Research Note

Control of Listeria monocytogenes on Cooked Cured Ham by Formulation with a Lactate-Diacetate Blend and Surface Treatment with Lauric Arginate

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ABSTRACT

Ready-to-eat (RTE) meat products have been identified as a significant source of listeriosis in humans in the United States. Meat processors in the United States are required to use one of three alternatives to control L. monocytogenes in RTE meats: (i) a postlethality inactivation treatment along with a L. monocytogenes growth inhibitor; (ii) a postlethality inactivation treatment or a growth inhibitor; or (iii) sanitation measures and intensive testing. Lauric arginate (LAE) has been proposed as an effective postlethality inactivation treatment. The present study was conducted to investigate the antimicrobial effect of a lactate-diacetate blend in the formulation combined with surface application of LAE on cooked cured ham inoculated with L. monocytogenes, vacuum packaged, and stored at 4°C for up to 90 days. The treatments evaluated were (i) control ham with no added antimicrobials (control); (ii) ham formulated with 1.68% potassium lactate and 0.12% sodium diacetate (PLSD); (iii) control ham with 0.07% LAE as a surface treatment (LAE); and (iv) ham formulated with PLSD and LAE surface treatment (sprayed in bag and distributed across meat surface during vacuum packing) (PLSD + LAE). Use of only LAE as a surface treatment resulted in an initial 1-log CFU/g reduction in levels of L. monocytogenes on ham; however, this reduction only delayed the growth of the pathogen to 8 log CFU/g by 12 days when compared with the control ham without added antimicrobials. Use of PLSD in the formulation of ham resulted in a complete inhibition of L. monocytogenes throughout storage. The combination of PLSD in the formulation and a surface treatment with LAE resulted in an initial 0.7-log CFU/g reduction of the pathogen on ham and complete inhibition of the pathogen at the reduced level throughout storage. Formulation of ham with a lactate-diacetate blend combined with lauric arginate as a surface treatment will allow RTE meat processors to effectively achieve alternative 1 status, as designated by the U.S. Department of Agriculture Food Safety and Inspection Service, in their facilities.

Ready-to-eat (RTE) meat or poultry products contaminated with Listeria monocytogenes, implicated in past multistate outbreaks, are still implicated in several sporadic outbreaks of listeriosis in the United States annually (10). In response to the frequency and magnitude of recalls and outbreaks associated with L. monocytogenes in RTE meat and poultry products, the U.S. Department of Agriculture Food Safety and Inspection Service (USDA/FSIS) has implemented regulations for meat processors. Such regulations include enforcement of a zero-tolerance rule for the pathogen’s presence in these products as well as the requirement that processors apply control measures if the products are exposed to the processing environment after the lethality processing step and may support growth of the pathogen (17, 18). Specifically, the USDA/FSIS requires processors to use one of three alternatives to control L. monocytogenes in RTE meats: (i) a postlethality inactivation treatment along with a L. monocytogenes growth inhibitor (least frequent testing); (ii) a postlethality inactivation treatment or a growth inhibitor (moderate testing frequency); or (iii) sanitation measures (most frequent testing) (17).

Generally recognized as safe (GRAS) compounds, such as organic acids and their salts employed as dipping solutions or as formulation ingredients, have been effectively used as antilisterial agents in RTE meat products to meet the regulatory requirements (1, 2, 14). Many combinations of various food antimicrobials are able to control L. monocytogenes in RTE meats; however, lactate and diacetate salts, especially when used in combination, appear to be particularly effective at controlling the pathogen in RTE meats held at refrigeration temperatures (9) and are recommended by the USDA/FSIS for control of the pathogen. However, to achieve better antilisterial control in their product or to achieve alternative 1 status, RTE meat processors need to consider inclusion of a listericidal treatment in addition to growth-inhibiting antimicrobials such as lactates and diacetates. Various compounds have been evaluated for their listericidal activity when applied as a surface treatment on RTE meats contaminated with L. monocytogenes, including organic acids (1, 14), acidified sodium chlorite (3), acidic calcium sulfate (9), sodium lauryl sulfate (4), nisin and/or lysozyme (7), and lauric arginate (6)
Lauric arginate has received increasing interest from the food industry in recent years due to its potent antimicrobial activity against a wide range of microorganisms (12).

Lauric arginate (N⁵-laurol-l-arginine ethyl ester monohydrochloride; LAE) is a cationic surfactant derived from lauric acid, l-arginine, and ethanol and has received GRAS status for many food applications in the United States (12). Notably, it has received approval by the USDA/FSIS for application on the surface (up to 44 ppm when applied as a “sprayed lethality in container” without labeling and up to 200 ppm surface treatment [provided it is labeled]) of RTE meat and poultry products (16). The antimicrobial mode of action of LAE is attributed to its destabilizing effect on the cytoplasmic membrane, where it alters metabolic processes (12). Furthermore, it tends to concentrate in the water phase of products (where most antimicrobial activity occurs) due to its low oil-water equilibrium partition coefficient (13).

It has been suggested that product purge in meat packages may be the primary reservoir of L. monocytogenes, so a method was developed to treat the purge (and the surface of the product) with an antimicrobial that was introduced into the package and then distributed when the meat was vacuum packaged (6). Recent investigations have provided evidence of the antilisterial efficacy of LAE as a postlethality treatment on the surface of frankfurters as well as its complementary interactions with lactate-diacetate formulations (8, 15). The objective of the present study was to investigate the antimicrobial effect of a lactate-diacetate blend in the formulation combined with surface application of LAE on cooked cured ham inoculated with L. monocytogenes, vacuum packaged, and stored at 4°C for up to 90 days.

MATERIALS AND METHODS

Preparation of inocula. Listeria monocytogenes strains NRRL B33028 (isolated from cooked chicken), NRRL B33039 (isolated from cooked beef jerky), NCTC 12480 (clinical isolate from outbreak linked to pate), NCIMB 13449 (isolated from ripened cheese), and LMG 23193 (isolated from crab salad) were activated individually in brain heart infusion (Oxoid, Basingstoke, UK) broth (24 h at 30°C) from frozen stock cultures. Activated cultures were transferred again into brain heart infusion broth and incubated for 24 h at 30°C. Equivalent populations of each isolate were combined to provide a five-strain mixture of L. monocytogenes and diluted in sterile diluent (0.85% [wt/wt] sodium chloride and 0.1% [wt/vol] bacteriological peptone) to yield a target level of approximately 3 log CFU/g of final product per package.

Preparation of cooked cured hams. The model ham formulation (control ham) consisted of pork topside (480 g) (80.00% w/w), water (11.70%), sodium chloride (2.30% w/w), Fisher Scientific, Houston), sodium tripolyphosphate (0.30% w/w; M212, Haifa Chemicals Ltd., Haifa Bay, Israel), sodium nitrite (0.01% w/w; Fisher Scientific), maltodextrin (1.00% w/w; C³Gel 20006, Cargill Benelux bv, Sas Van Gent, The Netherlands), modified corn starch (4.00% w/w; C³Polarex 06727, Cargill Benelux bv), and carrageenan (0.70% w/w; Gelgarin ME2251, FMC Biopolymer, Philadelphia). The model formulation was modified to produce two formulation batches: (i) control (formulation as above) and (ii) blend of 1.68% potassium lactate and 0.12% sodium diacetate (PLSD; PURASAL Opti.Form PD4, PURAC, Lincolnshire, IL). The pork topside was stored for 24 h at 0°C before use. On the day of production, the brines were prepared, and 20% brine solution was added to the pork topside. A portion of each batch (6% of total meat; 29 g) was ground (to aid in structuring) while the remainder was cut into pieces (5 by 5 cm). The brine and meat blends were subsequently placed in vacuum bags with partial vacuum (to avoid foam formation) (Turbovac, HFE Vacuum Systems, ‘s-Hertogenbosch, The Netherlands) and tumbled (five 30-min cycles with 3-h rest periods between) (LU25, Lumar Ideal Inc., Montreal, Quebec, Canada) at 4°C (batches were in vacuum bags since all treatments were tumbled together to save time). The tumbled meat was vacuum packaged in crimp bags (Cryovac HT3050, Cryovac Food Packaging, Duncan, SC) and cooked in a water bath (set to 80°C) to an internal temperature of 72°C (monitored using a digital thermometer; Test 926, Testo, Almere, The Netherlands). Hams were subsequently cooled in 13°C water for 45 min before being stored at 0°C for 24 h. After cooling, hams were removed from the bag and sliced (approximately 70-g pieces; one slice per bag) for further analysis.

Product inoculation and treatment. Approximately 50 µl of an appropriate dilution of the five-strain inocula mixture was surface spread over both sides of the ham slices to reach 3 log CFU/g and kept for 30 min at 4°C for attachment. Following attachment, slices from both formulations were packaged into aseptic vacuum bags (110 by 200 mm; Super Export Plus, Hevel, Haarlem, The Netherlands) and either left untreated or treated by adding 0.5 g of a 10% (equivalent to 0.07% active LAE) commercial LAE solution (Protect-M, PURAC) into the package containing inoculated ham slices 30 min after inoculation. Thus treatments for this study were (i) control ham with no antimicrobial treatments (control); (ii) ham formulated with 1.68% potassium lactate and 0.12% sodium diacetate (PLSD); (iii) control ham with 0.07% LAE as a surface treatment (LAE); and (iv) ham formulated with PLSD and LAE surface treatment (PLSD + LAE). Bags containing slices from the different treatments were then vacuum packaged and stored at 4°C for up to 90 days.

Chemical properties. The pH of formulated meat batches was measured by placing approximately 25 g of sample in a sterile filter bag (stomacher bag with lateral filter, M-Tech Diagnostics, Ltd., Warrington, Cheshire, UK), homogenizing it (Stomacher 400 Lab Blender, Seward Medical, London, UK) with distilled water (1:10), and immersing a pH electrode (744 pH, Metrohm, Herisau, Switzerland) in the bag containing the homogenate. The water activity of the samples was determined using an Aw Sprint TH 500 (Novasina, Talstrasse, Switzerland) by placing approximately 5-g portions of chopped meat into plastic sample cups and inserting them into the vapor chamber.

Microbiological properties. Triplicate samples of each treatment were analyzed on days 0, 1, 6, 14, 28, 35, 47, 76, and 90 on tryptic soy agar (Becton Dickinson, Sparks, MD) for total microbial populations and on Palcam agar (Difco) for L. monocytogenes populations. Day 0 samples were analyzed within 3 h of treatment. Each sample (70-g slice) was opened, transferred into stomacher filter bags (M-Tech) where sterile diluent (0.85% [wt/wt] sodium chloride and 0.1% [wt/vol] bacteriological peptone) was added in a ratio of 1:3 (meat:diluent), and homogenized for 60 s (Seward Medical). Additional dilutions were also made in the same sterile diluent. A 50-µl portion of the appropriate dilution for each sample was plated using a spiral plater (Eddyjet type 1.23, IUL Instruments, Barcelona, Spain). Plates
were incubated for 48 h at 30°C, and the colonies were counted using an automatic colony counter (Colyte Supercount, Synoptics, Cambridge, UK) and the associated software package.

**Statistical analysis.** The experiment was conducted twice, and for each replicate three individual samples were analyzed on each sampling day for each treatment (n = 6). The microbiological data were converted to log CFU per gram based on the sample weight analyzed and the volume of diluent added to each sample. Data for changes in populations were analyzed by one-way analysis of variance to determine significant differences among antimicrobial treatments at each day of sampling using Minitab version 14.2 statistical software (Minitab, Inc., State College, PA). Differences were considered significant when the associated P value was less than 0.05.

**RESULTS AND DISCUSSION**

Total microbial populations were below the limit of detection at day 0 before inoculation and did not differ (P ≥ 0.05) from those of the pathogen during storage; thus, discussion of results focuses on the inoculated pathogen populations.

The pH of ham formulated with PLSD (6.23 ± 0.010) did not differ from that of the control ham (6.27 ± 0.010); however, the increased salt level in ham with PLSD resulted in a lower (0.965 ± 0.001) water activity compared with that of the control (0.974 ± 0.001). The effect of the lower water activity in addition to the antimicrobial activity of PLSD resulted in complete inhibition of *Listeria monocytogenes* growth on cooked cured ham throughout the 90-day storage (Fig. 1). In contrast, the control ham with no added antimicrobials did not retard the growth of *Listeria monocytogenes* with the pathogen reaching 8 log CFU/g by day 35 (Fig. 1). Surface treatment of cooked cured ham using 0.07% LAE resulted in a significant (P < 0.05) initial reduction (by 1 log CFU/g) of *Listeria monocytogenes* when compared with starting levels (3.3 log CFU/g) of the pathogen on control ham and for that matter ham formulated with the growth-inhibiting PLSD blend (Fig. 1). Despite this initial reduction in *Listeria monocytogenes* populations on ham treated with LAE, the pathogen mirrored the growth pattern on control ham, reaching 8 log CFU/g by day 47

(Fig. 1). *Listeria monocytogenes* on ham formulated with PLSD and surface treated with LAE experienced a complementary antimicrobial effect of the two compounds. Lauric arginate caused an initial reduction (P < 0.05) of 0.7 log CFU/g in *L. monocytogenes* on the surface of ham to 2.6 log CFU/g, and formulation with PLSD maintained the pathogen at the same level throughout storage (Fig. 1). With the exception of day 35, the level of *L. monocytogenes* on ham treated with PLSD+LAE was significantly (P < 0.05) lower than on ham treated with only PLSD during storage, with final levels of 2.4 and 3.3 log CFU/g, respectively, on day 90. Levels of *L. monocytogenes* on product treated with PLSD+LAE ranged between 0.4 and 1.5 log CFU/g lower than on product treated with PLSD alone (0.7 log CFU/g on day 0 and 0.9 log CFU/g by day 90). The initial 0.7-log CFU/g bactericidal effect of LAE on *L. monocytogenes* on ham with PLSD translated into approximately a mean 1-log CFU/g (0.9-log CFU/g averaged over all days) lower pathogen count throughout storage. It is not established that this reduced pathogen level has a public health impact. However, based on the investigation by Chen et al. (5) suggesting that foods that contain less than 2 log CFU/g of the pathogen pose very little risk, any reduction of the pathogen toward the estimated 2 log CFU/g level should reduce the risk of listeriosis. Meat processors should use the results of this study to evaluate the benefit for their own process of decreasing the levels of the pathogen by almost 1 log CFU/g on hams by postlethality application of LAE.

The results demonstrate that a bactericidal treatment such as LAE alone is insufficient to reduce the growth and, thus, the risk of *L. monocytogenes* on meat products; without the residual effect of growth-inhibitors such as lactate-diacetate blends, when the lag phase is overcome, the pathogen’s growth rate is unaffected. Results from Martin et al. (8) support the finding that LAE results in an initial 1-log reduction in *L. monocytogenes* levels but the complementary action of the lactate-diacetate blend is necessary to suppress growth during storage. In addition, a mathematical simulation with a predictive model (11) was used to simulate the effect of a postlethality treatment alone compared with use of a lactate-diacetate blend alone (at
similar levels as in the present study) as a growth inhibitor in cooked cured ham (with similar properties as in the present study). Results of the simulation revealed that even with an initial 5-log kill in *L. monocytogenes* due to postlethality treatment, use of the lactate-diacetate blend alone controlled growth of the pathogen better (up to 80 days of storage at 4°C) than use of only a postlethality treatment resulting in 5-log kill of the pathogen (data not shown).

Use of a postlethality inactivation treatment such as LAE in combination with a growth inhibitor such as a lactate-diacetate blend in cooked cured ham results in reduction of the pathogen level and continued suppression of growth when stored under vacuum at refrigeration (4°C). Use of a postlethality treatment alone is insufficient to provide residual protection after further processing (such as slicing), and thus there is a need to include a growth inhibitor such as a lactate-diacetate blend to reduce risk of *L. monocytogenes*. These results provide RTE meat processors with a method for achieving alternative 1 status in their facilities and for reducing the risk of *L. monocytogenes* in their products.

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**REFERENCES**


