Center for Food Safety, University of Georgia, Griffin, GA (http://www.griffin.uga.edu/cfs)
2003 Highlights

THERMAL INACTIVATION OF PATHOGENS

HEAT INACTIVATION OF *LISTERIA MONOCYTOGENES*-CONTAINING BIOFILMS
(R. Chmielewski and J. F. Frank)

Recent outbreaks of *Listeria monocytogenes* have been associated with the consumption of contaminated processed meat products and refrigerated products and have raised concern that recontamination is occurring during or after processing. Possible sources of recontamination in food processing plants could be due to ineffective cleaning and redeposition of soil especially in stagnant areas such as joints and dead ends. The objective of this research was to develop a predictive model to determine the importance of time and temperature for predicting survival of *Listeria monocytogenes, Pseudomonas,* and *Listeria -Pseudomonas* mixed culture biofilms formed on stainless steel and buna-N rubber coupon surfaces.

Coupons were added to 10% TSB inoculated with 0.1% *Pseudomonas spp M21, L. monocytogenes,* or 1:4 *Pseudomonas spp M21- L. monocytogenes* mixed culture and incubated for 4 h at 25°C . After attachment, coupon surfaces were rinsed with phosphate buffer and transferred to 10% TSB and incubated for 48 h at 25°C. Duplicate coupons were tested for each heating time (1, 3, 5, or 15 min) and temperature (70, 72, 75, or 77°C). Heat treated samples were enumerated using the fraction negative enumeration method. Positive controls were vortexed with glass beads and enumerated using PCA and *Listeria* selective agar The experiment was repeated six times.

Time was the predominant predictive factor for biofilm survival on stainless steel while both temperature and time contributed equally to predicting the survival of biofilm on buna-N rubber. Overall, *Pseudomonas* was more heat resistant than *Listeria* on stainless steel, probably due to its higher initial load. On rubber, *Listeria* in the mixed culture biofilm had the greatest probability of survival. *Pseudomonas* in biofilms on stainless steel has a 16% probability of survival after heat treatment of 77°C for 15 min and 0.04% on buna-N rubber. For *Listeria* in biofilms, the probability of survival is 7% on stainless steel and 0.094% on buna-N while in mixed culture biofilms, the probability of survival of *Listeria* was 0.3% on stainless steel and 0.4% on buna-N rubber.

RADIO-FREQUENCY HEATING OF ALFALFA SEED FOR REDUCING HUMAN PATHOGENS
(S. O. Nelson, C.-Y. Lu, L. R. Beuchat, and M. A. Harrison)

The production of sprouts from alfalfa and other seeds for human consumption is a substantial industry; however, there have been several outbreaks of illness associated with sprouts, and contamination by *Salmonella* and *Escherichia coli* O157:H7 has been identified as the cause. Contaminated seed used for sprouting is considered the most likely source of these human pathogens. No sprout-related illness attributable to *Listeria monocytogenes* has yet been documented, but this pathogen also poses a potential threat. Because most of the outbreaks of infections have been attributed to contaminated sprouting seed, several methods have been studied for decontaminating seed. Treatment of alfalfa seed in hot water at 54°C significantly reduced seed viability. Several aqueous solutions of chemicals, including chlorine, chlorine dioxide, hydrogen peroxide, trisodium phosphate, ethanol, peracetic acid, and some commercial fruit and vegetable produce wash solutions have been studied for decontaminating alfalfa seed. None of these treatments eliminated *E. coli* O157:H7 or *Salmonella* from alfalfa seed intended for sprouting.

Earlier research has shown that radio-frequency (RF) and microwave dielectric heating treatments are effective for increasing the germination percentage of alfalfa seed lots containing high percentages of hard seed. Hard seeds occur naturally and are viable seeds with seed coats that are impermeable to water. Therefore, they will not germinate promptly when planted, but they may germinate several weeks, months, or years later when the seed coat becomes permeable through natural processes. Mechanical scarification of such seed lots to increase germination is common practice for alfalfa, but the abrasive process scratches the seed coat thus providing a favorable environment for bacterial attachment, which may make sanitization with liquids more difficult. Thus, it appeared reasonable to explore the possible use of dielectric heating for reduction of bacterial populations on alfalfa seed, especially since the improvement of germination and subsequent sprout yield can be achieved without mechanical abrasion of the seed coat. Similar consistent increases in alfalfa seed germination through hard seed reduction have been achieved by dielectric heating at frequencies of 5, 10, 39, or 2,450 MHz. Treatment at 39 MHz was selected

for this study because of equipment availability and because it provides a more uniform electric field for exposure of the samples than is commonly available in microwave ovens.

The potential for controlling human bacterial pathogens on alfalfa seed used in the production of sprouts by dielectric heating was studied by experimental exposure of alfalfa seed artificially contaminated with *Salmonella, E. coli* O157:H7, and *L. monocytogenes* to RF dielectric heating treatments at 39 MHz and different electric field intensities for varying time of exposure. Moisture content of alfalfa seed and final temperatures produced by the RF exposures were determined, and control and treated seed samples were analyzed in the laboratory for reduction of bacterial populations and effects on seed germination. Significant reductions in populations of all three pathogens were achieved without reductions in seed germination. However, exposures that provided substantial reductions in pathogen levels were not achieved without significant damage to seed germination. Treatments providing moderate reductions in bacterial pathogen populations also increased alfalfa seed germination through reductions in hard seed percentages, so the combined benefits need to be considered in evaluating dielectric heating treatments for practical use.