions in initial viable *L. monocytogenes* populations were observed when OA was combined with each of the natural antimicrobial ingredients evaluated after day 1 and compared to the control treatment. On day 1, compared to the control treatment, the *L. monocytogenes* populations were lower by 2.67, 2.52, and 2.33  $\log_{10}$  CFU per g when OA was combined with 90 MX, DV, and LV1 X, respectively. Burnett et al. [34] concluded that octanoic acid solutions acidified to pH 2.0 or 4.0, and applied to RTE meat and poultry, resulted in *L. monocytogenes* log reductions ranging from 0.85 to 2.89  $\log_{10}$  CFU per sample. The pH of the working solution of OA used in the current study was 3.01. It has been reported that the main mechanism by which medium and short chain fatty acids achieve microbial inactivation is through the diffusion of undissociated acids across the bacterial cells and the subsequent intracellular acidification [35]. Thus, it is likely that the bactericidal effects of OA on *L. monocytogenes* follow that mechanism.

Sustained inhibition of *L. monocytogenes* recovery and growth compared to the control was exhibited by treatments that combined OA with DV or LV1 X ( $P < 0.05$ ) but not with 90 MX ( $P > 0.05$ ), which

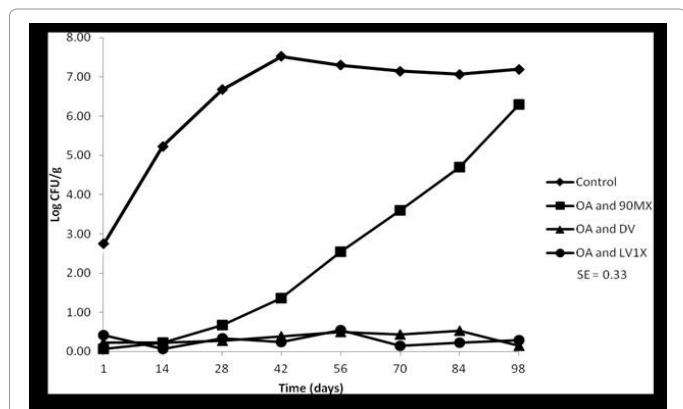


Figure 2: Effect of octanoic acid treatment in combination with natural antimicrobials on viable *Listeria monocytogenes* ( $\log_{10}$  CFU per gram) on alternatively-cured RTE ham stored at  $4 \pm 1^\circ\text{C}$ , using modified Oxford medium.

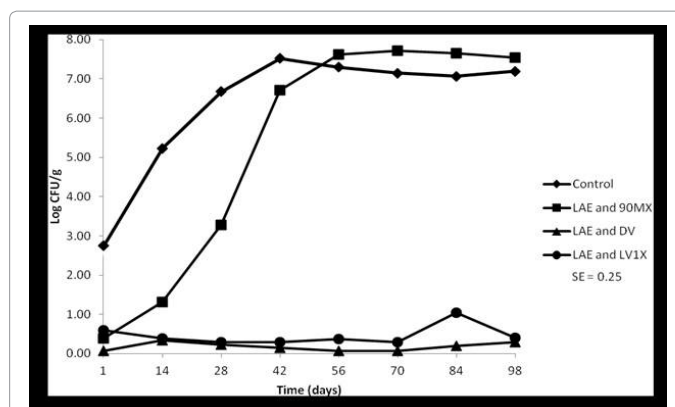


Figure 3: Effect of lauricarginate treatment in combination with natural antimicrobials on viable *Listeria monocytogenes* ( $\log_{10}$  CFU per gram) on alternatively-cured RTE ham stored at  $4 \pm 1^\circ\text{C}$ , using modified Oxford medium.

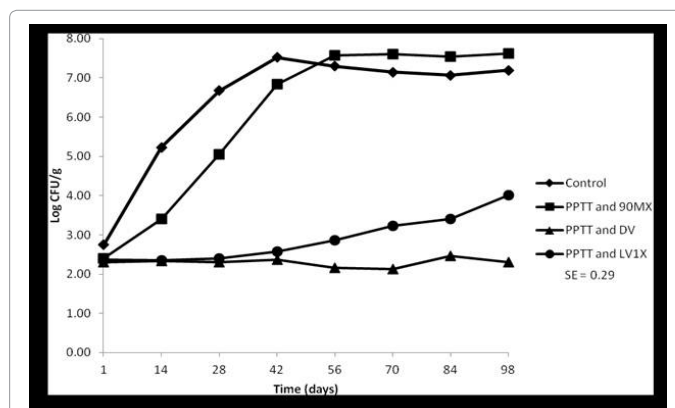


Figure 4: Effect of post-packaging thermal treatment in combination with natural antimicrobials on viable *Listeria monocytogenes* ( $\log_{10}$  CFU per gram) on alternatively-cured RTE ham stored at  $4 \pm 1^\circ\text{C}$ , using modified Oxford medium.

resulted in an increased population by over 6  $\log_{10}$  CFU per g after 98 days. Previous work in our laboratory [19] showed that OA, when applied alone to naturally-cured frankfurters and RTE ham using similar protocols, exerted an initial bactericidal effect on *L. monocytogenes* but failed to inhibit the organism's recovery and growth over the refrigerated life of the products. Thus, the use of OA in combination with DV or LV1 X, similar to the effect of HHP, represents a necessary multiple-hurdle approach for *L. monocytogenes* in alternatively-cured processed meats.

The effects of using lauricarginate in combination with natural antimicrobial ingredients on viable *L. monocytogenes* populations are shown in Figure 3. Again, on day 1 of the study, LAE in combination with DV, 90 MX, and LV1 X resulted in 2.67, 2.37, and 2.16  $\log_{10}$  CFU per g reductions, respectively, in viable *L. monocytogenes* populations ( $P < 0.05$ ) compared to the control but which were not different ( $P > 0.05$ ) from each other. Similar to patterns observed when combining HHP and OA with the specified antimicrobial ingredients, sustained inhibition of the recovery and growth of *L. monocytogenes* was only observed when LAE was combined with the DV or LV1 X ingredients. When LAE was used in combination with the 90 MX ingredients, on the other hand, significant ( $P < 0.05$ ) increases in viable *L. monocytogenes* populations were observed from day 0 to day 14 of the study, with the increase reaching more than 7  $\log_{10}$  CFU per g by 56 days and

after. These findings are similar to other reports that found lauric arginate will exert a bacteriostatic effect on the pathogen only when used in combination with lactate or diacetate [36,37]. Consequently, the combination of a LAE post-lethality intervention with DV or LV1 X, much like combining HHP and OA post-lethality interventions with those same natural antimicrobial ingredients represents another promising multiple-hurdle approach.

For the PPTT treatment, no significant reduction in viable *L. monocytogenes* populations was observed in any of the products with PPTT ( $P>0.05$ ) when compared to the control treatment (Figure 4). The PPTT treatment has been shown to be a potentially effective post-lethality treatment [38], but in the current study, a longer heating time or a higher final temperature probably would have been necessary for the products to achieve significant population reduction under the conditions used.

## Conclusions

As evidenced by our results, the use of high hydrostatic pressure at 400 MPa, octanoic acid, or lauric arginate as post-lethality interventions when used in combination with vinegar or vinegar and lemon juice concentrate represent effective multiple-hurdle approaches to control *L. monocytogenes* if post-processing contamination occurs in alternatively-cured RTE ham. These combination treatments inhibit the potential recovery and growth of those cells that might survive initial lethality treatments and that might remain viable during the refrigerated storage of the products. It should be noted that previous studies have shown that these post-lethality interventions will reduce the initial bacterial population but will not affect subsequent growth of survivors. Further, the antimicrobial ingredients used in this study did not affect initial population numbers but provided for suppression of subsequent growth. Thus, the combination of the appropriate post-lethality treatment with an effective bacteriostatic ingredient is necessary to assure control of *L. monocytogenes* on natural and organic ready-to-eat processed meat products. While these treatments did not independently achieve and sustain reduction of *L. monocytogenes* populations during product storage, the combination of these hurdles provides a means for manufacturers of natural and organic processed meat and poultry products to achieve control of *L. monocytogenes*.

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