Meat Science 97 (2014) 568–574

Contents lists available at ScienceDirect

Meat Science

journal homepage: www.elsevier.com/locate/meatsci

Investigating the control of *Listeria monocytogenes* on alternatively-cured frankfurters using natural antimicrobial ingredients or post-lethality interventions

Nicolas A. Lavieri [a], Joseph G. Sebranek [a,b,*], Byron F. Brehm-Stecher [b], Joseph C. Cordray [a], James S. Dickson [a], Ashley M. Horsch [a], Stephanie Jung [b], Elaine M. Larson [a], David K. Manu [b], Aubrey F. Mendonca [b]

[a] Department of Animal Science, Iowa State University, Ames, IA 50011, United States
[b] Food Science and Human Nutrition Department, Iowa State University, Ames, IA 50011, United States

ARTICLE INFO

Article history:
Received 17 September 2013
Received in revised form 18 January 2014
Accepted 11 March 2014
Available online 19 March 2014

Keywords:
Listeria monocytogenes
Alternatively cured frankfurters
Post-lethality intervention
Nitrite
Antimicrobial ingredient

ABSTRACT

The objective of this study was to investigate natural antimicrobials including cranberry powder, dried vinegar and lemon juice/vinegar concentrate, and post-lethality interventions (lauric arginate, octanoic acid, thermal treatment and high hydrostatic pressure) for the control of *Listeria monocytogenes* on alternatively-cured frankfurters. Lauric arginate, octanoic acid, and high hydrostatic pressure (400 MPa) reduced *L. monocytogenes* populations by 2.28, 2.03, and 1.88 log10 CFU per g compared to the control. *L. monocytogenes* grew in all post-lethality intervention treatments, except after a 600 MPa high hydrostatic pressure treatment for 4 min. Cranberry powder did not inhibit the growth of *L. monocytogenes*, while a dried vinegar and a vinegar/lemon juice concentrate did. This study demonstrated the bactericidal properties of high hydrostatic pressure, octanoic acid and lauric arginate, and the bacteriostatic potential of natural antimicrobial ingredients such as dried vinegar and vinegar/lemon juice concentrate against *L. monocytogenes*.

1. Introduction

In many parts of the world, natural and organic foods have recently been experiencing noticeable market growth (Organic Trade Association, 2012; Sebranek & Bacus, 2007; Winter & Davis, 2006). This growth is expected to continue despite estimated price premiums for organic products ranging from 10 to 40% (Winter & Davis, 2006) to greater than 200% for some product categories such as organic meat and poultry (Bacus, 2006).

Although many natural and organic products resemble their conventionally produced counterparts, the stringent regulations that apply to natural and organic foods do not allow the use of several specific ingredients. The use of nitrate and nitrite in the production of cured processed meat products such as ham and frankfurters, among others, is one such example, as neither ingredient is permitted when manufacturing natural and organic processed meat products. Because of the clear quality and safety benefits derived from meat curing, the indirect addition of nitrate or nitrite to natural and organic processed meat products, sometimes referred to as "natural or alternative curing," represents a new technology for cured meat products that has garnered interest from processors, consumers and researchers alike (Sebranek & Bacus, 2007; Sindelar & Milkowski, 2011).

Ready-to-eat (RTE) meat and poultry products that are manufactured following natural or organic requirements are potentially at a greater risk for post-contamination growth of *Listeria monocytogenes* than their conventional counterparts because the antimicrobials traditionally used to preserve conventional products cannot be used (Schrader, 2010; Sullivan, 2011). For example, the combination of lactate and diacetate, an effective antilisterial treatment used widely in RTE meat and poultry products is not permitted in natural or organic meat products. Therefore, the use of natural antimicrobials and "clean label" technologies or interventions to reduce the number of chemical additives used in the manufacture and on the label of these types of meat products has received significant attention from researchers (Schrader, 2010; Sebranek & Bacus, 2007; Sullivan, 2011; Sullivan, Jackson-Davis, Niebuhr, et al., 2012; Sullivan, Jackson-Davis, Schrader, et al., 2012).

The USDA Food Safety Inspection Service (USDA, 2012a) has defined 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." The use of post-lethality interventions to address the potential presence of *L. monocytogenes* in uncured, no-nitrate-or-nitrite-added, RTE natural or organic meat and poultry products is of interest because

* Corresponding author at: Department of Animal Science, Iowa State University, Ames, IA 50011, United States. Tel.: +1 515 294 1091; fax: +1 515 294 5066.
E-mail address: sebranek@iastate.edu (J.G. Sebranek).

http://dx.doi.org/10.1016/j.meatsci.2014.03.004
0309-1740/

some of these technologies are allowed for use in these categories of products.

The USDA FSIS lists lauric arginate (lauramide arginine ethyl ester or LAE) as a safe and suitable ingredient for the production of meat and poultry products and allows up to 44 mg/kg (plus or minus a 20% tolerance) of lauric arginate by weight of the product to be applied to the inside of a package as a processing aid (USDA FSIS, 2012b). The USDA FSIS also allows for octanoic acid to be used as a processing aid as long as it is applied to the surface of an RTE meat and poultry product at a rate not to exceed 400 mg/kg octanoic acid by weight of the final product (USDA FSIS, 2012b). Octanoic acid, sometimes referred to as caprylic acid, is a saturated ($C_{8:0}$) fatty acid (pK_a 4.89) naturally found in coconut oil and bovine milk (Jensen, 2002). Although promising against *L. monocytogenes* from the standpoint of initial reduction in numbers (initial lethality), longer-term effects of octanoic acid on the growth of this pathogen during an extended storage life have not been extensively studied.

Although post-lethality interventions might deliver an initial lethality and natural antimicrobials may have a bacteriostatic effect, some concerns still exist over the potential recovery and growth of sublethally injured *L. monocytogenes* during the storage life of the product. These concerns create a clear need for investigation of additional hurdles to fully address *L. monocytogenes* in RTE meat and poultry products.

Much emphasis has been placed on the investigation of natural sources of antimicrobials that could potentially replace chemical preservatives and synthetic antimicrobial ingredients as a means to address *L. monocytogenes* in the highly restrictive natural and organic categories. Several compounds derived from fruits, spices, oilseeds, and vegetables have been studied for bactericidal or bacteriostatic effects on *L. monocytogenes* and other foodborne pathogens. However, the antilisterial properties of natural antimicrobial ingredients used in RTE meat and poultry products are likely to vary based on product characteristics such as fat content, protein content, pH, a_w, and other ingredients added (Larson et al., 1996).

Investigating the use of natural antimicrobial ingredients and post-lethality interventions that are currently allowed for use with natural and organic meat and poultry products to inhibit the recovery and growth of *L. monocytogenes* on RTE frankfurters was, therefore, the focus of this study.

2. Materials and methods

2.1. Manufacture of frankfurters

Nine frankfurter formulations (eight experimental and one control formulation) were manufactured. Frankfurters were produced at the Iowa State University Meat Laboratory by blending 90% lean beef trimmings and 50% lean pork trimmings. The formulations consisted of 8.95 kg of 90% lean beef trimmings, 8.95 kg of 50% lean pork trimmings, 3.61 kg of ice/water, 0.40 kg of salt, 0.36 kg of dextrose, 0.32 kg of spices, 74.84 g celery powder plus the selected antimicrobials or post-lethality interventions. Pre-converted celery powder (VegStable 504, Florida Food Products, Inc., Eustis, FL) containing 1.5% (w/w) nitrite as ionic nitrite (NO_2^-) was used as the natural source of nitrite. All products were formulated to contain 50 mg/kg ingoing natural nitrite to represent the reduced ingoing nitrite concentration that is typical of many natural and organic processed meat products (Sebranek & Bacus, 2007). The beef and pork trimmings were obtained from a local processor and frozen prior to use to ensure uniformity of raw materials. The beef and pork trimmings were tempered to −2 °C and then were coarse ground through a plate with 9.53-mm-diameter holes (Biro MFG Co., Marblehead, OH). The ground beef and pork trimmings were then ground through a plate with 3.18-mm-diameter holes (Biro MFG Co.). The ground beef trimmings were then chopped (VSM65, Krämer & Grebe GmbH & Co. KG., Biendenkopf-Wallau, Germany) with the salt, natural nitrite, and half of the ice/water under vacuum until a temperature of 3 °C was achieved. Then, ground pork trimmings, dextrose, spices, the rest of the ice/water, and natural antimicrobial (if applicable) were added and chopping continued until a temperature of 14 °C was attained. The emulsion was then stuffed into 21-mm-diameter cellulose casings (RP 21/95, Viscofan, Danville, IL) using a rotary vane vacuum stuffer (RS 1040 C, Risco USA Corp., South Eaton, MA) and linked into approximately 7.4 cm units to accommodate later high hydrostatic pressure (HHP) treatments. All treatments were then placed in a single-truck smokehouse (MT EVD RSE 4, Alkar Engineering Corp., Lodi, WI) and heated to an internal temperature of 71.1 °C. The frankfurters were then placed in a 0 °C cooler overnight to stabilize. The next day (day 0 of the experiment), the frankfurters were stripped of the casing, placed into barrier bags (B2470, Cryovac Sealed Air Corporation, Duncan, SC; oxygen transmission rate of 3–6 cm^3/m^2 at 4 °C [0% RH, 24 h] and water vapor transmission rate of 0.5–0.6 g/0.6 m^2 at 38 °C [100% RH, 24 h]), and vacuum sealed (UV 2100, Multivac, Inc., Kansas City, MO). The frankfurters were individually packaged (1 frankfurter per package) to improve the consistency of inoculations and of the antimicrobial treatments. Frankfurters for physicochemical analyses were placed in boxes, transferred to a holding cooler in the Iowa State University Meat Laboratory, and stored at 4 ± 1 °C for the duration of the experiment. The frankfurters for microbial analyses were placed in boxes with vacuum packaged ice and transferred to the Iowa State University Microbial Food Safety Laboratory in the Food Science and Human Nutrition Department for immediate inoculation, which was accomplished within 30 min of the transfer. Frankfurters were packaged prior to transfer across campus to avoid contamination during the transfer, then opened for inoculation and repackaged. The inoculated samples were stored at 4 ± 1 °C for the duration of the experiment. Two independent replications were produced using the same facilities and procedures.

2.2. Mean weight and surface area calculations

On day 0, a total of five randomly selected frankfurter links from the control, 90MX, DV, and LV1X formulations were weighed and measured ($n = 20$ per replication) so as to obtain representative average weights and surface area measurements. The surface area (cm^2) of the frankfurter links was modeled by the equation of the surface area of a cylinder (area = $2\pi r^2$ [side only]) plus two half spheres (area = $4\ \pi r^2$), where r = radius, and h = height.

The mean weight of the frankfurters was 23.76 ± 0.92 g, while the mean diameter, length, and surface area were 1.95 ± 0.03 cm, 7.36 ± 0.23 cm, and 57.03 ± 1.64 cm^2, respectively (data not shown and $n = 40$ for all measurements). Average weight and surface area measurements were then used to calculate log CFU per g and octanoic acid (OA) and lauric arginate (LAE) volumes per link to be used in the study, respectively.

2.3. Natural antimicrobial ingredients

Three commercially available natural antimicrobial ingredients were evaluated in this study; cranberry powder (90MX; Ocean Spray International, Middleboro, MA), dried vinegar (DV; WTI Ingredients, Inc., Jefferson, GA), and vinegar/lemon juice concentrate (LV1X; WTI Ingredients, Inc., Jefferson, GA) (w/w). 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 90MX, DV, and LV1X ingredients were 3.89, 5.87, and 5.57, respectively.

2.4. Post-lethality interventions

Four post-lethality interventions were evaluated in this study; HHP, octanoic acid (OA), lauric arginate (LAE), and post-packaging thermal treatment (PPTT). Frankfurter links from the control formulation were randomly assigned to these post-lethality interventions. For

frankfurters that were to be subjected to microbial analyses, all post-lethality interventions were applied to the product within 2 h after inoculation.

HHP was evaluated under two different pressures; 400 MPa or 600 MPa with a 4 min dwell time at 12 ± 2 °C initial temperature of the pressurization. Frankfurters were transported on ice to the High Pressure Processing Laboratory at the Iowa State University Food Science and Human Nutrition Department and subjected to the appropriate HHP treatment using a FOOD-LAB 900 Plunger Press System (Stansted Fluid Power Ltd., Stansted, 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 ± 0.8 °C/100 MPa.

OA (Octa-Gone; EcoLab, Inc., Eagan, MN) was applied according to the supplier'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 per replication as previously described, the OA solution (1.06 ml) was aseptically dispensed into the bag containing the frankfurter link (1.86×10^{-2} ml per cm^2) and vacuum sealed. This OA solution volume resulted in a product/package OA concentration of 376.11 mg/kg.

LAE (Protect-M; Purac America, Lincolnshire, IL) was applied according to the supplier's recommendations. According to the manufacturer, Protect-M contains approximately 10.0% lauric arginate (v/v). A 2.5% Protect-M and 97.5% water solution (v/v) was prepared by mixing Protect-M with sterile de-ionized water at 4 ± 1 °C to achieve a final solution concentration of 0.25%. Based on average surface area measurements obtained per replication as previously described, the LAE solution (0.41 ml) was aseptically dispensed into the bag containing the frankfurter link (7.19×10^{-3} ml per cm^2) and vacuum sealed. The resulting LAE concentration on the products in the packages was 43.16 mg/kg.

PPTT was conducted by immersing packages of frankfurters in water at 71.0 ± 1.0 °C for 30 s using a water bath (Isotemp-228, Fisher Scientific). Seven packages were immersed as a group so as not to affect water temperature 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 after to chill before placement in refrigerated storage.

2.5. Proximate analysis

Proximate analysis was conducted for moisture, fat, and protein of homogenized control, 90MX, DV, and LV1X formulations on day 0 using AOAC methods 950.46, 960.63, and 992.15, respectively (AOAC, 1990a,b, 1993). Samples were prepared in duplicate for each frankfurter formulation.

2.6. pH

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 control, 90MX, DV, and LV1X formulations that were prepared by first blending the ground frankfurters 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). 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.

2.7. Water activity

Available moisture was determined using a water activity meter (AquaLab 4TE, 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 control, 90MX, DV, and LV1X formulations. Calibration was performed using 1.00 and 0.76 sodium chloride water activity standards.

2.8. Color measurements

External and internal color measurements were performed using a Hunterlab LabScan XE spectrocolorimeter (HunterLab, Reston, VA) at two randomly selected locations on the frankfurter links in duplicate, and the resulting average was used in data analysis. Color measurements were obtained at days 1, 14, 28, 42, 56, 70, 84, and 98. The colorimeter was calibrated using the same packaging material as used on the samples and placed over a white standard tile. Values for the white standard tile were X = 81.72, Y = 86.80, and Z = 91.46. External color of the frankfurter links was measured while they were still inside the packaging material under vacuum. Internal color of the frankfurter links was evaluated by slicing individual links longitudinally, followed by immediate measurement. Illuminant A, 10° standard observer with a 1.27 cm viewing area and a 1.78 cm port size was used to evaluate frankfurter samples. Commission International d'Eclairage (CIE) L* (lightness), a* (redness), and b* (yellowness) values were determined by reflectance ratio of wavelengths 650/670 nm.

2.9. Residual nitrite analysis

Residual nitrite was determined utilizing AOAC method 973.31 (AOAC, 1990c). Samples from each treatment were evaluated in duplicate and measurements were obtained at days 1, 14, 28, 42, 56, 70, 84, and 98.

2.10. Preparation of inoculum

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. 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) was performed prior to each experiment. The bacterial cells were harvested by centrifugation (10 min at 10,000 rpm 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 resuspended in 30.0 ml of sterile buffered peptone water (BPW; Difco, Becton Dickinson). The total concentration of the five-strain mixed culture was approximately 10^9 colony forming units (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 10^5 CFU per ml. This diluted five-strain mixed culture was used to inoculate frankfurters.

2.11. Sample inoculation

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 of the diluted five-strain mixed culture. The cell concentration at inoculation was approximately 10^3 CFU per g of frankfurter. This inoculation level was chosen to provide a recoverable number of organisms that would reflect the different potential effects of the treatments as previously reported (Xi, Sullivan, Jackson, Zhou, &

Sebranek, 2011). 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.

2.12. Microbial analysis

Microbial analysis of frankfurter samples for viable *L. monocytogenes* was conducted on days 1, 14, 28, 42, 56, 70, 84, and 98. On the appropriate day, two packages for each treatment were removed from the holding cooler, opened aseptically, and the contents of each placed inside sterile Whirl-Pak stomacher bags (Nasco, Ft. Atkinson, WI). Fifty ml of sterile BPW was added to each bag, and the bags were shaken by hand for approximately 30 s. The rinse solution from each frankfurter sample was then serially diluted (10-fold) in BPW to obtain predetermined dilutions of the samples according to the sampling day. 1.0 ml (for 10° dilution, divided into three ~0.33-ml aliquots plated on three separate plates) or 0.1 ml of the appropriate dilution was surface-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 10° dilution, 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 (1999) with some modifications. Within 48 h of 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 agar plates were incubated 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 $\log_{10}$ CFU per g using the average weight of the frankfurters from the two replications of the experiment ($n = 40$). The detection limit of our sampling protocols was ≥ 0.30 $\log_{10}$ CFU per g based on a sample weight of 25.0 g. Because the frankfurters were manufactured on-site in a thoroughly sanitized pilot plant, background bacterial contamination, based on extensive past experience, was expected to be minimal. Further, all treatments in each replication were processed simultaneously so that any background bacterial contamination that might have occurred would be the same in all cases. Consequently, background bacteria were not enumerated in this study because any of the limited numbers that might be observed would be expected to be of little consequence to the study.

2.13. 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 and physicochemical data for the frankfurters were analyzed for treatment effects within the day. Day and treatment × day interactions were also analyzed. 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.

3. Results and discussion

3.1. Frankfurter physicochemical traits

The only product trait for which significant ($P < 0.05$) differences were detected was product pH (Table 1), which is not surprising given that one of the expected product effects of the ingredients used was an impact on pH. The 90MX treatment, for example, resulted in the lowest pH, followed by the LV1X, the DV, and the control treatments. Cranberry has been reported to contain phenolic acids and exhibit a high titratable acidity (Lee, Reed, & Richards, 2006). Similar results were reported when using different ingoing levels of cranberry powder in a cooked meat model system and in frankfurters (Xi, Sullivan, Jackson, Zhou, & Sebranek, 2012; Xi et al., 2011). Similarly, the dried vinegar and vinegar/lemon juice concentrate used in this study are also likely reservoirs of phenolic and other acidic compounds, such as acetic and citric acid, and were expected to cause the observed lower pH in frankfurters made with those ingredients. The proximate composition and the available water values of the different frankfurter formulations manufactured were not significantly different ($P > 0.05$).

3.2. Residual nitrite concentration results

Although all frankfurter formulations were manufactured with 50 mg/kg natural nitrite on an ingoing basis, the highest residual nitrite concentration observed on day 1 of the study was 25.84 mg/kg (HHP 600 treatment, Table 2). This indicates that part of the ingoing nitrite was depleted in curing and in other reactions that took place during product manufacture. As much as 75% of the ingoing nitrite can be depleted during product manufacture (Honikel, 2008; Xi et al., 2011, 2012). Factors such as product pH, cooking temperature, and reducing agents affect residual nitrite concentrations in meat systems (Cassens, Ito, Lee, & Buege, 1978). For example, a decrease in pH as small as 0.2 units during product manufacture can result in a doubling of the rate at which curing reactions occur (Sebranek, 1979). Thus, the significant ($P < 0.05$) decrease in pH observed when adding the natural antimicrobial ingredients used in this study, especially cranberry powder, was expected to influence residual nitrite concentrations. No natural cure accelerator such as cherry powder (ascorbic acid) was used in this study which could also affect residual nitrite concentrations and the antimicrobial impact of nitrite.

On day 1, the OA, 90MX, and LV1X treatments exhibited lower ($P < 0.05$) residual nitrite concentrations than the control treatment, whereas the HHP400, HHP600, LAE, and DV treatments did not, probably due to the acidity of compounds included in the vinegar/lemon juice concentrate and the OA as well as the cranberry powder. Throughout the storage of the products at 4 ± 1 °C, all treatments showed significant ($P < 0.05$) decreases in residual nitrite concentrations as expected. The OA and 90MX treatments experienced significant ($P < 0.05$) decreases in residual nitrite concentrations relatively early, as early as day 14 of the study, and reached their lowest residual nitrite concentrations by day 70, indicating that the acidity of the cranberry powder and octanoic acid probably accelerated the rate at which residual nitrite was depleted compared to the other treatments.

Table 1
Effect of natural antimicrobial ingredients on physicochemical properties of alternatively-cured frankfurters.[a]

Treatment[b]	a_w	pH	Fat %	Moisture %	Protein %
Control	0.9703	6.11^{A}	25.39	55.30	13.29
90MX	0.9724	5.70^{B}	25.38	54.85	13.42
DV	0.9672	6.06^{C}	24.47	55.83	13.38
LV1X	0.9704	5.95^{D}	23.89	56.16	13.39
SE[c]	0.003	0.01	0.64	0.47	0.40

[a] Values are least squares means. Within a column, means with different superscripts (A through D) are significantly different ($P < 0.05$).
[b] Control, alternatively-cured control; 90MX, cranberry powder; DV, vinegar; LV1X, vinegar/lemon juice concentrate.
[c] Standard error of the differences of least squares means.

Table 2
Effect of treatment on residual nitrite concentrations of alternatively-cured frankfurters stored at 4 ± 1 °C.[a]

Treatment[b]	Day							
	1	14	28	42	56	70	84	98
Control	$25.01^{AD,Z}$	$26.68^{A,Z}$	$21.02^{AD,Y}$	$17.00^{A,X}$	$11.45^{AD,W}$	$9.33^{A,W}$	$9.36^{AD,W}$	$5.78^{ABD,V}$
HHP400	$24.76^{AD,Z}$	$25.82^{AB,Z}$	$19.89^{AD,Y}$	$15.81^{A,X}$	$14.38^{B,X}$	$9.39^{A,W}$	$8.34^{AB,W}$	$7.25^{AD,W}$
HHP600	$25.84^{A,Z}$	$24.77^{A,Z}$	$18.86^{D,Y}$	$15.01^{A,X}$	$12.88^{AB,X}$	$7.77^{AD,W}$	$7.02^{BE,W}$	$6.34^{ACD,W}$
OA	$22.39^{BE,Z}$	$18.45^{C,Y}$	$13.18^{B,X}$	$9.14^{BC,W}$	$8.05^{CE,W}$	$4.55^{BC,V}$	$4.30^{C,V}$	$4.97^{DE,V}$
LAE	$24.56^{AB,Z}$	$25.72^{AB,Z}$	$20.68^{AD,Y}$	$16.38^{A,X}$	$14.36^{B,X}$	$8.95^{A,VW}$	$10.68^{D,W}$	$7.34^{A,V}$
PPTT	$23.49^{DBE,YZ}$	$24.60^{A,Z}$	$21.28^{A,Y}$	$16.23^{A,X}$	$14.08^{B,X}$	$9.81^{A,W}$	$5.82^{CE,V}$	$3.72^{BE,V}$
90MX	$17.31^{C,Z}$	$13.99^{D,Y}$	$9.91^{C,X}$	$7.39^{B,W}$	$6.39^{E,W}$	$3.65^{C,V}$	$4.13^{C,V}$	$4.01^{BE,V}$
DV	$24.34^{AB,Z}$	$24.07^{B,Z}$	$18.96^{D,Y}$	$15.22^{A,X}$	$13.65^{AB,X}$	$8.88^{A,W}$	$7.56^{AE,W}$	$7.46^{A,W}$
LV1X	$21.58^{E,Z}$	$20.08^{C,Z}$	$14.55^{B,Y}$	$11.22^{C,X}$	$9.86^{DC,X}$	$5.97^{BD,W}$	$5.38^{CE,W}$	$4.86^{BCE,W}$
SE[c]	0.73							

[a] Values are least squares means. Within a column, means with different superscripts (A through E) are significantly different ($P < 0.05$). Within a row, means with different superscripts (V through Z) are significantly different ($P < 0.05$).
[b] Control, alternatively-cured control; HHP400, high hydrostatic pressure, 400 MPa; HHP600, high hydrostatic pressure, 600 MPa; OA, Octa-Gone; LAE, Protect-M; PPTT, post-packaging thermal treatment; 90MX, cranberry powder; DV, vinegar; LV1X, vinegar/lemon juice concentrate.
[c] Standard error of the differences of least squares means.

3.3. External and internal L*, a*, and b* values

Color was measured to determine if the treatments studied resulted in any obvious visual changes because previous reports (Xi et al., 2012) have indicated that cranberry powder, in particular, can impact meat color due to the anthocyanin pigments from cranberries. Other sensory qualities were not measured in this study because the primary focus was on microbiological effects of the treatments.

The 90MX treatment resulted in lower ($P < 0.05$) external L* values than the control treatment on days 42, 56, and 84 and, although not significant ($P > 0.05$) at other time points, exhibited numerically lower external L* values when compared to all other treatments evaluated throughout the duration of the study (data not shown). However, the post-lethality interventions studied did not affect external L* values of frankfurters in this study (data not shown). Neither a* nor b* values were significantly affected by the treatments during storage of the frankfurters (data not shown).

As with the external L* value results, the 90MX treatment consistently yielded the lowest internal L* values of all treatments across the duration of the study (data not shown). Further, no significant differences ($P > 0.05$) in internal a* values were detected between treatments during storage (data not shown), similar to external a* value results previously discussed. None of the post-lethality interventions exerted a significant ($P > 0.05$) effect on the internal b* values when compared to the control treatment (data not shown). These results suggest that a decrease in both external and internal lightness of frankfurters is likely to occur as a consequence of using cranberry powder as an ingredient, but other color values are not likely to be affected. Similar patterns were observed by Xi et al. (2012) when using cranberry powder in the manufacture of naturally-cured frankfurters. The other treatments included in this study did not impact product L*, a* or b* color values.

3.4. Viable L. monocytogenes

The growth mediums used did not significantly differ ($P > 0.05$) within treatment on any given day, indicating that the use of the TAL technique offered limited advantages compared to using traditional selective plating onto MOX. Thus, the discussion about viable *L. monocytogenes* populations is based on results obtained using MOX (Fig. 1).

Of the post-lethality interventions evaluated, all except PPTT resulted in a significant decrease in *L. monocytogenes* populations after 1 day of storage at 4 ± 1 °C ($P < 0.05$). The greatest reduction was seen in the LAE treatment, where a decrease of 2.28 $\log_{10}$ CFU per g compared to the untreated control treatment was observed. Porto-Fett et al. (2010) evaluated the effects of 22 and 44 mg/kg lauric arginate, with or without the addition of potassium lactate and sodium diacetate, on the growth of *L. monocytogenes* on commercially-produced frankfurters. These authors concluded that lauric arginate provides initial lethality towards *L. monocytogenes* when used alone (1.8 $\log_{10}$ CFU per package) or in combination with lactate and diacetate (2.0 $\log_{10}$ CFU per package). Similar results were obtained by Luchansky et al. (2005) when they researched the effects of lauric arginate on the growth of *L. monocytogenes* on hams.

Similarly, the OA treatment resulted in a 2.03 $\log_{10}$ CFU per g reduction in *L. monocytogenes* populations compared to the control treatment. In a study that evaluated the antilisterial effect of octanoic acid on the surface of several different RTE meats within their final packaging, Burnett et al. (2007) concluded that 1% octanoic acid solutions acidified to pH 2.0 or 4.0 and applied to RTE meat and poultry products at 1.9 ± 0.5 ml per 100 cm^2 of the product surface area which resulted in *L. monocytogenes* log reductions ranging from 0.85 to 2.89 $\log_{10}$ CFU per sample in the different RTE products following 24 ± 4 h of storage at 5 ± 2 °C. Furthermore, *L. monocytogenes* populations in all treated samples were significantly lower following treatment with octanoic solutions at either pH compared to the control in that study.

The HHP400 treatment resulted in a 1.88 $\log_{10}$ CFU per g reduction in *L. monocytogenes* populations compared to the control treatment while the HHP600 treatment resulted in a reduction of *L. monocytogenes* populations to levels below the detection limit of our sampling protocols ($\geq$0.30 $\log_{10}$ CFU per g) and completely prevented growth throughout the entire duration of the study. These results agree with those obtained by Myers, Montoya, Cannon, Dickson, and Sebranek (2013) and Myers, Cannon, Montoya, Dickson, Lonergan and Sebranek (2013), as these authors found that an HHP treatment of 600 MPa for 3 min and 17 °C resulted in a 3.9–4.4 and 3.9–4.3 $\log_{10}$ CFU per g reduction in *L. monocytogenes* populations on RTE sliced turkey and ham, respectively. The same authors, however, concluded that 400 MPa HHP treatment of RTE sliced ham for 3 min at 17 °C resulted in less than a 1 $\log_{10}$ CFU per g reduction in *L. monocytogenes* populations, while we observed close to 2 $\log_{10}$ CFU per g reduction in this study. The extent to which HHP will inactivate microorganisms depends on several factors including the specific bacterial strain and the growth phase at the time of treatment, the properties of the food matrix such as composition, pH and water activity, and for the HHP treatment, temperature of the transfer medium, the pressure level and the exposure time (Hugas, Garriga, & Monfort, 2002). When compared to broth systems, for example, organisms in nutrient-rich meat matrices are generally more resistant to HHP treatment (Hoover, Metrick, Papineau, Farkas, & Knorr, 1989; Simpson & Gilmour, 1997). Therefore, variables contributing to successful HHP outcome will include the food product type, the target organism and the conditions of the HHP process.

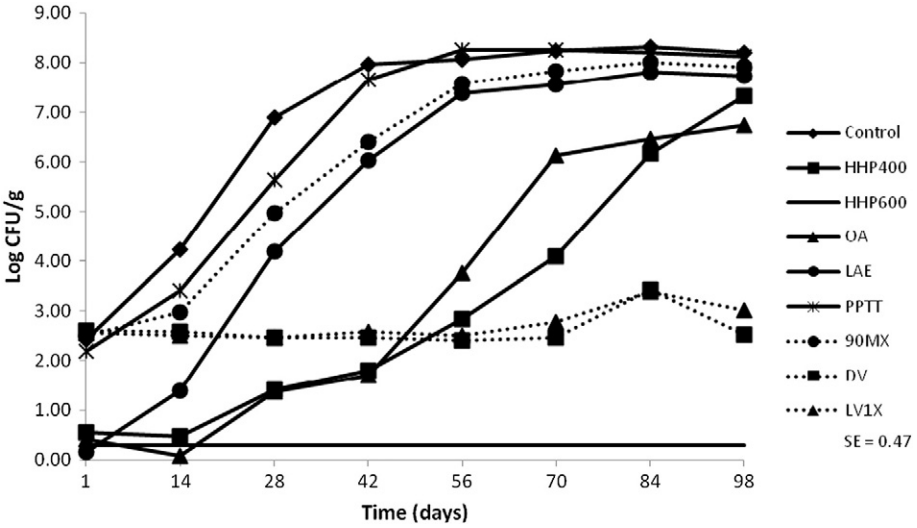

Fig. 1. Effect of treatment on viable *Listeria monocytogenes* (log CFU 1 per g) on modified Oxford medium on alternativelycured frankfurters stored at 4 ± 1 °C.

The post packaging thermal treatment did not significantly decrease initial *L. monocytogenes* populations ($P > 0.05$) and resulted in similar *L. monocytogenes* growth patterns compared to the control treatment throughout the duration of the study. These results contrast those obtained by Chen, Sebranek, Dickson, and Mendonca (2004), as these authors concluded that a post-packaging thermal treatment of 71 ± 1 °C for 30 s would result in a 1.4 $\log_{10}$ CFU per g reduction in *L. monocytogenes* populations on 1-link packages of frankfurters when using a 3.4 $\log_{10}$ CFU per g initial inoculation level. It is not clear why the same PPTT conditions had a more limited impact on *L. monocytogenes* in the current study.

Although an initial bactericidal effect of the HHP400, OA, and LAE treatments was clearly observed, our results indicate that these treatments did not lead to long-term suppression of growth. The HHP400 treatment resulted in an extended lag phase and slower increase in *L. monocytogenes* populations but growth occurred after 56 days of storage, and by day 98 of the study there was no significant difference between the control and the HHP400 treatments ($P > 0.05$). Similarly, after 400 MPa HHP treatment of RTE sliced ham for 3 min at 17 °C, which resulted in less than a 1 log CFU per g reduction in *L. monocytogenes* populations, the pathogen was able to grow to numbers above inoculation levels upon storage under refrigeration (Myers, Cannon, et al., 2013; Myers, Montoya, et al., 2013).

The OA and LAE treatments showed significant ($P < 0.05$) increases in *L. monocytogenes* populations by days 42 and 28, respectively, with the latter also showing no significant difference in *L. monocytogenes* populations compared to the control treatment on day 98 ($P > 0.05$). These findings are in agreement with previous reports on lauric arginate (Burnett et al., 2007; Luchansky et al., 2005; Porto-Fett et al., 2010). Thus, while they may provide an initial lethality, lauric arginate and octanoic acid alone do not inhibit the outgrowth of any *L. monocytogenes* cells that may survive. The results of our study indicate that, although they result in initial population reductions, under the conditions studied here, HHP400, OA, and LAE post-lethality interventions do not offer protection against the growth of surviving *L. monocytogenes* during product storage.

The natural antimicrobials evaluated in this study did not significantly affect *L. monocytogenes* populations after 1 day of storage ($P > 0.05$) when compared to the control treatment. The bacteriostatic properties of these ingredients, however, varied greatly. The 90MX treatment, for example, resulted in significantly ($P < 0.05$) increased *L. monocytogenes* populations as early as day 28 of the study. The populations continued to increase ($P < 0.05$) through day 56 and reached a maximum level on day 84. These results indicate that, at the level used and under the conditions of this study, cranberry powder does not exert bacteriostatic effects on *L. monocytogenes*. When studied using fish and beef slices, cranberry extract alone did not inhibit growth of inoculated *L. monocytogenes* during refrigerated storage (Lin, Labbe, & Shetty, 2004). Similarly, cranberry powder, when used at a level of 1.0% (w/w), did not completely inhibit the growth of *L. monocytogenes* (Xi et al., 2011).

The DV and LV1X treatments, on the other hand, exhibited strong bacteriostatic properties against *L. monocytogenes* and represent feasible options that could be used by manufacturers of organic and natural RTE processed meat and poultry products in their *L. monocytogenes* control plans. These natural antimicrobial ingredients, however, did not exhibit bactericidal properties under the conditions studied here. Additionally, although beneficial from the standpoint of initial lethality, the HHP400, OA, and LAE post-lethality interventions did not offer protection against the growth of surviving *L. monocytogenes* during product storage. Therefore, additional research on combining natural antimicrobial ingredients and post-lethality interventions for the most effective combination treatment suitable for use in the manufacture of organic and natural processed meat and poultry products is warranted because of the safety advantage of such treatments for natural and organic processed meat products. For those treatments found to be effective, additional studies on the organoleptic effects of each treatment is also warranted.

Acknowledgments

This project was supported by the American Meat Institute Foundation (AMIF). The authors would like to recognize the companies that donated research materials: Florida Food Products, WTI Ingredients, Ocean Spray International, EcoLab, Purac America, A.C. Legg, and Viscofan. Special thanks to Devin Maurer and Daniel Fortin for their invaluable contributions made throughout the conduction of these experiments.

References

AOAC, Association of Official Analytical Chemists (1990a). *Fat (crude) or ether extract in meat. Official method 960.39. Official methods of analysis*Arlington, VA: AOAC International.

AOAC, Association of Official Analytical Chemists (1990b). *Moisture in meat. Official method 950.46. Official methods of analysis*Arlington, VA: AOAC International.

AOAC, Association of Official Analytical Chemists (1990c). *Nitrites in Cured Meat. Official Method 973.31. Official methods of analysis*Arlington, VA: AOAC International.

AOAC, Association of Official Analytical Chemists (1993). *Crude protein in meat and meat products. Official method 992.15. Official methods of analysis*Arlington, VA: AOAC International.

Bacus, J. N. (2006). Natural ingredients for cured and smoked meats. *Proceedings of the 59th Reciprocal Meat Conference* (pp. 77–78). Champaign-Urbana, IL,: American Meat Science Association (18 to 21 June 2006).

Burnett, S. L., Chopskie, J. H., Podtburg, T. C., Gutzmann, T. A., Gilbreth, S. E., & Bodnaruk, P. W. (2007). Use of octanoic acid as a postlethality treatment to reduce *Listeria monocytogenes* on ready-to-eat meat and poultry products. *Journal of Food Protection*, *70*, 392–398.

Cassens, R. G., Ito, T., Lee, M., & Buege, D. (1978). The use of nitrite in meat. *Bioscience*, *28*, 633–637.

Chen, C. M., Sebranek, J. G., Dickson, J. S., & Mendonca, A. F. (2004). Combining pediocin (ALTA 2341) with postpackaging thermal pasteurization for control of *Listeria monocytogenes* on frankfurters. *Journal of Food Protection*, *67*, 1855–1865.

Honikel, K. -O. (2008). The use and control of nitrate and nitrite for the processing of meat products. *Meat Science*, *78*, 68–76.

Hoover, D.G., Metrick, C., Papineau, A.M., Farkas, D. F., & Knorr, D. (1989). Biological effects of high hydrostatic pressure on food microorganisms. *Food Technolology*, *43*, 99–107.

Hugas, M., Garriga, M., & Monfort, J. M. (2002). New mild technologies in meat processing: High pressure as a model technology. *Meat Science*, *62*, 359–371.

Jensen, R. G. (2002). The composition of bovine milk lipids: January 1995 to December 2000. *Journal of Dairy Science*, *85*, 295–350.

Kang, D. H., & Fung, D. Y. C. (1999). Thin agar layer method for recovery of heat-injured Listeria monocytogenes. *Journal of Food Protection*, *62*, 1346–1349.

Larson, A. E., Yu, R. R., Lee, O. A., Price, S., Haas, G. J., & Johnson, E. A. (1996). Antimicrobial activity of hop extracts against *Listeria monocytogenes* in media and in food. *International Journal of Food Microbiology*, *33*, 195–207.

Lee, C., Reed, J.D., & Richards, M. P. (2006). Ability of various polyphenolic classes from cranberry to inhibit lipid oxidation in mechanically separated turkey and cooked ground pork. *Journal of Muscle Foods*, *17*, 248–266.

Lin, Y. T., Labbe, R. G., & Shetty, K. (2004). Inhibition of *Listeria monocytogenes* in fish and meat systems by use of oregano and cranberry phytochemical synergies. *Applied and Environmental Microbiology*, *70*, 5672–5678.

Luchansky, J. B., Call, J. E., Hristova, B., Rumery, L., Yoder, L., & Oser, A. (2005). Viability of *Listeria monocytogenes* on commercially-prepared hams surface treated with acidic calcium sulfate and lauric arginate and stored at 4 °C. *Meat Science*, *71*, 92–99.

Myers, K., Cannon, J., Montoya, D., Dickson, J. S., Lonergan, S., & Sebranek, J. G. (2013). Effects of high hydrostatic pressure and varying concentrations of sodium nitrite from traditional and vegetable-based sources on the growth of *Listeria monocytogenes* on ready-to-eat (RTE) sliced ham. *Meat Science*, *94*, 69–76.

Myers, K., Montoya, D., Cannon, J., Dickson, J. S., & Sebranek, J. G. (2013). The effect of high hydrostatic pressure, sodium nitrite and salt concentration on the growth of *Listeria monocytogenes* on RTE ham and turkey. *Meat Science*, *93*, 263–268.

Organic Trade Association (2012). Consumer-driven U.S. organic market surpasses $31 billion in 2011. http://www.organicnewsroom.com/2012/04/us_consumerdriven_organic_mark.html (Accessed 04.07.2013)

Porto-Fett, A.C. S., Campano, S. G., Smith, J. L., Oser, A., Shoyer, B., Call, J. E., & Luchansky, J. B. (2010). Control of *Listeria monocytogenes* on commercially-produced frankfurters prepared with and without potassium lactate and sodium diacetate and surface treated with lauric arginate using the sprayed lethality in container (SLIC®) delivery method. *Meat Science*, *85*, 312–318.

Schrader, K. D. (2010). *Investigating the control of Listeria monocytogenes on uncured, no-nitrate- or-nitrite-added meat products.* (Ph.D. dissertation). Ames, IA: Iowa State University.

Sebranek, J. G. (1979). Advances in the technology of nitrite use and consideration of alternatives. *Food Technology*, *33*, 58–62.

Sebranek, J. G., & Bacus, J. N. (2007). Cured meat products without direct addition of nitrate or nitrite: What are the issues? *Meat Science*, *77*, 136–147.

Simpson, R. K., & Gilmour, A. (1997). The resistance of *Listeria monocytogenes* to high hydrostatic pressure in foods. *Food Microbiology*, *14*, 567–573.

Sindelar, J. J., & Milkowski, A. L. (2011). *Sodium nitrite in processed meat and poultry meats: A review of curing and examining the risk/benefit of its use. White Paper Series — Number 3*Champaign, IL: American Meat Science Association.

Sullivan, G. A. (2011). *Naturally cured meats: Quality, safety, and chemistry.* (Ph.D. dissertation). Ames, IA: Iowa State University.

Sullivan, G. A., Jackson-Davis, A. L., Niebuhr, S. E., Xi, Y., Schrader, K. D., Sebranek, J. G., & Dickson, J. S. (2012). Inhibition of *Listeria monocytogenes* using natural antimicrobials in no-nitrate-or-nitrite-added ham. *Journal of Food Protection*, *75*, 1071–1076.

Sullivan, G. A., Jackson-Davis, A. L., Schrader, K. D., Xi, Y., Kulchaiyawat, C., Sebranek, J. G., & Dickson, J. S. (2012). Survey of naturally and conventionally cured commercial frankfurters, ham, and bacon for physio-chemical characteristics that affect bacterial growth. *Meat Science*, *92*, 808–815.

USDA FSIS, U.S. Department of Agriculture Food Safety Inspection Service (2012a). FSIS Compliance guideline: Controlling *Listeria monocytogenes* in post-lethality exposed ready-to-eat meat and poultry products. http://www.fsis.usda.gov/shared/PDF/Controlling_LM_RTE_guideline_0912.pdf (Accessed 04.07.2013)

USDA FSIS, U.S. Department of Agriculture Food Safety Inspection Service (2012b). FSIS Directive 7120.1, revision 15. Safe and suitable ingredients used in the production of meat, poultry, and egg products. Available at: http://www.fsis.usda.gov/wps/wcm/connect/7f981741-94f1-468c-b60d-b428c971152d/7120_68.pdf?MOD=AJPERES (Accessed 04.07.2013)

Winter, C. K., & Davis, S. F. (2006). Organic foods. *Journal of Food Science*, *71*, R117–R124.

Xi, Y., Sullivan, G. A., Jackson, A. L., Zhou, G. H., & Sebranek, J. G. (2011). Use of natural antimicrobials to improve the control of *Listeria monocytogenes* in a cured cooked meat model system. *Meat Science*, *88*, 503–511.

Xi, Y., Sullivan, G. A., Jackson, A. L., Zhou, G. H., & Sebranek, J. G. (2012). Effects of natural antimicrobials on inhibition of *Listeria monocytogenes* and on chemical, physical and sensory attributes of naturally-cured frankfurters. *Meat Science*, *90*, 130–138.