RESEARCH REPORT
PORK SAFETY

Title: Microbiological Risk Factors with Moisture Enhanced Fresh Pork – **NPB #06-029**

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II. Industry Summary:

Fresh pork injected with brines or marinades have become very popular with the consumer. According to a 2004 nationwide retail survey (Anonymous, 2004), 45% of the retail fresh pork in the United States was labeled as “enhanced”, with enhanced products defined as “moisture added and could also be value added (flavored and/or contained additional ingredients”. The research in this report addresses two issues in moisture enhanced pork: the potential for cross-contamination between individual cuts of pork (either through equipment or through contaminated brine, and the survival of foodborne pathogens in re-circulating brines.

Pork loins were intentionally contaminated with *Escherichia coli* and then were moisture enhanced to a 10% level. The moisture enhancement process transferred bacteria from the surface of the meat to the interior of the meat. In addition, bacteria were transferred from the meat to both the injector needles and to the re-circulating brine. When non-inoculated loins were moisture enhanced immediately after processing a single inoculated loin, *E. coli* were recovered from the non-inoculated loins in populations comparable to those found on the original inoculated loin. Clearly. a single contaminated loin has the potential to cross-contaminate other loins, primarily through contamination of the equipment and the re-circulating brine.

To evaluate the survival of bacteria in the re-circulating brines, *Salmonella* and *Campylobacter* were inoculated into a re-circulating brine, and the populations were monitored over 48 hours. Although the populations of both bacteria declined, both survived for 24 hours. This emphasizes the potential for cross contamination attributable to the re-circulating brine, and suggests that the brines

should be periodically disposed of and the re-circulating systems cleaned to prevent the cross contamination of product.

III. Scientific Abstract:

Pork loins were surface inoculated with *Escherichia coli* Biotype I and moisture enhanced with a needle injector. The moisture enhancement process transferred bacteria from the surface to the interior of the pork loin. In addition, bacteria were transferred from the meat to the injector needles and the re-circulating brine. When additional, non-inoculated loins were processed on the same equipment without cleaning, the *E. coli* bacteria were recovered from both the surface and interior tissue of the meat. Additional experiments evaluated the survival of *Salmonella enterica* and *Campylobacter jejuni* in re-circulating brines, and found that both bacteria survived for at least 24 h at 4^{o}C.

IV. Introduction:

Moisture enhanced fresh pork is a category of product whose popularity is steadily increasing. Pork is moisture enhanced for a variety of reasons, including improved tenderness, moisture, flavor and consumer convenience. Moisture enhanced pork provides the consumer with a more desirable product, while increasing profitability and sales of branded product for the processor.

Although moisture enhanced products have been available for some time, relatively little is available within the public domain regarding the microbiological properties of the product. In a survey of retail pork conducted in 2000, enhanced pork products were not statistically different from pork packaged in the store on almost every category of microbiological analysis (Duffy et al., 2001). The only notable difference was that the incidence of *Yersinia* spp. was statistically lower in the moisture enhanced products than in the store-packaged products, although this could not be viewed as a cause and effect relationship. In another study, although moisture enhanced pork had higher microbiological populations than non-enhanced products, the shelf life was equivalent between the two categories of products (Bohaychuk and Greer, 2003)

The moisture enhancing solution (brine or marinade) is re-circulated during production of the products. During re-circulation, the populations of microorganisms in the solution increases. Greer et al., (2004) noted increases in most categories of microorganisms during the first hour of re-circulation in a commercial processing operation, but also noted that the populations tended to remain relatively

constant after that to two and ½ hours. These authors also reported a significant increase in *L. monocytogenes* during the sampling time of the study, although the significance of this bacterium in a fresh un-cooked product is open to discussion.

There has been a developing interest in this process by USDA-FSIS during the last year, in part due to the relative lack of information on the subject. Recently, USDA-FSIS began a sampling program to analyze re-circulating brines from processing establishments throughout the United States (Carl Custer, USDA-FSIS, Personal communication). In addition to the specific concern about the re-circulating brines, there are also some "crossover" issues with this process and the mechanical tenderization process commonly used with beef (Sporing, 1999; USDA-FSIS 2002). Specifically, the issues include the potential transfer of bacteria from the surface to the interior of the meat, as well as the potential for cross contamination between cuts of meat.

V. Objectives:

1. Determine the potential for cross contamination with needle injected brine in fresh, uncured pork.
2. Determine the survival of *Campylobacter jejuni* and *Salmonella enterica* in simulated brines used for fresh, uncured pork.

VI. Materials & Methods:

Cross Contamination of Pork Loins:

Bacterial Cultures: A mixed culture of five strains of non-pathogenic, *Escherichia coli* Biotype I was prepared. The strains were cultured separately in tryptic soy broth (TSB) at 37^{o}C, and then mixed in equal volumes. The strains were previously characterized and identified as be suitable surrogates for both *Escherichia coli* O157:H7 (Marshall et al., 2005)and *Salmonella enterica* (Niebuhr et al., 2008).

Inoculation and processing of pork loins: The mixed culture was inoculated on to the surface of whole boneless pork loins at a target population of 10^6 colony forming units/cm^2 using a foam paint brush. The pork was stored at 4^{o}C and then subjected to a single pass through a needle injector moisture enhancement process, with a target injection of 10% (wt/wt; P-10 Pokomat Injector, Quality Food Equipment, El Monte CA). The brine formulation used in the Iowa State University Meat Laboratory, consisting of 3.04% sodium tripolyphosphate, 2.17% sodium chloride, and water was used.

Experimental Design: A single inoculated pork loin was processed through the injector, and then four non-inoculated loins were processed in the same manner without cleaning the equipment.

Sample analysis: The populations of bacteria were enumerated on both the surface and internally at a depth of approximately 1 cm in the boneless pork at three locations on each pork loin; approximately 6 cm from the leading edge, 6 cm from the trailing edge and in the approximate geometric center of the loin. Samples were collected aseptically using a sterile scalpel and forceps. After the completion of the process, the injector needles were swabbed with sterile sponges to determine the extent of contamination on the equipment. Samples of the re-circulating brine were also collected and analyzed for the presence of *E. coli*. Samples were homogenized 1:10 in sterile buffered peptone water with a Tekmar Stomacher 400 Mk. II for 2 min (Tekmar, Cincinnati, OH). Bacterial populations were enumerated by surface plating on Violet Red Bile Glucose Agar (VRBA) using a Whitely Automated Spiral Plater (Microbiology International, Frederick MD). The plates were incubated at 37^{o}C for 24 hours, and then manually counted.

Survival in re-circulating Brines: The standard brine formulation used in the Iowa State Meat Laboratory was prepared and sterilized. A mixed culture, consisting of five strains of either *Campylobacter jejuni* or *Sallmonella enterica*, was inoculated into the brine, and re-circulated in a model system (see Gailey et al., 2003) at either 4^{o}C. The populations of the surviving bacteria were enumerated at 0,1,2,4,8,16, 24, and 48 hours. Samples of the re-circulating brine were also collected and analyzed for the presence of either *S. enterica* or *C. jejuni*. *S. enterica* populations were enumerated by surface plating on to Xylose Lysine Desoxycholate (XLD) agar. The plates were incubated at 37^{o}C for 24 hours, and colonies showing typical salmonellae morphology were enumerated. *C. jejuni* populations were enumerated by surface plating on to Charcoal Cephoperazone Desoxycholate Agar (CCDA) agar. The plates were incubated at 37^{o}C for 24 hours in a modified atmosphere incubator (5% O_2, 10% CO_2, 85% N_2), and colonies showing typical *Campylobacter* morphology were enumerated.

Statistical analysis: The detection limit of the assay was 10 cfu/g. Samples with undetectable populations were recorded as <10 cfu/g", but for statistical analysis entered as 10. The bacterial populations were transformed to $\log_{10}$ cfu/g, and analyzed using a one way analysis of variance using WINKS SDA Software (Texasoft, Cedar Hill, TX.). The reported results are the average of five replications. Statistical decisions were made at p=0.05 unless otherwise stated. Based on the

population data, mathematical models were developed to predict survival of these bacteria in re-circulating brines.

VII. Results:

Objective 1. Cross Contamination of Pork

Microbiological analysis of both the exterior and interior indicated that the non-inoculated loins were readily contaminated by both the brine and injection equipment, after the inoculated loin was injected (Fig.1). There was no statistically significant difference between locations within each loin, so the data from all locations within each loin were pooled. Further analysis indicated that both the brine and needles were contaminated, and that this contamination was likely the source of the subsequent cross contamination. After injecting the first pork loin, 1 – 2 log cycles of *E. coli* were recovered per needle, while the population in the re-circulating brine was approximately 4.6 log cfu/50 ml.

Objective 2. Survival of Campylobacter jejuni and Salmonella enterica in simulated re-circulating brines

A 5 strain mixture of *Salmonella enterica* was inoculated into a re-circulating brine system at 4^{o}C, and the population monitored over 48 hours (Fig. 2). The bacterial population declined approximately 1.5 log cycles within 8 hours, and a total of 2 log cycles over 48 hours.

A 5 strain mixture of *Campylobacter jejuni* was inoculated into a re-circulating brine system at 4^{o}C, and the population monitored over 48 hours (Fig. 3). The bacterial population declined approximately 1 log cycles within 8 hours, but declined to below the detectable limits of the assay over 48 hours.

The descriptive mathematical model of the *S. enterica* results is:

$$Y = 2.64 + 1.88e^{(-0.221x)} \qquad (1)$$

Where:

$Y = \log_{10}$ colony forming units/ml

And

X = time in hours

The correlation coefficient (r^2) value for this equation is 0.97.

This equation may be generalized as a predictive model as:

$$Y = (Y_0/4.52)(2.64+1.8888e^{(-0.221x)}) \quad (2)$$

Where:

$Y = \log_{10}$ colony forming units/ml

Y_0 = initial population in $\log_{10}$ colony forming units/ml

And

X = time in hours

The descriptive mathematical model of the *C. jejuni* results is:

$$Y = Y_0e^{(-0.037x)} \quad (2)$$

Where:

$Y = \log_{10}$ colony forming units/ml

Y_0 = initial population in $\log_{10}$ colony forming units/ml

And

X = time in hours

The correlation coefficient (r^2) value for this equation is 0.98.

VIII. Discussion:

While it is generally known that meat may be cross contaminated, it was surprising to see the extent of bacterial contamination on the non-inoculated loins. Previous research by Sporing (1999) had suggested that that relatively few bacteria (2-3%) were transferred from inoculated to un-inoculated beef when the beef was mechanically tenderized. In our study we found much higher percentages (~70%) transferred by moisture enhancement. In addition, the deep muscle tissue of non-inoculated was subsequently contaminated by the process.

Based on our data, the contamination was caused by both contaminated injection needles, as well as the contaminated brine. The bacterial populations in the deep muscle tissue of the non-inoculated loins were numerically (but not statistically) higher than those found in the inoculated loin. Moisture enhanced products, immediately after processing, are wet on the surface. Although attempts were made to reduce to transfer of contamination from the surface and brine to the interior muscle, it is likely that at least some of the brine did contaminate the muscle tissue.

The survival of both *S. enterica* and *C. jejuni* in the re-circulating refrigerated brines is consistent with the known biology of these bacteria. The mathematical models suggest that even low levels of contamination could survive for several hours in the brine, emphasizing the need for frequent replacement of the brine. These results indicate the potential for cross contamination by moisture enhancement, and may have implications in the recommended end point cooking temperatures for these products.

IX. References

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Figure 1. Cross contamination of pork loins by moisture enhancement. A pork loin surface inoculated with *E. coli* Biotype I was moisture enhanced, followed by four non-inoculated pork loins.

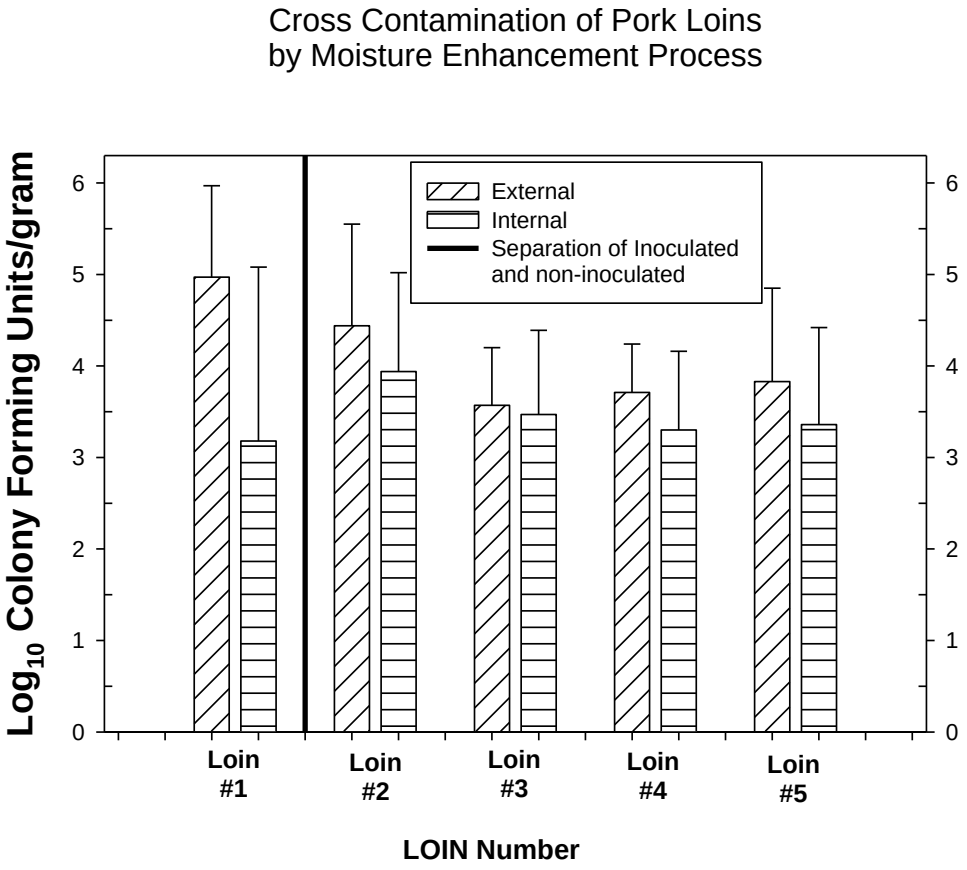

Figure 2. Survival of *Salmonella enterica* in a re-circulating brine at 4°C.

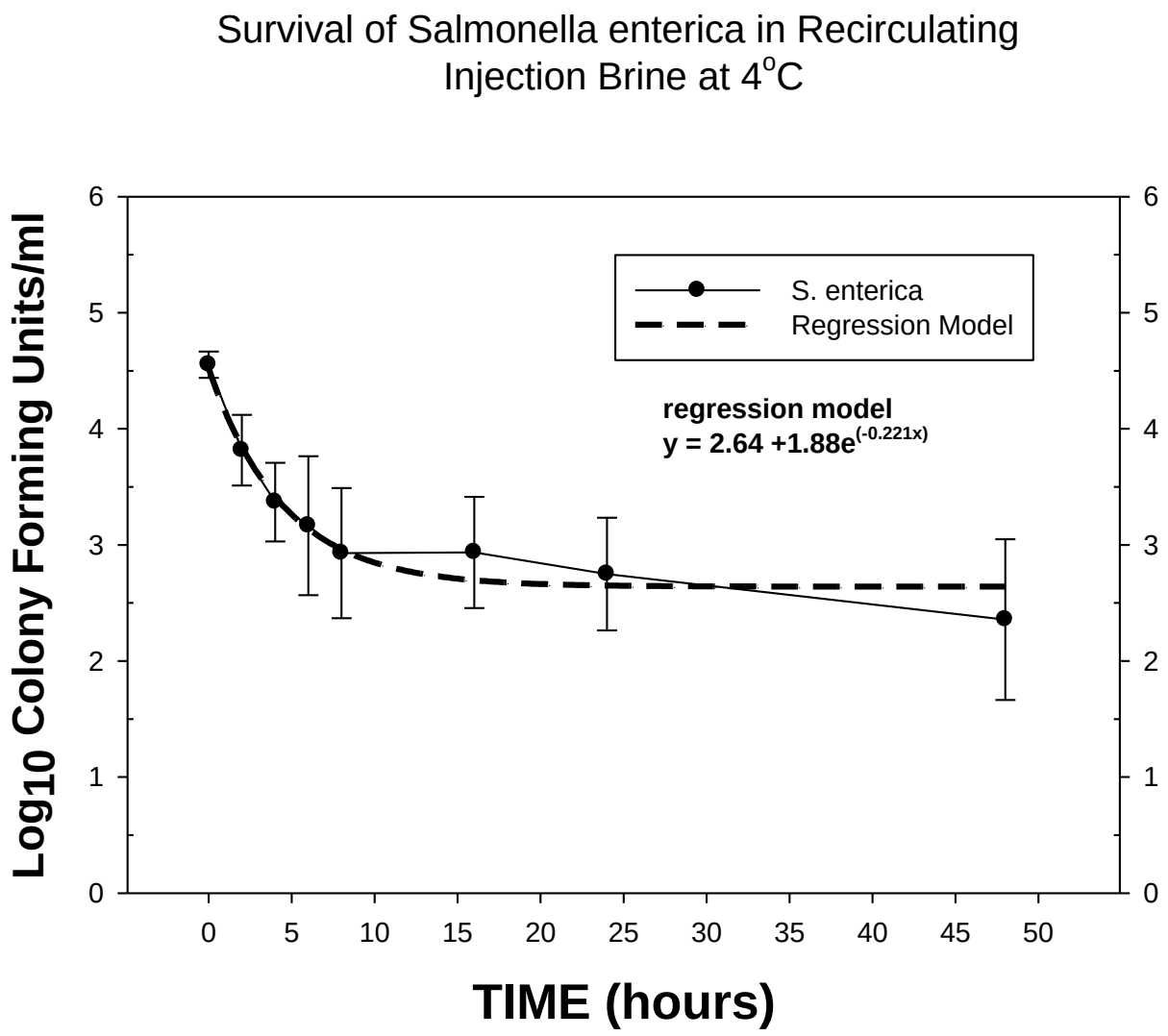

Figure 3. Survival of *Campylobacter jejuni* in a re-circulating brine at 4°C.