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Dry Heat Thermal Inactivation of *Listeria innocua* on Deli Slicer Components

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ABSTRACT

A thermal kill step was evaluated as a method of obtaining an additional margin of safety for retail deli meat slicers following cleaning and sanitizing. Retail deli slicers were cut into coupons or marked off in grids, cleaned and sanitized. *Listeria innocua*, an established thermal-resistant *Listeria* species, was inoculated at 10^7 CFU/cm^2. The inoculated components or stainless steel coupons were placed in a dry heat oven at 66°C or 80°C and sampled at 0.5, 1, 3 and 15 h. There was no statistically significant difference in survival between the stainless steel and the cast aluminum portions of the slicer. At 66°C, there was an initial drop of approximately 1.5 log CFU after 30 min of treatment, but recovery of *L. innocua* remained at more than 4 log CFU 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 an overnight treatment that might be considered practical for a retail deli. From these results, it appears that dry thermal treatments at temperature of 80°C and times up to 15 h are not sufficient to produce a 5-log reduction of residual *L. innocua* that may have survived improper cleaning and sanitizing of the deli slicer.

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INTRODUCTION

Listeria monocytogenes causes a rare but potentially fatal disease, listeriosis. The mortality rate from this disease is approximately 28%, particularly in immunocompromised individuals, who are most at risk *(3, 5)*. The economic burden for US citizens for a single case of listeriosis is estimated to be $1,659,143 *(23)*. The prevalence of *L. monocytogenes* in ready-to-eat (RTE) deli meat and poultry products has steadily decreased from 4.61% positive in 1990 to only 0.42% positive in 2008 *(10)*, but the incidence of *L. monocytogenes* infections has not changed substantially in the past 3 years *(3)*.

It has been apparent for many years that meat slicers in retail establishments can harbor large populations of bacteria *(12)* and that these bacteria have the potential to cross contaminate sliced, ready-to-eat foods *(11)*. A few outbreaks of salmonellosis have also been linked to meat slicers *(1, 17)*. Although no cases of listeriosis have been directly linked to meat slicers, slicers have been found to be contaminated with *L. monocytogenes (16)*, and the 2008 listeriosis outbreak in Canada was ultimately traced to the meat slicers in the RTE meat plant *(15)*. In addition, a large study by Gombas and others *(13)* found that prevalence of *L. monocytogenes* was approximately 7 times higher in RTE meats sliced in retail deli as compared to commercially manufactured, sliced and packaged RTE meats. The fact that nearly 75% of consumers purchase deli sliced meats rather than commercially packaged meats *(9)* implies that significant numbers of consumers are exposed to *L. monocytogenes*.

Foegeding and Stanley *(7)* proposed the use of *Listeria innocua* ATCC 33091 as a thermal-processing indicator for *L. monocytogenes* in milk because this organism is 1.3 times more heat resistant than *L. monocytogenes*. Fairchild and Foegeding *(6)* generated a natural mutation of *L. innocua* 33091 that was resistant to rifampin and streptomycin and designated it strain M1. Resistance to these two antibiotics allows this strain to be counted directly on nonselective medium with these antibiotics added, without interference from the normal background microflora present. Murphy and others *(19)* extended this research to poultry and determined the $D_{(67.5°C)}$ in chicken breast meat was 0.35 min for *L. innocua* M1 and 0.29 min for *L. monocytogenes*. *L. innocua* also responds similarly to *L. monocytogenes* to thermal processing, ultraviolet-C light, flash pasteurization, and ionizing radiation on the surface of RTE meats *(8, 24, 25, 26)*. Studies conducted in a food pilot plant setting, using flash pasteurization, have used *L. innocua* cocktails as a nonpathogenic surrogate in place of *L. monocytogenes (18, 20, 21, 24)*.

Dry heat has been used for many years as a means to sterilize materials, especially for such medical commodities as assembled non-disposable syringes, where dry heat can penetrate into the sealed container, in contrast to steam, which cannot be relied upon to reach the interior of the container *(4)*. In 1972, NASA used dry heat sterilization on the Mars landing craft to prevent forward contamination of Mars *(14)*. Commercial manufacturers of RTE meats sometimes place their cleaned and sanitized commercial scale size slicers in their smoke houses at 60 to 80°C overnight in an effort to inactivate any residual *L. monocytogenes* (personal communication, John Butts, May 2009). The objective of this study was to attempt to recreate this treatment, using the existing delicatessen ovens to provide a lethal thermal treatment to destroy *L. innocua* on food contact surfaces. The objective was to mimic the use of bread proofing ovens, in which temperatures do not reach the typical dry heat sterilization temperatures of 160 to 180°C. We also assessed the effectiveness of studying coupons cut from a slicer rather than the entire slicer, as well as the difference in survival of *L. innocua* on the slicer's stainless steel blade versus the cast aluminum guard.

MATERIALS AND METHODS

Bacterial cultures

Listeria innocua M1 resistant to streptomycin (250 mg/L), and rifampicin (50 mg/L), both generated by selective enrichment *(6)*, was originally obtained from Dr. P. M. Foegeding (Department of Food Science, North Carolina State University, Raleigh, NC). Stock cultures were maintained frozen (–80°C). The working culture was started from frozen stock inoculated into tryptic soy broth (Bacto, Becton Dickinson Co., Sparks, MD) supplemented with 0.6% yeast extract (TSBYE) and was incubated at 37°C overnight.

Preparing deli slicer coupons and components

Stainless steel components from the blade of a Hobart heavy duty slicer (Hobart Food Equipment, Australia) were cut into 2 × 2.5 cm coupons, using a Flow Waterjet Cutting System (Flow International Corporation, Kent, WA). This cutting system was used to prevent heat-induced stress, which could cause a change in the physical properties of the stainless steel. From the blade guard of the same slicer, cast aluminum coupons (2 × 2 × 0.5 cm) were cut, using a Milwaukee Heavy-Duty metal cold-cutting metal saw (Brookfield, WI) and a Wellsaw metal-cutting band saw (Wells Manufacturing Corporation, Three Rivers, MI). Coupons were washed thoroughly in Micro 90 cleaning solution (International Products Corp., Burlington, NJ) prepared as per directions of the manufacturer and then rinsed in sterile deionized water. Coupons were sterilized by autoclaving for 15 min at 121°C. In addition, from another slicer, disassembled stainless steel and cast aluminum deli slicer food contact surfaces were marked off into 2 × 2 cm grids, using permanent markers. Gridded areas were serially numbered in a random fashion. Slicer components were wrapped in aluminum foil and autoclaved at 121°C for 15 minutes.

Inoculation of coupons and components

Sterile coupons were laid individually on sterile glass microscope slides and forty microliters (40 µl) of the *L. innocua* culture was pipetted into the middle of each coupon and carefully spread over the area with a sterile inoculation loop. The inoculum was allowed

FIGURE 1. Comparison of recovery of *L. innocua* from stainless steel blade, coupon cut from blade, cast aluminum guard or coupon cut from guard after 3 hours dry heat at 66°C. [a]values are not significantly different by *t* test ($P > 0.05$). Each value is the mean of duplicate samples from six experiments.

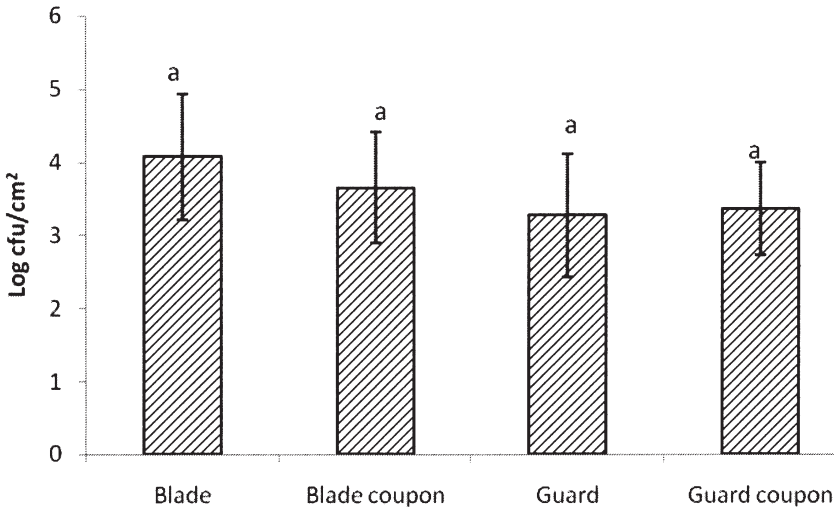
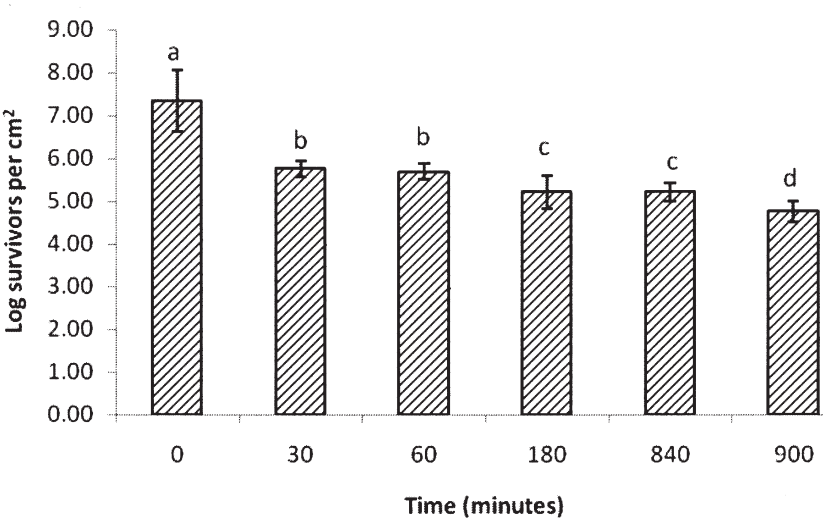


FIGURE 2. Thermal inactivation of *L. innocua* on cast aluminum guard of a deli meat slicer in a dry oven at 66°C for up to 15 h (900 min). [a-d]values with different superscripts are significantly different by *t* test ($P < 0.05$). Each value is the mean of duplicate samples from 3 experiments.



to air dry for 2 h. Forty microliters (40 µl) of the *L. innocua* culture was pipetted into the middle of each 2 × 2 cm gridded area of the components of the slicer, and spread carefully over the gridded area with an inoculation loop, and allowed to air dry for 2 h.

Thermal inactivation

Each deli slicer component was wrapped in Heavy Duty Reynolds Wrap® (Reynolds Kitchens, Richmond, VA) to prevent contamination, and coupons were placed in sterile petri dishes before being placed in a convection oven (Power-O-matic 60, Blue M. Electric Company, Blue Island, IL) at 66°C or 80°C. Components or coupons were sampled at 0.5, 1, 3, and 15 h. Thermocouples (Type J, Iron-Constantan) were placed in thin sleeves and then taped under the aluminum foil right next to each component and to the interior of the oven during runs. Results were logged onto a 21X Micrologger (Campbell Scientific, Inc., Logan, UT).

Sampling after thermal inactivation

After each oven run, gridded areas or coupons were swabbed with sterile cotton-tipped swabs, which were then placed in 10 ml sterile phosphate buffered saline, vortexed, serially diluted, plated on TSAYE agar, and incubated at 37°C for 24 h. Colonies were enumerated and data entered into Microsoft Excel (Microsoft Corporation, Redmond, WA) spreadsheets and analyzed.

Statistical analysis

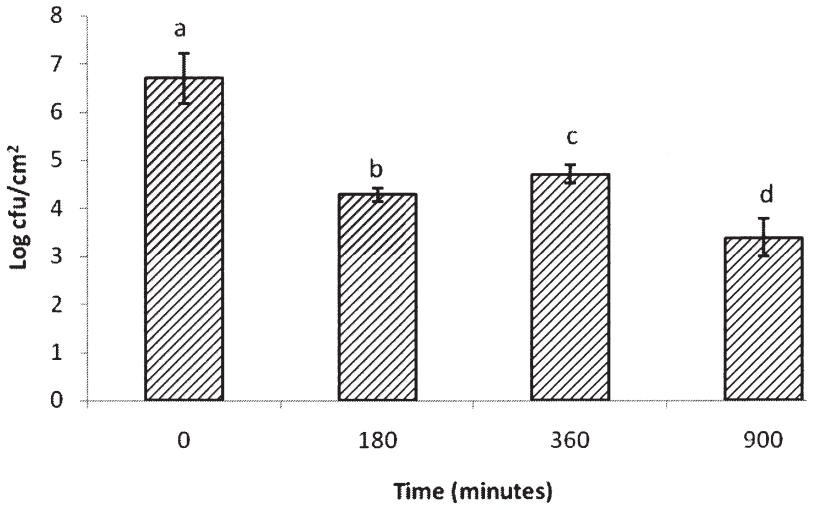
Each experiment was repeated 3 to 6 times. Mean number of colonies per ml (survivors) was converted to log CFU/cm² and means were calculated. Statistical significance of differences was determined by Students *t* test, with significance assigned at $P < 0.05$.

RESULTS AND DISCUSSION

Comparisons of the two different construction materials of the slicer, aluminum and stainless steel, as well as comparison of whole components versus coupons, are shown in Fig. 1. As can be seen, there was no significant difference ($P > 0.05$) in the recovery of *L. innocua* from the different materials, nor was there a difference between results with use of the whole slicer components and the coupons. In contrast, Wilks and others *(27)* found that *L. monocytogenes* survived better on stainless steel than on a copper-base alloy. They detected viable cells on stainless steel after 24 h incubation at room temperature, as opposed to no viable cells on copper, brass, aluminum bronze and silicon bronze after 60 min incubation. Bremer and others *(2)* found that *L. monocytogenes* on stainless steel coupons held at 15°C exhibited a D value of 1.2 days.

Dry heat at 66°C resulted in a small initial drop at 30 minutes, but recovery of *L. innocua* remained at high levels even after 15 h (Fig. 2). Although there were statistically sig-

FIGURE 3. Thermal inactivation of *L. innocua* on cast aluminum guard of a deli meat slicer in a dry oven at 80°C for up to 15 h. [a-d]values with different superscripts are significantly different by *t* test ($P < 0.05$). Each value is the mean of duplicate samples from three experiments.



nificant differences in survivors as time progressed, levels of survivors never declined below 4 logs. Temperature of the oven was increased to 80°C and the experiment was repeated. Results of the second experiment are shown in Fig. 3, where it can be seen that there was recovery of over 3 log CFU/cm^2 of *L. innocua* survivors. The decrease at 80°C was over 4 log, but the thermal treatment time required to achieve this level of reduction (15 h) is most likely not practical for use in a working delicatessen. However, it should also be noted that the thermal resistance of this *L. innocua* is 1.3 times greater than that of *L. monocytogenes*, so it is possible that this temperature overnight would be sufficient to cause a 5-log reduction of *L. monocytogenes.* Because of the nonlinear nature of the curves obtained, it was not possible to calculate D- and z-values for the slicer materials. We chose a 5-log reduction target because food regulations routinely require that treatments reduce pathogens by this amount in food products. In the case of the deli slicer, it should be noted that we did achieve a reduction of the *L. innocua* in the first three hours of heating that could be adequate, given that levels of residual *L. monocytogenes* on equipment are likely not as high as the levels in the inocula we used.

Rodriguez and others *(22)* found that as *Listeria* biofilms dried on stainless steel, they were more able to transfer *Listeria* to food. Although we did not study *L. innocua* biofilms on these slicers, results of our tests indicate that contaminated slicers subjected to dry heat might be more likely to transfer residual *Listeria* to foods. Although dry heat is economical and could easily be used with typical dry heat ovens found in most delis, it appears that it is not well suited to sterilization of deli slicers. An alternative avenue of exploration would be the use of moist heat applied on the contaminated slicer at similar temperatures as those used in the current study. With moist heat, it is critical for the moisture to penetrate to the contaminated areas, but sterilization is usually achieved over shorter time periods at lower temperatures.

CONCLUSION

Dry thermal treatment at 80°C and times up to 15 h are not sufficient to achieve a 5-log reduction of residual *L. innocua* that may have survived improper cleaning and sanitizing of the deli slicer. However, a three-hour treatment at 80°C produced a 2 or 3 log reduction, which would likely be adequate for a machine that had been cleaned and sanitized prior to heating. Dry thermal heating overnight could provide an extra hurdle for *Listeria* contamination in the worst case scenario of an inadequately cleaned and sanitized slicer.

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