Combining Organic Acid Treatment with Steam Pasteurization To Eliminate *Listeria monocytogenes* on Fully Cooked Frankfurters

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ABSTRACT

An organic acid solution of 2% acetic, 1% lactic, 0.1% propionic, and 0.1% benzoic acids was combined with steam surface pasteurization to treat frankfurters during vacuum packaging to eliminate potential postcook contamination with *Listeria monocytogenes*. The thermal lethality of *L. monocytogenes* from steam was evaluated at an inoculation concentration of 1 to 6 log CFU/cm². About 3-log reductions of *L. monocytogenes* were achieved when frankfurters were treated by steam for 1.5 s. Combining organic acid treatment with steam pasteurization further inhibited the growth of surviving *L. monocytogenes* cells for 19 and 14 weeks when the packaged frankfurters were stored at 4 and 7°C, respectively. The results from this study provide meat processors with useful information for controlling *L. monocytogenes* on ready-to-eat meats.

Listeriosis occurs sporadically and has been traced to different foodstuffs contaminated by a ubiquitous pathogen, *Listeria monocytogenes* (26). At least 90 million pounds (41 million kilograms) of ready-to-eat (RTE) meat products have been recalled since 1998 in the United States because of suspected contamination with *L. monocytogenes* (1). *L. monocytogenes* is a facultative anaerobic, non—spore-forming, psychrophilic, and intracellular gram-positive rod. It is able to survive in different food-processing environments (3, 23), multiply at refrigeration temperatures, and grow in the presence or absence of oxygen (7, 27). *L. monocytogenes* tolerates a pH range of 4.6 to 9.2 and salt concentrations up to 12 to 13% (7, 27).

Although the infectious dose of *L. monocytogenes* is unknown, the widespread occurrence, high infection-associated mortality, and ability to form biofilms on food-processing equipment make *L. monocytogenes* of continued worldwide interest to the food industry, regulatory agencies, scientists, and consumers (23). The final rule published by the U.S. Department of Agriculture Food Safety and Inspection Service (USDA-FSIS) in October 2003 has established three alternative means for regulating RTE meat and poultry products that are exposed to the environment after cooking (25).

Establishments that manufacture RTE meat or poultry under alternative 1 use a postlethality (postcooking) treatment that reduces or eliminates *L. monocytogenes* and an antimicrobial agent or process that suppresses or limits *L. monocytogenes* growth throughout the product shelf life. The RTE meat or poultry producers that are operating under alternative 1 are not required to have a microbiological testing program. An RTE meat or poultry product that is manufactured under alternative 2 is either subjected to a postcook lethality treatment or is formulated with antimicrobial ingredients. Meat producers using alternative 3 rely solely on good manufacturing practices and sanitation programs to control *L. monocytogenes*. The USDA-FSIS concentrates its sampling effort for *L. monocytogenes* detection most heavily on meat and poultry products manufactured under alternative 3 because these products are considered to be the highest risk to public health (25).

Researchers and processors have been working for years developing and implementing postcook (postprocess) lethality treatments for at-risk RTE meats such as frankfurters (12, 14, 16). The industry has options for both pre- and postpackaging lethal treatments, including steam, hot water, radiant heat, and high-pressure processing. Application of steam surface pasteurization in vacuum-packaging systems allows postprocess lethality treatments to be achieved at a production line speed that is comparable to that of commercial packaging for RTE foods (9, 17).

Certain food ingredients, such as organic acids and their salts, spices, and natural flavoring agents, provide an antimicrobial effect by reducing the concentrations of pathogenic microorganisms such as *Escherichia coli* O157:H7, *Salmonella*, and *Listeria* (5). Since December 1999, the USDA-FSIS and the U.S. Food and Drug Administration have worked jointly to evaluate and approve a list of antimicrobial agents that are safe and suitable for use in the production of meat and poultry products (24). Antimicrobial agents such as organic acids and their salts have been reported to increase the sensitivity of *Listeria* to lethal treatments such as irradiation (1). A mixture of salts of acetic acid and lactic acid in bologna formulations decreased from 3.0 to 2.5 kGy the radiation dose needed to inactivate

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99.999% of \textit{L. monocytogenes} inoculated onto the meat. The mixture also prevented growth of spoilage microorganisms for 2 months (1).

Organic acids occur naturally in some foods and may also be added as an ingredient. Liquid smoke (containing about 10% acetic acid) has been used as antimicrobial agent to inhibit pathogens such as \textit{E. coli} O157:H7, \textit{Salmonella}, \textit{Listeria}, and \textit{Streptococcus} (10, 21). Combined use of liquid smoke and steam had a synergistic thermal lethality effect on \textit{L. monocytogenes} and inhibited potential growth of \textit{L. monocytogenes} on frankfurters (18). Organic acids, such as acetic, citric, propionic, and sorbic acids, and their soluble salts (i.e., calcium, potassium, or sodium) have been used to control the growth of select microorganisms by lowering the pH of foods (4).

When steam surface pasteurization treatment is applied during vacuum packaging of foods and combined with antimicrobial applications, potential outgrowth of any surviving pathogen cells during postpackage storage may be suppressed or eliminated. Vacuum applied during food packaging may assist in an even distribution of antimicrobial solution in the food package so that a small amount of antimicrobial solution may effectively coat the food surfaces without the need for a large amount of liquid in the package. Surface application of antimicrobial solution should also minimize the formulation changes of the food. In this study, an organic acid solution containing acetic, lactic, benzoic, and propionic acids was used in combination with steam surface pasteurization to control \textit{L. monocytogenes} on RTE frankfurters.

**MATERIALS AND METHODS**

**Frankfurters.** RTE frankfurters (2.6 cm diameter by 12.7 cm length) were obtained from a processor. The frankfurters contained about 52% moisture, 27% fat, 11% protein, and 2% salt and were made of beef, pork, mechanically separated turkey, water, salt, corn syrup, partially hydrolyzed beef stock, sodium phosphates, flavorings, ascorbic acid, extractives of paprika, and sodium nitrite. The formulation of each ingredient was proprietary to the processor.

**Culture preparation.** Nine strains of \textit{L. monocytogenes} (ATCC 19115, LM 51777, LM 984, MHL9, 01b, LMGP 39, Scott A, L-47, and LM 04b; Deibel Laboratories, Madison, Wis.) were used to include heat-resistant strains and isolates derived from foodborne illness outbreaks, different RTE food sources, and different food-processing environments. For comparison, separate cultures were also conducted for \textit{Listeria innocua} M1 (Deibel Laboratories) because of a report that \textit{L. innocua} is more heat resistant than \textit{L. monocytogenes} (6).

Each strain was individually maintained on tryptic soy agar (TSA) + 0.6% yeast extract (YE). To prepare each stock culture for test trials, a loopful of each strain was transferred from TSAYE to 5 ml of tryptic soy broth (TSB) + 0.6% YE and incubated at 35°C for 24 h to make stock cultures. A 0.1-ml aliquot of each stock culture was transferred to 9 ml of TSBYE and incubated at 35°C for 24 h to make substock cultures. The bacterial concentration in each substock culture was estimated at 10^8 CFU/ml.

**Surface inoculation.** A mixture of \textit{L. monocytogenes} culture was used as inoculum. Before bacterial inoculation, equal volumes of each substock culture were mixed together to obtain a cocktail of \textit{L. monocytogenes} inoculation culture. Each frankfurter (4°C) was submersed in a sterile pan containing 300 ml of the \textit{L. monocytogenes} cocktail (15°C) for 2 min and then removed, and the excess fluid was allowed to drip off. The inoculated frankfurters were kept at 4°C for 60 min before further treatment. At each test trial, inoculated and untreated frankfurters that were prepared according to the same procedure were used as controls to calculate the initial inoculation concentration of bacterial cells. \textit{L. innocua} inoculation was conducted separately following the same procedure.

**Treatment by organic acids and steam.** The inoculated frankfurters (4°C) were loaded onto formed-bottom packaging films at the inlet of a continuous packaging machine (RapidPak-SP 211, Alkar-RapidPak, Lodi, Wis.) in a double-layer arrangement (four frankfurters per layer and eight frankfurters per package). The organic acid solution (0.05 ml) containing 2% acetic, 1% lactic, 0.1% propionic, and 0.1% benzoic acids was dripped on to each end of the frankfurters, and steam (114°C) was applied for 1.5 s. The treated frankfurters were immediately covered with top films, vacuum sealed, and stored at 4 or 7°C. The packaged frankfurters were equilibrated to the storage temperature within 1 min.

**Microbial enumeration.** For microbial enumeration, entire packages of frankfurters were evaluated. Sterile phosphate buffer solutions (50 ml, pH 7.0) were poured into each package and hand massaged for 2 min to allow the buffer solution to contact all surfaces of the frankfurters. Serial dilutions of this rinseate were made and pour plated onto TSAYE overlaid with modified Oxford medium to resuscitate heat-injured cells. The viable colonies were counted after incubating the plates at 35°C for 48 h. For low numbers of bacteria, an enrichment procedure was used following the USDA-FSIS method for \textit{Listeria} detection (8). All chemicals used in this study were purchased from Becton Dickinson (Sparks, Md.).

**Data analysis.** About 500 lb (227 kg) of frankfurters were inoculated and tested in this study. Six replicated sampling units (1 lb [0.45 kg] per unit sample) were analyzed in each treatment. The concentration of surviving bacteria on the frankfurters was expressed as CFU per square centimeter of frankfurter surface area, and the means and standard deviations were calculated. Temperature measurements were made with thermocouple probes (type T) before and after treatment and during storage. The surface temperature of frankfurters during the 1.5-s steam treatment was difficult to measure and therefore was simulated by a computer program (ThermoPro PPP, FPS Technologies, LLC, Fayetteville, Ark.). Significant differences were identified based on a paired comparison using SAS (version 8.1, Cary, N.C.) at α = 0.5.

**Calculation for log kill.** The log kill for \textit{L. monocytogenes} was calculated by dividing process lethality (F) by the thermal decimal reduction time (D) of \textit{L. monocytogenes} at a reference temperature of 70°C: F/D_{10}. The process lethality (in minutes) was calculated with the following equation: \[10^{-T_{ref}}\Delta t\], where \(T\) is frankfurter temperature at time \(t\), \(T_{ref}\) is the reference temperature (70°C), \(\Delta t\) is the time interval, and \(z\) is temperature change caused by a 10-fold change in the decimal reduction time. In this study, a \(D_{10}\) of 0.17 min and a \(z\)-value of 5.91°C were obtained experimentally for \textit{L. monocytogenes} in frankfurters according to the procedures described by Murphy et al. (13–15).

**RESULTS AND DISCUSSION**

**Lethality.** The initial temperature of frankfurters was 4°C before treatment. Because a short steam treatment time...
(1.5 s) was used in this study, meat temperature changes over time at a specific position on the frankfurter was difficult to monitor. Therefore the meat temperature during steam treatment was simulated by a computer program (ThermoPro). Figure 1 shows the simulation for the changes in frankfurter temperature versus time during steam treatment within a 2-mm depth below the frankfurter surface. After steam treatment, the meat temperature measured at this position was about 82°C, which was in agreement with the value obtained by simulation.

The changes in concentrations of *L. monocytogenes* before and after steam pasteurization were also evaluated at initial inoculation concentrations of 1, 2, 4, and 6 log CFU/cm² (Fig. 2). At initial concentrations of 1 and 2 log CFU/cm², no *L. monocytogenes* survivors were detected on frankfurters after steam treatment. At initial concentrations of 4 and 6 log CFU/cm², a 3-log reduction was obtained for *L. monocytogenes* on frankfurters. The log kill for *L. monocytogenes* on frankfurters during steam treatment also was calculated using the simulated temperature-time curve (Fig. 1). The calculated log kill was not significantly different from the results obtained by inoculation tests up to a reduction of 3.5 log units for *L. monocytogenes*.

The inoculum could be trapped or protected in the folds of the frankfurter ends, where the casing was twisted. However, zero detection after enrichment from the low-concentration samples (1 and 2 log) indicates that the calculated and measured 3.5-log reduction is accurate for these protected areas.

*L. innocua* has been used often as an indicator in challenge studies for *L. monocytogenes* because it is slightly more heat resistance than *L. monocytogenes* (6). In this study, the lethality of *L. innocua* M1 was evaluated at initial inoculation concentrations of 1, 2, 4, and 6 log CFU/ml. The log reductions for *L. innocua* were not significantly different from those for *L. monocytogenes* at any of these initial concentrations (Fig. 3).

In a previous study, liquid smoke was used as an antimicrobial agent and was combined with steam pasteurization (100°C for 1.5 s) to treat frankfurters (18). This combination had a synergistic effect, reducing *L. monocytogenes* by 3 log units (18). In the present study, organic acid

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**FIGURE 1.** Temperature changes versus time within 2 mm of the surface of frankfurters, and the predicted log kills for *L. monocytogenes* from the equation \( F/D_{70} \), where \( F \) is process lethality calculated by \( \left[ 10^{(T-T_{ref})/z} \right] \Delta t \). \( D_{70} \) is the decimal reduction time of *L. monocytogenes* at a reference temperature of 70°C. The \( z \)-value is the temperature rise when the \( D \)-value of *L. monocytogenes* is changed 10-fold. \( T_{ref} \) is the reference temperature (i.e., 70°C), and \( \Delta t \) is the time interval in minutes. \( D_{70} = 0.17 \) min, and \( z = 5.91°C \) for *L. monocytogenes* in RTE frankfurters.

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**FIGURE 2.** Changes in the concentration of *L. monocytogenes* (log CFU per square centimeter) on frankfurters after steam pasteurization (SP). The initial inoculation concentrations were 6, 4, 2, and 1 log CFU/cm².

**FIGURE 3.** Changes in the concentration of *L. innocua* (log CFU per square centimeter) on frankfurters after steam pasteurization (SP). The initial inoculation concentrations were 6.0, 4.0, 2.0, and 1.0 log CFU/cm².
solution was dripped onto each frankfurter at both ends (0.05 ml at each end). With a combined treatment of steam and organic acids, *L. monocytogenes* was reduced about 3.4 log units (Fig. 4). The addition of organic acid solution contributed about a 0.4-log reduction of *L. monocytogenes* on frankfurters.

**Growth inhibition.** Organic acids have been used deliberately or as by-products of fermentation to acidify a variety of foods to control foodborne pathogens. However, *L. monocytogenes* is more acid tolerant than most foodborne pathogens. The optimum pH for *L. monocytogenes* growth is 7 to 8, but this organism may grow in a pH range of 5 to 10 (22). *L. monocytogenes* has been shown to survive and grow at a pH as low as 4.4 (2). Previous studies also indicated that a mixture of organic acids was superior to addition of individual organic acids for inhibiting *L. monocytogenes* (19, 20). Because of the loss of H+–ATPase when bacteria are exposed to low pH, sublethal injury may occur to the cells by disrupting proton motive force across cell membranes (11). Therefore, after low pH exposure, *L. monocytogenes* cells may be more susceptible to heat and other antimicrobial chemicals.

Following a combined treatment of organic acids and steam, *L. monocytogenes* numbers declined from 6 to 1.5 log CFU after the packaged frankfurters were stored at 4°C for 122 days (Fig. 4). The studies were also conducted at initial inoculation concentrations of 3.4 and 4.8 log CFU to evaluate the potential growth of heat-injured or surviving *L. monocytogenes* cells during refrigerated storage following a combined treatment with organic acids and steam pasteurization. A storage temperature of 7°C was used in this study. By combining steam pasteurization with organic acid treatment, the heat-injured or surviving *L. monocytogenes* cells continued to decrease during storage at 7°C (Fig. 5). After 11 weeks (77 days) at 7°C, *L. monocytogenes* was reduced to below the detection limit (<10 cells). After 14 weeks at 7°C, no growth of *L. monocytogenes* was detected in the packaged frankfurters. These results indicate that steam pasteurization has a lethal effect on *L. monocytogenes* cells and organic acids retarded the growth of any surviving *L. monocytogenes* cells during refrigerated storage.

Palumbo and Williams (19) evaluated prepackaging organic acid dips for frankfurters as a secondary lethal step to destroy *L. monocytogenes* and observed that 2.5% acetic acid combining with 2.5% citric acid restricted the growth of *L. monocytogenes* on vacuum-packaged frankfurters stored at 5°C for 90 days. Samelis et al. (21) evaluated the effectiveness of different organic acids and their salts as dipping solutions to control *L. monocytogenes* on sliced pork bologna stored at 4°C in vacuum packages and found that 2.5 or 5% acetic acid, 5% sodium diacetate, or 5% potassium benzoate inhibited the growth of *L. monocytogenes* for 120 days. In the present study, *L. monocytogenes* at 3.4 log CFU/ml was inoculated onto RTE frankfurters, and after a combined treatment with organic acids and steam no growth was detected after 19 weeks of storage at 4°C (Fig. 6). Thus, the combined use of organic acids and steam inhibited the outgrowth of heat-injured *L. monocytogenes* cells at a storage temperature of 4°C.
In this study, organic acids were used in conjunction with steam to reduce *L. monocytogenes* and inhibit the growth of heat-injured *L. monocytogenes* cells on RTE frankfurters during refrigerated storage. By combining organic acid treatment with steam pasteurization, no growth of *L. monocytogenes* was detected after the frankfurters were stored at 7°C for 14 weeks or at 4°C for 19 weeks. The results from this study indicate that organic acids may be combined with steam pasteurization to eliminate *L. monocytogenes* from RTE frankfurters.

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