51st International Congress of Meat Science and Technology
August 7-12, 2005 – Baltimore, Maryland USA

DEVELOPMENT OF A METHOD TO MONITOR REAL-TIME THERMAL INACTIVATION OF PATHOGENS IN MEAT AND POULTRY PRODUCTS

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Introduction

This work is part of an overall research program, which is aimed at developing information and tools to ensure/improve the safety of meat and poultry products. Previous and current work by our research team (Warsow et al. 2003) has demonstrated the potential for contamination in marinated turkey breast when *Salmonella* penetrates intact, whole muscle during vacuum tumbling marination. Moreover, once *Salmonella* gained access to the inner portions of the muscle, we observed an enhanced thermal resistance as compared to the ground product (Orta-Ramirez et al. 2005). Although our previous work was designed to evaluate the degree of penetration and to quantify the effects of product structure (whole-muscle vs. ground) on thermal resistance of *Salmonella*, it did not evaluate "how" and "why" these phenomena occur. In order to develop the most effective intervention approach, we need to elucidate the mechanisms of pathogen penetration and enhanced resistance in these marinated whole-muscle products.

Pathogen migration into intact, whole-muscle products and the thermal resistance of these pathogens are clearly relevant to a wide and growing market sector of marinated products, ranging from value-added fresh retail products to ready-to-eat foodservice products. If pathogens are indeed able to migrate into vacuum marinated products and consequently exhibit enhanced thermal resistance, then the ramifications might be significant; the common assumption that intact products have pathogen-free interiors undoubtedly affects attitudes and behaviors associated with preparing these products for consumption. In fact, the Food Safety Inspection Service (FSIS) has distinguished between intact beef cuts (e.g., steaks, roasts) and non-intact products that have been "…injected with solutions, mechanically tenderized by needling, cubing, or pounding devices, or reconstructed into formed entrees," when establishing policies regarding *E. coli* O157:H7 contamination (FSIS, 1999a). In doing so, FSIS also stated (FSIS, 1999a), "In these intact cuts the interior remains protected from pathogens that may exist on the exterior. It is highly unlikely that pathogens would migrate below the surface." Although this is clearly a widely accepted assertion, our preliminary work has indicated that pathogens can indeed migrate into the interior of intact products, particularly if the product is subjected to vacuum tumbling.

With regards to thermal resistance of pathogens, FSIS recently established lethality performance standards for ready-to-eat (RTE), whole-muscle meat and poultry product (FSIS, 1999b), and has proposed to extend these standards to all RTE meat and poultry products (FSIS, 2001). These regulatory standards essentially state that a process must achieve a 6.5-$\log_{10}$ or 7.0-$\log_{10}$ reduction in *Salmonella* for meat and poultry products, respectively. Processors are no longer held to specific endpoint temperatures; however, they "must validate new or altered process schedules by

scientifically supportable means" (FSIS, 1999b). This new regulatory paradigm puts significant pressure on the industry to document process lethality for any new product or process.

This problem is also economically important, given that the meat and poultry industry is the largest component of U.S. agriculture, contributing over $116 billion in annual sales to the GNP (AMI, 2003). Consumer trends for RTE products suggest continued rapid growth in this particular category (Russell, 2002), which includes a lot of marinated products. Therefore, given the economic importance of RTE products, there is an urgent need for information and tools that will enable the industry to design and operate value-added processes that ensure the safety of marinated, whole-muscle products.

Objectives

Based on previous work and preliminary results, our hypothesis is that *Salmonella* cells follow a non-random penetration pathway and exhibit preferential attachment within whole muscle after marination, which results in greater heat resistance than in ground products during thermal processing. To test this hypothesis, the overall goal of our research is to document, at the microscopic level, the penetration, attachment, location, and distribution of *Salmonella* within the muscle after marination as well as the thermal inactivation using confocal laser scanning microscopy (CLSM). The specific objectives are:

1. To determine the relationship between bacterial thermal inactivation and fluorescence loss using fluorescent-labeled bacterial strains
2. To test a custom-designed/built heating apparatus that can be operated under the confocal microscope to visualize thermal inactivation of bacteria using CLSM

The overall novelty of this project is our plan to visualize the destruction of pathogens in whole-muscle products, during thermal processing, by using a powerful microscopic technique. This project will make a unique contribution to the literature and to the safety of whole-muscle meat and poultry products, as this is the first project (to our knowledge) to study the thermal inactivation of bacteria on a cell-by-cell basis.

Methodology

Bacterial Cultures

Although the ultimate target of study is *Salmonella*, for the development of this technique we used Green Fluorescent Protein (GFP)-labeled *E coli* O157:H7 that was readily available to us. GFP-transformed *E. coli* O157:H7 E318 (GFP-ECO157:H7) was obtained from Mansel Griffith, University of Guelph, Ontario, Canada. The stock culture was maintained at -80ºC in water with 10% glycerol (Seo and Frank 1999). Cultures were activated by streaking on tryptic soy agar (TSA) containing 100 μL ampicillin per mL (TSA-amp) and incubation at 37ºC for 24 h. To prepare the *E. coli* suspension for inoculation, 10-mL sterile deionized water was added to each of two TSA-amp plates and colonies were disrupted with a sterile bent glass rod. Culture controls for CSLM were prepared by building a round well of petroleum jelly on a clean microscopy slide with a syringe, adding 0.5 mL of bacterial suspension, then

positioning a coverslip with enough pressure to adhere to the petroleum jelly, therefore sealing the sample.

Meat Preparation

Turkey breasts and beef roasts were purchased from a local processor. Muscle-to-muscle variability should be minimal with these cuts and we assume animal-to-animal variability will not influence muscle structure. Immediately after receiving the meat, it was trimmed of excess fat, cut into appropriate sized roasts, vacuum packaged, and rapidly blast frozen (-28ºC) at the Michigan State University pilot meat processing facility. A frozen roast or breast was partially thawed and one core was aseptically removed (cross section) using a sterile stainless steel corer (1 cm diameter). With a sterile knife, we cut slices (1-2 mm), mounted them on 2.5 cm diameter plastic dishes (Decagon, Pullman, WA) and dispensed petroleum jelly around the outer edges of the sample with a syringe. GFP-ECO157:H7 suspension (0.5. mL) was added to the slice and a coverslip was positioned to seal the sample. All mounted samples were placed in Petri dishes to provide additional containment while examined with CSLM.

Confocal Scanning Laser Microscopy

Samples were observed using a LSM 5 Pascal (Carl Zeiss, Inc., Thornwood, NY) under a 40x dry objective with an excitation wavelength of 488 nm. Random locations from each slice were sampled for penetration and attachment and to avoid photobleaching, no more than 5 fields per location were examined (Vodovotz et al. 1996, Prachaiyo and McLandsborough 2000).

Results & Discussion

Visualization of GFP-E. coliO157:H7 in Inoculated Whole-Muscle Turkey Breast and Beef Roast

GFP-expressing *E. coli*O157:H7 in deionized water (culture controls) were easily visualized as fluorescent rods (Fig. 1). CSLM images of inoculated turkey muscle before and after excitation at 488 nm (Fig. 2A and B, respectively) allowed visualization of certain muscle structures (muscle fibers, fat globules) while bacterial cells were easily recognizable.

However, in order to quantitatively describe the relationship between tissue structures and the location of bacteria within the muscle, it is necessary to more objectively analyze the images. Therefore, we applied several image enhancement tools to demonstrate that we could utilize these types of images to achieve the objectives of the proposed project. First, Gaussian and smoothing (3x3) filters were applied to the non-excited image (Fig. 2C), in order to highlight the image features associated with the interfaces between muscle fibers, and eliminate extraneous background information (such as the out-of-focus regions that show up as large black dots in Fig. 2A but are eliminated in Fig. 2C). The image processing results in Fig. 2C have not been optimized, in terms of achieving the maximum differentiation of image features; however, the preliminary results clearly show that the tissue features can be enhanced, relative to the original image. Subsequently, the excited image (Fig. 2B) was subjected to a basic thresh holding process in the green color band and then laid over the unexcited image to yield Fig. 2D, which shows that clusters of the bacteria

are clearly “aligned” near the tissue features highlighted in Fig. 2C, rather than being randomly distributed across the sample. The purpose of these images is to demonstrate that basic image processing techniques can be used to enhance the utility of the microscopy results. Once the image processing technique has been optimized, it will be possible to conduct statistical analyses of the image files (which are just matrices of numerical information) in order to determine a correlation between muscle fiber interfaces and location of the bacteria.

Thermal inactivation experiments

A unique, custom heating apparatus has been designed and built (and it is currently under testing) to hold the small sample “discs” in place on the microscopy stage (Fig. 3). The heating apparatus consist of a heating element (~3 cm diameter) placed on a 100 x 20 mm glass Petri dish. The electrodes connect to this heating pad through holes on the dish wall, and a fine-gauge thermocouple is also placed through the side of the Petri dish to maintain accurate readings of the sample temperature. The electrodes are attached to a power source via a PID electronic controller. The control is programmed as a variable power source, so that the temperature can be reached at different time periods and then can be held at the target temperature. When the sample is placed inside the Petri dish, the top can then be placed over to contain the inoculated sample. This apparatus enables precise control of sample temperature (±0.5ºC), as the sample is heated and images are collected.

Because “macroscopic” isothermal inactivation tests are being conducted in a separate leveraging study, this heating unit is not designed to generate data for estimating thermal inactivation parameters. Rather, the primary goal is to observe, document, and quantify any effect of bacterial location on the time-to-inactivation. Therefore, the computer program is designed to produce non-isothermal heat treatments, consisting of a linear increase in temperature of ~8ºC/min and three endpoint/holding temperatures (55, 60 and 65ºC). This apparatus is currently fully operational, and experiments are being conducted to determine the relationship between bacterial thermal inactivation and fluorescence loss of GFP-labeled bacterial strains during cooking.

Conclusions

The results of this experiment confirm CSLM as a powerful technique to study the interaction of bacteria and muscle structural components during thermal inactivation. Only viable bacteria are visible on the microscopy images, thus, by heating an inoculated meat sample in place on the microscope stage, and collecting a time series of images without moving the sample, this method will be able to track when individual cells cease to fluoresce and are therefore inactivated. Overall, utilization of the new apparatus will provide fundamental information impacting pathogen penetration and enhanced thermal resistance in marinated meat and poultry products, with these findings eventually leading to a series of practical intervention strategies that can be applied in industry.

References

AMI. 2003. Meat & Poultry Facts. American Meat Institute. Watt Publishing, Mt. Morris, IL.

FSIS. 1999a. FSIS policy on non-intact raw beef products contaminated with E. coli O157:H7. Backgrounder, available at http://www.fsis.usda.gov/OA/background/O157policy.htm.

FSIS. 1999b. Performance standards for the production of certain meat and poultry products. Federal Register. 64(3):732-749. Food Safety Inspection Service, U.S. Department of Agriculture, Washington, DC.

FSIS. 2001. Performance standards for the production of processed meat and poultry products; proposed rule. Federal Register. 66(39):12590-12636 Food Safety Inspection Service, U.S. Department of Agriculture, Washington, DC.

Orta-Ramirez, A., Marks, B.P., Warsow, C.R., Booren, A.M. and Ryser, E.T. 2005. Enhanced thermal resistance of Salmonella in whole muscle vs. ground Beef. Submitted to the Journal of Food Science

Pearson, A.M. and Dutson, T.R.(eds). 1987. Advances in Meat Research. Vol. 3. Van Nostrand Reinhold Company Inc. New York, NY.

Prachaiyo, P.,P., and McLandsborough, L.A. 2000. A microscopic method to visualize Escherichia coli interaction with beef muscle. J. Food Prot. 63:427-433.

Russell, J. 2002. Annual Deli Report: Fresh Start. The National Provisioner. 216(7):14.

Seo, K.H., and Frank, J.F. 1999. Attachment of Escherichia coli O157:H7 to lettuce leaf surface and bacterial viability in response to chlorine treatment as demonstrated by using confocal scanning laser microscopy. J. Food Prot. 62:3-9.

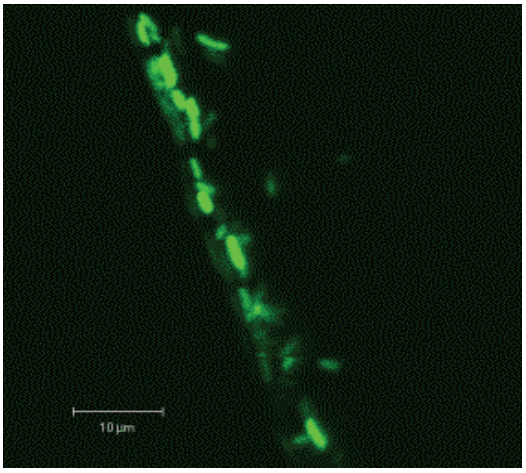
Vodovotz, Y., Vittadini, E., Coupland, J., McClements, D.J., and Chinachoti, P. 1996. Bridging the gap: Use of confocal microscopy in food research. Food Technol. 50:74-82.

Warsow, C.R., Orta-Ramirez, A., Marks, B.P., Ryser, E.T, and Booren, A.M. 2003. Effects of vacuum tumbling on Salmonella migration into the interior of intact, marinated turkey breasts. Abstracts of the Institute of Food Technologists Annual Meeting, Abstract No. 76-F22.

Acknowledgements

This material is based upon work supported by the Cooperative State Research, Education, and Extension Service, U.S. Department of Agriculture under Agreement 2003-35201-13796, and by the Michigan Agricultural Experiment Station.

Tables and Figures



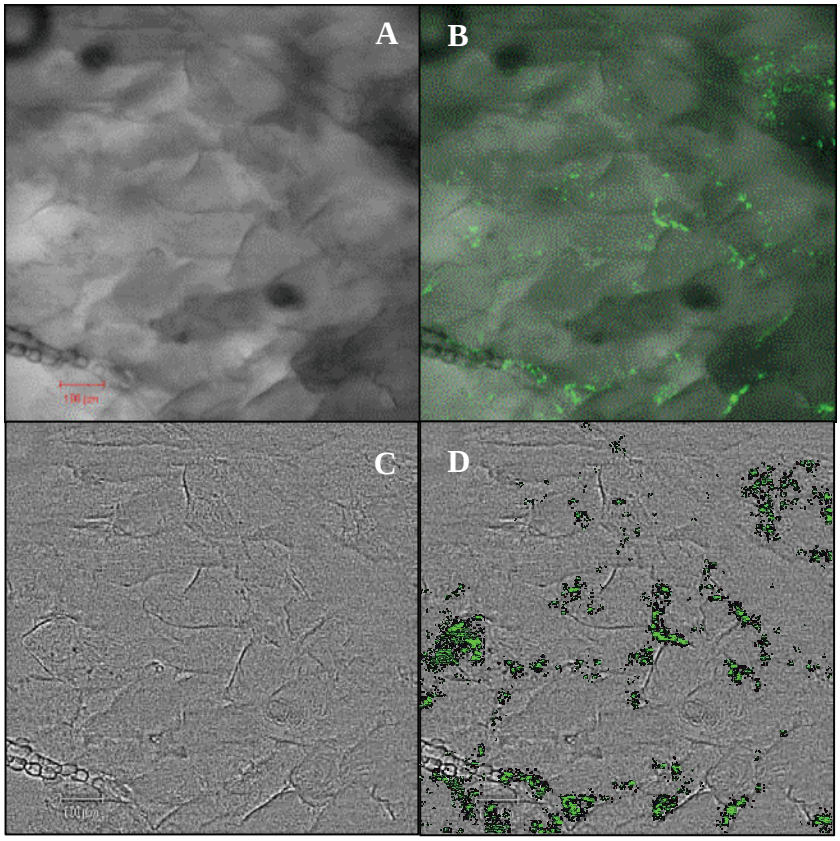


Figure 2. CSLM complementary images of surface-inoculated turkey breast before (A) and after (B) excitation at 488 nm, and after image enhancement of (C) the transmitted image and (D) the excited image. GFP-transformed E. coli O157H7 are colored fluorescent green.

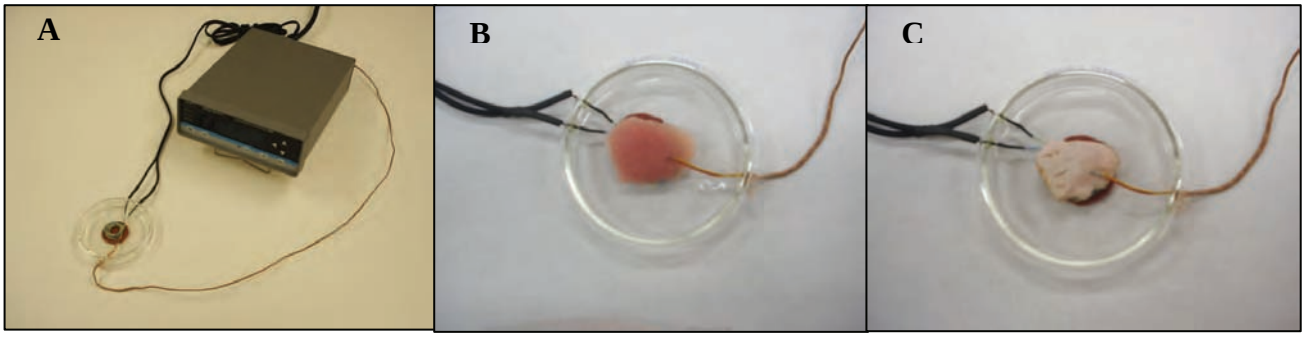


Figure 3. A custom-built apparatus allows cooking of meat samples under the microscope: (A) overview of heating unit and controller, (B) the raw meat is placed on the heating pad, and (C) the meat is cooked under contained conditions