EFFECT OF PACKAGING AND STORAGE TIME ON SURVIVAL OF *LISTERIA MONOCYTOGENES* ON SHELF-STABLE MEAT SNACKS

by

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Abstract

The United States Department of Agriculture's Food Safety and Inspection Service require that processors of ready-to-eat (RTE) meat and poultry products implement post-processing intervention strategies for controlling *Listeria monocytogenes.* The objective of our study was to determine the effect of packaging methods and storage time on reducing *L. monocytogenes* in shelf-stable meat snacks. Commercially available kippered beef steak strips (14 × 2.5 cm rectangle piece) and turkey tenders (4 × 4 cm square piece) were dipped into a five-strain *L. monocytogenes* cocktail, and dried at 23°C until a water activity of approximately 0.80 was achieved. Inoculated samples were packaged with four treatments: 1) vacuum, 2) nitrogen flushed with oxygen scavenger, 3) heat sealed with oxygen scavenger, and 4) heat sealed without oxygen scavenger. Samples were stored at 23°C and evaluated for *L. monocytogenes* levels at 0, 24, 48, and 72 h. Initial levels (time 0) of *L. monocytogenes* were approximately 5.7 log CFU/cm^2 for steak and tenders. For kippered beef steak, there was no interaction among packaging treatments and storage times ($P > 0.05$) whereas, storage time was different ($P < 0.05$). A 1 log reduction of *L. monocytogenes* was observed at 24 and 48 h at 23°C for all packaging treatments and a 2.1 log CFU/cm^2 reduction occurred at 72 h. A 1 log CFU/cm^2 reduction of *L. monocytogenes* was observed after 24 h of storage for turkey tenders for all packaging treatments. After 48 h of storage time turkey tenders showed >1 log CFU/cm^2 reduction of *L. monocytogenes* for all packaging treatments except for vacuum packaged where only 0.9 log CFU/cm^2 reduction was observed. Log reductions at 72 h for all packaging treatments for turkey tenders ranged from 1.5 to 2.2. Processors of kippered beef steak and turkey tenders could use vacuum, nitrogen-flushing, or heat sealed with an oxygen scavenger packaging methods and hold product 24 h prior to shipping to reduce potential *L. monocytogenes* numbers by ≥1 log. However, processors should be encouraged to hold packaged product a minimum of 72 h to enhance the margin of safety for *L. monocytogenes* control.

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Chapter 1 - Introduction

Listeria monocytogenes is a food pathogen that has the highest mortality rate of foodborne pathogens (Buzby 2002; Donnelly 2001). In 2002, the Centers for Disease Control and Prevention (CDC) reported that there were about 2,500 cases annually from listeriosis, including 500 deaths and around $200 million in monetary loss in the United States (U.S.) (CDC 2002). Sporadic cases of human listeriosis occur with an annual incidence of approximately 0.27 per 100,000 populations in the U.S. (CDC 2007). In the 1990's, there were a number of foodborne illness outbreaks linked to ground beef products and ready-to-eat (RTE) meat and poultry products due to *L. monocytogenes* cross contamination.

The main concern of the food industry is that *L. monocytogenes* can grow at refrigeration temperatures and is resistant to various environmental conditions, allowing it to survive longer under adverse conditions. Many efforts have been made to eliminate the organism from ready-to-eat foods (RTE) (Tompkin and others 1999; Tompkin 2002). The implementation of the Hazard Analysis Critical Control Points (HACCP) system for food processors has allowed for careful evaluation on how to control, reduce, or eliminate *L. monocytogenes* in processed RTE foods (CFR 2009). The cooking step in the manufacturing of a RTE meat or poultry product is a critical control point (CCP) for reducing or eliminating *L. monocytogenes*. However, post-lethality microbial contamination is a serious concern for processors of RTE meat and poultry products.

The United States Department of Agriculture's Food Safety and Inspection Service (USDA/FSIS) has applied regulations that address *L. monocytogenes* in RTE meat and poultry products. For instance, USDA/FSIS has a zero-tolerance policy for *L. monocytogenes* in RTE meat and poultry products due to the severity of this organism (USDA/FSIS 2000). The USDA/FSIS also requires that processors of RTE meat and poultry products implement post processing intervention strategies for controlling *L. monocytogenes.* The compliance guidelines from USDA/FSIS state that an effective post-lethality treatment must reduce numbers of *L. monocytogenes* by at least one log. The regulation requires that post-lethality treatments must be scientifically validated (USDA/FSIS 2003).

Kippered beef steak and turkey tenders are RTE meat snacks that are exposed after

cooking (post-lethality) to the environment during the cutting and packaging process; this is often when contamination with *L. monocytogenes* can occur (USDA/FSIS 2003). In both of these products reduction of water activity through the addition of salt and thermal processing could serve as an antimicrobial process (Samelis and others 2001, 2005). Packaging also can serve as a post-lethality treatment. Research has shown that vacuum packaging of jerky can generate a 1 log reduction of *L. monocytogenes* following 1 or more weeks of storage at ambient temperature and that *L. monocytogenes* was not able to survive on a jerky product during its shelf life (Ingham and others 2004, 2006ab; CFR 2009).

There is a lack of information in evaluating effectiveness of a post-lethality packaging treatment that will allow products to be held for a shorter time period. Combining modified atmosphere packaging with short-term storage prior to product distribution could be an effective antimicrobial process that would serve as a post-lethality treatment. Therefore, the objective of our study was to determine the effect of four packaging treatments: heat sealed (HS), heat sealed with oxygen scavenger (HSOS), nitrogen flushed with oxygen scavenger (NFOS), and vacuum (VAC) with four ambient temperature storage times: 0, 24, 48, and 72 h on reducing *L. monocytogenes* in kippered beef steak and turkey tenders.

References

Buzby JC. 2002. Economics of foodborne disease: *Listeria monocytogenes*. Available from: http://www.ers.usda.gov/Briefing/FoodborneDisease/listeria/. Accessed July 10, 2007.

[CDC] Centers for Disease Control and Prevention. 2002. Public health dispatch: outbreak of listeriosis – northeastern United States, 2002. MMWR. 51:950-951.

[CDC] Centers for Disease Control and Prevention. 2007. Preliminary FoodNet Data on the Incidence of Infection with Pathogens Transmitted Commonly Through Food --- 10 States, 2007. MMRW. 57:366-370.

[CFR] Code of Federal Regulations. 2009. Control of *Listeria monocytogenes* in post- lethality exposed ready-to-eat products. 9CFR430: 635-637. Available from:

http://www.gpoaccess.gov/CFR/. Accessed December 15, 2009.

Donnelly CW. 2001. *Listeria monocytogenes*. In: Labbé RG, Garcia S, editors. Guide to Foodborne Pathogens. John W& Sons, Inc: New York. p 99-132.

Ingham SC, Buege DR, Dropp BK, Losinski JA. 2004. Survival of *Listeria monocytogenes* during storage of ready-to-eat meat products processed by drying, fermentation, and/or smoking. J Food Prot. 67: 2690-2702.

Ingham SC, Searls G, Buege DR. 2006a. Inhibition of *Salmonella serovars*, *Escherichia coli* O157:H7 and *Listeria monocytogenes* during dry-curing and drying of meat: A case study with basturma. J Food Safety. 26:160-172.

Ingham SC, Searls G, Mohanan S, Buege DR. 2006b. Survival of *Staphylococcus aureus* and *Listeria monocytogenes* on vacuum-packaged beef jerky and related products stored at 21°C. J Food Prot. 69:2263-2267.

Samelis J, Sofos J, Kain J, Scanga M, Belk K, Smith G. 2001. Organic acids and their salts as dipping solutions to control *Listeria monocytogenes* inoculated following processing of sliced pork bologna stored at 4°C in vacuum packages. J Food Prot. 64:1722-1729.

Samelis J, Bedie GK, Sofos JN, Belk KE, Scanga JA, Smith GC. 2005. Combinations of nisin with organic acids or salts to control *Listeria monocytogenes* on sliced pork bologna stored at 4◦C in vacuum packages. LebenWissen Technol 38:21-28.

Tompkin RB. 2002. Control of *Listeria monocytogenes* in the food-processing environment. J Food Prot. 65:709-725.

Tompkin RB, Scott VN,Bernard DT, Sveum WH, Gombas KS. 1999. Guidelines to prevent post-processing contamination from *Listeria monocytogenes*. Dairy, Food and Environ. Sanit. 19:551-562.

[USDA/FSIS] United States Department of Agriculture, Food Safety and Inspection Service. 2000. FSIS strategies for addressing *Listeria monoctyogenes*. Available from: http://www.fsis.usda.gov/OA/background/lmplan.htm. Accessed December 17, 2010.

[USDA/FSIS] United States Department of Agriculture, Food Safety and Inspection Service. 2003. Control of *Listeria monocytogenes* in ready-to-eat meat and poultry products; final rule. Federal Register 68:34207-34254.

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Chapter 2 - Review of Literature

Introduction

Food eating habits in the United States (U.S.) have undergone a drastic change during the last decade. Consumers demand foods that are low in calories, fat, and sodium. Consumers are also demanding microbiologically safe food from processors (Sloan 2001). In the 1990's there were a number of foodborne illness outbreaks linked to ground beef products and ready-to-eat (RTE) meat and poultry products such has hot dogs and deli-meats due to *Listeria monocytogenes* cross contamination (Low and Donachie 1997). There is a great need to understand the survival and growth of *L. monocytogenes* on RTE meat and poultry products.

It is estimated that microbial pathogens in foods are the cause for 6.5 to 33 million cases of human illness and up to 9,000 deaths in the U.S. each year (Buzby 2002). Over 40 different foodborne microbial pathogens, including fungi, viruses, parasites, and bacteria, are believed to cause human illnesses. For six bacterial pathogens, *Campylobacter* species, *Salmonella* species, *Yersinia enterocolitica*, *Escherichia coli*, *Escherichia coli* O157 non-O157 STEC, and *L. monocytogenes*, the costs of human illnesses are estimated to be $9.3 to $12.9 billion annually. Of these costs, $2.9 to $6.7 billion is attributed to foodborne bacteria. To estimate medical costs and productivity losses, United States Department of Agriculture's (USDA) Economic Research Service (ERS) uses four severity categories for acute illnesses: those who did not visit a physician, visited a physician, were hospitalized, or died prematurely. The lifetime consequences of chronic disease are included in the cost estimates for *E. coli* O157:H7 and fetal listeriosis causing *L. monocytogenes.* The ERS estimates that, each year in the U.S., the costs of acute illness from foodborne *Listeria* are $2.3 billion (Buzby and others 1996). *Listeria monocytogenes* is a food pathogen that has the highest mortality rate of foodborne pathogens (Buzby 2002; Donnelly 2001).

Characteristics of *Listeria monocytogenes*

Listeria monocytogenes is widely found in the environment and can gain entrance into a processing plant by way of raw materials, air, and people. *Listeria monocytogenes* exists as a short rod measuring 0.4 to 0.5 μm by 0.5 to 2.0 μm with rounded ends, and is ubiquitous in nature (Seeliger and Jones, 1986). It is a gram positive, facultative aerobe with both psychotropic and mesophillic characteristics. *Listeria* species are found in the intracellular state within monocytes and neutrophils (Gray and Killinger 1966). The bacteria may be curved in single or short chains, often in a "V" shape. *Listeria monocytogenes* is motile due to one to five peritrichous flagella, which may be lost as the bacteria enter the human cell. Movement still is possible because the bacteria polymerize into long actin tails that propel the bacteria through the cytoplasm (Salyers and Whitt 2002). The organism has a growth temperature range of approximately 1 to 45°C (Junttila and others 1988) classifying it as a psychrotroph and mesophile.

However, there are growth factors which are temperature dependent. For example, at 20 to 25°C peritrichous flagella are formed and the organism becomes motile, whereas at 37°C the organism is weakly or non-motile (Galsworthy and others 1990). Additionally, its ability to not only survive but to grow as a psychrotroph at 4°C makes *L. monocytogenes* unique from other commonly found foodborne pathogens which are usually inhibited from growth at refrigeration temperatures.

Regulation of *Listeria monocytogenes*

The contamination by *L. monocytogenes* in RTE meat and poultry primarily occurs after cooking (post-lethality) during slicing and packaging. Therefore, USDA's Food Safety and Inspection Service (FSIS) implemented a final rule to control *L. monocytogenes* in RTE meat and poultry products. This rule went into effect October 6, 2003 and has had a major effect on processors of these products (USDA/FSIS 2003).

The rule only applies to RTE meat and poultry products exposed to the environment after cooking (post-lethality) which is often when contamination with *L. monocytogenes* occurs. Processors of RTE products must choose one of the following alternatives to prevent *L. monocytogenes* contamination of the final product. The first alternative (Alternative 1) uses a post-lethality treatment that reduces or eliminates *L. monocytogenes* and an antimicrobial agent or process that suppresses or limits *L. monocytogenes* growth throughout shelf-life. The second alternative (Alternative 2) uses either a post-lethality treatment that reduces or eliminates *L. monocytogenes* or an antimicrobial agent or process that suppresses or limits *L. monocytogenes* growth throughout shelf-life. The third alternative (Alternative 3) uses only sanitation measures to prevent *L. monocytogenes* contamination. Government regulators are conducting more verification testing on Alternative 3 since it is perceived as riskiest and less verification of Alternative 1 which is perceived as safest (USDA/FSIS 2003).

USDA/FSIS requires that processors of RTE meat and poultry products implement post processing intervention strategies for controlling *L. monocytogenes.* The concept of intervention technology is based on the application of preservatives to achieve microbiological safety and stability of foods (Leistner and Gould 1978). In the processing of RTE products, the use of antimicrobial agents is a common intervention technology. Antimicrobial agents are substances in or added to RTE product that has the effect of reducing or eliminating a microorganism, or that has the effect of suppressing or limiting growth in the product throughout the shelf-life of the product (USDA/FSIS 2003).

For many RTE meat and poultry products, the reduction of water activity through the addition of salt and thermal processing could serve as an antimicrobial process. Similarly, the reduction of pH via fermentation or addition of an acidulant could act as an effective antimicrobial process. Products with these types of antimicrobial processes would best be protected by the methods stated in Alternative 2. However, some RTE meat and poultry products could be better protected by the methods stated in Alternative 1, when the standard antimicrobial processing techniques are combined with a post-lethality treatment that would reduce *L. monocytogenes* after thermal processing. The compliance guidelines from USDA/FSIS state that an effective post-lethality treatment must reduce numbers of *L. monocytogenes* by at least one log. The regulation requires that post-lethality treatments must be scientifically validated (USDA/FSIS 2003).

Due to the perceived antimicrobial complexity of implementing post-lethality treatments, most small-scale meat processors have adopted the method of sanitation only as stated in Alternative 3 to control *L. monocytogenes*. However, this strategy provides the least assurance of food safety and is less economical (Ingham and others 2004).

Methods to Inhibit the Growth of *Listeria monocytogenes*

Hurdle technology is using more than one food preservation technique to prevent growth of pathogenic organisms in food products (McMeekin and others 2000). Altering the product chemistry or inherent parameters such as low water activity, low pH, absence of oxygen and thermal processing temperatures are some examples of hurdle technology. Addition of antimicrobials or packaging methods is also used in hurdle technology since *L. monocytogenes* has the ability to grow in refrigerated RTE products.

Hot-water post-packaging pasteurization (PPP) is a post-lethality treatment that can be used by small and very small processors to operate under Alternatives 1 or 2 of the USDA/FSIS regulations. This post-lethality treatment was studied for use with beef snack sticks and natural-casing wieners, two products commonly made by small and very small processors (Muriana and others 2002). In the processing of beef snack sticks, the addition of salt and the processes of thermal processing and smoking were effective antimicrobial interventions that made the finished product unsuitable for *L. monocytogenes* growth (Ingham and others 2006).

The PPP decontamination is a valid intervention strategy that processors can use to control *L. monocytogenes* in RTE products. The PPP method consists of packaging product in a thermally stable plastic packaging film and then immersing packages in 2.8 liters of boiling water (100°C) in a sauce pan on a hot plate (Muriana and others 2002). For the PPP decontamination method, an average reduction of >2 logs of *L. monocytogenes* populations was obtained using heating times of 1.0 min for individually packaged beef snack sticks (three brands), 4.0 min for four-per-package beef snack sticks (two brands) and seven-per-package beef snack sticks (three brands). Average product surface temperatures, measured as soon as possible after PPP and opening the package, were 47 to 51.5°C, 58 to 61.5°C, and 58.5 to 61°C for the beef snack sticks packaged one, four, and seven pieces per package, respectively. A treatment of

7.0 minutes for four-per-package natural casing wieners (three brands) achieved *L. monocytogenes* reductions of >1.0 log and average product surface temperature of 60.5 to 63.5°C. However, this method may not be economically feasible for food industry application.

Listeria monocytogenes has the ability to grow in wide pH ranges (4.5 to 9.6), and it has a minimum water activity of 0.92 for growth. Therefore, an acidification of foods with organic acids is important to adversely affect this microorganism by lowering the pH (Barker and Park 2001; Doyle 1999). Some fruits (raisins and cranberries) may be added to a RTE products' marinade thus bringing the pH and water activity down, and acting as an antimicrobial agent. For example, 4% raisins in beef jerky marinade brought down the pH to 4.5 and water activity to 0.62 (Bower and others 2003).

Antimicrobials when used in combination with irradiation at 1.0 kGy were effective in suppressing the growth of *L. monocytogenes* in fine emulsion sausage storage at 4°C for about 6 wk (Sommers and Fan 2003). No growth of *L. monocytogenes* occurred in turkey breast rolls during 42 d of storage when irradiated at 2.0 kGy (Zhu and others 2009).

Packaging films coated with a cellulose-based solution containing nisin significantly decreased *L. monocytogenes* populations on the surface of individually wrapped hot dogs by greater than two logs CFU per package throughout 60-d study (Franklin and others 2004). This study gives manufacturers a tool of using packaging films coated with nisin as an antimicrobial agent to control *L. monocytogenes* and subsequently fall under Alternative 1 (Franklin and others 2004).

A study conducted by McCormick and others (2005) investigated the inhibition of *L. monocytogenes* in turkey bologna by in-package pasteurization combined with biocide infused films. The combination of these techniques was effective against growth of *L. monocytogenes*. Another part of this research investigated the efficacy of in-package pasteurization combined with pre-surface application of nisin and/or lysozyme to reduce and prevent the subsequent recovery and growth of *L. monocytogenes* during refrigerated storage on the surface of low-fat turkey bologna. Sterile bologna samples were treated with solutions of nisin (2 mg/ml=5000 AU/ml), lysozyme (10 mg/ml=80 AU/ml) and a mixture of nisin and lysozyme (2 mg nisin+10 mg lysozyme/ml) before in-package pasteurization at 65°C for 32 s. In-package pasteurization resulted in an immediate 3.5 to 4.2 log CFU/cm^2 reduction in *L. monocytogenes* population for all treatments. In another study, control of *L. monocytogenes* in frankfurters by

post-package irradiation (2.3 kGy) with pediocin was evaluated (Chen and others 2004). Results showed that both of the techniques had a synergistic effect on growth, achieving 50% reduction of *L. monocytogenes*. The effect of a sodium lactate in combination with fat replacer was found to inhibit the growth of *L. monocytogenes* on the texture of low-fat sausages. The results showed that 3.33% sodium lactate with fat replacer had an antimicrobial affect on *L. monocytogenes* without affecting the textural properties of low-fat sausage (Choi and others 2003).

Listeria monocytogenes has been inhibited by using juice concentrates (Nogueira and others 2003), thermal inactivation in broth (Jesús and Whiting 2003) and through pH enhancement with ammonia gas on beef trimmings (Niebuhr and Dickson 2003). These methods or use of ingredients for RTE products let processors adopt Alternative 2.

Presence of *Listeria monocytogenes* in RTE Meat and Poultry Products

To date, poultry jerky has not been associated with foodborne illnesses or recalls. Between 1990 and 1999, the USDA/FSIS reported a cumulative prevalence of *Salmonella* and *L. monocytogenes* for meat and poultry jerky produced in federally inspected plants of 0.31 and 0.52%, respectively (Levine and others 2001). In response to recalls and outbreaks epidemiologically linked to beef jerky and other RTE red meat and poultry products, USDA/FSIS requires that commercial jerky manufacturers validate that their processes deliver a 5.0 log reduction for *E. coli* O157:H7 in beef and poultry, a 6.5 log reduction of *Salmonella* in beef, or a 7.0 log reduction in poultry, as well as meet the zero-tolerance policy or current compliance guidelines for *L. monocytogenes*, or both (USDA/FSIS 1999, 2004).

Conclusion

As the literature review has shown, *L. monocytogenes* is a potential hazard for RTE meat and poultry products. However, as long as production and processing of RTE products are according to food safety standards and prerequisites, these products are safe to consume. In order

to maintain the RTE product market there is still a need to study the survival mechanism of *Lm* in RTE products.

References

Barker C, Park SF. 2001. Sensitization of *Listeria monocytogenes* to low pH, organic acids, and osmotic stress by ethanol. Appl Eviron Microbiol. 67:1594-1600.

Bower CK, Schilke KF, Daeschel MA. 2003. Antimicrobial properties of raisins in beef jerky preservation. J Food Sci. 68: 1484-1489.

Buzby J, Roberts T, Lin C, MacDonald J. 1996. Bacterial foodborne disease: Medical costs and productivity losses. Pub. No. AER 741, USDA/Economic Research Service, Washington DC, August 1996.

Buzby JC. 2002. Economics of foodborne disease: *Listeria monocytogenes*. Available from: http://www.ers.usda.gov/Briefing/FoodborneDisease/listeria/. Accessed July 10, 2007.

Chen CM, Sebranek JG, Dickson JS. 2004. Combining pediocin with postpackaging irradiation for control of *Listeria monocytogenes* on frankfurters. J Food Prot. 67:1866-1875.

Choi SH, Kim KH, Eun JB, Chin KB. 2003. Growth suppression of inoculated *Listeria monocytogenes* and physicochemical and textural properties of low-fat sausages as affected by sodium lactate and a fat replacer. J Food Sci. 68:2542-2546.

Donnelly CW. 2001. *Listeria monocytogenes*. In: Labbé RG, Garcia S, editors. Guide to Foodborne Pathogens. John W& Sons, Inc: New York. p 99-132.

Doyle ME. 1999. Use of organic acids to control *Listeria* in meat. AMI Foundation. Available from: http://www.amif.org/AMIFResearch/1AMIorganic.pdf. Accessed July 9, 2007.

Franklin NB, Cooksey KD, Getty KJK. 2004. Inhibition of *Listeria monocytogenes* on the surface of individually packaged hot dogs with a packaging film coating containing nisin. J Food Prot. 67:480-481.

Galsworthy SB, Girdler S, Koval SF.1990. Chemotaxis in *Listeria monocytogenes.* Acta Microbiol. Hung. 37:81-85.

Gray M L, Killinger AH. 1966. *Listeria monocytogenes* and listeric infections. J. Bacteriol. 30: 309-382.

Ingham SC, Buege DR, Dropp BK, Losinski JA. 2004. Survival of *Listeria monocytogenes* during storage of ready-to-eat meat products processed by drying, fermentation, and/or smoking. J Food Prot. 67:2690-2702.

Ingham SC, Searls G, Mohanan S, Buege DR. 2006. Survival of *Staphylococcus aureus* and *Listeria monocytogenes* on vacuum-packaged beef jerky and related products stored at 21°C. J Food Prot. 69:2263-2267.

Jesús AJ, Whiting RC. 2003. Thermal inactivation, growth, and survival studies of *Listeria monocytogenes* strains belonging to three distinct genotypic lineages. J Food Prot. 66: 1611-1617.

Junttila JR , Niemela SI, Hirn J. 1988. Minimum growth temperatures of *Listeria monocytogenes* and non-haemolytic *Listeria*. J. Appl Bacteriol. 65:321-327.

Leistner L, Gould GW. 1978. Hurdle effect and energy saving. In: Downey WK (ed). Food Quality and Nutrition. London: Appl Sci. Publishers. p 553-557.

Levine PB, Rose B, Green S, Ransom G, Hill W. 2001. Pathogen testing of ready-to-eat meat and poultry products collected at federally-inspected establishments in the United States, 1990 to 1999. J Food Prot. 64:1188–1193.

Low JC, Donachie W. 1997. A Review on *Listeria monocytogenes* and listeriosis. Science Direct. Vet J. 153:9-29.

McCormick KE, Han IY, Acton JC, Sheldon BW, Dawson PL. 2005. In-package pasteurization combined with biocide-impregnated films to inhibit *Listeria monocytogenes* and *Salmonella* Typhimurium in turkey bologna. J Food Sci. 70:52-57.

McMeekin TA, Presser K, Ratkowsky D, Ross T, Salter M, Tienungoon S. 2000. Quantifying the hurdle concept by modeling the bacterial growth/no growth interface. Int J Food Microbiol. 55: 93-98.

Muriana PM, Quimby W, Davidson CA, Grooms J. 2002. Postpackage pasteurization of ready-to-eat deli meats by submersion heating for reduction of *Listeria monocytogenes.* J Food Prot. 65:963-969.

Niebuhr SE, Dickson JS. 2003. Impact of pH enhancement on populations of *Salmonella*, *Listeria monocytogenes*, and *Escherichia coli* O157:H7 in boneless lean beef trimmings. J Food Prot. 66:874-877.

Nogueira, MCL, Oyarzábal OA, Gombas DE. 2003. Inactivation of *Escherichia coli* O157:H7, *Listeria monocytogenes*, and *Salmonella* in cranberry, lemon, and lime juice concentrates. J Food Prot. 66:1637-1641.

Salyers AA, Whitt DD. 2002. *Listeria monocytogenes*, a doubly motile pathogen. Bacterial Pathogenesis, A Molecular Approach, 2nd ed. ASM Press: Washington, D.C. 579 p.

Seeliger HPR, Jones D. 1986 . Bergey's Manual of Systemic Bacteriology. 2(383):1940.

Sloan, A.E. 2001. Ethnic foods in the decade ahead. Food Technol. 55(10):18-19.

Sommers C, Fan X. 2003. Gamma irradiation of fine-emulsion sausage containing sodium diacetate. J Food Prot. 66:819-824.

[USDA/FSIS] United States Department of Agriculture, Food Safety and Inspection Service. 1999. Performance standards for the production of certain meat and poultry products: final rule. Fed. Regist. 64:732-749.

[USDA/FSIS] United States Department of Agriculture, Food Safety and Inspection Service 2003. Control of *Listeria monocytogenes* in ready-to-eat meat and poultry products: final rule. Fed. Regist. 68:34207–34254.

[USDA/FSIS] United States Department of Agriculture, Food Safety and Inspection Service. 2004. Compliance guidelines for meat and poultry jerky. Available from: http://www.fsis.usda.gov/PDF/Compliance_Guideline_Jerky. Accessed November 29, 2010.

Zhu MJ, Mendonca A, Ismail HA, Ahn DU. 2009. Fate of *Listeria monocytogenes* in ready-to-eat turkey breast rolls formulated with antimicrobials following electron-beam irradiation. Poultry Sci.88:205-213.

Chapter 3 - Effect of Packaging and Storage Time on Survival of *Listeria monocytogenes* on Kippered Beef Steak and Turkey Tenders

Abstract

The objective of our study was to determine the effect of packaging method and storage time on reducing *Listeria monocytogenes* in shelf-stable meat snacks. Commercially available kippered beef steak strips (14 × 2.5 cm piece) and turkey tenders (4 × 4 cm square piece) were dipped into a five-strain *L. monocytogenes* cocktail, and dried at 23°C until a water activity of approximately 0.80 was achieved. Inoculated samples were then packaged with four treatments: 1) vacuum, 2) nitrogen flushed with oxygen scavenger, 3) heat sealed with oxygen scavenger, and 4) heat sealed without oxygen scavenger. Samples were stored at 23°C and evaluated for *L. monocytogenes* levels at 0, 24, 48, and 72 h. Initial levels (time 0) of *L. monocytogenes* were approximately 5.7 log CFU/cm^2 for steak and tenders. For kippered beef steak, there was no interaction among packaging treatments and storage times ($P > 0.05$) whereas, time was different ($P < 0.05$). For kippered beef steak, there was 1 log reduction of *L. monocytogenes* at 24 and 48 h of storage times at 23°C for all packaging treatments and a 2.1 log CFU/ cm^2 *L. monocytogenes* reduction at 72 h. A 1 log CFU/cm^2 reduction of *L. monocytogenes* was observed after 24 h of storage for turkey tenders for all packaging treatments. After 48 h of storage time turkey tenders showed >1 log CFU/cm^2 reduction of *L. monocytogenes* for all packaging treatments except for vacuum packaged where only 0.9 log CFU/cm^2 reduction was observed. After 72 h of storage, reductions for all packaging treatments for turkey tenders ranged from 1.5 to 2.4 log CFU/cm^2.

Practical Applications

Processors of kippered beef steak and turkey tenders could use a combination of vacuum or nitrogen-flushing or heat sealed with an oxygen scavenger packaging methods and a holding time of 24 h prior to shipping to reduce potential *L. monocytogenes* numbers by ≥1 log. However, processors should be encouraged to hold packaged product a minimum of 72 h to enhance the margin of safety for *L. monocytogenes* control.

Introduction

The United States Department of Agriculture's Food Safety and Inspection Service (USDA/FSIS) has applied a zero-tolerance policy to *L. monocytogenes* in RTE meat and poultry products due to the severity foodborne illness from this organism (USDA/FSIS 1999). To date, poultry jerky has not been associated with foodborne illnesses or recalls, or both. Between 1990 and 1999, the USDA/FSIS reported a cumulative prevalence of *Salmonella* and *L. monocytogenes* for meat and poultry jerky produced in federally inspected plants of 0.31 and 0.52%, respectively (Levine and others 2001). In response to recalls and outbreaks epidemiologically linked to jerky and other RTE red meat and poultry products, the USDA/FSIS requires that commercial jerky manufacturers validate that their processes deliver a 5.0 log reduction for *E. coli* O157:H7 in beef and poultry, a 6.5 log reduction of *Salmonella* in beef, or a 7.0 log reduction in poultry, as well as meet the zero-tolerance policy and current compliance guidelines for *L. monocytogenes* (USDA/FSIS 2000, 2004).

The USDA/FSIS has implemented a regulation that requires processors to control *L. monocytogenes* in RTE meat and poultry products (USDA/FSIS 2003a). This regulation went into effect October 6, 2003, and has had major impact on how processors of these products control *L. monocytogenes* in their plants. The regulation only applies to RTE meat and poultry products exposed to the environment after cooking (post- lethality) (USDA/FSIS 2003a). Processors of RTE meat and poultry products must choose one of the following alternatives to prevent *L. monocytogenes* contamination of the final product: the first alternative is using a post-

lethality treatment that reduces or eliminates *L. monocytogenes* and an antimicrobial agent or process that suppresses or limits *L. monocytogenes* growth throughout shelf-life. The second alternative is using either a post-lethality treatment that reduces or eliminates *L. monocytogenes* or an antimicrobial agent or process that suppresses or limits *L. monocytogenes* growth throughout shelf-life. The third alternative is using only sanitation measures to prevent *L. monocytogenes* contamination.

For many RTE meat and poultry products, the reduction of water activity through the addition of salt and thermal processing could serve as an antimicrobial process. Similarly, the reduction of pH via fermentation or addition of an acidulant could act as an effective antimicrobial process (Samelis and others 2001, 2005). These types of fermented products, that have a low pH, can be processed under Alternative 2. However, processors could safely process under Alternative 1 which is more economical, if there are antimicrobial processing techniques, for example low pH and water activity, involved in the process along with short-term storage prior to distribution that effectively serves as a post-lethality treatment (Ingham and others 2004).

The compliance guidelines from USDA/FSIS state that an effective post-lethality treatment must reduce numbers of *L. monocytogenes* by at least one log. The regulation also requires that post-lethality treatments must be scientifically validated (USDA/FSIS 2003a). Post-lethality cross contamination of *L. monocytogenes* is also possible in products such as kippered beef steak that have a higher than 0.75:1 moisture-to-protein ratio (MPR) (USDA/FSIS 2005). Kippered beef produced on September 4, 2003 with code of 121 or 122 was subject to recall for product being contaminated with *L. monocytogenes* (USDA/FSIS 2003b). In a study by Ingham and others (2004), vacuum packaged products with characteristics similar to turkey tenders and kippered beef steak that was held 1-week in storage prior to distribution were able to inhibit growth of *L. monocytogenes*. This study evaluated both a cured (kippered beef) and un-cured (turkey tender) RTE meat and poultry snacks. It was noted that *L. monocytogenes* contamination of cured and non-cured RTE meat is a major safety concern (USDA/FSIS 2006*)* because RTE meats have a long shelf-life and are consumed without further heating *(*Leistner 2000*)*.

Research also has shown that vacuum packaging of jerky can generate a 1 log reduction of *L. monocytogenes* following one or more weeks of storage at ambient temperature and that *L. monocytogenes* was not able to survive on a jerky product during its shelf life of approximately

one year (Ingham and others 2004, 2006b; CFR 2009).

There is a lack of information evaluating the effectiveness of a post-lethality treatment that will allow products such as kippered beef steak and turkey tenders to be held for a shorter time period. Combining modified atmosphere packaging with short-term storage prior to distribution could be an effective antimicrobial process that would serve as a post-lethality treatment. Therefore, the objective of our study was to evaluate the effect of packaging methods and storage time on reducing *L. monocytogenes* in commercially prepared kippered beef steak (cured with sodium nitrite) and turkey tenders (uncured product).

Materials and Methods

Experimental Design

This study consisted of four packaging treatments: heat sealed (HS), heat sealed with oxygen scavenger (HSOS), nitrogen flushed with oxygen scavenger (NFOS), and vacuum (VAC) with four storage times (0, 24, 48, and 72 h). For each packaging treatment and storage time, two samples of inoculated kippered beef steak and turkey tenders were evaluated for *L. monocytogenes* populations. A replication consisted of commercially processed kippered beef steak and turkey tenders making up a single lot. Three replications were completed for each product.

Product Description

Three different production lots of kippered beef steak and turkey tenders were commercially obtained from a single manufacturer. Each lot was assigned to one of the three replications. Commercial kippered beef steak and turkey tenders samples were obtained and packaged in a nitrogen-flushed, O_2 impermeable, re-sealable, 5-mil-thick, low-linear-density polyethylene (LLDPE) clear pouch (19 cm × 33.5 cm; TPG Co., Ltd, Korea) that contained an O_2 absorber (O_2-Zero, BJ100; TPG Co., Ltd, Korea).

Kippered beef steak ingredients included beef, dextrose, water, flavorings, salt, natural smoke flavor, hydrolyzed corn and soy protein, yeast extract, soy sauce, monosodium glutamate, sodium erythorbate, and sodium nitrite. According to the manufacturer's data, the kippered beef steak had 38.3% moisture, 29.2% protein, 6.1% fat, 5.4% salt, 1.31 moisture-to-protein ratio

(MPR), and pH 6.0. Turkey tenders ingredients included turkey breast, brown sugar, soy sauce, corn syrup, sugar, flavorings, salt, pineapple juice concentrate, vinegar, water, natural smoke flavor, molasses, caramel color and citric acid. According to the manufacturer's data, the tenders had 32.1% moisture, 35.9% protein, 2.5% fat, 4.0% salt, 0.89 MPR, and pH 5.6.

Inoculum Preparation

The inoculum consisted of a five-strain *L. monocytogenes* cocktail (Table 3.1). Stock cultures of *L. monocytogenes* were obtained (Kwik-Stik; Microbiologics, Inc., Grenobel Cedex 2, France.), and a pure subculture of each strain was prepared by transferring a loopful of stock culture into 10 mL of sterilized tryptic soy broth (TSB; Difco BD and Company, Sparks, MD, U.S.A.) and incubating at 37 °C for 24 h. One liter of the five-strain L. monocytogenes cocktail was prepared aseptically by transferring 0.5 mL of each pure culture isolate to 200 mL of pre-sterilized TSB and then incubating at 37 °C for 24 h. Next, each strain (200 mL total) was transferred to a sterile 2-liter beaker to obtain 1 liter of the five-strain cocktail containing 8 to 9 log CFU/mL of *L. monocytogenes*. A total of 1 liter of *L. monocytogenes* cocktail was prepared for this study.

Table 3.1-Strains of microorganisms used for *Listeria monocytogenes* inoculum.

Strain	**Original Source**
ATCC 7644	Human
ATCC 19115	Human
ATCC 19118	Chicken (England)
ATCC 19112	Spinal fluid of man (Scotland)
SLR 2249 Cornell University	Human

Inoculation, Packaging, and Enumeration

Kippered beef steak and turkey tenders were aseptically removed from pouches. Kippered beef steak strips (14 × 2.5 cm piece) and cut pieces (4 × 4 cm^2) of turkey tenders were

dipped into inoculum, and held for 1 min. Pieces were removed from the inoculum and allowed to air dry at 23°C for approximately 1 to 2 h to an a_w level of approximately 0.80.

A sample consisted of kippered beef steak strip (14 × 2.5 cm piece) and a 4 × 4 cm square cut piece of turkey tenders. Two strips of kippered beef steak and two 4 × 4 cm cut pieces of turkey tenders were evaluated for each packaging treatment and time combination for a total of 32 samples per replicate for each product. Samples were then assigned to one of the four packaging treatments: HS, HSOS, NFOS, or VAC. All samples were placed in a 5-mil-thick, clear, LLDPE pouch cut to 19 × 35 cm. Samples assigned to NFOS were flushed for 10 s with food grade 100% N_2 and vacuum packaged by using a vacuum packager (Multivac C100; Gepufte Sicherheit, Germany) with 600 mm Hg. The HS and HSOS samples were packaged with an impulse sealer (Model H-1029; ULINE, Chicago, IL). An O_2 absorber was added to each sample packaged in NFOS or HSOS prior to heat sealing or N_2 flushing. Each of the four packaging treatments was subsequently held at 23°C for 0, 24, 48, and 72 h.

For all time periods including time 0 (initial *L. monocytogenes* level), two samples of kippered beef steak and turkey tenders were used for enumeration. After each holding period, a kippered beef steak strip and a 4 × 4 cm piece of turkey tenders were placed into filtered stomacher bags (Fisher Scientific, Pittsburg, PA, U.S.A.), and 34 mL of 0.1% peptone water (Difco, Detroit, MI, U.S.A.) was added to the bags. The samples and diluent were then pummeled in a stomacher (Stomacher Mix 1 Lab Blender; Microbiology International, Frederick, MD, U.S.A.) for 1 min. Serial dilutions were prepared. *Listeria monocytogenes* populations were enumerated after spread plating 0.1-mL of diluent onto duplicate modified oxford agar plates (Difco BD and Company, Sparks, MD, U.S.A.) and incubating for 48 h at 35°C.

Water Activity (a_w) and O_2 Determination

Water activity was measured to determine if changes in a_w resulted due to the dipping procedure. Kippered beef steak and turkey tenders pieces dipped into sterile TSB were used to measure a_w. Two samples from the HSOS treatment were measured before dipping, immediately after drying, and after 24, 48, and 72 h of storage at 23°C.

Water activity was determined with an a_w meter (AQUALAB CX2 series 3TE; Decagon, Pullman, WA, U.S.A.) calibrated using a 0.760 NaCl verification standard (6.0 M in water,

Decagon, Pullman, WA, U.S.A.) and distilled water at 25.5 °C. Kippered beef steak and turkey tenders were cut into a hexagonal shape with a diameter of approximately 3.2 cm and placed in a sample container (Harper and others 2010). For each sample, duplicate readings were taken at 25.5 °C.

Oxygen content of the HS, HSOS, and NFOS packages was measured after 0, 24, 48, and 72 h of storage at 25.5 °C with an O_2 analyzer (Checkpoint-O_2; PBI Dansensor, DK-400 Kingsted, Denmark). Oxygen concentration was measured prior to *L. monocytogenes* enumeration by piercing the package 1.0 cm away from the seal with the O_2 detector needle inserted at a 45° angle.

Statistical Analysis

The experiment was a completely randomized design using a 4 × 4 factorial with kippered beef steak and turkey tenders as the experimental unit. The model included the main effects of packaging treatment and storage time and the interaction of packaging treatment and storage time. The random effects were replication and treatment by time by replication. Analysis of variance was performed using the PROC MIXED procedure of SAS (SAS Inst. Inc., Cary, NC.). Least squares means were calculated for each independent variable. Statistical significance was $P < 0.05$.

Results and Discussion

The initial mean a_w of kippered beef steak and turkey tenders was 0.81 and 0.77 respectively, prior to inoculation. The a_w levels did not change throughout the sampling times (Table 3.2). The residual O_2 concentration was less than 0.5% for non-inoculated steaks and tenders dipped in sterile TSB media and packaged in NFOS. Across all storage times, HSOS and NFOS had an average O_2 concentration of less than 0.2%, and HS averaged 19.3% O_2.

Table 3.2-Mean water activity (a_w) and standard deviations during storage at 23°C.

	Kippered Beef Steak	**Turkey Tenders**
Time (h)	Mean a_w ± Standard Deviation	Mean a_w ± Standard Deviation
Prior to Dip	0.81 ± 0.01	0.77 ± 0.02
0	0.80 ± 0.01	0.81 ± 0.02
24	0.80 ± 0.01	0.81 ± 0.02
48	0.82 ± 0.01	0.82 ± 0.02
72	0.81 ± 0.01	0.81 ± 0.02

There was no difference ($P > 0.05$) in packaging treatments on the reduction of *L. monocytogenes* on kippered beef steak, however storage time was different ($P < 0.05$). Therefore, all packaging treatments means were pooled for each time period. After 24 and 48 h of 23°C storage, 1.0 log CFU/cm^2 reductions of *L. monocytogenes* were observed for all packaging treatments (Table 3.3). For the 72 h holding time, log reductions for *L. monocytogenes* populations were 2.1 log CFU/cm^2 for all packaging treatments for kippered beef steak.

Table 3.3-Least squares means and standard errors of *Listeria monocytogenes* populations (log CFU/cm^2) and log reductions for kippered beef steak at 0, 24, 48, and 72 h storage at 23°C for all packaging treatments (heat sealed only, heat sealed with oxygen scavenger, nitrogen flushed with oxygen scavenger, and vacuum) combined (n=24).

Time (h)	**Least squares means ± standard error (log CFU/cm^2)**	**Log Reduction (log CFU/cm^2)**
0	5.6 ± 0.21^a	0
24	4.6 ± 0.22^b	1.0
48	4.6 ± 0.23^b	1.0
72	3.5 ± 0.21^c	2.1

$^{a-c}$ Indicates differences ($P > 0.05$) for storage times within a column.

A whole muscle beef jerky with a_w of <0.81 was sufficient to prevent growth of *L. monocytogenes*, which has a minimum a_w for growth of 0.92 (Ingham and others 2004). Other studies investigating longer storage times have shown reduction of *L. monocytogenes* in RTE meat products (Ingham and others 2004, 2006ab). *Listera monocytogenes* populations decreased 2.4 log CFU after 1 week in 21°C storage on beef jerky having an a_w of 0.75 that was vacuum packaged, and after 4 weeks of storage, no surviving cells were recovered (Ingham and others 2004). Greater reductions of *L. monocytogenes* populations were observed in vacuum packaged beef jerky products (a_w ranging from 0.47 to 0.87), declining by 0.6 and 2.3 log CFU after 21°C storage for 1 and 4 weeks, respectively (Ingham and others 2004).

Griffith and others (2009) observed that fully cooked cured turkey bacon when inoculated with *L. monocytogenes* had essentially the same levels when bacon was stored at 10°C and 4°C for up to 34 and 45 d. This study suggests that if post-process contamination were to occur in the turkey bacon the environment would be unfavorable for subsequent outgrowth of *L. monocytogenes* during storage at either refrigeration or mildly abusive temperatures. In our study, we observed that the population numbers actually decreased during storage of kippered beef at ambient temperature also indicating that the packaging environment and product characteristics are not favorable for *L. monocytogenes* outgrowth.

In our study of turkey tenders, a difference ($P < 0.05$) in *L. monocytogenes* populations was observed among four packaging treatments (HS, HSOS, NOFS, and VAC) along with a difference ($P < 0.05$) for populations among storage times of 0, 24, 48 and 72 h at 23°C (Table 3.4). Turkey tenders showed >1.0 log CFU/ cm^2 for all packaging treatments at 24 and 48 h storage time at 23°C except for vacuum packaged which showed 0.9 log reduction of *L. monocytogenes* at 48 h of storage time (Table 3.5). After 72 h of storage at 23°C, log reductions ranged from 1.5 to 2.2 for all packaging treatments.

In our study, oxygen restriction via vacuum showed consistently less reduction of inoculated *L. monocytogenes* populations at 23°C for turkey tenders. Similar findings were reported in another study by Buchanan and Klawitter (1999). They found that oxygen restriction also enhanced *L. monocytogenes* growth at 19°C. We observed that vacuum packaging treatment was less effective in reducing *L. monocytogenes* for both products as compared to heat sealed (no oxygen scavenger), heat sealed with oxygen scavenger and nitrogen flushed packaging treatments under 24 h, 48 h and 72 h of storage times.

Table 3.4-Least squares means and standard errors of *Listeria monocytogenes* populations (log CFU/cm^2) for turkey tenders in different packaging environments after 0, 24, 48, and 72 h storage at 23°C (n=6).

	Least Squares Means ± Standard Error (log CFU/cm^2)			
	Type of Packaging			
Time (h)	**Heat Sealed**	**Heat Sealed with Oxygen Scavenger**	**Nitrogen Flushed with Oxygen Scavenger**	**Vacuum**
0	5.8 ± 0.23^{ax}	5.8 ± 0.23^{ax}	5.8 ± 0.23^{ax}	5.8 ± 0.23^{ax}
24	4.4 ± 0.23^{bxy}	4.2 ± 0.23^{bcx}	4.8 ± 0.23^{by}	4.6 ± 0.23^{bxy}
48	3.8 ± 0.23^{cx}	4.3 ± 0.23^{bxy}	4.7 ± 0.25^{bcy}	4.9 ± 0.23^{by}
72	3.9 ± 0.23^{bcxy}	3.6 ± 0.26^{cx}	4.2 ± 0.23^{cxy}	4.3 ± 0.23^{by}

[a-c] Indicates differences ($P < 0.05$) in column for different storage times.

[xy] Indicates differences ($P < 0.05$) in rows for different packaging treatments.

Table 3.5-Log reductions (CFU/cm^2) for turkey tenders in different packaging environments after 24, 48, and 72 h storage at 23°C.

	Log Reduction (CFU/cm^2)			
	Type of Packaging			
Time (h)	**Heat Sealed**	**Heat Sealed with Oxygen Scavenger**	**Nitrogen Flushed with Oxygen Scavenger**	**Vacuum**
24	1.4	1.6	1.0	1.2
48	2.0	1.5	1.1	0.9
72	1.9	2.2	1.6	1.5

Conclusions

Processors of kippered beef steak and turkey tenders could use a storage time of 24 h prior to shipping in combination with heat sealed, nitrogen flushed with oxygen scavenger, or vacuum packaging to reduce potential *L. monocytogenes* populations by at least 1 log. However, processors should be encouraged to hold packaged product a minimum of 72 h to enhance the margin of safety for *L. monocytogenes* control.

References

Buchanan RL, Klawitter LA. 1990. Effects of temperature and oxygen on the growth of *Listeria monocytogenes* at pH 4.5. J Food Sci. 55:1754–1756.

[CFR] Code of Federal Regulations. 2009. Control of *Listeria monocytogenes* in post-lethality exposed ready-to-eat products. 9CFR430: 635-637. Available from: http://www.gpoaccess.gov/CFR/. Accessed December 15, 2009.

Griffith E, Porto Fett AC, Oser A, Call, JE, Shoyer BA, Luchansky JB. 2009. Survival of Listeria monocytogenes on slices of turkey bacon during extended storage at 4 Degrees and 10 Degrees C. [abstract]. American Society for Microbiology's Annual Meeting. p.1. Available from: http://www.ars.usda.gov/research/publications/publications.htm?seq_no_115=235317. Accessed December 19, 2010.

Harper NM, Getty KJK, and BoyleEAE. 2010. Evaluation of sample preparation methods for determination of water activity in jerky and kippered beef: A research note. Meat Sci. 86:527-528.

Ingham SC, Buege DR, Dropp BK, Losinski JA. 2004. Survival of *Listeria monocytogenes* during storage of ready-to-eat meat products processed by drying, fermentation, and/or smoking. J Food Prot. 67: 2690-2702.

Ingham SC, Searls G, Buege DR. 2006a. Inhibition of *Salmonella serovars*, *Escherichia coli* O157:H7 and *Listeria monocytogenes* during dry-curing and drying of meat: A case study with basturma. J Food Safety. 26:160-161.

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Ingham SC, Searls G, Mohanan S, Buege DR. 2006b. Survival of *Staphylococcus aureus* and *Listeria monocytogenes* on vacuum-packaged beef jerky and related products stored at 21°C. J Food Prot. 69:2263-2267.

Leistner L. 2000. Basic aspects of food preservation by hurdle technology. Int J Food Microbiol. 55:181-186.

Levine PB, Rose B, Green S, Ransom G, Hill W. 2001. Pathogen testing of ready-to-eat meat and poultry products collected at federally-inspected establishments in the United States, 1990 to 1999. J Food Prot. 64:1188-1193.

Samelis J, Sofos J, Kain J, Scanga M, Belk K, Smith G. 2001. Organic acids and their salts as dipping solutions to control *Listeria monocytogenes* inoculated following processing of sliced pork bologna stored at 4°C in vacuum packages. J Food Prot. 64:1722-1729.

Samelis J, Bedie GK, Sofos JN, Belk KE, Scanga JA, Smith GC. 2005. Combinations of nisin with organic acids or salts to control *Listeria monocytogenes* on sliced pork bologna stored at 4°C in vacuum packages. LebenWissen Technol 38:21-28.

[USDA/FSIS] United States Department of Agriculture, Food Safety and Inspection Service. 1999. Performance standards for the production of certain meat and poultry products: final rule. Fed. Regist. 64:732-749.

[USDA/FSIS] United States Department of Agriculture, Food Safety and Inspection Service. 2000. FSIS strategies for addressing *Listeria monoctyogenes*. Available from: http://www.fsis.usda.gov/OA/background/lmplan.htm. Accessed December 17, 2010.

[USDA/FSIS] United States Department of Agriculture, Food Safety and Inspection Service 2003a. Control of *Listeria monocytogenes* in ready-to-eat meat and poultry products; final rule. Fed. Regist. 68:34207-34254.

[USDA/FSIS] United States Department of Agriculture, Food Safety and Inspection Service 2003b. Washington State firm recalls beef products for possible *Listeria* contamination. Available from: http://www.fsis.usda.gov/OA/recalls/prelease/pr050-2003.htm. Accessed December 20, 2010.

[USDA/FSIS] United States Department of Agriculture, Food Safety and Inspection Service. 2004. Compliance guidelines for meat and poultry jerky. Available from: http://www.fsis.usda.gov/PDF/Compliance_Guideline_Jerky. Accessed November 29, 2010.

[USDA/FSIS] United States Department of Agriculture, Food Safety and Inspection Service. 2005. Food standards and labeling policy book. Available from: http://www.fsis.usda.gov/OPPDE/larc/Policies/Labeling_Policy_Book_082005. Accessed November 28, 2010.

[USDA/FSIS] United States Department of Agriculture, Food Safety and Inspection Service. 2006. Compliance guideline to control *Listeria monocytogenes* in post-lethality exposed read-to-eat meat and poultry products. Available from: http://www.fsis.usda.gov/oppde/rdad/FRPubs/97013F/LM_Rule_Compliance_Guidelines_May_2006.pdf. Accessed 10 February 2009.

Appendix A - Statistical Analysis for Kippered Beef Steaks

Below is the SAS statistical analysis program (Statistical Analysis Systems software, (SAS Institute, Inc., Cary, NC) and data that was used to determine differences among *Listeria monocytogenes* populations for packaging environments and storage times. The data is listed in seven columns. The first column is a code where the first number represents the replication and the second letter represents the product (kippered beef steak), the third, fourth, and fifth letters represent the packaging treatment (HO=heat sealed oxygen scavenger; HNO=heat sealed no oxygen scavenger; VO=vacuum; and NO=nitrogen flushed oxygen scavenger), and the number at the end represents the sample. The second column represents the packaging treatment. The third column represents the storage times of 0, 24, 48, or 72 h, the forth column is the sample number, the fifth column is the replication, and the sixth column is the log cfu/g average count for two duplicate plates with the seventh column being a corrected value. Missing values also were determined as needed for the analysis.

```
data steaks;
input Code $   trt $    Time   sample rep     logcfu1 logcfu2;
if logcfu1 = . then logcfu1 = 0;
logcfu_avg = (logcfu1 + logcfu2)/2;
datalines;
1SHO1       HO    0      1      1      5.6411 5.6411
1SHO2       HO    0      2      1      5.4387 5.4387
1SHO1       HO    24     1      1      4.3735 4.3735
1SHO2       HO    24     2      1      5.3587 5.3587
1SHO1       HO    48     1      1      5.2472 5.2472
1SHO2       HO    48     2      1      4.9643 4.9643
1SHO1       HO    72     1      1      4.3751 4.3751
1SHO2       HO    72     2      1      4.1551 4.1551
1SHNO1      HNO   0      1      1      5.6411 5.6411
1SHNO2      HNO   0      2      1      5.4387 5.4387
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1SHNO1	HNO	24	1	1	4.3906	4.3906
1SHNO2	HNO	24	2	1	4.2612	4.2612
1SHNO1	HNO	48	1	1	4.0453	4.0453
1SHNO2	HNO	48	2	1	4.0453	4.0453
1SHNO1	HNO	72	1	1	4.625	4.625
1SHNO2	HNO	72	2	1	4.0581	4.0581
1SVO1	VO	0	1	1	5.6411	5.6411
1SVO2	VO	0	2	1	5.4382	5.4382
1SVO1	VO	24	1	1	4.1445	4.1445
1SVO2	VO	24	2	1	5.221	5.221
1SVO1	VO	48	1	1	4.5737	4.5737
1SVO2	VO	48	2	1	5.2784	5.2784
1SVO1	VO	72	1	1	4.2602	4.2602
1SVO2	VO	72	2	1	4.0606	4.0606
1SNO1	NO	0	1	1	5.6411	5.6411
1SNO2	NO	0	2	1	5.4382	5.4382
1SNO1	NO	24	1	1	5.1834	5.1834
1SNO2	NO	24	2	1	5.0657	5.0657
1SNO1	NO	48	1	1	5.5655	5.5655
1SNO2	NO	48	2	1	5.5693	5.5693
1SNO1	NO	72	1	1	4.3751	4.3751
1SNO2	NO	72	2	1	4.1676	4.1676
2SHO1	HO	0	1	2	5.8751	5.8751
2SHO2	HO	0	2	2	5.2659	5.2659
2SHO1	HO	24	1	2	<2.477	2.447
2SHO2	HO	24	2	2	<2.477	2.447
2SHO1	HO	48	1	2	4.8827	4.8827
2SHO2	HO	48	2	2	4.5563	4.5563
2SHO1	HO	72	1	2	4.4983	4.4983
2SHO2	HO	72	2	2	3.4058	3.4058
2SHNO1	HNO	0	1	2	5.8751	5.8751

2SHNO2	HNO	0	2	2	5.2659	5.2659
2SHNO1	HNO	24	1	2	5.3512	5.3512
2SHNO2	HNO	24	2	2	4.1761	4.1761
2SHNO1	HNO	48	1	2	3.6232	3.6232
2SHNO2	HNO	48	2	2	<2.477	2.447
2SHNO1	HNO	72	1	2	2.5737	2.5737
2SHNO2	HNO	72	2	2	<1.477	1.477
2SVO1	VO	0	1	2	5.8751	5.8751
2SVO2	VO	0	2	2	5.2659	5.2659
2SVO1	VO	24	1	2	4.6796	4.6796
2SVO2	VO	24	2	2	4.4583	4.4583
2SVO1	VO	48	1	2	5.8751	5.8751
2SVO2	VO	48	2	2	4.0375	4.0375
2SVO1	VO	72	1	2	3.8607	3.8607
2SVO2	VO	72	2	2	3.3979	3.3979
2SNO1	NO	0	1	2	5.8751	5.8751
2SNO2	NO	0	2	2	5.2659	5.2659
2SNO1	NO	24	1	2	4.4771	4.4771
2SNO2	NO	24	2	2	<2.477	2.447
2SNO1	NO	48	1	2	4.5147	4.5147
2SNO2	NO	48	2	2	4.3982	4.3982
2SNO1	NO	72	1	2	1.4771	1.4771
2SNO2	NO	72	2	2	1.9542	1.9542
3SHO1	HO	0	1	3	5.6691	5.6691
3SHO2	HO	0	2	3	5.5589	5.5589
3SHO1	HO	24	1	3	<2.477	2.447
3SHO2	HO	24	2	3	5.3934	5.3934
3SHO1	HO	48	1	3	5.8315	5.8315
3SHO2	HO	48	2	3	5.8751	5.8751
3SHO1	HO	72	1	3	<1.477	1.477
3SHO2	HO	72	2	3	1.9542	1.9542

```
3SHNO1      HNO  0   1   3   5.6214 5.6214
3SHNO2      HNO  0   2   3   5.6041 5.6041
3SHNO1      HNO  24  1   3   4.662  4.662
3SHNO2      HNO  24  2   3   <2.477 2.447
3SHNO1      HNO  48  1   3   <2.477 2.447
3SHNO2      HNO  48  2   3   <2.477 2.447
3SHNO1      HNO  72  1   3   3.4234 3.4234
3SHNO2      HNO  72  2   3   3.9236 3.9236
3SVO1       VO   0   1   3   5.6691 5.6691
3SVO2       VO   0   2   3   5.5589 5.5589
3SVO1       VO   24  1   3   4.7743 4.7743
3SVO2       VO   24  2   3   4.3551 4.3551
3SVO1       VO   48  1   3   3.9906 3.9906
3SVO2       VO   48  2   3   4.7531 4.7531
3SVO1       VO   72  1   3   3.8751 3.8751
3SVO2       VO   72  2   3   3.0949 3.0949
3SNO1       NO   0   1   3   5.6691 5.6691
3SNO2       NO   0   2   3   5.6041 5.6041
3SNO1       NO   24  1   3   3.592  3.592
3SNO2       NO   24  2   3   3.1702 3.1702
3SNO1       NO   48  1   3   4.5147 4.5147
3SNO2       NO   48  2   3   4.3982 4.3982
3SNO1       NO   72  1   3   <1.477 1.447
3SNO2       NO   72  2   3   3.9278 3.9278
proc print data = steaks;
title 'steaks';
run;
proc mixed data = steaks;
class trt time sample rep;
model logcfu2 = trt time trt*time;
random rep rep*trt*time;
```

```
lsmeans trt time trt*time/pdiff;
run;
proc mixed data = steaks;
class trt time sample rep;
model logcfu1 = trt time trt*time;
random rep rep*trt*time;
lsmeans trt time trt*time/pdiff;
run;
proc mixed data = steaks;
class trt time sample rep;
model logcfu_avg = trt time trt*time;
random rep rep*trt*time;
lsmeans trt time trt*time/pdiff;
run;
*/

data missing_steaks;
input Code $    trt $     Time    sample rep      logcfu1 logcfu2;
if logcfu1 = . then logcfu1 = 0;
logcfu_avg = (logcfu1 + logcfu2)/2;
datalines;
1SHO1       HO    0     1     1     5.6411 5.6411
1SHO2       HO    0     2     1     5.4387 5.4387
1SHO1       HO    24    1     1     4.3735 4.3735
1SHO2       HO    24    2     1     5.3587 5.3587
1SHO1       HO    48    1     1     5.2472 5.2472
1SHO2       HO    48    2     1     4.9643 4.9643
1SHO1       HO    72    1     1     4.3751 4.3751
1SHO2       HO    72    2     1     4.1551 4.1551
1SHNO1      HNO   0     1     1     5.6411 5.6411
1SHNO2      HNO   0     2     1     5.4387 5.4387
```

1SHNO1	HNO	24	1	1	4.3906	4.3906
1SHNO2	HNO	24	2	1	4.2612	4.2612
1SHNO1	HNO	48	1	1	4.0453	4.0453
1SHNO2	HNO	48	2	1	4.0453	4.0453
1SHNO1	HNO	72	1	1	4.625	4.625
1SHNO2	HNO	72	2	1	4.0581	4.0581
1SVO1	VO	0	1	1	5.6411	5.6411
1SVO2	VO	0	2	1	5.4382	5.4382
1SVO1	VO	24	1	1	4.1445	4.1445
1SVO2	VO	24	2	1	5.221	5.221
1SVO1	VO	48	1	1	4.5737	4.5737
1SVO2	VO	48	2	1	5.2784	5.2784
1SVO1	VO	72	1	1	4.2602	4.2602
1SVO2	VO	72	2	1	4.0606	4.0606
1SNO1	NO	0	1	1	5.6411	5.6411
1SNO2	NO	0	2	1	5.4382	5.4382
1SNO1	NO	24	1	1	5.1834	5.1834
1SNO2	NO	24	2	1	5.0657	5.0657
1SNO1	NO	48	1	1	5.5655	5.5655
1SNO2	NO	48	2	1	5.5693	5.5693
1SNO1	NO	72	1	1	4.3751	4.3751
1SNO2	NO	72	2	1	4.1676	4.1676
2SHO1	HO	0	1	2	5.8751	5.8751
2SHO2	HO	0	2	2	5.2659	5.2659
2SHO1	HO	24	1	2	<2.477	2.447
2SHO2	HO	24	2	2	<2.477	2.447
2SHO1	HO	48	1	2	4.8827	4.8827
2SHO2	HO	48	2	2	4.5563	4.5563
2SHO1	HO	72	1	2	4.4983	4.4983
2SHO2	HO	72	2	2	3.4058	3.4058
2SHNO1	HNO	0	1	2	5.8751	5.8751

2SHNO2	HNO	0	2	2	5.2659	5.2659
2SHNO1	HNO	24	1	2	5.3512	5.3512
2SHNO2	HNO	24	2	2	4.1761	4.1761
2SHNO1	HNO	48	1	2	3.6232	3.6232
2SHNO1	HNO	72	1	2	2.5737	2.5737
2SHNO2	HNO	72	2	2	<1.477	1.477
2SVO1	VO	0	1	2	5.8751	5.8751
2SVO2	VO	0	2	2	5.2659	5.2659
2SVO1	VO	24	1	2	4.6796	4.6796
2SVO2	VO	24	2	2	4.4583	4.4583
2SVO1	VO	48	1	2	5.8751	5.8751
2SVO2	VO	48	2	2	4.0375	4.0375
2SVO1	VO	72	1	2	3.8607	3.8607
2SVO2	VO	72	2	2	3.3979	3.3979
2SNO1	NO	0	1	2	5.8751	5.8751
2SNO2	NO	0	2	2	5.2659	5.2659
2SNO1	NO	24	1	2	4.4771	4.4771
2SNO1	NO	48	1	2	4.5147	4.5147
2SNO2	NO	48	2	2	4.3982	4.3982
2SNO1	NO	72	1	2	1.4771	1.4771
2SNO2	NO	72	2	2	1.9542	1.9542
3SHO1	HO	0	1	3	5.6691	5.6691
3SHO2	HO	0	2	3	5.5589	5.5589
3SHO2	HO	24	2	3	5.3934	5.3934
3SHO1	HO	48	1	3	5.8315	5.8315
3SHO2	HO	48	2	3	5.8751	5.8751
3SHO1	HO	72	1	3	<1.477	1.477
3SHO2	HO	72	2	3	1.9542	1.9542
3SHNO1	HNO	0	1	3	5.6214	5.6214
3SHNO2	HNO	0	2	3	5.6041	5.6041
3SHNO1	HNO	24	1	3	4.662	4.662

3SHNO1	HNO	48	1	3	<2.477	2.447
3SHNO2	HNO	48	2	3	<2.477	2.447
3SHNO1	HNO	72	1	3	3.4234	3.4234
3SHNO2	HNO	72	2	3	3.9236	3.9236
3SVO1	VO	0	1	3	5.6691	5.6691
3SVO2	VO	0	2	3	5.5589	5.5589
3SVO1	VO	24	1	3	4.7743	4.7743
3SVO2	VO	24	2	3	4.3551	4.3551
3SVO1	VO	48	1	3	3.9906	3.9906
3SVO2	VO	48	2	3	4.7531	4.7531
3SVO1	VO	72	1	3	3.8751	3.8751
3SVO2	VO	72	2	3	3.0949	3.0949
3SNO1	NO	0	1	3	5.6691	5.6691
3SNO2	NO	0	2	3	5.6041	5.6041
3SNO1	NO	24	1	3	3.592	3.592
3SNO2	NO	24	2	3	3.1702	3.1702
3SNO1	NO	48	1	3	4.5147	4.5147
3SNO2	NO	48	2	3	4.3982	4.3982
3SNO2	NO	72	2	3	3.9278	3.9278

```
/*proc mixed data = missing_steaks;
class trt time sample rep;
model logcfu2 = trt time trt*time;
random rep rep*trt*time;
lsmeans trt time trt*time/pdiff;
run;
proc mixed data = missing_steaks;
class trt time sample rep;
model logcfu1 = trt time trt*time;
random rep rep*trt*time;
lsmeans trt time trt*time/pdiff;
run;
```

```
*/
proc mixed data = missing_steaks;
class trt time sample rep;
model logcfu_avg = trt time trt*time;
random rep rep*trt*time;
lsmeans trt time trt*time/pdiff;
run;
```

Appendix B - Statistical Analysis for Turkey Tenders

Below is the SAS statistical analysis program (Statistical Analysis Systems software, (SAS Institute, Inc., Cary, NC) and data that was used to determine differences among *Listeria monocytogenes* populations for packaging environments and storage times. The data is listed in seven columns. The first column is a code where the first number represents the replication and the second letter represents the product (kippered beef steak), the third, fourth, and fifth letters represent the packaging treatment (HO=heat sealed oxygen scavenger; HNO=heat sealed no oxygen scavenger; VO=vacuum; and NO=nitrogen flushed oxygen scavenger), and the number at the end represents the sample. The second column represents the packaging treatment. The third column represents the storage times of 0, 24, 48, or 72 h, the forth column is the sample number, the fifth column is the replication, and the sixth column is the log cfu/g average count for two duplicate plates with the seventh column being a corrected value. Missing values also were determined as needed for the analysis.

```
*data tenders;
input Code $   trt $    Time   sample rep     logcfu1 logcfu2;
if logcfu1 = . then logcfu1 = 0;
logcfu_avg = (logcfu1 + logcfu2)/2;
datalines;
1THO1       HO    0      1      1      5.4879 5.4879
1THO2       HO    0      2      1      5.7406 5.7406
1THO1       HO    24     1      1      4.8326 4.8326
1THO2       HO    24     2      1      4.2403 4.2403
1THO1       HO    48     1      1      4.6587 4.6587
1THO2       HO    48     2      1      4.7164 4.7164
1THO1       HO    72     1      1      4.0971 4.0971
1THO2       HO    72     2      1      <1.477 1.447
1THNO1      HNO   0      1      1      5.4879 5.4879
1THNO2      HNO   0      2      1      5.7406 5.7406
1THNO1      HNO   24     1      1      3.5638 3.5638
```

1THNO2	HNO	24	2	1	5.0499 5.0499
1THNO1	HNO	48	1	1	4.0273 4.0273
1THNO2	HNO	48	2	1	3.9085 3.9085
1THNO1	HNO	72	1	1	4.1717 4.1717
1THNO2	HNO	72	2	1	4.1656 4.1656
1TVO1	VO	0	1	1	5.4879 5.4879
1TVO2	VO	0	2	1	5.7406 5.7406
1TVO1	VO	24	1	1	4.9699 4.9699
1TVO2	VO	24	2	1	4.9158 4.9158
1TVO1	VO	48	1	1	4.6939 4.6939
1TVO2	VO	48	2	1	4.3606 4.3606
1TVO1	VO	72	1	1	4.2500 4.2500
1TVO2	VO	72	2	1	4.3338 4.3338
1TNO1	NO	0	1	1	5.4879 5.4879
1TNO2	NO	0	2	1	5.7406 5.7406
1TNO1	NO	24	1	1	4.4846 4.4846
1TNO2	NO	24	2	1	4.1930 4.1930
1TNO1	NO	48	1	1	<2.477 2.447
1TNO2	NO	48	2	1	5.3463 5.3463
1TNO1	NO	72	1	1	4.3751 4.3751
1TNO2	NO	72	2	1	4.3533 4.3533
2THO1	HO	0	1	2	6.2797 6.2797
2THO2	HO	0	2	2	6.1889 6.1889
2THO1	HO	24	1	2	3.9395 3.9395
2THO2	HO	24	2	2	4.1911 4.1911
2THO1	HO	48	1	2	4.3692 4.3692
2THO2	HO	48	2	2	4.9788 4.9788
2THO1	HO	72	1	2	4.2028 4.2028
2THO2	HO	72	2	2	4.0889 4.0889
2THNO1	HNO	0	1	2	6.2797 6.2797
2THNO2	HNO	0	2	2	6.1889 6.1889

2THNO1	HNO	24	1	2	3.8921 3.8921
2THNO2	HNO	24	2	2	4.9209 4.9209
2THNO1	HNO	48	1	2	4.0334 4.0334
2THNO2	HNO	48	2	2	3.3445 3.3445
2THNO1	HNO	72	1	2	4.2404 4.2404
2THNO2	HNO	72	2	2	3.9216 3.9216
2TVO1	VO	0	1	2	6.2797 6.2797
2TVO2	VO	0	2	2	6.1889 6.1889
2TVO1	VO	24	1	2	4.9775 4.9775
2TVO2	VO	24	2	2	4.2354 4.2354
2TVO1	VO	48	1	2	4.8205 4.8205
2TVO2	VO	48	2	2	5.1103 5.1103
2TVO1	VO	72	1	2	3.8751 3.8751
2TVO2	VO	72	2	2	4.3588 4.3588
2TNO1	NO	0	1	2	6.2797 6.2797
2TNO2	NO	0	2	2	6.1889 6.1889
2TNO1	NO	24	1	2	5.5406 5.5406
2TNO2	NO	24	2	2	4.2298 4.2298
2TNO1	NO	48	1	2	4.3692 4.3692
2TNO2	NO	48	2	2	4.9788 4.9788
2TNO1	NO	72	1	2	4.0887 4.0887
2TNO2	NO	72	2	2	4.1259 4.1259
3THO1	HO	0	1	3	5.3372 5.3372
3THO2	HO	0	2	3	5.6421 5.6421
3THO1	HO	24	1	3	3.6527 3.6527
3THO2	HO	24	2	3	4.0770 4.0770
3THO1	HO	48	1	3	4.0569 4.0569
3THO2	HO	48	2	3	3.1048 3.1048
3THO1	HO	72	1	3	1.4771 1.4771
3THO2	HO	72	2	3	2.3222 2.3222
3THNO1	HNO	0	1	3	5.3372 5.3372

```
3THNO2      HNO   0     2     3     5.6421 5.6421
3THNO1      HNO   24    1     3     4.6839 4.6839
3THNO2      HNO   24    2     3     4.0086 4.0086
3THNO1      HNO   48    1     3     3.2553 3.2553
3THNO2      HNO   48    2     3     4.3222 4.3222
3THNO1      HNO   72    1     3     3.4216 3.4216
3THNO2      HNO   72    2     3     3.6626 3.6626
3TVO1       VO    0     1     3     5.3372 5.3372
3TVO2       VO    0     2     3     5.6421 5.6421
3TVO1       VO    24    1     3     4.0493 4.0493
3TVO2       VO    24    2     3     4.1319 4.1319
3TVO1       VO    48    1     3     5.2054 5.2054
3TVO2       VO    48    2     3     4.9108 4.9108
3TVO1       VO    72    1     3     4.3139 4.3139
3TVO2       VO    72    2     3     4.3878 4.3878
3TNO1       NO    0     1     3     5.3372 5.3372
3TNO2       NO    0     2     3     5.6421 5.6421
3TNO1       NO    24    1     3     4.7583 4.7583
3TNO2       NO    24    2     3     5.7307 5.7307
3TNO1       NO    48    1     3     4.3440 4.3440
3TNO2       NO    48    2     3     4.4334 4.4334
3TNO1       NO    72    1     3     3.8751 3.8751
3TNO2       NO    72    2     3     4.1067 4.1067
;
*proc print;
*title 'tenders';
*run;
*input Code $ trt $     Time    sample rep      logcfu1 logcfu2;
/*proc mixed data = tenders;
class trt time sample rep;
model logcfu2 = trt time trt*time;
```

```
random rep rep*trt*time;
lsmeans trt time trt*time/pdiff;
run;
proc mixed data = tenders;
class trt time sample rep;
model logcfu1 = trt time trt*time;
random rep rep*trt*time;
lsmeans trt time trt*time/pdiff;
run;
proc mixed data = tenders;
class trt time sample rep;
model logcfu_avg = trt time trt*time;
random rep rep*trt*time;
lsmeans trt time trt*time/pdiff;
run;
```