Effect of Inhibitory Liquid Smoke Fractions on *Listeria monocytogenes* during Long-Term Storage of Frankfurters

SARITHA GEDELA,¹ J. ROY ESCOBAS,¹,² AND PETER M. MURIANA¹,²,*

¹Department of Animal Science and ²the Oklahoma Food and Agricultural Products Research and Technology Center, Oklahoma State University, Stillwater, Oklahoma 74078-6055, USA

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ABSTRACT

*Listeria monocytogenes* is a potential health hazard that sometimes finds habitation in facilities that manufacture ready-to-eat meats, including frankfurters. Our objectives were to examine the effect of select liquid smoke extracts on control of *L. monocytogenes* on frankfurters. Frankfurters were either obtained locally at retail (containing lactate-diacetate) or manufactured for us in-house or by a local processor (without added lactate-diacetate). In challenge studies of retail franks containing lactate-diacetate, low levels of *L. monocytogenes* were able to increase by 2 to 8 log on 5 of 10 brands tested when held at 1.6°C (35°F). Treatments with liquid smoke extracts were able to reduce and control growth of *L. monocytogenes* on the most permissive franks for 10 weeks when treated for as long as 90 s to as little as 5 s versus untreated controls. Effective control of *L. monocytogenes* was also obtained when dipped for as short as 1 s or when dropped through an atomized mist produced by a pressurized spray canister. Frankfurters manufactured without lactate-diacetate by a large commercial manufacturer of franks were sprayed with liquid smoke by using a commercial device as they exited the peeler. When inoculated at three different levels (10⁵, 10⁶, and 10⁷ CFU) with a four-strain cocktail of *L. monocytogenes* and stored at 6°C (43°F), the smoke-treated samples again demonstrated effective control of *L. monocytogenes* relative to untreated control samples. Frankfurters produced in-house without lactate-diacetate and treated while still in the casing also showed suppression of *Listeria* compared with controls. The data show that surface application of liquid smoke extracts by dipping or spraying may inhibit the growth of *L. monocytogenes* on frankfurters during shelf life and should facilitate a claim as an alternative 2, and possibly alternative 1, process for (U.S. Food and Drug Administration) hazard analysis and critical control point purposes.

*Listeria monocytogenes* is a foodborne pathogen that causes listeriosis in humans as well as animals (11). It is a rod-shaped, gram-positive, non–spore-forming, facultatively anaerobic bacterium (7). *L. monocytogenes* can enter host cells and spread both intra- and intercellularly, resulting in the spread of infection. Groups that are particularly at risk are the elderly, the immunocompromised, and pregnant women and their unborn fetuses.

*L. monocytogenes* is widely distributed in nature and has been found on decaying vegetation, in soils, animal feces, sewage, silage, water, as well as in food-processing environments (8). It has the ability to grow on most nonacid foods. These characteristics offer *L. monocytogenes* plenty of opportunity to enter the food chain and multiply (1). *L. monocytogenes* can grow over a wide range of temperatures and even at refrigeration temperature, especially if abused. It is generally more heat resistant than are other gastrointestinal foodborne pathogens (i.e., *Salmonella, Escherichia coli* O157:H7) and can survive freezing and drying. In the processed meat industry, a major health concern has been the consumption of *Listeria*-contaminated ready-to-eat (RTE) meat products that are not expected to be cooked by consumers prior to consumption.

According to the Centers for Disease Control and Prevention, 76 million illnesses, 325,000 hospitalizations, and 5,000 deaths occur annually in the United States because of various foodborne diseases (5). Of those, *L. monocytogenes* is estimated to be responsible for 2,500 illnesses and 500 deaths each year (10). In a recent outbreak, seven deaths and three stillbirths occurred because of consumption of *L. monocytogenes*-contaminated sliced turkey deli meat products in the northeast United States, resulting in the recall of 27.4 million pounds of fresh and frozen RTE poultry products (5).

The seriousness of listeriosis and its involvement with RTE meats demonstrates there is a need for better control of *Listeria* in food processing plants, and especially in those producing RTE products. According to U.S. Department of Agriculture–Food Safety and Inspection Service (USDA-FSIS) Directive 10.240.3, products may be placed in high/medium or low-risk product categories (2). Based on this directive, products could be changed from high-risk to low-risk if using a postprocess lethality step or antimicrobials to control *Listeria*. The USDA-FSIS final rule identified three process category alternatives for manufacturers of RTE meat products (3). An alternative 3 process controlled *L. monocytogenes* by sanitation alone and thereby had the highest level of FSIS product testing. An alternative 2 process had either a postprocess lethality step or included an-
timicrobial ingredients in addition to sanitation, and therefore had less product testing than an alternative 3 process. An alternative 1 process included both a postprocess lethality step and ingredients to control L. monocytogenes during shelf life in addition to sanitation and therefore, received the least product testing. If an ingredient provides for both reduction of L. monocytogenes and control of growth during shelf life, it is possible for its use alone to achieve alternative 1 status. Due to the incentives provided by the recent directives and final rule (i.e., less FSIS testing), efforts are currently being made by the industry to identify new and emerging processes and antimicrobials that are effective in suppressing or eliminating L. monocytogenes on processed meat products.

The traditional smoking of meat and meat products by heating in the presence of burning wood chips imparts a smoky flavor to meat. The advent of liquid smoke condensates has hastened the development of smoke flavor on treated meat products and shortened the overall processing time. Although the main functions of liquid smoke are to develop aroma, flavor, and color, recent work indicates additional attributes such as antioxidant properties and inhibition of microorganisms, especially L. monocytogenes (6, 9, 12, 13). The main advantage of liquid smoke is that it can be applied quickly and inexpensively. The purpose of this study was to examine the efficacy of select fractions of liquid smoke condensates chosen for their inhibitory activity on L. monocytogenes.

**MATERIALS AND METHODS**

**Bacterial strains.** The four strains of L. monocytogenes used in this study were Scott A-2 (serotype 4b, clinical isolate), V7-2 (serotype 1/2a, milk isolate), 39-2 (retail frankfurter isolate), and 383-2 (ground beef isolate). Cultures were transferred twice from frozen stocks stored at −75°C into individual brain heart infusion broth tubes at 1% inoculum level, and incubated overnight at 30°C. Overnight cultures were then mixed in equal proportions, and 1 ml of mixed culture was used for making dilutions in 0.1% buffered peptone water to obtain specific inoculum levels for various trials. These strains were resistant to both streptomycin (100 μg/ml; Sigma, St. Louis, Mo.) and rifamycin S/V (10 μg/ml; Sigma), and platings were performed on tryptic soy agar (Difco, Becton Dickinson, Sparks, Md.) containing these antibiotics to exclude the detection of indigenous contaminating bacteria from nonsterile food products.

**Smoke extracts.** A commercial supplier of liquid smoke condensates (Mastertaste, Inc., Monterey, Tenn.) provided the liquid smoke extracts (Zesti-B and AM-3) used in this study. Zesti-B is Zesti-AM2 (cat no. 1042004) that has been adjusted to pH 4.2 to 4.4, contains 3.5 to 5.6% acidity, and has a specific gravity of 1.1 to 1.2 g/ml. AM-3 is a refined liquid smoke fraction that has significantly reduced color and smoke flavor than does Zesti-B, while still retaining inhibitory activity. It is designed for use in a broader array of meat products. Products treated with liquid smoke extracts are generally listed as “smoke flavor” on ingredient labels (4). The extract was stored at room temperature and used directly at full strength as received.

**Sample preparation and treatments.** Frankfurters used in this study were either purchased locally and contained lactate and/or diacetate, or were manufactured for us without lactate-diacetate. For our retail challenge study, retail franks from the same product/brand, and of the same use-by date, were comingle and rinsed briefly with a liter of chilled sterile water, then bagged, inoculated, vacuum packaged, and stored for periodic shelf life testing.

**Smoke treatments.** The frankfurter brand that allowed high-level growth of L. monocytogenes in the retail challenge study was used for testing various dip times with liquid smoke extract. Frank’s were rinsed with sterile water and held briefly in a refrigerator before treatment with liquid smoke. In one study, frankfurters were not dipped (control), or dipped for 5, 15, 30, 60, and 90 s, and placed two per bag into vacuum packaging bags. In another study, frankfurters were not dipped (control), or dipped for 1 or 5 s, or applied by spray with a pressurized spray canister in which franks were dropped through a continuous mist of liquid smoke (Fig. 1A). In both studies, bagged samples were inoculated with ~10^4 CFU of L. monocytogenes, vacuum packaged, stored at 1.7°C (35°F) for 10 weeks, and plated weekly.

Franks that were dipped in the liquid smoke extracts were allowed to drip dry for 5 min in the refrigerator to allow excess liquid smoke to run off to standardize the amount of free liquid entering the packaging bags. Untreated controls were dipped in sterile water and held for a similar drip period before bagging. After bagging (two per bag), 0.5 to 1.0 ml of inoculum was added, massaged to distribute, and then vacuum sealed and placed in temperature-controlled refrigerated storage at 1.7°C (35°F) for shelf life testing at various weekly intervals.

Frankfurters were manufactured without lactate and diacetate,
sprayed with liquid smoke, and challenged with different levels of *L. monocytogenes*. The effect of liquid smoke against different levels of *L. monocytogenes* was tested. Frankfurters (30% fat) were manufactured without lactate and diacetate for this study by a large commercial processor and sprayed with liquid smoke extract by using a commercial spraying device as they exited the peeler (Fig. 1B). The manufactured franks were packaged in retail packages for transfer to our facility, where they were stored frozen until used. Upon use, frankfurter samples were transferred to new vacuum bags to which 0.5 ml of a four-strain mixture of *L. monocytogenes* was added at one of three different inoculation levels (10^3, 10^4, or 10^5 CFU). Purge from smoke-treated packages was also distributed to the new packages in order to minimize loss of smoke extract. Controls were also done at each inoculation level without liquid smoke treatment. Sample bags were vacuum sealed and stored at 6.1°C (43°F) for 10 weeks. Treatments inoculated with 10^3 CFU were plated weekly while the other inoculation levels were sampled biweekly. Each treatment was performed in triplicate.

Frankfurters were also made in-house in our meat pilot plant, using a similar generic formulation for frankfurters, but without added lactate or diacetate. Frankfurters were treated by dipping in liquid smoke (AM-3) for 30, 60, or 120 s while still in the casing, peeled, and then placed into vacuum-packaged bags to which a low level inoculum later determined to be 15 CFU was added. Frankfurters were incubated at 6.1°C (43°F) and tested weekly for 2 weeks and then biweekly thereafter until 10 weeks. Each treatment was performed in triplicate.

**Microbiological analysis.** A minimal rinse volume of 3 ml of 0.1% buffered peptone water (considered the 10^0 dilution) was added to opened sample packages of frankfurters, which were massaged for a few minutes to resuspend surface bacteria into the rinse buffer, of which 2 ml was pour plated and 1 ml used for additional dilutions, giving a detection limit of 0.50 log CFU/ml. Additional serial dilutions were either pour plated or spiral plated, depending on the level of cells expected from the prior weeks' testing. Spiral plating was done using the Eddy Jet (IUL Instruments, Cincinnati, Ohio). Plates were then incubated for 48 h at 30°C (86°F). Colony counts were obtained using an automatic colony counter (IUL Countermat Flash 4.2, IUL Instruments).

**Statistical analysis.** All trials were performed in triplicate. For most studies different replications were done on separate days with different lots of the same product. The means obtained from three replicate trials were subjected to one-way repeated measures analysis of variance to determine significant differences between different liquid smoke treatments using Sigma Stat 3.1 (Systat Software, Inc., Richmond, Va.). All pairwise multiple comparisons were done using the Holm-Sidak method.

**RESULTS AND DISCUSSION**

When we challenged retail frankfurters that contained lactate and diacetate inoculated with a low level of *L. monocytogenes* (i.e., ~10^3 CFU) and incubated them at 1.7°C (35°F), *L. monocytogenes* was effectively suppressed on five brands tested, indicating that the antimicrobial ingredients included in the formulation were sufficient to prevent the growth of contaminating *Listeria* that may find their way into finished packages of frankfurters (Fig. 2A). However, in five additional brands tested, levels of *L. monocytogenes* rose to significantly higher levels, indicating ineffective inhibition of *Listeria* and demonstrating the need for more effective control on such products (Fig. 2B). Currently, potassium lactate and sodium diacetate are the industry standard in chemical preservatives that are widely used by the processed meat industry, especially for frankfurters. The use of these preservatives allows processors to attain alternative 2 process category status (or alternative 1 if it possesses both lethality and growth inhibition). Although levels of lactate-diacetate in the various retail brands were not determined by analytical testing, the main point remains that even low levels of added *Listeria* increased to higher levels under proper refrigeration conditions on some brands. We would recommend that manufacturers perform inoculated challenge studies through offsite testing laboratories when relying on antimicrobials to perform specific tasks (i.e., prevent growth of *L. monocytogenes* to achieve an alternative 2 product risk category) to ensure they are working as intended.

For these reasons, we examined the use of liquid smoke extracts on one of the brands that was most permissive for growth of *L. monocytogenes* in our retail challenge study (i.e., brand F; a low-fat frankfurter). The liquid smoke extract was effective in preventing growth of *L. monocytogenes* when dipped for 90, 60, 30, or 15 s in the liquid smoke fraction, and even with as short a dip time as 5 s, whereas prolific growth of *L. monocytogenes* occurred on untreated controls (Fig. 3A). No significant differences were observed between treatments dipped for 5, 15, 30, 60, and 90 s. However, a significant difference (*P* < 0.05) was observed between untreated controls and all smoke treatments in which viable counts were reduced below detection limits (Fig. 3A).

After these initial results, we were interested in examining even shorter treatment times that could be easily applied in the industry. Since a 5-s dip time was effective in reducing and preventing outgrowth of *L. monocytogenes*, we proceeded to examine the shortest possible treatment times of 1 s and spray treatments. Effective control of *L. monocytogenes* was observed for as long as 10 weeks when using either a 1-s dip time or a simple spray device to deliver the active agent, compared with untreated controls (Fig. 3B). Significant differences (*P* < 0.05) were observed between controls versus dip or spray treatments but not between dip and spray treatments. Hence, reduction or control of the growth of *L. monocytogenes* has been achieved either by dipping or spraying the retail frankfurters with liquid smoke that would otherwise allow prodigious growth of *L. monocytogenes*.

The effect of liquid smoke extract was next examined on commercial franks manufactured without lactate and diacetate in which the smoke was applied by using a commercial spray applicator that would spray frankfurters as they were shot out through the peeler apparatus (Fig. 1B). The commercial sprayer uses a conical recovery section that catches and recirculates unused liquid smoke. In this study, trials were performed at three different levels of inoculation, each in triplicate, and using a higher shelf life temperature of 6.1°C (43°F), representing slightly abused storage conditions. However, because of limitations with commercial processor downtime, all frankfurters were manufactured during one shift and used for the three replica-
FIGURE 2. Inoculation of 10 retail brands of frankfurters that contain lactate-diacetate, with a low-level inoculum mixture (15 CFU) of four strains of L. monocytogenes, incubated at 1.7°C (35°F), and tested weekly for L. monocytogenes. (A) Suppression of growth of L. monocytogenes among five brands tested for 6 weeks. (B) Growth of L. monocytogenes within 5 weeks on 5 of 10 brands tested. The data represent the means of three replications, and error bars represent standard deviation of the means.

The results showed that with frankfurters inoculated at ~10^1 CFU, L. monocytogenes declined quickly to undetectable levels and remained that way for the entire test period (Fig. 4A). At the 10^2 CFU inoculation level, the levels slowly declined over a 4-week period, and at the 10^3 CFU inoculum level, Listeria slowly increased over 10 weeks to only 0.8 log higher than initial (high) inoculation levels, whereas untreated controls increased by nearly 7 log (Fig. 4B and 4C).

This study demonstrated control of L. monocytogenes with liquid smoke extracts; however, further evaluation of several conditions may even yield better results. For instance, the level of liquid smoke applied to the franks by the commercial sprayer may have been less than the treatment received from our manual spray applicator. The commercial peeler ejects franks at a much faster rate through the commercially sprayed liquid smoke “curtain” than the rate at which we manually dropped franks through our pressurized bottle spray mist (Fig. 1). This could be improved by having franks ejected through a longer orifice, requiring passage through several curtains of liquid smoke. Also, the frankfurters for this last trial series were manufactured, sprayed, and vacuum packaged at the manufacturer’s facilities, from which we would later open the packages (smoke-treated and untreated controls) and repackaged them with the challenge inoculum at our facilities. The frankfurters used in Figure 4 were kept frozen prior to use, indicating that the effectiveness of the inhibitory properties of the liquid smoke remained intact over time, as one might expect some adsorptive dilution of its concentration at the frankfurter surface. We would also expect that due to some loss of the antimicrobial during transfer from the original packaging by repackaging during the challenge inoculation, as well as loss due to adsorptive dilution at the surface, we may have had better results had we used them quickly after manufacture and/or reinoculated them in their original packaging. However, the results still demonstrate effective control at higher levels of L. monocytogenes than would be expected from typical contact contamination.

We also examined the effect of dipping frankfurters that were still in their casings into liquid smoke extract with frankfurters manufactured in-house without lactate or diacetate in challenge studies against low levels of L. monocytogenes. Untreated control samples demonstrated a 2-log increase in L. monocytogenes within 2 weeks, resulting in up to a 7.6-log level by the end of the 10-week shelf life period (Fig. 5). Dipping of encased frankfurters for any of the dwell times we used prevented growth of L. monocy-
FIGURE 3. Retail frankfurters shown to allow prodigious growth of *L. monocytogenes* (brand F) were used to test various treatment times with Zesti-B liquid smoke extract. (A) Frankfurters not dipped (control), and dipped for 5, 15, 30, 60, and 90 s in liquid smoke extract and inoculated with a low level of a four-strain mixture of *L. monocytogenes*. (B) Same brand of retail frankfurters used in the prior study were again used to examine shorter treatment time and/or spray application of the liquid smoke extract, using a pressurized spray canister. All treatments were done in triplicate. Samples were stored at 1.7°C (35°F) for 10 weeks. Error bars represent standard deviation of the mean.

FIGURE 4. Treatment of retail frankfurters with Zesti-B liquid smoke extract. Frankfurters were manufactured for this study without lactate and diacetate and sprayed with liquid smoke extract as they exited the peeler with a commercial spraying device. Untreated controls and smoke-treated samples were inoculated with three different levels of a four-strain mixture of *L. monocytogenes* and held at 6°C (43°F) for 10 weeks. (A) Inoculated with $3 \times 10^1$ CFU, (B) inoculated with $3 \times 10^2$ CFU, (C) inoculated with $3 \times 10^3$ CFU. Data points represent the mean of triplicate replications.
Inhibition of Listeria by liquid smoke extracts

FIGURE 5. Liquid smoke treatment of frankfurters manufactured without lactate-diacetate while retained in casings. Frankfurters were dip treated for 30, 60, or 120 s with liquid smoke extract (AM-3) prior to peeling; control frankfurters were not dipped. Peeled frankfurters were placed in bags into which 0.5 ml of a low-level inoculum (15 CFU) of a four-strain mixture of L. monocytogenes was added, vacuum sealed, and held at 6°C (43°F) for 10 weeks. Data points represent the mean of triplicate replications.

togenes for up to 4 weeks before increasing, whereas dip treatment of 120 s prevented growth for the entire 10-week period (Fig. 5). Such treatments could be done as either part of a postprocess chill regimen, or to "sanitize" the casings with an antimicrobial prior to peeling. Frankfurter peeler equipment is generally considered to be a potential collection point for Listeria contamination. Treatment of encased frankfurters could result in reduction of L. monocytogenes that could collect at the peeler as well as prevent the growth of L. monocytogenes during retail shelf life by leaching through the permeable cellulose casings. Although the length of treatment employed here on encased product may be considered long or impractical for some processors, it could be shortened if combined with additional application on peeled product downstream in the process, prior to packaging.

Frankfurters are just one RTE product category that currently employs widespread use of inhibitors throughout the volume mass of product for a problem that is largely restricted to the surface via potential contact contamination after cooking. We have examined the use of surface application of select inhibitory liquid smoke extracts on both encased and peeled frankfurters to reduce and inhibit the growth of L. monocytogenes contamination that may occur on RTE frankfurter products, and feel that the data show there is merit in consideration of these types of inhibitors toward reducing risk of L. monocytogenes on RTE meat products to provide alternative 2, and possibly alternative 1, product categories.

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REFERENCES