



Use of a Moist-heat Bread Proofer for Thermal Inactivation of *Listeria* on Deli Slicers

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SUMMARY

Risk assessments have identified ready-to-eat deli meats sliced at the retail level as one of the categories at highest risk of being contaminated with the foodborne pathogen *Listeria monocytogenes*. The deli slicer can harbor *L. monocytogenes* on food contact surfaces of the slicer blade, food chute or base and potentially in the motor compartment. Objectives of this study were to determine (1) whether a moist heat treatment can cause a significant reduction of *L. monocytogenes* inoculated onto aluminum and stainless deli slicer components and (2) whether the deli slicer would remain fully operable after repeated moist thermal treatments. A bread proofer with controlled relative humidity (RH), operated at 65°C for 7 hours, achieved a 5-log reduction of a cocktail of *L. monocytogenes* inoculated onto the deli slicer metal surfaces. The RH in the interior of the proofer was maintained at 95% for 45 min, and subsequently at below 10% RH within 2 h, to dry out the electrical components and minimize any potential damage to the slicer. The motor and motor components were not damaged when subjected to repeated moist and dry heat cycles. It should be noted that these cultures were suspended in a laboratory medium. Such media are not representative of the high-fat and high-protein material normally found as contamination on a deli slicer, which would experience considerably less inactivation of *L. monocytogenes* than we report in this study. Future studies will utilize a slurry of turkey-based luncheon meat that will more accurately represent contamination occurring in commercial settings.

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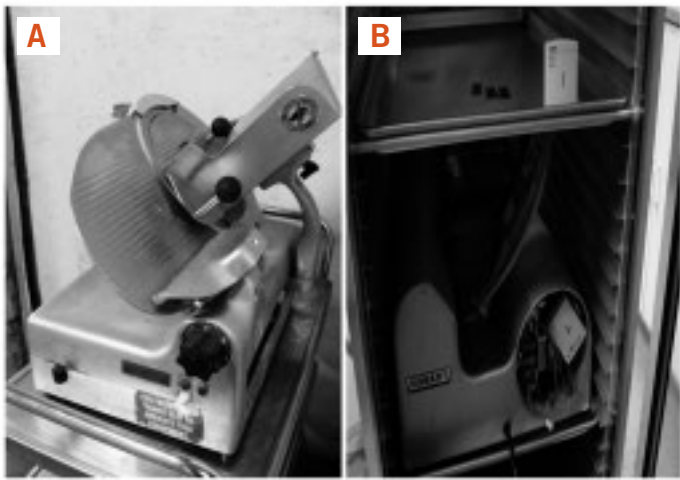


FIGURE 1. (A) Hobart Deli slicer, assembled. (B) Deli slicer placed in bread proofer, shown with Hobo data loggers 1 and 2, placed to record exterior (#1 – open in the bread proofer, set beside inoculated coupons) and interior (#2 inside motor compartment, where 3 other test coupons have been placed). The motor compartment was closed prior to thermal inactivation.

INTRODUCTION

Listeria monocytogenes was first recognized as a foodborne pathogen in the early 1980s (20). Although foodborne illness caused by *L. monocytogenes* is rare, the fatality rate for immune-compromised individuals is above 29%, among the highest for any foodborne pathogen (5). Ready-to-eat (RTE) foods have been determined to be the main source of human listeriosis (22). Commercially produced deli meats are given a lethal thermal kill step as part of their manufacturing process, but post-process contamination has been identified as the main risk factor for *L. monocytogenes* in RTE foods (1, 17, 21). Gombas et al. (11) gathered data that specifically indicated that RTE deli meats sliced at retail stores had a higher prevalence of *L. monocytogenes* contamination than RTE deli meats that are sliced and prepackaged by the manufacturer and not handled in stores. Two risk assessments conducted for *L. monocytogenes* in deli meats indicated that most illnesses and deaths from listeriosis are likely due to post-process contamination of products at retail (7, 16). Studies of retail environments indicate that *L. monocytogenes* can be present and thus could cross-contaminate RTE meats (18, 19).

L. monocytogenes occupying a niche on a food contact surface at retail can allow for cross-contamination of RTE foods. Meats that arrive free of contamination at retail facilities may become contaminated by cross-contamination from other *L. monocytogenes*-contaminated RTE products or by cross-contamination from the environment during slicing. It is known that *L. monocytogenes* on a contaminated deli slicer blade can easily contaminate multiple luncheon meat servings during mechanical slicing (10, 21).

The typical deli slicer is composed of numerous components, only some of which were designed for ease of disassembly; typically, the slicer blade, motor compartment and base are not easy to

disassemble. The slicer components are made of either stainless steel or cast aluminum, the surfaces of which can become pitted and porous with repeated washings and through the use of harsh chemical sanitizers. These degraded metal surfaces create niches for *L. monocytogenes* where cleaners and sanitizers cannot reach. Developing an additional lethal process to eliminate/reduce *L. monocytogenes* contamination from these degraded equipment food contact surfaces is important. Our previous work (4) reported that thermally treating a deli slicer in a dry oven at temperatures up to 82°C for 15 h did not sufficiently decrease *L. monocytogenes* inoculated onto deli slicer components. Moist heat may be necessary to increase heat transfer and aid in the reduction of foodborne pathogens (12).

Bread proofers, heated metal cabinets with metal racks in which the bread rises, are common pieces of equipment in many commercial delis and have the ability to run in either heat alone or moist-heat proofer mode. In the proofer mode, water is placed in an interior pan and the bread proofer produces moist heat which we hypothesized would aid in the reduction/elimination of *L. monocytogenes*. The objectives of this study were to determine: (1) whether moist heat treatment, using a commercial bread proofer, could cause a significant reduction of *L. monocytogenes* inoculated onto deli slicer components and coupons and (2) whether the deli slicer would remain fully operable after repeated moist thermal treatments.

MATERIALS AND METHODS

Deli bread proofer and deli slicer

A commercial-scale deli slicer (Model 1812, Hobart Food Equipment, Troy, OH) was used in these experiments (Fig. 1A). The slicer was partially disassembled so that it would fit into a bread proofer (Model NHPL-1836, Win-Holt Equipment, Syosset, NY; Fig. 1B). This would be typical of the disassembly needed in a routine thorough cleaning.

Data logging

Temperature and relative humidity (RH) were logged with two portable data loggers (HOB0® U12 Temp/RH2; Onset Computer Corporation, Buzzards Bay, MA) (Fig. 1B), one data logger was placed in the bread proofer on an open shelf where inoculated test coupons were placed; the other logger was placed in the motor compartment (MC) along with other test coupons. Preliminary work had shown the MC was the location on the slicer that was the hardest to heat, typically referred to as the “cold-point.” The MC was reassembled to a normal working configuration prior to thermal testing to simulate an internal niche.

Bacterial strains and growth conditions

Six strains of *L. monocytogenes* from the culture collection of the Center for Food Safety of the University of Arkansas were used in this study. The strains were *L. monocytogenes* 4b (CDC), 1/2 c (ATCC 19112), 4b (CDC from cheese outbreak), 1/2 a (Cornell, sliced

TABLE 1. Log of *Listeria* survivors on deli slicer aluminum feed-chute, inoculated with 40 μ L of cocktail per 4 cm² gridded area. The feed chute was placed on a shelf in a bread proofer with 100 mL DI water in water pan, operated in proofer mode at 65°C, for 7-h. Numbers represent average of three independent experiments, 3 replications per experiment

	Inoculum	Positive control	Negative control	Test grid
Average	6.9	5.9	< 1 CFU/cm ²	< 1 CFU/cm ²
St Dev	0.2	1.2		

turkey associated with an outbreak), 1/2 a (Cornell, human illness), and 1/2 a (Cornell, human illness). In addition, *Listeria innocua* M1, known for its heat resistance (8, 9) was added to the cocktail. Stock cultures were maintained frozen at -80°C in tryptic soy broth containing 0.6% yeast extract (TSBYE; Bacto, Becton Dickinson Co., Sparks, MD) supplemented with 16% glycerol. Working cultures were grown from stock by streaking on tryptic soy agar with 0.6% yeast extract (TSAYE) and incubation at 37°C for 48 h. Single colonies were placed in 10 mL of TSBYE and incubated at 37°C for 18 to 20 h. The cocktail for inoculation was prepared by placing 1 mL of each of the 7 cultures in a single sterile centrifuge tube and mixing the suspension with a vortex mixer (Thermo Fisher Scientific, Waltham, MA). The cocktail was then centrifuged and the pellet re-suspended in 1.5 mL of TSBYE to yield approximately 10⁹ CFU/mL.

Preparing deli slicer components and coupons

The aluminum feed-chute was marked off with permanent markers into 2 × 2 cm gridded areas. The stainless steel blade of a second deli slicer was cut into 2 × 2.5 cm coupons by use of a Flow Waterjet Cutting System (Flow International Corporation, Kent, WA). From the blade guard of the same slicer, cast aluminum coupons (2 × 2 cm) were cut with a cold-cutting saw (Milwaukee Heavy-Duty, Brookfield, WI) and a metal-cutting band saw (Wells Manufacturing Corporation, Three Rivers, MI). The deli components cut into coupons were washed by hand prior to autoclaving for 15 min at 121°C for sterilization.

Inoculation of components and coupons

Gridded areas of the RTE meat feed-chute were inoculated with 40 μ L of the cocktail. Coupons were used, rather than directly inoculating the deli slicer motor compartment, because we wanted to place the inoculated coupons in exactly the same location inside the small motor compartment. This allowed us to reproducibly inoculate known levels of *Listeria* on both aluminum and stainless steel deli components within the motor compartment and required us to simultaneously place duplicate coupons on the shelves of the bread proofer for comparison of the two environmental conditions (i.e., internal and external to the motor compartment). Sterile aluminum and stainless coupons cut from the deli slicer components were placed in Petri dishes and inoculated with 40 or 100 μ L of the concentrated cocktail, by use of a pipette. The inocula were allowed to air dry in a biosafety cabinet for 2 h prior to moist heat treatment.

Each experiment was repeated three times, with triplicate samples in each experiment. Controls were (1) positive controls, consisting of coupons inoculated as described and left in the biosafety cabinet during the experiment times, and (2) negative controls, consisting of coupons not inoculated and left in the biosafety cabinet during the experiment times. Initial inoculum levels were measured for each experiment.

Thermal treatment

Bread proofers are pieces of equipment consisting of heated metal cabinets with metal racks where bread rises. Bread proofers have the ability to run either in dry heat alone or in the proofer mode. In the proofer mode, a measured amount of water is placed in the water pan and is evaporated during the initial heating cycle. After the water evaporates, the bread proofer operates in the dry heat cycle for the duration of the cycle. We hypothesized that the bread proofers' moist heat and following drying cycle would aid in the reduction or elimination of *L. monocytogenes* while still protecting the electronic components of the slicer. The chute of the partially disassembled deli slicer was placed in the bread proofer at room temperature and 500 mL of deionized (DI) water was added to the water pan, after which the proofer was set on "proofer" setting and the internal temperature set to 65°C, with a total run time of 7 h.

Coupons were placed in the proofer with the slicer. The coupons were transferred with sterile tweezers from the biosafety cabinet to the proofer; three randomly selected, inoculated coupons were placed on a shelf in the proofer (OUT) and three inoculated coupons were placed in the interior of the motor compartment (MC). The coupons were randomly selected as either aluminum or stainless steel. The internal temperature of the bread proofer was set at 65°C in proofer mode, the amount of water in the proofer pan reduced to 100 mL, and time was set at 7 h. After each experimental run, the deli slicer was reassembled, plugged in and tested to ensure that it was operating normally.

Microbial sampling after thermal inactivation

After each thermal processing run, gridded areas on the chute component or coupons were swabbed in a prescribed manner with sterile cotton swabs saturated with phosphate buffered saline (PBS), 5 strokes horizontally and then 7 strokes vertically and horizontally. Each swab was then placed in 9 mL of PBS (approximated as a 1:10 dilution); these suspensions were serially diluted and plated onto TSAYE agar and modified oxford agar (MOX; EMD Millipore, Billerica, MA) and then enumerated after incubation at 37°C for 24 h and 48 h, respectively.

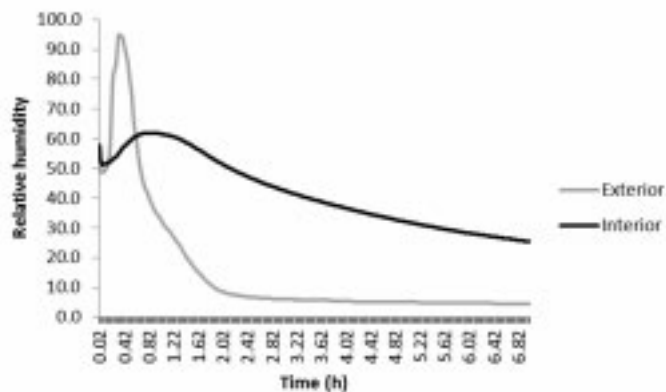


FIGURE 2. Relative humidity on the exterior of the slicer and interior of the motor compartment, in a bread proofer with 100 mL water in water pan, operated in proofer mode at 65°C, for 7 h.

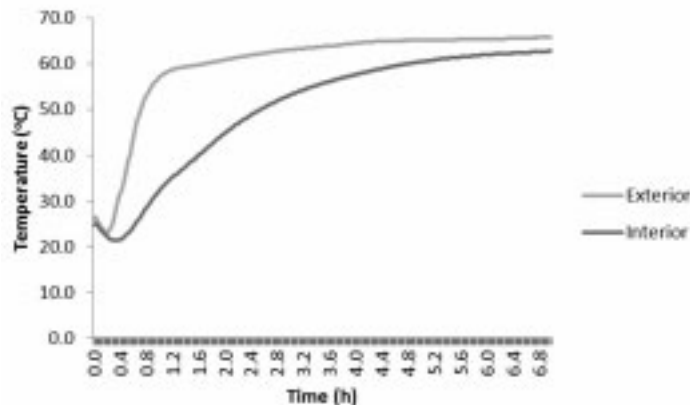


FIGURE 3. Temperature at the exterior of the slicer and the interior of the motor compartment during thermal treatment in a bread proofer with 100 mL water in water pan, operated in proofer mode at 65°C, for 7 h.

RESULTS AND DISCUSSION

Temperature and humidity

Relative humidity in the motor compartment and at the exterior of the slicer is presented in Fig. 2. In the “proof” mode in the bread proofer, the humidity on the exterior of the slicer rose quickly to over 90%, but decreased within two hours to below 10%. In the motor compartment, however, RH rose to only around 60% and fell slowly during the 7-h treatment time, still being above 20% at the end of the treatment time. Temperature rose more slowly in the motor compartment than at the exterior, and never quite reached the cabinet temperature of 65°C (Fig. 3).

Reduction of *Listeria* cocktail due to thermal treatment

The 7 h experimental runs with the inoculated chute given a heat treatment using 100 mL water and a temperature of 65°C in the bread proofer chamber, reduced the counts of the *Listeria* cocktail to below detection limits (Table 1). This met our initial design requirements of designing RH and thermal conditions that produced at least a 5 log reduction of *Listeria* on the exterior of the slicer, where the food contact surfaces are.

Because the recovery of *Listeria* was below detectable levels in the initial experiments, the initial inoculum was increased to 100 µL. Additionally, samples were plated on both TSAYE and MOX agar in order to get an indication of the differential recovery of thermally injured but viable *Listeria* that could be recovered on the TSAYE plates, compared with the MOX plates containing additional inhibitors. Results of these experiments are shown in Tables 2 and 3. Counts were still very low for all the test coupons, but the greatest log reductions occurred in the enclosed motor compartment, possibly because the RH remained higher in this area for a longer period during the 7 h moist thermal treatment, compared with the exterior coupons. Although the counts from the motor compartment are numerically lower, they are not statistically significantly lower ($P > 0.05$). No statistically significant differences in TSAYE recovery of thermally injured cells, as opposed to MOX recovery, were detected, and no significant differences were noted between AL or SS food contact materials.

In previous studies, we found that dry heat at temperatures of 66, 71, 77, or 82°C for up to 15 h were ineffective in eliminating *L. monocytogenes*/*L. innocua* (4). At 66°C, there was an initial drop of approximately 1.5 log CFU/cm² after 30 min of treatment, but recovery was more than 4 log CFU/cm² even after 15 h. When temperatures were increased to 80°C, the decrease was over 4-log, but the thermal treatment times (15 h) were longer than the duration of the overnight treatment that might be considered practical for a retail deli. Other researchers have found that a combination of UV light and dry heat (50°C) resulted in more than a 5 log reduction in *L. monocytogenes* in 3 h on both stainless steel and polypropylene (2). A study of cleaning and drying holding chillers used for postcook commercial processed meats (RTE) used dry heat (50°C) for 2 hours and demonstrated a reduction from 8% *Listeria* positive samples before drying to none detected after drying (6). However, these moist thermal inactivation studies and studies by others indicate that moist conditions significantly increase the thermal lethality over dry conditions (3, 13, 14, 15). This research extends our previous findings by placing actual pieces of the deli slicer that are very difficult to clean, the motor compartment and structural elements of the slicer, into the proofer for a lethal, moist heat kill step.

This group of researchers is currently developing a prototype portable or collapsible pasteurizer for use in retail venues that do not have a bread proofer available. Our ultimate goal is to minimize the risk of *L. monocytogenes* contamination from all deli equipment by thermally treating any possible harborages in the equipment and thereby minimizing future cross-contamination.

Verification of no damage to motor components

We verified that the proposed moist thermal treatment did no damage to the deli motor by continually starting and operating the slicer after each moist thermal treatment. To date, this slicer has successfully started more than 25 times after being subjected to the proposed moist thermal inactivation cycle. Visual inspection of the motor housing, motor windings and brushes for signs of corrosion indicated that their appearance compared quite favorably to the pictures we had taken before initiating this study.

TABLE 2. Log CFU/cm² of *Listeria* survivors on TSAYE plates from deli slicer coupons, stainless steel (SS) or aluminum (AL) placed on a shelf in a bread proofer with 100 mL deionized water in water pan, operated in proofer mode at 65°C, for 7-h. Positive controls are inoculated coupons that were left at room temperature in a biosafety cabinet for the duration of the experiment

TSAYE						
	AL Pos	AL out	AL MC	SS Pos	SS out	SS MC
Run 1	5.4	*	*	5.5	1.4	*
Run 2	5.4	1	*	5.7	1.1	*
Run 3	6.3	2	*	6.3	2.9	2.6
Run 4	6.3	2.7	*	6.0	2.5	*
Run 5	6.0	2.8	1.8	6.0	2.9	*
Avg	5.9 ^a	1.7 ^{bc}	0.4 ^c	5.9 ^a	2.2 ^b	0.5 ^c
Std Dev	0.5	1.2	0.8	0.3	0.9	1.2

*Below the detection limit (10 CFU/cm²). Out = exterior of slicer, MC = motor compartment, Pos is positive control. Numbers with different letters are statistically significantly different by Student's *t*-test (*P* < 0.05).

TABLE 3. Log CFU/*Listeria* survivors on Modified Oxford agar plates from deli slicer coupons, stainless steel (SS) or aluminum (AL) placed on a shelf in a bread proofer with 100 mL deionized water in water pan, operated in proofer mode at 65°C, for 7-h. Positive controls are inoculated coupons that were left at room temperature in a biosafety cabinet for the duration of the experiment

MOX						
	AL Pos	AL out	AL MC	SS Pos	SS out	SS MC
Run 1	4.1	*	*	5.2	*	*
Run 2	5.2	*	*	5.4	*	*
Run 3	6.2	1.7	*	6.3	2.3	1.4
Run 4	6.1	1.6	*	5.6	1.9	*
Run 5	6.1	2.4	1.6	5.8	2.6	*
Avg	5.5 ^a	1.1 ^b	0.3 ^b	5.7 ^a	1.4 ^b	0.3 ^b
Std Dev	0.9	1.1	0.7	0.4	1.3	0.6

*Below the detection limit (10 CFU/cm²). Out = exterior of slicer, MC = motor compartment, Pos is positive control. Numbers with different letters are statistically significantly different by Student's *t*-test (*P* < 0.05).

CONCLUSIONS

Regulators, the food service industry, and consumer groups all want safer food. The deli environment has been identified as a high-risk location for harboring the food pathogen *L. monocytogenes*. Therefore, additional procedures that go beyond the daily SSOPs, cleaning routines and sanitizing of deli equipment food contact surfaces are needed. The results of this research indicate that using a bread proofer to deliver moist heat to a deli slicer for 7 h can result in a 4 to 5 log reduction of *Listeria* on the slicer internal surfaces. Additionally, this thermal treatment has not been shown to affect the operation of the deli slicer motor adversely. Although our experiments resulted in a high level of thermal inactivation, these cultures were suspended in a laboratory TSAYE, which is not representative of contamination that would occur on slicers in commercial settings. A suspension high in fat and protein, as

normally found on slicers, would result in considerably lower log/CFU inactivation of *L. monocytogenes* than we report in this study. Future studies will utilize a slurry prepared from a turkey-based luncheon meat that will more accurately represent natural contamination occurring in commercial delis.

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