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

LOCATION OF SALMONELLA SPP. IN WHOLE-TURKEY MUSCLE AFTER MARINATION

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Key Words: marination, turkey muscle, *Salmonella*, transmission electron microscopy

Introduction

Poultry marketing in the U.S. has changed considerably in the past 20 years, and poultry products are now the second most consumed animal-based protein behind pork (Roenigk 1999). Consumer trends show an increasing demand for ready-to-eat (RTE) products, which include marinated whole-muscle products (Russell, 2002). Value-added processing, such as marination, is frequently used to improve the quality of whole-muscle meat and poultry products.

The processes of marination and mechanical tenderization are used throughout the meat industry to increase palatability of lesser quality cuts of meat (Johnston 1978). Methods implicated for meat marination/tenderization include blade tenderization, vacuum tumbling, and needle injection. One of the concerns that arise with these procedures is the introduction of bacterial pathogens from the meat surface into the interior of value-added meat products (Phebus et al., 1999). In addition, when internalized, these bacteria may exhibit enhanced thermal resistance, which depends upon various factors, including meat species, muscle type, pH, fat content, and additives (Orta-Ramirez et al., 2003). Warsow (2003) concluded that bacteria could penetrate into intact muscle with or without the aid of vacuum during marination.

Salt and phosphates, which induce changes in muscle structure, are commonly incorporated in marinades to increase product yield and palatability. As the marinade penetrates into the interior of the muscles, it may act as a vehicle for microbial contamination. Water in the marinade may contribute to bacterial penetration by increasing the water content between muscle fibers and therefore increasing penetration (Thomas et al., 1987). Phosphates in the marinade could also contribute to an increase water absorption. Pyrophosphates act as a fluidizing agent in muscle, dissociating actin and myosin, which leads to increased water uptake (Xiong and Kupski 1999).

Salmonella is responsible for an estimated 1.4 million cases of foodborne illness each year in the U.S. *Salmonella* is the second most common cause of foodborne illness behind *Campylobacter*, both of which are commonly found in poultry products (CDC, 2001). According to the Food Safety Inspection Service, raw turkey is a common source of *Salmonella* in the U.S. food supply (FSIS, 2002a). About 30% of ground turkey sampled from July 1999 to June 2000 was positive for *Salmonella* (FSIS 2002b). An infective dose can be as little as 10 to 100 cells in susceptible persons leading to symptoms of salmonellosis (nausea, abdominal cramps, diarrhea and vomiting).

Therefore, the overall goal of this research was to improve the safety of marinated poultry products. In order to observe bacterial penetration during marination process,

the effects of marinade composition on muscle microstructure need to be studied. Transmission electron microscopy (TEM) was used to observe changes in muscle structure and also the attachment of *Salmonella* spp. after marination.

Hypothesis

Marination of turkey muscle with a salt, phosphate marinade containing an 8-strain *Salmonella* cocktail results in penetration of *Salmonella* into the intact muscle.

Objectives

Determine the location of *Salmonella* in whole-muscle turkey, with respect to tissue structure, after marination with a *Salmonella*-inoculated marinade.

Specific aims: 1) To document changes in the turkey muscle microstructure after marination and 2) To view the position of *Salmonella* in marinated turkey muscle using TEM

Methodology

Marinade preparation

The marinade solution contained 95.8% water (filtered and deionized), 3.2% NaCl, and 1% mixed phosphate solution (w/w). Salt was incorporated into the water before adding the phosphate solution, in order to ensure total dispersal. Aliquots (525 mL) of marinade were poured into glass bottles with plastic screw caps and autoclaved for 15 min at 121 °C to ensure sterility.

Inoculum preparation

The *Salmonella* cultures were propagated by transferring frozen culture to 9 mL of tryptic soy broth (TSB). The strains were maintained separately by transfer to fresh TSB followed by 24 hr of incubation at 37°C. On the day of experiment, the cultures were combined and centrifuged at 6000 x g for 20 min at 4°C. The supernatant was removed and the bacterial pellet was resuspended in marinade to give a final concentration of ~10^8 CFU/mL.

Turkey preparation

Frozen turkey breasts (whole, intact, irradiated) were thawed 24 hr at 4°C. A hand-coring device was used to aseptically remove cores from the turkey breast (2-3 cores/treatment) aseptically. Cores were submerged in treatments including water, marinade, and an 8-strain *Salmonella*-inoculated marinade for 20 min at 4 °C. Treated cores were then removed from the treatments placed in Petri dishes and sectioned into smaller pieces measuring approximately 5 x 5 x 2 mm (W x L x H). Samples were then processed for electron microscopy.

Sample preparation for bright-field and transmission electron microscopy

Samples for bright field and electron microscopy were fixed in a mixture of 2.5% glutaraldehyde and 2.0% paraformaldehyde in 0.1 M cacodylate buffer (pH 7.4) overnight at 4°C. After fixation, samples were washed with cacodylate buffer and postfixed with 2% osmium tetroxide and dehydrated using a graded series of acetone and embedded in Poly/Bed 812 resin. Thin sections were obtained with a diamond knife (90-110 nm thickness) on a Power Tome XL ultramicrotome (RMC). Uranyl acetate (2% in 50% ethanol) and lead citrate (Reynolds formula) were used as a positive stain for the thin sections. After staining, turkey muscle samples were observed under a transmission electron microscope, JEOL 100CX, at an accelerating voltage of 100 kV.

For bright-field microscopy, in order to determine the location of the bacteria in the muscle, thick sections (~ 500 nm thickness) were cut using a glass knife and stained with 1% toluidine blue. Bright-field transmitted images were taken with a Zeiss LSM5 Pascal microscope using a 633 nm laser.

Results & Discussion

The ultrastructure of whole turkey breast muscle subjected to marinade provides an initial view of the marination effects on turkey muscle structure. The changes of muscle structure after each treatment were observed using TEM. Figures 1 and 2 illustrate representative micrographs of turkey samples that were subjected to water and marinade treatments, respectively. The TEM images of the longitudinal section of muscle fibers indicate greater fiber size (width) in the marinated sample, compared to the water-treated sample. The changes in muscle fiber size of marinated turkey samples may be due to the contribution of salt and phosphate incorporated in the marinade solution. Offer and Trinick (1983) explained that increased moisture retention ability by phosphates is achieved through muscle fiber expansion (swelling) caused by electrostatic repulsions, which allows more water to be immobilized in the myofibril lattices. The action of phosphates in improving water holding capacity (WHC) appears to be twofold: (1) raising the pH; and (2) causing an unfolding of muscle proteins, consequently making more sites available for water binding (Pearson and Gillett 1999). In addition, the combination of salt and phosphate in marinade recipes has the primary function of WHC.

TEM is also a useful method to locate *Salmonella* attached inside turkey muscle. Figure 3 is a transmission electron micrograph of a longitudinal section of whole muscle turkey breast subjected to *Salmonella*-inoculated marinade for 20 min at 4°C. We observe the attachment of *Salmonella* to be parallel to the orientation of the muscle fibers. This result is also supported by Gill and Penney (1977), who indicated that *Salmonella* likely penetrated the tissue between muscle fibers. Thomas and others (1987) described changes in poultry muscle post-slaughter where gaps between muscle fibers are created by radial shrinkage of the muscle fibers due to increases in muscle osmolality from lactic acid formation post-slaughter. Water in the marinade may contribute to bacterial penetration as the water content between muscle fibers increases and therefore increases penetration of the *Salmonella*.

In order to obtain an overall view of the bulk tissue samples for bacterial penetration/attachment, bright-field transmitted images of longitudinal and cross sections of turkey muscle bundles were also taken from thick sections (Figures 4 and 5). Turkey samples were subjected to the *Salmonella*-inoculated marinade treatment

for 20 min at 4°C. The images indicate where *Salmonella* are likely to attach between turkey muscle bundles.

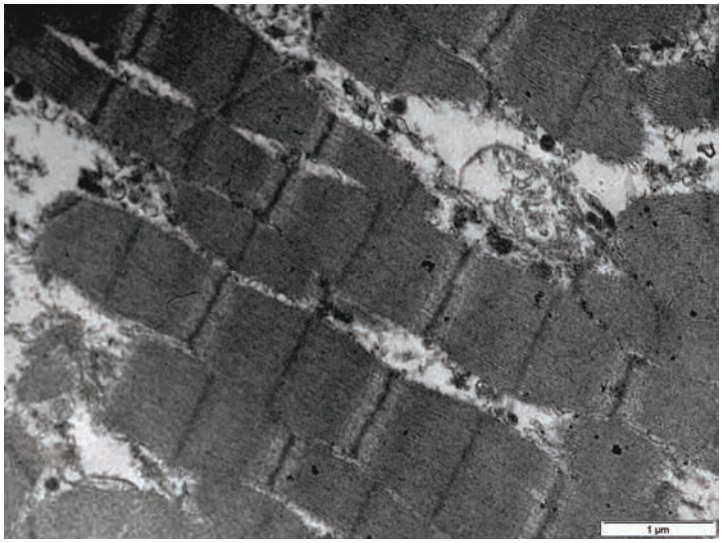

Figure 1. Transmission electron micrograph of a longitudinal section of whole muscle turkey breast subjected to water-only marination for 20 min at 4°C. Magnification 20,000x; bar indicates 1 µm.

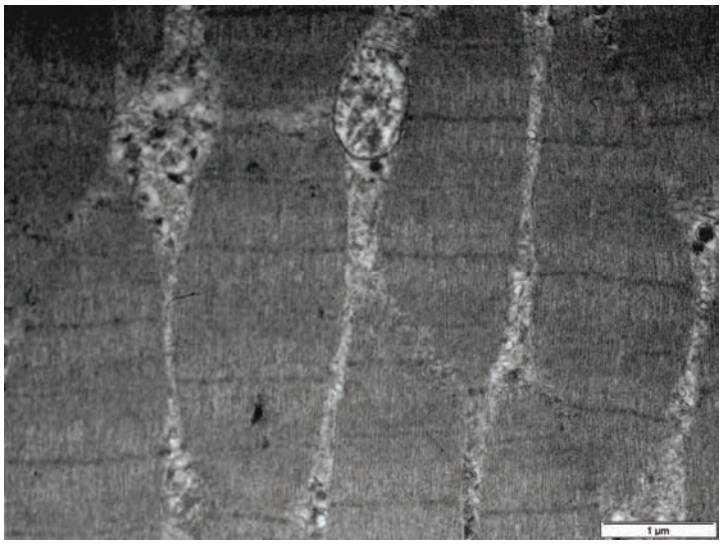

Figure 2. Transmission electron micrograph of a longitudinal section of whole muscle turkey breast subjected to marination treatment for 20 min at 4°C. Magnification 20,000x; bar indicates 1 µm.

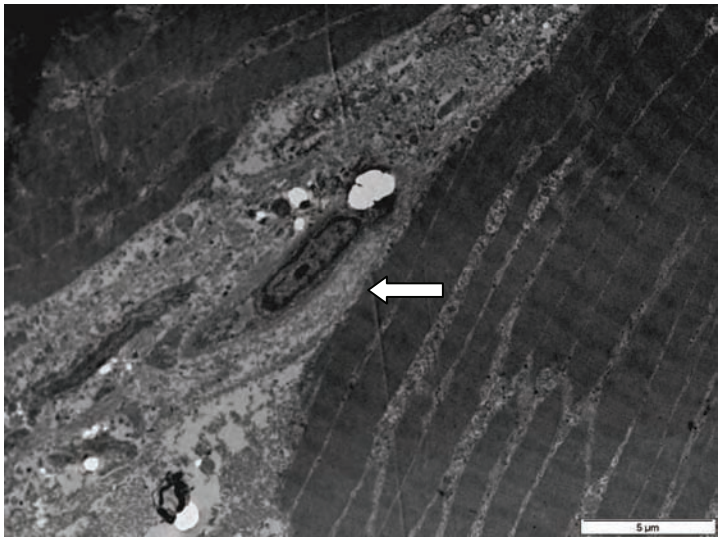

Figure 3. Transmission electron micrograph of longitudinal section of whole muscle turkey breast subjected to *Salmonella*-inoculated marinade (with salt and phosphate) treatment for 20 min at 4°C. Magnification 5,000x; bar indicates 5 µm. The arrow points to *Salmonella*, which has penetrated the muscle.

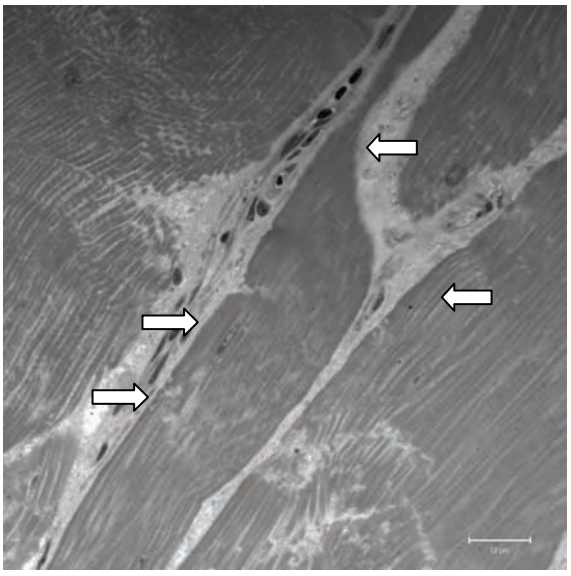

Figure 4. Bright-field transmitted image of longitudinal section of turkey muscle bundles subjected to *Salmonella*-inoculated marinade treatment for 20 min at 4°C. Bar indicates 10 µm. The arrows point to *Salmonella*, which has penetrated the muscle.

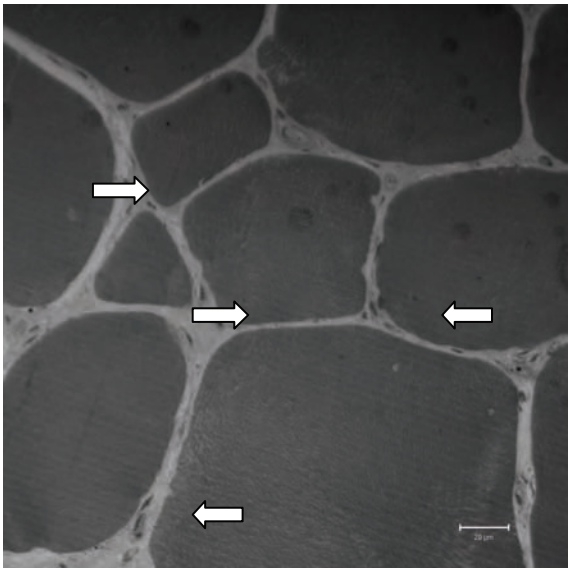

Figure 5. Bright-field transmitted image of cross section of turkey muscle bundles subjected to *Salmonella*-inoculated marinade treatment for 20 min at 4°C. Bar indicates 20 μm. The arrows point to *Salmonella*, which has penetrated the muscle.

Conclusions

The TEM technique conducted in this study provides a way to observe marinade absorption and *Salmonella* attachment in turkey at the microscopic level. *Salmonella* present in inoculated marinade migrated into the interior of intact, whole-muscle turkey breast during marination. Marinade composition, especially salt and phosphate, was a significant factor contributing to marinade penetration and absorption. Pathogen migration into whole muscle meat products may be characterized by the increase in water uptake and may be a function of the numbers of *Salmonella* present. As a result, the cooking times and temperatures currently recommended for pathogen inactivation, especially for marinated value-added meat products, may need to be reevaluated.

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. We are also grateful for support from the Michigan Agricultural Experiment Station.

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