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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 ☆

A.C.S. Porto-Fett [a], S.G. Campano [b], J.L. Smith [c], A. Oser [c], B. Shoyer [a], J.E. Call [a], J.B. Luchansky [a,*]

[a] US Department of Agriculture, Agricultural Research Service, Eastern Regional Research Center, Microbial Food Safety Research Unit, Wyndmoor, PA 19038, USA
[b] Hawkins Inc., Minneapolis, MN 55413, USA
[c] Oser Technologies, Blacksville, WV 26521, USA

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ABSTRACT

Viability of *Listeria monocytogenes* was monitored on frankfurters formulated with or without potassium lactate and sodium diacetate at a ratio of ca. 7:1 and treated with lauric arginate (LAE; 22 or 44 ppm) using the Sprayed Lethality in Container (SLIC®) delivery method. Without antimicrobials, pathogen numbers remained relatively constant at ca. 3.3 log CFU/package for ca. 30 d, but then increased to ca. 8.4 log CFU/package over 120 d. Regardless of whether or not lactate and diacetate were included, when treated with LAE, pathogen numbers decreased from ca. 3.3 log CFU/package to ca. 1.5 log CFU/package within 2 h, but then increased to 7.3 and 6.7 log CFU/package, respectively, after 120 d. When frankfurters were formulated with lactate and diacetate and treated with LAE, pathogen numbers decreased by ca. 2.0 log CFU/package within 2 h and remained relatively unchanged over the 120 d. These data confirm that LAE provides an initial lethality towards *L. monocytogenes* and when used in combination with reduced levels/ratio of lactate and diacetate as an ingredient for frankfurters provides inhibition throughout shelf life.

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1. Introduction

As evidenced by a 2008 outbreak in Canada associated with ready-to-eat (RTE) deli-meats that resulted in 57 cases of listeriosis and 22 deaths (Anonymous, 2009), as well as the occurrence of several recent, albeit smaller, recalls of RTE meats across North America (USDA–FSIS, 2009), *Listeria monocytogenes* remains a considerable threat to public health. In response to costly product recalls and to outbreaks epidemiologically linked to RTE meat and poultry products, the United States Department of Agriculture–Food Safety and Inspection Service (USDA–FSIS) established policies to control *L. monocytogenes* on RTE meat and poultry products that requires manufacturers to validate that their processes achieve "zero tolerance", as well as to include a post-process lethality treatment and/or to suppress outgrowth during shelf life (Anonymous, 2003b).

The antimicrobial effectiveness of organic acids and their salts, mostly lactate and diacetate, for controlling *L. monocytogenes* in RTE meats has been well established (Barmpalia et al., 2004; Bedie et al., 2001; Lu, Sebranek, Dickson, Mendonça, & Bailey, 2005; Mbandi & Shelef, 2002; Porto et al., 2002; Porto-Fett, Call, Muriana, Freier, & Luchansky, 2010, chap. 6; Samelis et al., 2005). Levels of lactates varying from 1.5% to 3.0%, added either alone or in combination with sodium diacetate levels ranging from 0.125% to 0.25%, are widely used by the meat industry as antilisterial ingredients in RTE meat and poultry products (Thompson, Carpenter, Martini, & Broadbent, 2008; Tompkin, 2002). It is well documented that these two food grade antimicrobials are quite effective at suppressing outgrowth of *L. monocytogenes* during an extended refrigerated shelf life, but are not that effective at delivering initial lethality towards the pathogen. Regarding the latter, previous studies evaluated the effectiveness of the Sprayed Lethality in Container (SLIC®) method to deliver different volumes and concentrations of lauric arginate (LAE) to the surfaces of various RTE meats to control *L. monocytogenes* (Luchansky, Call, Smith, Smith, & Oser, 2007; Luchansky, Smith, Oser, & Porto-Fett, 2009; Luchansky et al., 2005;

☆ Mention of trade names or commercial products in this publication is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the US Department of Agriculture. Portions of this research were presented at the 62nd Reciprocal Meat Conference, Rogers, Arkansas, June 21–24, 2009 (Campano et al., 2009).

* Corresponding author. Tel.: +1 215 233 6676; fax: +1 215 233 6581.
E-mail address: John.Luchansky@ars.usda.gov (J.B. Luchansky).

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Martin et al., 2009; Santiago-Connolly et al., 2008; Smith, Oser, Porto-Fett, Call, & Luchansky, 2009; Taormina & Dorsa, 2009). Results established that LAE applied via SLIC® was effective at delivering an initial lethality (ca. 1.1–6.5 log CFU/package) of *L. monocytogenes* within 24 h at refrigeration temperature, but was not effective at inhibiting outgrowth of the pathogen during shelf life. Thus, the objective of this study was to evaluate the potential for delivering both an initial lethality towards surface inoculated *L. monocytogenes*, as well as subsequent inhibition of the pathogen during shelf life, by formulating frankfurters with different levels of potassium lactate and sodium diacetate and then surface treating with different levels of LAE. These findings will be of great value to both large and small processors of red meat and poultry products especially if it can be validated that lower levels/ratios of these antimicrobials display appreciable antilisterial activity.

2. Material and methods

2.1. Bacterial strains

An equal volume of freshly grown cells of each of the five strains of *L. monocytogenes* (MFS2, MFS102, MFS104, MFS105, and MFS110) used in this study were combined and used to surface inoculate frankfurters. These strains were confirmed, cultured, and maintained as described previously by Porto et al. (2002).

2.2. Product

One batch (ca. 200 kg for each formulation) of freshly-manufactured, vacuum-packaged, commercially-produced frankfurters (pork, water, beef, corn syrup, salt, spices/seasoning, phosphate, and sodium nitrite) formulated with no, low, or high levels of potassium lactate and sodium diacetate (UltraLac KL6810; Hawkins Inc., Minneapolis, MN; low = 0.68% lactate and 0.097% diacetate and high = 1.36% lactate and 0.19% diacetate; dry solids basis, wt./wt. formula, ratio of 7:1 of lactate:diacetate) was used in each of the two trials. Briefly, the meat, phosphate, and sodium nitrite were added to a bowl chopper with a portion of water (as ice) and mixed thoroughly, followed by the addition of the salt, remaining water, and seasonings. Lastly, the potassium lactate and sodium diacetate syrup was added to the batter and mixed thoroughly. The batter was chopped under vacuum to achieve an emulsion at a final temperature of 20 °C, and then vacuum stuffed into a cellulose casing. Frankfurters were smoked and thermally processed to a minimum internal temperature of 74 °C in a batch smokehouse. Product was chilled to below 4 °C in cold room, peeled, and commercially packaged as one lb packages (eight links per lb). The product, obtained directly from a producer/collaborator, was transported on ice to the USDA/ARS Eastern Regional Research Center (ERRC; Wyndmoor, PA) and stored at 4 °C for up to 2 d prior to being inoculated with *L. monocytogenes*.

2.3. Treatment of frankfurters via SLIC®

Frankfurters were aseptically removed from the original package, repackaged (eight links per package, "4 on 4"; ca. 454 g total per package) into nylon–polyethylene bags (3 mil standard barrier, 6 in. by 8 in., $O_2 < 0.6\ cm^3/100\ in.^2/24$ h at 0 °C relative humidity with a moisture vapor transmission rate of 0.6 g of H_2O per 100 $in.^2$ per 24 h at 38 °C; Koch Supplies, Kansas City, MO), and then surface inoculated with 2 ml of the five-strain cocktail of *L. monocytogenes* using a pipet to achieve a target level of ca. 3.3 log CFU/package. At the outset, it was not clear if growth or lethality would be observed. Thus, we reasoned that using an initial inoculum of ca. 3.0 log CFU/package would allow us to quantify either scenario. Also, it would not be uncommon for contaminated packages to harbor between 10 and 1000 CFU/g of this pathogen (Johnson, Doyle, & Cassens, 1990). Each package was massaged by hand for ca. 20 s to ensure for adequate coverage of the inoculum on all surfaces, and then 4 ml of lauric arginate (LAE; ethyl-*N*-dodecanoyl-L-arginate hydrochloride; CytoGuard LA®; a 10% solution of LAE; A&B Ingredients Inc., Fairfield, NJ), diluted to a final concentration of 22 ppm (2.5%; 2.5 parts LAE:97.5 parts sterile distilled water) or 44 ppm (5% of LAE; 5 parts LAE:95 parts sterile distilled water), were delivered into each package via SLIC®. Briefly, SLIC® delivers an antimicrobial purge directly into the package just prior to the introduction of the food product and then the vacuum produced by the packaging system subsequently distributes the LAE across the entire surface of the packaged food (Luchansky et al., 2005). The USDA/FSIS list of safe and suitable ingredients for production of meat and poultry products (http://www.fsis.usda.gov/OPPDE/rdad/FSISDirectives/7120.1_Amend_8.pdf) allows processors to use SLIC® to deliver up to 44 ppm (plus or minus a process tolerance of 20%) of LAE (not to exceed 53 ppm) by weight of the product. We also evaluated 22 ppm of LAE to determine if a lower level of LAE would have appreciable antilisterial activity when used either alone or in combination with lactates. When applied in this manner and at these levels, LAE would be considered a processing aid and would not have to be disclosed on the product label. Control packages were treated with 4 ml of sterile 0.1% peptone water. Packages were then vacuum sealed to 950 mBar using a Multivac A300/16 vacuum-packaging unit (Sepp Haggemuller KG, Wolfertschwenden, Germany) and stored for up to 120 d at 4 °C in a temperature-controlled incubator. The shelf life of frankfurters can range from 65 to 110 d for smaller to larger processors, respectively. The shelf life was extended to 120 d in the present study to quantify inhibition to the fullest extent possible. From our experience, it is expected and readily achievable for such products to be held at $\leqslant$4 °C over the duration of the projected shelf life.

2.4. Microbiological analyses

L. monocytogenes cells were recovered using the USDA/ARS package rinse method (Luchansky, Porto, Wallace, & Call, 2002). The outside surface of each package was wiped with an ethanol-soaked (70% vol./vol.) paper towel, and the package was opened with the aid of ethanol-sterilized scissors. Nineteen milliliters of Dey/Engley neutralizing broth (D/E broth; Difco, Becton, Dickinson Co., Franklin Lakes, NJ) were added, and the packages were massaged by hand for ca. 1 min before the resulting rinsate was transferred to a sterile 15-ml screw-capped conical centrifuge tube with the aid of a sterile pipette. Pathogen numbers were enumerated by directly spread-plating the rinsate or dilutions thereof onto duplicate polymyxin B, acriflavin, lithium chloride, ceftazidine, esculin, D-mannitol (PALCAM; Difco) agar plates, which were subsequently incubated at 37 °C for 48 h. When pathogen levels decreased to below the detection limit ($\leqslant$1.40 log CFU/package) by direct plating, samples were enriched as previously described in Porto-Fett, Call, and Luchansky (2008). The total aerobic plate counts (TPC) and total lactic acid bacteria counts (LAB) were enumerated on days 0 and 120 by spread-plating 100 μl of the control rinsate or dilutions thereof onto brain heart infusion (BHI; Difco) and De Mann, Rogosa and Sharpe (MRS; Difco) agar plates, respectively. The MRS agar plates were incubated anaerobically (10.1% carbon dioxide, 4.38% hydrogen and balance nitrogen; Bactron IV Anaerobic/Environmental Chamber, Sheldon Manufacturing Inc., Cornelius, OR) at 37 °C for 48 h and the BHI agar plates were incubated at 30 °C for 72 h. Typical colonies of TPC on BHI, LAB on MRS, and *L. monocytogenes* on PALCAM were counted and bacterial numbers were expressed as log CFU/package.