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

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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

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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