DISSERTATION

THE SURVIVAL OF INOCULATED POPULATIONS OF *LISTERIA MONOCYTOGENES* AND *STAPHYLOCOCCUS AUREUS* ON SHELF-STABLE MEAT BARS DURING VACUUM-PACKAGED STORAGE

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ABSTRACT

THE SURVIVAL OF INOCULATED POPULATIONS OF *LISTERIA MONOCYTOGENES* AND *STAPHYLOCOCCUS AUREUS* ON SHELF-STABLE MEAT BARS DURING VACUUM-PACKAGED STORAGE

Shelf-stable meat snacks are at risk for post-processing contamination with pathogens. Understanding the magnitude of these risks is important due to the increasing popularity of these products. Two studies were conducted to evaluate the survival of inoculated populations of *Listeria monocytogenes* and/or *Staphylococcus aureus* on ready-to-eat, shelf-stable, beef and/or poultry meat bars during vacuum packaged storage. Study I evaluated the survival of inoculated populations of *L. monocytogenes* and *S. aureus* on commercially-available, beef- and poultry-based meat bars during vacuum-packaged storage. Three different brands of commercially available beef and turkey meat bars were obtained in their original commercial packaging (brand [1, 2] and beef or turkey [B, T]: 1B, 1T, 2B, 2T, 3B, and 3T). A total of 120 bars were collected for each beef and turkey bar within brand (N = 720; study repeated for two trials). Two inocula were utilized, a five-strain mixture of *L. monocytogenes* or a five-strain mixture of *S. aureus.* Bars were removed from their commercial packaging and inoculated (both sides) for a target inoculation level of 6 to 7 log CFU/g. Following a 15-min cellular attachment time per side, bars were individually vacuum packaged and stored at 25ºC for 50 d. Microbiological analyses were conducted to enumerate surviving *L. monocytogenes* (Modified Oxford Agar; MOX) and *S. aureus* populations (Baird Parker Agar; BPA). Water activity (a_w) and pH were obtained for each bar and proximate analyses were conducted on a subset of each of the formulations (six

formulations). Surviving bacterial counts for were fitted with the Baranyi and Roberts mathematical model (DMFit version 3.5, ComBase) to determine shoulder periods (the time in days where the levels of pathogen remain at the level of inoculation) and inactivation rates (log CFU/g/day) for each pathogen on each bar type. Differences were assessed using a Mixed Models Procedure of SAS with significance reported at $P < 0.05$. Bars 1B, 1T, 2B, 2T, 3B, and 3T had average pH and a_w values of 5.25 and 0.855, 5.51 and 0.861, 4.41 and 0.877, 4.54 and 0.891, 5.20 and 0.835, and 5.26 and 0.845, respectively. In general, the turkey bars (bars 1T, 2T, 3T) had slower inactivation rates compared to their beef counterparts. Turkey bars supported survival of *S. aureus* longer ($P < 0.05$) than *L. monocytogenes*. Both pathogens survived longest ($P < 0.05$) on bar 1T; shoulder periods and inactivation rates were 22.2 days and -0.08 log CFU/g/day, respectively, for *S. aureus*, and 9.6 days and -0.16 log CFU/g/day, respectively, for *L. monocytogenes*. Additionally, of the beef bars, *S. aureus* survived the longest with a shoulder period of 12.4 days on bar 1B followed by an inactivation rate of -0.27 log CFU/g/day compared to the other beef bars. Bar 2B exhibited the highest ($P < 0.05$) death rate compared to the other five bars, with an inactivation rate of -1.20 log CFU/g/day for *S. aureus* and -0.91 log CFU/g/day for *L. monocytogenes* and no shoulder periods. Regardless of bar type, both pathogens present were after enumeration on MOX and BPA following 50 d of vacuum packaged storage. Survival of these pathogens stored at 25ºC under vacuum-packaged conditions indicates further research may be needed to assess the risk of meat bars with differing a_w parameters as a controlled factor. These data provide awareness of the survival behavior of post-processing contamination of pathogens on commercially available shelf-stable meat bar snacks.

Study II was conducted to evaluate the effects of meat bar water activity (a_w) and high-pressure processing (HPP) as a post-lethality treatment on the survival of inoculated *L.*

monocytogenes populations on shelf-stable vacuum-packaged turkey-based meat bars stored at 25ºC. A five-strain mixture of *L. monocytogenes* was used in this study. The study was repeated twice on separate start days with separate cooked batches (two a_w level $\leq 0.91, \leq 0.85$) of meat bars for each trial. The study was designed as a 2 x 2 factorial, with factors of water activity ($\leq 0.91, \leq 0.85$) and treatment (control, HPP) for two different inoculation levels (3 log CFU/g, 6 log CFU/g).There were N = 240 (n = 120 each trial) bars inoculated for the $a_w \leq 0.91$ group; half were inoculated at a target level of 6 to 7 log CFU/g, while the other half were inoculated at a target of 3 to 4 log CFU/g. Additionally, bars with $a_w \leq 0.85$ were inoculated the same as those in the higher a_w group. Following inoculation, all meat bars were individually vacuum packaged. Half of the bars from each a_w group and inoculation level were labeled for HPP treatment, while the other half were labeled as "control" and were not exposed to HPP treatment. Bars were placed into foam coolers without ice and shipped over night for HPP-treatment 18 to 20 h post-inoculation. Cornell University, Department of Food Science, HPP Validation Center, treated the bars using a Hiperbaric 55 HPP machine for 180 s at 586 MPa (5ºC). Once shipped back, treated and control vacuum packaged bars were stored in an incubator (25ºC) for 40 or 50 d. The Mixed Models Procedures of SAS version 9.4 were utilized to determine differences between treatments within inoculation level on each storage day. Least squares mean differences were reported using a significance level of $\alpha = 0.05$. Surviving *L. monocytogenes* counts were modeled as a function of storage time (day) using the model by Baranyi et al. (*7*). Surviving *L. monocytogenes* counts for each treatment were fitted to assess shoulder periods (log CFU/g/day) and inactivation rates (log CFU/g). Storage day affected ($P < 0.05$) the *L. monocytogenes* populations recovered from bars inoculated at both levels; populations tended to decrease over time. Additionally, irrespective of inoculation level, a_w ($\leq 0.91, \leq 0.85$) and post-processing treatment (control, HPP)

differed ($P < 0.05$) in *L. monocytogenes* populations during storage. For the 6 log CFU/g inoculation level, a_w was a significant effect for shoulder period and inactivation rate of the pathogen in each of the treatment combinations during storage; there were no significant effects observed for bars inoculated at 3 log CFU/g. The HPP treatment didn't ($P \geq 0.05$) affect the survival of *L. monocytogenes* compared to the control; it only reduced ($P < 0.05$) the initial and/or end of storage counts. Initial pathogen reductions obtained with HPP ranged from 0.2 to 0.6 log CFU/g (6 log CFU/g inoculation) and 0.5 to 1.0 log CFU/g (3 log CFU/g inoculation). When inoculated to 6 log CFU/g, bars with $a_w \leq 0.91$ had longer ($P < 0.05$) shoulder periods (6.5 and 8.8 days) compared to bars dried to $a_w \leq 0.85$ (1.9, 1.8 days). Likewise, bars dried to $a_w \leq 0.91$ had slower ($P < 0.05$) pathogen inactivation rates (-0.06, -0.08 log CFU/g/day) compared to bars dried to $a_w \leq 0.85$ (-0.12, -0.10 log CFU/g/day). Regardless of treatment, *L. monocytogenes* populations were recovered from all bars following 40 or 50 d of storage at 25ºC. High pressure processing of bars with $a_w \leq 0.85$ showed the greatest potential for increased control of *L. monocytogenes* presence starting with 3 log CFU/g of post-processing contamination. The a_w impacted pathogen inactivation and surviving counts on shelf-stable meat bars. Parameters of HPP should be further investigated to better understand the most effective time and temperature to increase inactivation of *L. monocytogenes* on meat bars.

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DEDICATION

This dissertation is dedicated to my husband, Joseph Bullard. I am so grateful for all your support and encouragement during my very long educational career. Thank you for standing by my side through all the highs and lows, I love you.

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CHAPTER 1

Introduction

Shelf-stable meat snacks represent a growing sector of the meat and poultry industry (*97*). Shelf-stable meat and poultry products have grown in popularity in the US, as they satisfy many consumer preferences as a snack food (*12*, *97*, *142*). There are several factors that contribute to increased demand: convenience, shelf stability, nutrient dense, and high in protein among others (*97*, *142*). Shelf-stable meat snacks are at risk for post-processing contamination of foodborne pathogens such as *Listeria. monocytogenes* and *Staphylococcus aureus*. Researchers have investigated *L. monocytogenes* and *S. aureus* on shelf-stable meat and poultry jerky products; however, demand for convenient meat snacks has driven innovation of niche dried meat products that aren't jerky (*10*, *15*, *16*, *19*, *37*, *52*, *56*, *62*, *64*, *65*, *66*, *83*, *88*, *132*, *144*, *146*). Shelf-stable meat bars are a newer product being produced that contain significant amounts of non-meat ingredients compared to their traditional dried jerky and sausage counterparts. These non-meat ingredients are often unique in nature and might include fruits, vegetables, seeds, rice and nuts. The condition of, and ingredients in, these meat bars differ depending on brand and formulation. There is a regulatory need for validated evidence of pathogen survival behavior on currently utilized water activity parameters in meat bars.

The primary pathogen of concern in ready-to-eat (RTE) shelf-stable meat and poultry products is *L. monocytogenes* (*42*, *51*, *55*, *70*, *77*, *125*, *135*, *147*). *Listeria monocytogenes* is the public health concern in RTE meat products due to its ubiquity in meat processing environments, potential for post-processing contamination, ability to grow in refrigerated environments, and it's disease severity, including high mortality (*17*, *31*, *80*, *91*, *113*, *125*). Another ubiquitous pathogen, *Staphylococcus aureus,* is a pathogen that can be introduced onto shelf-stable meat

products during handling post-lethality treatment. *S. aureus* and its toxins can tolerate harsh environments such as low water activity foods, which makes it a concern in RTE shelf-stable meat products due to their extended shelf lives under ambient temperature conditions. This pathogen is referenced in USDA-FSIS jerky compliance and other jerky research because its a_w growth limits have been used to support the a_w safe harbors for jerky production and are the standard for the production of jerky; however, these safe harbors will not necessarily prevent the survival of *S. aureus* and *L. monocytogenes*. According to the Centers for Disease Control and Prevention (CDC), *S. aureus* was involved in 13 foodborne disease outbreaks and two outbreaks were associated *Listeria monocytogenes* in 2015 (*21*). Although *L. monocytogenes* was only associated with 2 outbreaks reported in the 2015 CDC annual report, there were 19 deaths, whereas the outbreaks associated with *S. aureus* resulted in only 3 deaths (*21*). The presence of these pathogens must be addressed and controlled in shelf-stable meat products.

Currently, USDA-FSIS considers *L. monocytogenes* an adulterant in all ready-to-eat meat and poultry products (*40*, *134*). *L. monocytogenes* and *S. aureus* can only be present in dried meat products as a result of post-lethality treatment contamination from the processing environment or employees (*134*, *136*). The USDA-FSIS requires effective thermal lethality of vegetative pathogenic bacteria; however, shelf-stable meat products must have conditions unfavorable to *S. aureus* and *L. monocytogenes* to be safe from post-processing contamination (*134*, *136*). Additionally, they require establishments to have validated evidence assessing the critical parameters needed to prevent, eliminate or reduce the presence of pathogenic bacteria from post-processing contamination (*134*, *136*). The prevention of growth has been thoroughly investigated in dried shelf-stable meat snacks. However, little research has been done assessing pathogens' ability to survive during extended storage on these products produced under critical

parameters that prevents growth, but not necessarily presence. *Staphylococcus aureus* is most tolerant of lower a_w compared to *L. monocytogenes*; therefore, research investigating *S. aureus* in addition to *L. monocytogenes* in shelf-stable meat products is common (*66*, *136*).

There are several federal standards for the composition and condition of ready-to-eat and shelf-stable meat products (*65*). Historically, USDA-FSIS considered a meat and poultry product to be shelf-stable if it met a moisture:protein level of 0.75:1 (MPR; *125*). Recently, USDA-FSIS has been assessing shelf-stability more commonly by measuring water activity (*134*, *136*). Water activity is a more accurate and appropriate way to assess shelf stability in meat products, because it is an indicator of available water for microbial growth (*136*). Therefore; the aforementioned a_w safe harbors, ≤ 0.85 in oxygenated environment are required to be or ≤ 0.91 in an anaerobic environment, are the critical parameters the industry uses as drying limits, but this does not necessarily control the presence of these pathogens (*136*). These limits were developed based on the growth limits for *S. aureus* under optimal conditions; however, other factors in dried meat products might contribute to inhibition of growth and survival, such as pH (*63*, *65*, *66*, *132*). It appears that the consumer today is demanding more "moist" meat snacks; therefore, many producers have chosen to produce dried meat products with $a_w \leq 0.91$ packaged in anaerobic environments to meet their demands (*36*). While intrinsic properties can be manipulated to control post-processing growth of pathogens, there also are post-processing treatments that can be applied to reduce or eliminate presence of pathogens.

Interventions to control post-processing contamination in RTE meat products may include chemical antimicrobials added to formulations, such as acetates and lactates, natural plant based antimicrobials, packaging material with immobilized antimicrobials, and thermal pasteurization before or after packaging (*2*, *8*, *9*, *11*, *28*, *47*, *48*, *77*, *114*, *115*, *116*, *125*, *143*,

148). There are other more novel technologies such as irradiation or high-pressure processing (HPP) that may control post-processing pathogenic contamination on RTE meat and poultry (*29*, *32*, *44*, *45*, *69*, *76*). These novel technologies are increasingly investigated as alternatives for control as well as clean label options. "Clean label" is a colloquial term used to describe an ingredient list that contains minimal ingredients, free of artificial ingredients which consumers perceive as "unhealthy" or not "natural" (*6*). However, novel technologies either have consumer acceptance issues or are currently a more expensive option compared to other available interventions. Trends in consumer demands have often dictated the type of interventions being used to control pathogens in meat products, with particular interest in keeping a "clean label", which is desirable to today's consumer. One of the trending intervention technologies that allows processors to provide post-processing control of pathogens and keep a clean label, is high pressure processing (HPP).

There have been several research studies published investigating dried meat (whole muscle and ground) products and the fate of post-processing contamination of *S. aureus* or *L. monocytogenes* during aerobic and vacuum-packaged storage (*19*, *37*, *55*, *56*, *63*, *65*, *66*, *132*, *146*). Some of these studies have assessed differing a_w and pH effects on survival of *S. aureus* and *L. monocytogenes* in shelf-stable dried meat snacks (*63*, *65*, *66*, *132*). These studies have been used as scientific support for establishments as evidence inhibition of pathogen growth and survival on shelf-stable meat products (*63*, *65*, *66*, *132*). Whole muscle and ground jerky literature may not be representative for these new shelf-stable "meat bar" snacks for the producers to draw the same conclusions about the conditions of their products in their hazard analysis.

Meat bars have commonly been dried between a_w of 0.85 and 0.91 due to consumer preferences and due to the safe harbors to control growth of post-processing pathogen contamination. These safe harbors might be appropriate to prevent growth; however, there is no indication of the effects on the pathogens' ability to survive on these products during shelf-stable storage. It might be necessary for meat bar producers to understand the survival characteristics of *S. aureus* and *L. monocytogenes* under the conditions of commercially available meat bars. Currently, there is no literature investigating pathogen survival in ready-to-eat dried shelf-stable meat bars. Therefore, two experiments were conducted to evaluate survival of inoculated populations of *Listeria monocytogenes* and/or *Staphylococcus aureus* on vacuum-packaged commercially available meat bars.

CHAPTER 2

Review of Literature

2.1 History of Regulatory Requirements for Ready-to-Eat Meat & Poultry Products

Currently, the primary pathogen of concern in ready-to-eat (RTE) meat and poultry products is *Listeria monocytogenes* (*42*, *51*, *55*, *70*, *77*, *125*, *135*, *147*). *Listeria monocytogenes* is a public health concern in RTE meat products due to its ubiquity in meat processing environments, potential for post-processing contamination, ability to grow in refrigerated environments, and its disease severity (*17*, *31*, *80*, *91*, *113*, *125*). In the 1980's, *L. monocytogenes* developed as an emerging public health issue in fully cooked processed meat and poultry products (*51*, *70*, *125*, *135*). In 1987, USDA – FSIS issued a rule that *L. monocytogenes* was an adulterant in RTE meat and poultry products and there was zero tolerance for presence in product, requiring establishments to control the pathogen in products and in their environment (*70*). Although deemed an adulterant in RTE meat and poultry products, there were a few key outbreaks that followed this rule.

An outbreak occurred in 1998 which 101 people fell ill with listeriosis (*14*, *17*, *51*, *53*, *135*). The outbreak was investigated by local health departments and the Centers for Disease Control and Prevention (CDC), and was ultimately linked to hotdogs and possibly deli luncheon meat (*51*, *53*, *135*, *139*). The *L. monocytogenes* strain was isolated from both an opened and unopened package of hotdogs from the same processing plant (*20*, *51*, *53*, *135*, *139*). Eventually, the final report indicated that, in addition to the illnesses, 15 deaths, and 6 miscarriages/stillbirths associated with the outbreak (*17*, *20*, *51*, *53*, *135*, *139*). Severity of the disease makes this pathogen, although less commonly involved in foodborne outbreaks compared to other pathogens, a major public health concern in RTE foods.

Following the 1998 outbreak associated with hotdogs and deli luncheon meat, USDA-FSIS decided to have establishments reassess their Hazard Analysis and Critical Control Points (HACCP) plans in order to address the risk of *L. monocytogenes* in their process; this FSIS notice was issued addressing RTE facilities specifically (*20*, *135*, *139*). In 2002, FSIS determined that not all facilities were adequately addressing *L. monocytogenes* in their products and process, and concluded that changes needed to be made in HACCP, Sanitation Standard Operating Procedures (SSOP), Good Manufacturing Practices (GMP) and other controls in these RTE meat and poultry facilities (*17*, *20*, *135*). Therefore, in December 2002, FSIS issued a directive outlining steps that needed to be taken by USDA inspectors to ensure that the RTE meat and poultry establishments were taking appropriate measures to control *L. monocytogenes* contamination in their products (*135*, *148*). This directive outlined that facilities producing hotdogs and deli meats, without validated programs that support elimination of *L. monocytogenes* on product, the food contact surfaces, and the environment, must be subjected to an intensified testing program with FSIS (*135*). This testing program consisted of increased product and food contact testing, environmental testing in the processing facility, as well as increased review of the establishments' records and data (*135*). In 2003, following a risk assessment of *L. monocytogenes* in RTE meat and poultry released by FSIS, a public meeting was held to discuss results of the assessment (*51*, *79*, *135*). Following the assessment and public comment, FSIS updated the final rule for *L. monocytogenes* in RTE meat and poultry products (*79*, *135*).

Along with HACCP programs, FSIS expects establishments to control *L. monocytogenes* using one of 3 alternatives. Alternative 1, a plant applies a post-lethality treatment that reduces or eliminates presence of *L. monocytogenes* and also applies an antimicrobial agent or a process

that can suppress or limit growth of *L. monocytogenes*; this alternative is the most stringent method for control (*40*, *134*, *135*). Under Alternative 2, the plant can apply either a post-lethality treatment OR an antimicrobial agent or process that limits or suppresses growth of *L. monocytogenes* (*40*, *134*, *135*). Lastly, Alternative 3, completely relies on sanitation to control *L. monocytogenes* in the environment to prevent cross-contamination of the pathogen to the RTE products. The alternatives outline two different requirements of "controlling" post-processing contamination, suppressing "growth" and preventing "presence" of pathogens. The USDA-FSIS requires manufacturers RTE meat and poultry products to have validated evidence outlining the critical parameters needed to prevent the growth of any pathogenic bacteria post processing to ensure this bacterial growth is prevented when stored without refrigeration.

Although *L. monocytogenes* is the primary pathogen of concern in RTE meat products and an considered an adulterant by USDA-FSIS, there are other pathogens of concern such as *Staphylococcus aureus* and *Salmonella* (*2*, *79*, *101*, *105*, *109*, *112*, *125*, *126*). *Staphylococcus aureus* poses a similar risk as *L. monocytogenes* due to risk of post-processing contamination; this typically occurs from employees and the processing environment (*1*, *2*). *Staphylococcus aureus* is a risk in RTE meat products not only because of its potential to contaminate products post-processing, but because it is also highly tolerant of increased salt levels and decreased water activity (a_w), making it particularly risky to common RTE meat and poultry products (*1*, *4*, *16*, *109*). Ready-to-eat meat and poultry products are fully cooked and ready to consume. The cooking process, according to USDA-FSIS, should have a lethality step, killing any potential pathogens making them safe to consume. The biggest risk of pathogen contamination in RTE to eat meat and poultry is the contamination post-processing because they will not necessarily undergo a second kill step before entering commerce. The USDA-FSIS continues to provide

guidance to the industry to address control of post-processing contamination of pathogens in RTE products. However, understanding the characteristics of pathogens implicating RTE shelf-stable meat products can help in further research to prevent, eliminate, or reduce their prevalence in the final product.

2.2 *Listeria monocytogenes*

Listeria spp. are gram-positive, facultative anaerobes that are non-spore forming, rod-shaped with low genetic G + C content (*60*, *74*, *82*). The taxonomy is broken into seventeen recognized species: *L. monocytogenes, L. seeliger, L. ivanovii, L. welshimeri, L. marthii, L. innocua, L. grayi, L. fleischmanni, L. floridensis, L. aquatica, L. newyorkensis, L. cornellensis, L. rocourtiae, L. weihenstephanensis, L. grandensis, L. riparia,* and *L. booriae* (*103*). Of these, two are considered pathogenic, *L. monocytogenes* and *L. ivanovi;,* the only one considered dangerous relevant to humans, is *L. monocytogenes* (*31*, *50*, *54*, *60*, *74*, *82*, *103*, *108*). According to Scallan et al. (*117*) *L. monocytogenes* is the third leading cause of death due to microbiological related foodborne deaths in the US. *Listeria monocytogenes* is catalase positive and oxidase negative, and expresses ß-hemolysin which produces clear zones around colonies when cultured on blood agar; this hemolysin is similar to the *Staphylococcus aureus* on blood agar (*38*). *Listeria monocytogenes* is characteristic as a pathogen with its peritrichous flagella, which gives the pathogen tumbling motility (*38*, *82*). This pathogen is widely found in plants, soil, water, and foods making it a very ubiquitous organism and high risk in food production. Another characteristic that makes *L. monocytogenes* risky in food production is its higher acid tolerance compared to other food-borne pathogens; *L. monocytogenes* can grow at a pH as low as 4.5 whereas other foodborne pathogens tend to reduce or cease growth at 4.6 or less (*38*). In

addition to its acid tolerance, it is also able to grow at refrigerated temperatures as low as -1.5ºC or 29.3ºF. These characteristics make this pathogen particularly risky in food production and, although it is widely recognized as a food-borne pathogen now, it was not always recognized as risk.

In 1924, E. G. D. Murray isolated a gram-positive rod-shaped bacterium from the blood of laboratory rabbits, but did not know how to classify it at the time; therefore, he called them *Bacterium monocytogenes* (*38*, *54*, *60*). Then, in 1940, Pirie (*107*) isolated this gram-positive rod-shaped bacterium and named the genus *Listeria* for the catalase-positive rods. During this time, *Listeria* was not only being isolated from animal models, but also from humans as well as from food and environments (*38*, *54*, *60*). However, this microorganism was not identified as a pathogen of concern in human health until later (*25*, *27*, *60*, *74*). The realization that there was a pathogenic species of *Listeria* did not occur until an epidemic of listeriosis in Germany in 1949 (*38*, *54*, *60*). There were 85 newborns or stillborn infants that had granulomas detected histopathologically in several of their organs, including the liver, brain, spleen, lungs and skin (*38*, *54*, *60*). In some cases, scientists believed this to be *Corynebacterium*; however, H. P. R. Seeliger detected the motility *L. monocytogenes*, which was not consistent with *Corynebacteria* which lead him to believe it was different (*38*, *54*, *60*). This realization marked the start of the new era of research in regard to *Listeria* as a pathogen and its disease, listeriosis.

In 1961 Seeliger invested time and effort to inform the public about the risks and dangers of *Listeria* and Listeriosis and published the first book that outlined Listeriosis. With his and other's efforts, *L. monocytogenes* is now known today as the severe human and animal intracellular pathogen that can causes listeriosis, a fatal disease (*25*, *38*, *50*, *54*, *60*, *74*, *82*). From a food safety perspective, *L. monocytogenes* has the greatest potential in post-processing

contamination, particularly in refrigerated RTE foods due to its growth and survival characteristics. Many listeriosis outbreaks have been linked to *L. monocytogenes* in cold-stored RTE foods such as dairy, vegetables, fruits and meat products (*17*, *20*, *23*, *26*, *51*, *53*, *74*, *96*, *102*).

Listeria monocytogenes has 12 serotypes that have been known to cause disease; however, approximately 95% of the serotypes that have been linked to human listeriosis cases, sporadic and outbreak, are serotypes 1/2a, 1/2b, and 4b (*74*). *Listeria monocytogenes* associated with meat and poultry outbreaks in the US have decreased since 1998 after the implementation of USDA-FSIS regulatory and industry *L. monocytogenes* control initiatives (*17*). Other food industries such as dairy products (ice cream) and produce have not seen a decrease in outbreaks and in fruits and vegetables they have seen an increase (celery, lettuce, cantaloupe, sprouts, stone fruit, caramel apples) (*17*). Since 2010, these particular food products have been involved in a number of listeriosis outbreaks that have been previously considered low or moderate risk foods in risk assessments (*17*).

An outbreak occurred that was associated with pre-cut celery in 2010, in a chicken salad served at a hospital (*17*, *46*). There were 10 cases and five of which resulted in death due to listeriosis or complications associated with their pre-existing conditions and the disease; the average age of the infected individuals was 80 (*17*, *46*). A different outbreak was identified in March 2015 as a result of regular surveillance and was tied back to listeriosis cases that had occurred between 2010 and 2015 (*17*). There were nine cases associated with this outbreak and the source was determined to be consumption of contaminated ice cream (*17*, *22*). The ice cream outbreak was atypical as it was associated with a few different serotypes of *L. monocytogenes*; serotypes 1/2b, 3b, and 1/2a were associated with the contaminated product (*17*, *22*). A different

outbreak associated with cantaloupes occurred in 2011 linked to a farm in Colorado, and impacting patients in what ended up being a multistate outbreak (*17*, *23*). This ended up being one of the largest listeriosis outbreaks in the US associated with food; there were a total of 147 illnesses, 143 hospitalizations, 33 deaths and one miscarriage (*17*, *23*). The median age of the patients was 78, 99% were hospitalized and seven of the cases were pregnancy related or were newborns (*17*, *23*). This particular outbreak was caused when the producers unknowingly inoculated cantaloupes with *L. monocytogenes* using a produce scrubber that was previously used as a potato scrubber. This particular machine was not easily cleanable, and biofilm of *L. monocytogenes* caused a "perfect storm" for cross-contamination of the cantaloupes. The Food and Drug Administration (FDA) was able to detect two serotypes 1/2a and 1/2b, in the food product and in the processing environment (*17*, *23*). There were two other outbreaks that occurred between 2014 and 2016 and were associated with sprouts, stone fruits, and caramel apples (*17*). Since 2010, it is evident that the majority of the issues seen with *L. monocytogenes* were less from meat and poultry products and more with dairy or produce.

The persons at risk for listeriosis infection from contaminated foods are those with a suppressed immune system, the elderly, children and pregnant women; all of these groups have been implicated in listeriosis outbreaks associated with food products. It appears that 90% of adults have been exposed to *L. monocytogenes,* because there is presence of immune lymphocytes (*60*, *84*, *94*, *119*). Infectious dose response is variable, which explains why many adults have immune lymphocytes, but do not necessarily present disease symptoms (*17*, *31*, *60*, *84*, *94*, *108*). *Listeria monocytogenes* is very prevalent in the soil and invade and mobilize within eukaryotic cells; therefore, it has not specifically adapted as a human pathogen making it more opportunistic having multiple routes of infection and disease presence, another reason infectious

dose varies (*74*, *94*). Listeriosis can cause gastrointestinal distress in infected adult patients, as well as sepsis, meningitis (central nervous infection), and endocarditis (*38*, *118*, *119*). *Listeria monocytogenes* is an intracellular pathogen which is why it is found in many tissues and disease symptoms and manifestation varies. Listeriosis is much more severe in pregnant women, neonates, infants and elderly people.

After pregnant women have eaten *L. monocytogenes* contaminated foods, the pathogen colonizes in their small intestines, they can develop sepsis which can result in chorioamnionitis and deliver a septic infant or fetus (*118*). There are two types of neonatal listeriosis infection, one is "early onset" and "late onset". The early onset is developed from maternal sepsis and chorioamnionitis which can result in abortion, stillbirth, or premature delivery of a severely affected infant (*17*, *38*, *118*, *119*). The mortality rate of the infants born alive with listeriosis is approximately 20% and the abortion and stillbirth frequency is greater than 50% (*118*). *Listeria monocytogenes* can be found in the infant's blood, central nervous system, placenta, skin, and multiple other organs marking the characteristic intracellular nature of the pathogen. The late onset of neonatal listeriosis manifests as typical meningitis and can occur 7 to 20 days following delivery (*38*, *118*). The mortality rate of late-onset of disease is approximately 10%, but in some cases have resulted in brain damage similar to other types of neonatal meningitis (*38*, *118*). The pathogenesis of *L. monocytogenes* makes the pathogen unique in terms of how it manifests the disease depending on the state of the host and route of transmission (*38*, *118*).

Typically, the route of transmission of *L. monocytogenes* in human cases is contaminated food products. The incubation period of this pathogen can be long, which can make investigation of the food source more challenging. Once the contaminated food is ingested, it travels to the high acid environment of the stomach (*94*). Differing foods and strains of *L. monocytogenes* can

dictate the pathogens ability to survive the acidic environment of the stomach and move to the site of invasion in the small intestines (*94*).

Following entry into the small intestines, hemolysin (listeriolysin O), two phosphlipases, and protein (ActA) are all key genes for virulence of *L. monocytogenes* and essential for intracellular motility of *Listeria monocytogenes* in host cells (*74*). Once *L. monocytogenes* has infected the host cell, the pathogen internalizes in the vacuole (*74*). Listeriolysin O is expressed and promotes the escape of the pathogen from the vacuole into the cytoplasm; that is where *L. monocytogenes* can replicate (*74*, *118*, *119*). In the cytoplasm, *L. monocytogenes* cells use the actin of the host cell along with the protein ActA to create actin filaments for movement of the pathogen (*74*, *133*). These filaments allow the pathogen to propel through the host cell cytoplasm allowing it to be motile (*133*). Once the pathogen has reached the host plasma membrane, the energy created during motility allows for the *L. monocytogenes* to push through the membrane and form a protrusion that allows for invasion into the neighboring cell (*38*, *74*, *133*). This process allows for formation of a two-membrane vacuole where the pathogen can escape and allow for the start of a new replication process into other neighboring cells.

Direct cell-to-cell movement allows *L. monocytogenes* to disseminate into various tissues and organs of the host making disease symptoms differ depending on which tissues are afflicted by the *L. monocytogenes* invasion (*35*). The intracellular nature of this pathogen not only allows it to move into many tissues but also protects it from several host defenses (*35*, *108*). *Listeria monocytogenes* depends on this process of internalization into different host cells to protect it and allow it to disseminate across barriers such as placenta and blood-brain barriers (*35*). This is what makes *L. monocytogenes* a dangerous pathogen; its pathogenesis and its ultimate disease severity when it is able to infect the host. Although *L. monocytogenes* does not rank in the top

five of most common foodborne pathogens according to CDC, its severity and risk in ready-to-eat meat and poultry products makes it a significant hazard.

2.3 *Staphylococcus aureus*

Staphylococcus aureus is a gram-positive, facultative anaerobe that is sphere shaped, non-spore forming, and a non-motile pathogen (*58*). *Staphylococcus aureus* is an opportunistic and commensal pathogen that has been known to cause a wide variety of infections from superficial skin issues to severe and fatal invasive disease (*4*, *72*, *85*). This pathogen has some similarities to *L. monocytogenes* because it is opportunistic and is considered a ubiquitous as well. This pathogen is unique because of its toxin-mediated virulence, invasiveness, and antibiotic resistance (*72*). Although not the most common foodborne pathogen, *S. aureus* has emerged as a major pathogen for community acquired and nosocomial infections (*72*, *85*, *104*). *Staphylococcus aureus* has been considered a major human pathogen since the 1880s when Sir Alexander Ogston first proposed that it was the source for a major wound infection (*4*, *104*). Additionally, in 1884, the first documented event of staphylococcal foodborne disease was linked to contaminated cheese in Michigan (*72*). In 1941, Skinner and Keefer (*123*) further provided evidence of *S. aureus* virulence when they reported that 122 infected Boston City Hospital patients had 82% mortality. Although not a spore-forming organism, *S. aureus* can become contaminated in food products, typically during food preparation handling and processing (*72*, *104*).

Although reportedly not as prevalent a foodborne pathogen such as *Salmonella*, *S. aureus* is a significant cause of foodborne illness, causing approximately 241,000 illness per year in the United States (*72*). However, there are several factors that contribute to the incidence of *S.*

aureus foodborne disease to be under reported and under recognized. Today, sporadic foodborne disease cases caused by *S. aureus* are not reportable in the US, only the ones that are associated with outbreaks (*13*, *72*, *95*). Additionally, other reasons *S. aureus* foodborne disease may be under reported are misdiagnosis, improper sample collection, lack of seeking medical attention by the afflicted patient, lack of routine surveillance of clinical stool specimens for *S. aureus* or the enterotoxins it produces (*4*, *5*, *13*, *58*, *104*). Not only are there issues with reporting details of possible ill patients, but also confirming possible implicated foods during the time of outbreaks (*13*, *72*).

Between 1998 and 2008, there were approximately 458 foodborne outbreaks associated with *S. aureus,* 167 of which were confirmed and 291 suspected (*13*). In 2016, 86 children may have been infected with *S. aureus* toxins found in the food that was served to them at daycares in Montgomery, AL; 35 of the children were hospitalized (*41*). The toxin was found in the foods served at the daycare centers by the Alabama Department of Public Health (*41*). Other outbreaks have involved large gatherings of people at events where contaminated food was served; this is a common scenario since cross contamination from people (found in the nose of 25% of adults) to food and temperature abuse can result in growth of *S. aureus* production of toxins which are the causative agent for *S. aureus* food poisoning (*34*, *71*). In *S. aureus* related outbreaks in the US, 77.8% of the toxins found were staphylococcal enterotoxin (SE) A, then 37.5% by SED and 10% by SEB (*72*). There was an outbreak associated with coleslaw in the US which was linked to SEC produced by a methicillin-resistant *S. aureus* (MRSA) from a person handling the coleslaw that was asymptomatic (*72*). Many humans and animals carry *S. aureus* with no symptoms, and typically people are more familiar with the skin infection rather than the food related illness.

The onset of *S. aureus* intoxication can be very rapid following ingestion of contaminated food as soon as 30 min to 6 hours and immediately presents as a gastrointestinal illness (*4*, *5*, *24*, *72*). This quick onset of symptoms is due to the production of one or more toxins by *S. aureus* during growth (*24*, *72*). These symptoms might include vomiting, nausea, stomach cramps and diarrhea and is not considered contagious to others (*5*, *24*). Although most cases are self-limiting, and symptoms improve within 24 to 48 hours, it can be severe in immunocompromised individuals, including infants, the elderly and pregnant women (*72*). Approximately 10% of people who become ill will go to a hospital (*5*, *13*, *72*). As discussed earlier, detection and sampling issues contribute to underreporting, this includes foods that were contaminated prior to cooking, formed toxins and then were cooked, so that the pathogen was no longer present but the toxins were (*5*, *72*). In order to conclude using diagnostics that a foodborne illness was related to *S. aureus* it has to be based on detecting the toxins in the food (*5*, *13*, *34*, *104*). On March 19, 2018 at a FSIS-Industry meeting with the US Food Safety and Inspection Affairs Committee, Scientific Affairs, Tiffany Lee reported that Dr. David Goldman updated FSIS's confirmatory step in the detection of *S. aureus* and will now only test for the presence of the toxin.

Staphylococcus aureus enterotoxins are the causative agent for gastrointestinal exotoxins synthesized by the pathogen during its logarithmic phase of growth or during transition from phases, exponential to the stationary phase (*5*, *72*). The toxins that *S. aureus* can produce are part of nine major serological categories; all the toxins are heat stable making them challenging when raw food is contaminated they can tolerate the cooking process (*5*, *13*, *72*, *104*). The toxins are staphylococcal enterotoxins (SEs) and are SEA, SEB, SEC, SED, SEE, SEG, SHE, SEI, and SEJ all that belong to the large family of pyrogenic toxin superantigens (*5*, *72*). These type of toxins cause superantigenic activity like immunosuppression and nonspecific T-cell proliferation (*72*).

This type of behavior is said to allow the toxins to enter the blood stream which ultimately triggers intestinal inflammatory response (*72*).

The staphylococcal enterotoxins, as previously mentioned, are resistant and very stable during heat treatment of foods, as well as other extreme conditions such as freezing as well as drying (*5*, *72*). There are other types of harsh environments such as low pH, which make the toxins functional in the GI tract once it is ingested (*72*). With these characteristics it makes *S. aureus* a significant hazard in food industries and the preparation of foods (*4*, *5*, *13*, *58*, *72*, *104*).

Improper and poor food handling practices in retail food industries are believed to be the reason for high foodborne disease outbreaks. Some studies have conveyed that the majority of outbreaks associated with *S. aureus* resulted from poor handling practices (*72*, *104*). In the US, between 1975 and 1998, 42% of the foodborne outbreaks were food contacted by persons with contamination on their hands; in some processing plants, *S. aureus* is the most prominently detected pathogen in their food products (*72*, *87*, *104*). Additionally, *Staphylococcus aureus* toxins are as tolerant in harsh environments which make it a risk to food processing facilities.

S. aureus toxins are able to survive what might be consider inhospitable environments such as high temperature, freezing, drying and low pH environments. Water activity (a_w), pH, temperature, and percent salt are all key factors that dictate whether or not *S. aureus* is able to survive, grow and produce these stable toxins. The range of water activity that *S. aureus* can survive and grow under is much larger compared to other foodborne pathogens (*58*). Aerobically, *S. aureus* can grow between 0.83 and 0.99 a_w, and 0.85 to 0.99 for toxin formation (*58*). The optimal pH range for *S. aureus* is 6 to 7, but it can grow and form toxins between 4 and 10 (*58*). The pH tolerance of *S. aureus* also depends on the state of other characteristics such as oxygen or water activity; for example, when anaerobically cultured, *S. aureus* produced toxins

no lower than 4.6 (*5*, *58*, *104*). The temperature range for *S. aureus* growth and toxin formation is 7 to 48ºC (*58*). Lastly, the range for *S. aureus* growth in foods with salt, are 0% to 20% salt and 0% to 10% for toxin formation; most processed meat products range from 1% to 3% depending on the product, making them reasonable targets for this pathogen and its toxin formation along with other food products (*5*, *58*). The tolerance of *S. aureus* and its toxins under harsh environments makes it a concern in RTE meat products due to their extended shelf lives. Critical Parameters are cautiously used when creating these products to avoid pathogen contamination of both *S. aureus* and *L. monocytogenes.*

2.4 Ready-to-Eat Meat Safety and Pathogen Control

The primary way to control post-processing contamination of *L. monocytogenes* and other pathogens is through sound GMPs and SSOPs that are properly implemented into daily production (*80*, *81*, *125*, *126*, *145*). These two foundational programs will aid in prevention of *L. monocytogenes* in the processing environment and prevent cross-contamination from the employees. There are other activities that can help inhibit presence and growth of pathogens including physical, chemical and biological interventions that can be applied in the products and/or process (*9*, *29*, *59*, *76*, *126*).

Interventions to control *L. monocytogenes* post-processing in RTE meat and poultry include chemical antimicrobials such as acetates and lactates, natural plant-based antimicrobials, packaging material with immobilized antimicrobials, and thermal pasteurization before or after packaging (*2*, *8*, *9*, *11*, *28*, *47*, *48*, *77*, *114*, *115*, *116*, *125*, *143*, *148*). There are other novel technologies, such as, irradiation or high pressure processing that are used to control post-processing contamination of pathogens on RTE meat and poultry (*29*, *32*, *44*, *45*, *69*, *76*). These

novel technologies are being increasingly investigated as alternatives for control as well as clean label options. However, the novel technologies either have consumer acceptance issues or are currently a more expensive option compared to other available interventions. Therefore, the most common forms of control, are antimicrobial ingredients incorporated into the formulation of RTE meat and poultry products (*8*, *9*, *11*, *49*, *115*, *126*, *143*, *148*).

Antimicrobial ingredients typically used are salt based (sodium, potassium, etc.) and organic acids such as lactic, acetic and other acids (*8*, *9*, *11*, *18*, *49*, *92*, *115*, *120*, *127*, *143*). They are Generally Recognized as Safe (GRAS), easy to use and effective at controlling post-processing contamination of *L. monocytogenes* in RTE meat and poultry (*115*). Following the 1998 outbreak of listeriosis involving meat products, FSIS increased the allowable level of sodium lactate, sodium acetate, and sodium diacetate in formulations in response (*115*). The levels allowed for these antimicrobials in meat formulations is 3% of commercially available sodium lactate, and 0.25% of sodium acetate or sodium diacetate (*138*). Research has shown that using these antimicrobials are effective at controlling post-processing contamination of *L. monocytogenes*, other pathogenic bacteria, and spoilage organisms for extended storage periods (*8*, *11*, *47*, *48*, *49*, *92*, *115*, *127*).

A study published by Bedie et al. (*11*) investigated the effect of antimicrobials incorporated into formulations on inoculated *L. monocytogenes* populations during refrigerated storage (4ºC) of cooked vacuum-packaged frankfurters. The frankfurter formulations included either sodium lactate (3 or 6% as a pure substance of a commercial liquid), sodium acetate (0.25 or 0.5%), or sodium diacetate (0.25 or 0.5%) as treatments (*11*). After 20 days of storage, control frankfurters (containing no antimicrobials) grew from 10^3 CFU/cm^2 to 10^6 CFU/cm^2 (*11*). The antimicrobial treatments decreased the rate of growth of *L. monocytogenes*, but there were two

treatments that were able to inhibit growth and also reduce the presence of *L. monocytogenes* populations (*11*).

In this study, the sodium lactate at 6% and sodium diacetate at 0.5% were bacteriostatic in the frankfurter formulations and also reduced the *L. monocytogenes* populations during 120 days of refrigerated vacuum storage (*11*). The treatments that were within the FSIS allowable limit (sodium lactate 3%, sodium diacetate 0.25%, and sodium acetate 0.25%) controlled growth for up to 70 days for 3% sodium lactate, and 25 to 50 days for 0.25% sodium lactate and sodium diacetate, respectively (*11*). Bedie et al. (*11*) concluded that the current allowable levels of these antimicrobials may inhibit growth of *L. monocytogenes* between 35 and 70 days depending upon the antimicrobial.

Similarly to the previous study, Samelis et al. (*115*) observed that sodium lactate (1.8%; 3% of a 60% commercial solution) inhibited growth of *L. monocytogenes* on frankfurters for 35 to 50 days under vacuum packaged refrigerated storage (4ºC). However, in the Bedie et al. (*11*) study, treatments with higher levels than those permitted by USDA-FSIS, provided greater control for growth and bacteriocidal properties which continued to reduce the presence of *L. monocytogenes* for as long as 120 days (*11*). Results of Seman et al. (*120*) agreed and found that both sodium diacetate and sodium lactate resulted in significant reductions in the growth rate constants of inoculated populations of *L. monocytogenes* on cured RTE meat products. The individual effects of these antimicrobial ingredients were effective at inhibiting growth rates during storage; however, they may not provide bacteriocidal affects against *L. monocytogenes* contamination. In a different study, Samelis et al. (*115*) determined the effect of combinations of antimicrobials included in a frankfurter formulation as a control method for post-processing contamination of *L. monocytogenes* for up to 120 days of refrigerated storage (*115*). They found

that, when sodium lactate (1.8%; 3% of a 60% commercial solution) was used in combination with 0.25% sodium acetate, sodium diacetate, or glucono-δ-lactone (GDL), sodium lactate in combination with the other compounds inhibited growth of *L. monocytogenes* throughout storage to 120 days on (*115*).

Researchers also investigated effects of antimicrobial ingredients in RTE meat products against post-processing contamination of *L. monocytogenes* when the RTE products were stored at abusive temperatures (*9*, *47*, *48*). In an attempt to understand the effects of these antimicrobials when temperature abuse occurs, a study was conducted by Geornaras et al. (*48*) to compare the antilisterial activity of frankfurters formulated with and without potassium lactate and sodium diacetate combinations as processing aids and were stored at 10ºC. The combinations of potassium lactate and sodium diacetate inhibited growth of *L. monocytogenes* on frankfurters when they were stored under vacuum at 10ºC (*48*). Use of these antimicrobials increased the lag phase of *L. monocytogenes* to 10.1 days and had a lower growth rate of 0.154/day compared to the control with no lag phase and a growth rate of 0.485/day (*48*). Another study by Barmpalia et al. (*9*), compared antilisterial effects of sodium lactate, sodium diacetate and GDL, used individually and in combination, in pork bologna formulations stored at refrigerated (4ºC) and abusive (10ºC) temperatures (*9*). Combinations of 1.8% sodium lactate and 0.25% sodium diacetate were most effective at controlling the growth of post-processing contamination of *L. monocytogenes* at both storage temperatures on pork bologna (*9*). Hence, data from multiple studies indicated that combinations of antimicrobial formulation ingredients are the most effective way to control post-processing contamination of *L. monocytogenes* at refrigerated and abusive storage temperatures (*9*, *11*, *48*, *49*, *69*, *92*, *115*, *120*, *127*).

Other interventions have been explored to determine their ability to inhibit growth or reduce the presence of post-processing contamination of *L. monocytogenes*. A study by Amèzquita and Brashears (*3*) investigated inhibitory effects of naturally occurring lactic acid bacteria (LAB) on RTE meat products contaminated with *L. monocytogenes*. They found three strains that expressed antimicrobial properties, *Pediococcus acidilactici*, *Lactobacillus casei*, and *Lactobacillus paracasei;* where their mode of action was suspected to be competitive inhibition (*3*). It is also thought that some of the antimicrobial properties of LAB populations result from bacteriocin production or organic acid production, which might explain their antilisterial effects (*3*). Other researchers have utilized specific bacteriocins as an intervention on RTE meat products to determine their antilisterial effects to control post-processing contamination (*43*, *44*, *47*, *114*).

A study by Franklin et al. (*43*) explored effects of packaging films coated with solution containing 10,000, 7,500, 2,500, or 156.3 IU/ml of nisin, a bacteriocin, to control *L. monocytogenes* on the surface of vacuum-packaged hot dogs during refrigerated (4ºC) storage. The packages containing 10,000 and 7,500 IU/ml of nisin decreased *L. monocytogenes* populations on the surface of the hot dogs by greater than 2 log CFU per package (initial inoculation 5 log CFU per package) throughout 60 d of refrigerated storage (*43*). Packages with 2,500 IU/ml of nisin also reduced *L. monocytogenes* populations on the surface of hot dogs, but did not have as large of a reduction as the higher concentrations (*43*). Another study investigated a different application of nisin as an immersion treatment, with and without other organic acids, to inhibit *L. monocytogenes* on sliced pork bologna stored at 4ºC for 120 days in vacuum packaging (*114*). Samelis et al. (*114*) utilized nisin at 5000 IU/ml and treated the bologna by dipping the product into the nisin solution. Nisin, alone, reduced *L. monocytogenes* by 1.0 to 1.5

log CUF/cm^2 on day-0 immediately following treatment (*114*). Samelis et al. (*114*) concluded that, of all treatments, the combination of nisin and 3 g/100ml of sodium diacetate was the most favorable treatment for controlling *L. monocytogenes* on sliced RTE pork bologna.

In addition to chemical and biological interventions utilized for inhibition of post-processing contamination of *L. monocytogenes*, researchers also have investigated the capabilities of thermal treatments to control *L. monocytogenes* (*148*). Effects of surface pasteurization temperatures on survival and destruction of *L. monocytogenes* on low fat turkey bologna showed that exposure at 85ºC in a water bath for 10 s reduced populations by > 6 log CFU/ml (*93*). However, McCormick et al. (*93*) observed viable *L. monocytogenes* cells after 10 min of heating at 61ºC. In a different study, Muriana et al. (*100*) investigated use of a steam injected water bath to pasteurize large packages of RTE deli meats by submersion to reduce post-processing contamination of *L. monocytogenes*. Muriana et al. (*100*) reported that submersion in steam injected water at 90.6ºC, 93.3ºC, and 96.1ºC between 2 and 10 min, resulted in 2 to 4 log CFU/g reduction in *L. monocytogenes* populations, depending on the time and temperature combination. The challenges observed for heat treating larger packages of deli meats were the inconsistencies in the contamination on cut surfaces, folds, grooves and skin (*100*). The authors concluded that the most consistent and effective intervention was heating to 90.6 and 96.1ºC for 2 min for most RTE deli meats to reduce *L. monocytogenes* populations (*100*). Overall, the literature varies on efficacy of thermal pasteurization treatments against *L. monocytogenes*; variables that dictate efficacy were package size, surface variability, and initial population level.

Other less common interventions, such as irradiation, have been explored to reduce and inhibit *L. monocytogenes* contamination. Irradiation is very effective at controlling food-borne pathogens in RTE meat products; however, it has some quality defects and has negative

consumer perception making it uncommon in the meat industry (*148*). Irradiation, or ionizing radiation, is a process where products are exposed to radiant energy such as gamma rays, electron beams, and X-rays (*148*). Specifically, gamma irradiation uses high-energy gamma rays from cesium 137 or cobalt 60, which have the ability to treat bulky foods, including pallets of food (*148*). In contrast, electron beam, otherwise referred to as "E-beam", uses streams of high-energy beta rays that penetrate fairly shallow surfaces of up to 5 cm (*148*). Lastly, X-irradiation is intermediate to other technologies and penetrates less than gamma rays, but deeper than E-beam (*148*). Use of these technologies, although not common in the meat industry, have been widely investigated.

Miyahara et al. (*99*) reported that gamma-ray irradiation was effective at decreasing *Bacillus cereus* and *E. coli* O157:H7 populations in Hexane and fatty acid solutions; however, was not very effective at reducing *L. monocytogenes* populations. Lamb et al. (*76*) investigated use of low-dose gamma irradiation against a different pathogen, *S. aureus,* in RTE ham and cheese sandwiches. They concluded that use of low-dose gamma irradiation proved to be effective at inhibiting growth of *S. aureus* during refrigerated storage (*76*). A different study investigated use of gamma irradiation to control post-processing contamination of *L. monocytogenes* in RTE ham and cheese sandwiches (*33*) and concluded that, in order to achieve an initial 5-log reduction of *L. monocytogenes*, gamma rays needed to be applied at 3.5 to 4.0 kGy, and would continue to provide antimicrobial properties during frozen storage compared to a control that did not change. Resistance of *L. monocytogenes* to irradiation varied depending on physiological state of cells, and generally showed greater resistance when cells were stressed (*98*, *148*). Mendonca et al. (*98*) investigated effects of E-beam irradiation against *L. monocytogenes* strain Scott A and the effects of starvation in NaCl saline in ground pork.

Following exposure of E-beam at 2.5 kGy on ground pork, *L. monocytogenes* was reduced by 6.0 log, but when the cells were starved only *L. monocytogenes* was reduced by only 3.8 log (*98*). In addition the state of the cells (i.e. stressed or stable), other researchers found that effects of irradiation differed depending on the type of the food product (*129*, *130*, *131*). A study by Thayer et al. (*131*) reported that effects of gamma irradiation on *L. monocytogenes* differed between cooked and raw turkey breast meat. Other researchers have found that combinations of antimicrobials, such as sodium lactate as a product formulation ingredient in combination with irradiation application, provided additional control against *L. monocytogenes* on meat products (*148*).

There are many interventions that can be utilized to control post-processing contamination of pathogens on RTE meat products. Control of *L. monocytogenes* has been a primary focus of research in RTE meat products in the US due to its disease severity, high mortality, ubiquity, ability to grow under refrigeration, and FSIS zero tolerance in RTE meat products. Depending on product type and risk of contamination, there are many interventions that have proven to be effective against controlling presence and growth of *L. monocytogenes.* Combinations of these interventions used in a multiple-hurdles food safety system provide the most control against post processing contamination of pathogens.

Trends in consumer demands have tended to influence the type of interventions being used to control pathogens in meat products, with particular interest in keeping a "clean label", to meet the desires of today's consumer. One of the trending intervention technologies that allows processors to provide post-processing control of pathogens while keeping a clean label, is high pressure processing (HPP).

2.5 High Pressure Processing in Ready-to Eat Meat Products

High pressure processing is a newer technology that is a non-thermal method of food preservation which has attracted interest in the last several decades because of its ability to inactivate microorganisms while still maintaining the original taste, odor, nutritional, and flavor properties of the food (*29*, *44*, *45*, *57*, *75*, *110*, *111*, *124*, *128*, *141*). High pressure processing involves uniform distribution of high pressure throughout the food material, regardless of its size and shape (*61*). High pressure processing was first adopted as a technology used in the chemical, ceramic steel and plastic industries. More recently, it was utilized in the food industry to control pathogen presence in food products (*61*, *75*). This technology is appealing as a preservation method because it is mild, eliminates pathogenic and spoilage microorganisms, interest in this technology has increased in the meat industry (*45*). The first reported use of HPP as a method for microbial control was in 1899, Hite and his researchers (*30*) reported this technology's ability to inactivate microbial populations; however, it wasn't until the 1980s when Farkas with the University of Delaware, reported that HPP reduced microbiological load of foods but still maintained other natural characteristics of the food (*29*, *44*, *45*, *61*).

The mode of action of HPP to inactivate microorganisms is likely the result of cell membrane damage, but also a combination of factors such as damages inside the cell (*45*, *73*, *122*). The very high pressure of HPP is necessary to damage bacterial spores, but has been documented to have some negative color effects as it can impact enzymes and protein structure (*73*). Cell death increases with pressure, but does not necessarily follow first-order kinetics; decrease in inactivation can occur (*45*, *73*). Pressure between 30 and 50 MPa can influence gene expression and protein synthesis and it is thought to be able to interfere with replication of DNA (*124*). Research shows that cells subjected to prior stress, for example heat stress or cells in

stationary phase, tend to be more resistant to pressure (*124*). Factors such as temperature can play an vital role in the efficacy of HPP against pathogens (*45*). When pressure is applied at optimal bacteria growth temperatures, reduced inactivation is observed compared to higher or lower temperatures due to the fluidity of the bacterial cell membrane at high and low temperatures becoming more easily disrupted (*45*, *124*). The food matrix can also dictate the efficacy of HPP against specific microorganisms. Ability of pathogens to survive greatly increases depending on the composition of the food during the time of treatment, particularly in nutrient rich foods such as meat and poultry products (*45*, *121*).

There are intrinsic characteristics, such as pH and aw that my enhance the inactivation of pathogens during HPP such as pH and water activity (*61*, *124*). Most foodborne pathogens are sensitive to pH 4.6 or less and can enhance the inactivation of the HPP treatment and may provide continual control during storage of these foods (*124*). There are general differences associated with osmotic effects depending on water activity on the cell, and there are also specific effects of factors that may influence the water activity (*61*, *124*). Salt content of a food tends to be less protective than carbohydrates. The lower the water activity, the more protection it provides the cells from pressure, but bacteria injured by pressure are more sensitive to low water activity which will aid in inactivation (*61*, *67*, *106*, *124*). Additionally, recovery of pressure treated bacterial cells tend to be much lower when in the presence of 2% salt; however, the efficacy of pressure on bacteria in a low water activity environment is challenging to predict (*61*, *67*, *106*, *124*). Therefore, research evaluating control of these specific pathogens on specific foods is needed to truly asses the efficacy of HPP.

Jofré et al. (*67*) conducted a study that evaluated the effect of HPP at 600 MPa on three convenience meat products (sliced cooked ham, sliced dry cured ham, and marinated beef)

against multiple foodborne pathogens, including *L. monocytogenes*. In this study, meat products were stored up to 120 days post treatment at 4ºC (*67*). The researchers reported that dry-cured ham with a_w of 0.918 had lower inactivation levels of *L. monocytogenes* compared to the cooked ham and beef loin. However, the researchers reported that the immediate effect may have been less on the dry cured hams compared to the higher water activity product, but it maintained an inhibitory effect during storage (*67*). Pressure resistance of the *L. monocytogenes* on the dry cured ham is likely due to the stable state of macromolecules at the low water activity (*67*, *121*).

A different study investigated HPP effects in combination with a packaging containing an antimicrobialn(bacteriocins) to target *L. monocytogenes* inoculated on cooked ham during storage at 6ºC (*89*). In this study, Marcos et al. (*89*) reported that untreated control packages of ham allowed for growth of *L. monocytogenes* reaching 8.6 log CFU/g within 22 days of storage, thus providing evidence of the need for additional interventions to control *L. monocytogenes*. Antimicrobial packaging alone delayed growth of *L. monocytogenes* on ham until day 8 of storage, however, again still providing evidence of the need for multiple hurdle interventions (*89*). When inoculated ham samples were treated with HPP (400 MPa, 10 min, 17ºC), researchers observed an immediate 3.4 log CFU/g reduction in *L. monocytogenes* populations which inhibited growth until day 8 of storage, but then did not achieve levels higher than the initial inoculation until day 22 of storage (*89*). Although HPP in combination with antimicrobial packaging proved to maintain the lowest counts during storage at 6ºC, it was not able to completely prevent growth of *L. monocytogenes* on inoculated ham slices (*89*).

Marcos et al. (*90*) investigated similar treatment parameters, but with storage at 1ºC as well as 6ºC, and antimicrobial packaging with lactate-diacetate compounds. The lactate-diacetate exerted control against *L. monocytogenes* during the three-month storage period at 1ºC and 6ºC,

even after temperature abuse (*90*). Marcos et al. (*90*), reported that the combination of low storage temperature (1ºC), HPP, and the lactate-diacetate reduced the inoculated *L. monocytogenes* on cooked ham during storage by 2.7 log CFU/g. Multiple interventions with the inclusion of HPP provided the most control on refrigerated, high-moisture cooked ham products. Similar results were seen in other research studies with objectives investigating HPP effects against *L. monocytogenes* on cooked ham products (*45*, *68*).

Lucore et al. (*86*) investigated effects of HPP (300, 500, 700 MPa) on *L. monocytogenes* inoculated vacuum packaged frankfurters. Treatments included HPP exposure at 300 MPa for 0, 1, 3, 5, or 7 min, 500 MPa for 0, 0.5, 1.5, 3, or 6 min, and 700 MPa for 0, 15, 30, 45, or 60 s (*86*). Lucore et al. (*86*) reported that the higher HPP pressure treatment (700 MPa) resulted in greater inactivation. Overall, there was > 5 log reduction during the come-up time and greater than 1 log decrease for 300 MPa and > 3 log for 500 MPa. Holding frankfurter packages at each treatment pressure of 300 and 500 MPa allowed additional inactivation of *L. monocytogenes* (*86*). When packages were held at 300 MPa, continual inactivation was observed up to 7 min resulting ultimately in > 1.3-log decrease in *L. monocytogenes* populations (*86*). Similarly, packages held at 500 MPa for 6 min resulted in approximately a 6-log decrease in *L. monocytogenes*. Lucore et al. (*86*) concluded that 700 MPa resulted in the greatest inactivation of *L. monocytogenes* in the shortest period of time; however, all treatment parameters were effective at reducing inoculated populations of *L. monocytogenes* on packaged frankfurters.

Few studies have evaluated efficacy of HPP against *Staphylococcus aureus* and *L. monocytogenes* on RTE shelf-stable meat products. This is likely due to the fact that HPP efficacy has been known to decrease when water activity of the food is less than 0.92.

2.6 Food Safety Regulatory Requirements for Shelf-Stable Meat Products

Following several outbreaks of listeriosis beginning in the 1980s, FSIS and FDA teamed up to implement strategies to decrease foodborne illness from *L. monocytogenes* (*135*). In 2003 USDA-FSIS issued 9 CFR part 430, Control of *Listeria monocytogenes* in Post-lethality Exposed Ready-To-Eat Products (Listeria Final Rule) in response to a couple risk assessments related to RTE meats and *L. monocytogenes* (*29*, *93*). The *Listeria* Rule requires producers who manufacture RTE products to comply to the regulation in order to produce safe products.

As previously discussed, this rule outlines the hazards of *L. monocytogenes* and how plants must control the risk of this pathogen in their products and process (*40*). The FSIS considers *L. monocytogenes* an adulterant in all RTE meat and poultry products as well as food contact surfaces, and they expect the establishments to control this pathogen with their HACCP plan, SSOPs, GMPs, and other prerequisite programs (*40*, *135*). Along with HACCP programs, FSIS expects establishments to control *L. monocytogenes* using one of the 3 alternatives. The aforementioned alternatives focus on both the "presence" and "growth" of post-processing contamination of pathogens. The USDA-FSIS requires manufacturers of shelf-stable, RTE meat and poultry products to have validated evidence outlining the critical parameters needed to prevent the growth of any pathogenic bacteria post processing to ensure this bacterial growth is prevented when stored without refrigeration. Although inhibiting growth of pathogens in shelf-stable products is crucial, often times the importance of preventing presence of these pathogens is overlooked.

Preventing growth of a pathogen will keep the presence of a pathogen from increasing in number. Water activity, temperature, pH and oxygen presence are a few examples of ways to prevent pathogens from growing by making the product environment not favorable for growth.

These parameters are closely monitored in order to meet these requirements, however there is little consideration of these safe harbors' ability to prevent survival of pathogens. Addressing the presence is not the same as controlling growth of pathogens, although they do have a relationship. For example, if a product is produced to be shelf-stable and control the growth of post-processing contamination of pathogens such as *S. aureus* and *L. monocytogenes*, it does not necessarily mean that there is not a possibility for presence and survival of that pathogen. Therefore, preventing and eliminating presence of pathogens is equally as imperative for producing safe shelf-stable products, because they can support survival of pathogens making post lethality treatments helpful to reduce potential presence.

Post-lethality treatments might include steam pasteurization, hot water pasteurization, radiant heating, HPP, UV treatment, Infrared treatment, drying, and other validated processes (*134*). Drying reduces the water activity of the final RTE product such as jerky, dried and fermented sausages, snack sticks, etc. Most commonly shelf-stable meat snacks would fall under Alternative 2b at minimum as long as the drying process reduces the water activity of the product to a point which suppresses the growth of *L. monocytogenes*; however, if these products also have an antimicrobial agent that reduces the "presence", then they would be classified as Alternative 1 (*134*).

In order for water activity to suppress the growth of *L. monocytogenes,* it must be low enough to consider the product shelf-stable. Although *L. monocytogenes*, from a regulatory stand point, is considered an adulterant in RTE, there are other pathogens such as toxigenic *Staphylococcus aureus* that need to be addressed when making shelf-stable meat and poultry products (*136*). There is not a specific regulatory limit for what FSIS considers shelf-stable; however, they do expect establishments to provide scientific support that justifies that the a_w

limit they are using scientifically supports suppression of *L. monocytogenes* as well as any other identified significant hazards such as *S. aureus* (*136*). As previously discussed, the industry typically produces shelf-stable meat snacks to meet the limits required to inhibit growth of pathogens, but it does not necessarily equally address presence.

The target water activity limits generally used to support shelf-stable meat products is drying the product to less than or equal to 0.85 when the products are stored in aerobic/oxygenated environments such as ambient air (*136*). This limit scientifically suppresses *L. monocytogenes* growth, and is generally used because it also suppresses the toxigenic growth of *S. aureus* and is the limit for that pathogen (*58*, *136*). If products are stored in an anaerobic or reduced oxygen environments, then the critical limit generally recognized to suppress growth of these pathogens is 0.91 or less (*136*). The characteristic of low a_w might indicate that shelf-stable products are at a lower risk of post processing contamination of pathogens compared to their higher a_w RTE product counterparts, this is not true. These safe harbors inhibit growth of pathogens during long shelf lives, but do not prevent or eliminate the presence/survival of pathogens. Understanding the survival capabilities of pathogens on shelf-stable meat products produced under these safe harbors is needed. Although a_w and pH are one way to control growth of pathogens in shelf-stable meat products, investigation of pathogen behavior and control is still needed as these products have an extended shelf life which can make them risky if not produced properly and safely.

2.7 Pathogen Control in Shelf-Stable Meat Products

Listeria monocytogenes is an adulterant in all RTE meat and poultry products including shelf-stable meat snacks. The pathogen that has the highest tolerance for reduced a_w is

Staphylococcus aureus (*66*). Research shows that *S. aureus* does not grow aerobically at a a_w of 0.85 or less, and 0.88 or less anaerobically (*66*). *Listeria monocytogenes* has a higher a_w limit for growth, 0.92; the limits for *S. aureus* are used as a guide to produce shelf-stable meat snacks because if they prevent *S. aureus* growth, it will also prevent other microbial growth since it is one of the most resistant to low a_w (*66*).

The production of shelf-stable meat and poultry products has grown in popularity in the US meat (*97*). There are several factors that contribute to the increase demand: convenience, shelf stability, nutrient dense, and high in protein among others. In response to the popularity of these products, the regulatory need for validated evidence of critical parameters that will prevent, eliminate or reduce presence and or growth of pathogens, research has been conducted investigating the fate of *L. monocytogenes* and *S. aureus* on shelf-stable meat and poultry products (*10*, *15*, *16*, *19*, *37*, *52*, *56*, *62*, *64*, *65*, *66*, *83*, *88*, *132*, *144*, *146*).

A very common shelf-stable meat snack is jerky, typically made with whole muscle beef products. Historically the standards to define shelf stability in beef jerky were moisture:protein ratios (MPR); however, research has demonstrated that controlling a_w and pH have been more appropriate and consistent parameters to control (*64*, *132*). Ingham et al. (*65*) conducted a study investigating the growth potential of *S. aureus* in various RTE meat products with known MPR, a_w, pH and % salt to evaluate the growth outcome of the pathogen.

Ingham and others (*65*), obtained 34 samples of four types of jerky, two types of beef snack sticks, three pepperoni, six dried salamis, and twelve summer sausages from differing producers (*65*). The meat products were inoculated with a three-strain mixture of *S. aureus* to approximately 6 log CFU/cm^2 and then the products were vacuum packaged and stored at 21ºC for 4 weeks (*65*). Meat products with a pH of ≤ 5.1, *S. aureus* decreased by 1.1 to 5.6 log CFU

depending the salt concentration and moisture level (*65*).Very similarly, products that were dried ($a_w \leq 0.82$; MPR ≤ 0.80), but did not have an acidified pH, decreased by 3.2 to 4.5 on the jerky products (*65*). Both acidified and dried products were able to suppress growth of *S. aureus* under vacuum storage; however, the products that were neither, clearly supported growth of *S. aureus* during vacuum packaged storage at room temperature and could not be considered shelf-stable (*65*). In this study, pH and either MPR or a_w provided the most useful predictive guidance for *S. aureus* survival and growth (*65*).

A second study by Ingham et al. (*62*) evaluated survival of inoculated populations of *L. monocytogenes* during storage of RTE meat products made from drying, fermenting, and/or smoking techniques. The researchers aimed to collect information that could be used as evidence for meat processors to implement FSIS alternatives 1 or 2 in RTE production systems to control *L. monocytogenes* (*62*). Product was provided by six processors which made up different products, including jerky, summer sausage, snack sticks, sausage (elk, beef, bison), pork rinds, pork cracklings and beef pieces and slices (*62*). Water activity and pH varied widely amongst the product types; a_w ranged from 0.27 to 0.98 and pH values were between 4.7 and 6.7 pH uites (*62*). All the products were vacuum packaged following inoculation (ca. 3 to 4 log CFU/sample) of *L. monocytogenes* (except the pork rinds which were stored in an aerobic environment in a zip-lock bag) and then stored either at 5 or 21ºC for 4 to 11 weeks (*62*).

In this study, Ingham et al. (*62*) observed that the pork rinds inhibited the growth of *L. monocytogenes* since a_w was 0.27 to 0.29, with a pH of approximately 6 pH units; this product showed immediate death of *L. monocytogenes* in the first week and near the analysis detection limit after 5 weeks (*62*). Similar results were observed for the beef jerky product, which had a a_w of 0.75 and a pH of 5.6 (*62*). The researchers concluded that, based on this study and these data,

products with similar a_w, pH and water phase salt % reduced in the first week of storage at ambient temperatures; therefore, a pre-shipment hold period of one week (post-lethality treatment) can be utilized in combination with the pH and a_w values (suppress growth) to serve as a post-lethality treatment allowing the processors to operate under USDA's alternative 1 to control *L. monocytogenes* (*62*).

A different study (*83*) also investigated the potential of pre-shipment storage days and differing packaging environments on the inactivation of *L. monocytogenes* populations on whole-muscle beef jerky and smoked pork and beef snack sticks. Lobaton-Sylabo et al. (*83*) evaluated four packaging systems, heat sealed without vacuum, heat sealed with oxygen scavenger, heat sealed with nitrogen flush and oxygen scavenger, and traditional vacuum packaging. The shelf-stable meat snacks were inoculated with *L. monocytogenes* and then packaged and stored at ambient temperature (25.5ºC) for 0, 24, 48, and 72 h as well as 30 days after packaging (*83*). All packaging systems were effective at reducing *L. monocytogenes* populations on meat snack sticks following 24 h of storage (*83*). Results indicated that beef jerky processors could utilize heat sealed (HS) with oxygen scavenger or vacuum in conjunction with a 24-h pre-shipment hold time to serve as an antimicrobial process to reduce *L. monocytogenes* > 1 log CFU/cm2 or use a 48-h hold time for HS with no oxygen scavenger and nitrogen flush and oxygen scavenger packaged jerky (*83*). This study provided additional evidence of using packaging and pre-shipment hold times as a means to provide additional control against *L. monocytogenes* contamination post-processing.

There are some methods that can be applied prior to lethality to serve as a post-lethality intervention to prevent growth of pathogens. Calicioglu et al. (*18*) investigated the fate of acid-adapted or non-adapted *L. monocytogenes* during storage of beef jerky treated with differing

marinades before drying and then inoculated (~ 5.7 log CFU/cm^2) after drying and stored aerobically for 60-days. The marinades tested in this study included i) control (C), ii) traditional marinade (TM), iii) modified marinade (double of TM with 1.2% sodium lactate, 9% acetic acid, and 68% soy sauce with 5% ethanol (MM), iv) dipping into 5% acetic acid and then the TM (AATM), and v) dipping into Tween 20 and then into 5% acetic acid followed by the TM (TWTM; *18*). Results following storage showed that the marinades TWTM, AATM, and MM resulted in lower *L. monocytogenes* compared to Control and TM until 42 days of aerobic storage; after 60 days of storage, the populations in all the treatments did not differ regardless of *L. monocytogenes* acid-adaption or not (*18*). There were no major difference between the acid-adapted and non-adapted inocula, except for the control and TM which had higher *L. monocytogenes* populations at days 60 and 24, respectively (*18*). The earliest observation of no presence of *L. monocytogenes* was observed on day 28 for the AATM inoculated with acid-adapted culture and by day 42 for TWTM and AATM in products inoculated with non-adapted culture (*18*). The researchers concluded that, the results of using modified marinades in jerky processing, in combination with drying to low water activity (~ 0.589 to 0.674), provided antimicrobial effects against post-processing contamination of *L. monocytogenes* (*18*). This may also indicate inhibitory control or antimicrobial control under FSIS Alternative 1 or 2 (*18*).

Other post-lethality treatments for RTE shelf-stable meat products were evaluated by Ingham et al. (*64*). The authors evaluated a small-scale hot water post-packaging pasteurization (PPP) as a post-lethality treatment for *L. monocytogenes* on RTE beef snack sticks (*64*). There were three brands of snack sticks tested, brand-A had a a_w of 0.91 and pH of 4.5, brand-B a a_w 0.86 and pH of 5.0, and brand-C a a_w 0.89 and pH of 5.0 (*64*). Three types of packages were treated, 1 stick per package, 4 sticks per package, and 7 sticks per package. These packages were

placed into 2.8 L of boiling water for 0.5, 1.0, 1.5, 3, 4, 5, or 6 min and then analyzed for remaining *L. monocytogenes* populations (*64*). The snack sticks packaged one per bag, decreased in *L. monocytogenes* populations by 1.9, 2.8, and 3.4 log CFU/sample following 0.5, 1.0, and 1.5 minutes of PPP (*64*). Sticks packaged with 4 sticks were subjected to PPP for 3, 4, and 5 min, where 5 min was the most effective at decreasing *L. monocytogenes* populations by 4.0 log CFU/sample (*64*). Packages with 7 sticks per bag exhibited greater variation in remaining populations of *L. monocytogenes*; after 6 minutes of PPP, *L. monocytogenes* decreased only by 2.8 log CFU/sample (*64*). The hot-water pasteurization post-lethality package treatment was effective at reducing *L. monocytogenes* populations on snack sticks; however, number of sticks per packaged impacted the efficacy of the intervention (*64*). The authors also reported that, although increasing time tended to increase antimicrobial effect, it also had negative quality outcomes such as moisture and fat excreting in the packaged following treatment (*64*). Not only does amount of product per package dictate the behavior of post-processing pathogens, but also compositional components of products might affect pathogenic characteristics even within appropriate water activity limits.

Several research studies investigating the fate of post-processing contamination of pathogens on shelf-stable meat products report and consider a_w and pH attributes; however, there is debate whether combinations of the intrinsic properties truly provide shelf stability and what the critical values might be to achieve it. A different study, involving snack sticks, was conducted to identify combinations of pH and a_w that provide shelf stability for acidified, RTE meat products, particularly to control *S. aureus*; additionally, the researchers wanted to obtain information on which factor, pH or a_w, contributes more toward shelf stability (*132*).

Tilkens et al. (*132*) conducted this study designed as a 3 × 3 factorial design with pH (5.6, 5.1, and 4.7) and a_w (0.96, 0.92, and 0.88) as the factors. All treatment groups were inoculated with *S. aureus* starting at approximately 3.5 log CFU/g (*132*). When stored aerobically, sticks with a pH of 5.6 and a_w of 0.96 grew to 6.36 log CFU/g by storage day 7, and similarly sticks with a pH of 5.6 and a_w of 0.92 grew to 4.49 log CFU/g and the sticks with pH 5.1 and a_w 0.96 grew to 4.15 log CFU/g ; this indicated that these parameters did not provide shelf stability in aerobic storage (*132*). All the other treatment combinations suppressed growth of *S. aureus* during aerobic storage; the treatments with a pH of 4.7 all reduced by approximately 2 log CFU/g regardless of water activity (*132*). The aerobic storage was only sampled up to 7 days because excessive mold growth grew on all of the samples that supported *S. aureus* growth and were considered spoiled (*132*).

Tilkens et al. (*132*) also evaluated these parameters under anaerobic storage at room temperature to determine shelf stability on snack sticks. Treatments with pH values 5.1 or 4.7 and $a_w \leq 0.96$, inhibited the growth of *S. aureus* throughout the 28-day study when stored under vacuum in a reduced oxygen environment (*132*). In this study, the researchers utilized current pH and a_w parameters (pH 5.2 and < 0.95 a_w) suggested for shelf stability (*78*) and found agreeable results with these suggestions for inhibition of pathogen growth. Tilkens et al. (*132*) found similar results to a study by Borneman et al. (*16*) that performed a 28-d storage study on two products with similar critical a_w and pH parameters (pH 5.1 and a_w 0.88; and pH 5.1 and a_w 0.92) and observed the same growth inhibition of *S. aureus* as Tilkens et al. (*132*). Lastly, Tilkens et al. (*132*), observed that reducing the pH to 4.7 regardless of the water activity, appeared to provide the most control against *S. aureus* growth, which may indicate pH level at or below this level my serve as a sufficient parameter to make a product shelf-stable.

To better understand the appropriate a_w levels to suppress pathogenic growth, specifically in beef jerky, Ingham et al. (*66*), conducted a study evaluating the fate of both *S. aureus* and *L. monocytogenes* on 15 brands of beef jerky as a response to FSIS Compliance Guideline for Jerky Processors and provide data to support processors using Alternatives 1, 2, or 3 in similar jerky products. Most processors produce jerky ≤ 0.88 which is the limit for *S. aureus* growth; therefore, the researchers obtained jerky with a_w ranging from 0.47 to 0.87 with pH values between 5.3 and 6.3, to determine fate of the pathogens (*66*). In general, all jerky products suppressed growth of pathogens during vacuum package storage at ambient temperatures (*66*). However, the jerky with the lowest a_w (0.47) exhibited the least amount of pathogen death during storage; clearly there are compositional attributes that dictate pathogen behavior such as non-meat ingredient content (*66*).

Studies show that water activity and pH are sufficient critical parameters that can suppress growth of post-processing contamination of pathogens such as *L. monocytogenes* and *S. aureus* on RTE shelf-stable meat products when they are within scientifically acceptable limits either alone or in combination with one another. There are other factors that dictate the fate of these pathogens such as product type, compositional characteristics, packaging, storage environment, among others. Although inhibition of growth is clearly researched in today's typical shelf-stable meat products, understanding the survival pathogens on emerging products and the risk associated during the long-term storage is needed. The use of a_w and pH critical limit safe harbors to prevent growth of post-processing contamination of pathogens is a widely used standard when producing shelf-stable meat snacks. These safe harbors may prevent growth of pathogens, but it does no necessarily address the survival of pathogens on these products.

CHAPTER 3

Study I

Summary

Study I evaluated the survival of inoculated populations of *L. monocytogenes* and *S. aureus* on commercially-available, beef- and poultry-based meat bars during vacuum-packaged storage. Three different brands of commercially available beef and turkey meat bars were obtained in their original commercial packaging (brand [1, 2] and beef or turkey [B, T]: 1B, 1T, 2B, 2T, 3B, and 3T). A total of 120 bars were collected for each beef and turkey bar within brand (N = 720; study repeated for two trials). Two inocula were utilized, a five-strain mixture of *L. monocytogenes* or a five-strain mixture of *S. aureus.* Bars were removed from their commercial packaging and inoculated (both sides) for a target inoculation level of 6 to 7 log CFU/g. Following a 15-min cellular attachment time per side, bars were individually vacuum packaged and stored at 25ºC for 50 d. Microbiological analyses were conducted to enumerate surviving *L. monocytogenes* (Modified Oxford Agar; MOX) and *S. aureus* populations (Baird Parker Agar; BPA). Water activity (a_w) and pH were obtained for each bar and proximate analyses were conducted on a subset of each of the formulations (six formulations). Surviving bacterial counts for were fitted with the Baranyi and Roberts mathematical model (DMFit version 3.5, ComBase) to determine shoulder periods (the time in days where the levels of pathogen remain at the level of inoculation) and inactivation rates (log CFU/g/day) for each pathogen on each bar type. Differences were assessed using a Mixed Models Procedure of SAS with significance reported at $P < 0.05$. Bars 1B, 1T, 2B, 2T, 3B, and 3T had average pH and a_w values of 5.25 and 0.855, 5.51 and 0.861, 4.41 and 0.877, 4.54 and 0.891, 5.20 and 0.835, and 5.26 and 0.845, respectively. In general, the turkey bars (bars 1T, 2T, 3T) had slower inactivation rates compared to their beef

counterparts. Turkey bars supported survival of *S. aureus* longer ($P < 0.05$) than *L. monocytogenes*. Both pathogens survived longest ($P < 0.05$) on bar 1T; shoulder periods and inactivation rates were 22.2 days and -0.08 log CFU/g/day, respectively, for *S. aureus*, and 9.6 days and -0.16 log CFU/g/day, respectively, for *L. monocytogenes*. Additionally, of the beef bars, *S. aureus* survived the longest with a shoulder period of 12.4 days on bar 1B followed by an inactivation rate of -0.27 log CFU/g/day compared to the other beef bars. Bar 2B exhibited the highest ($P < 0.05$) death rate compared to the other five bars, with an inactivation rate of -1.20 log CFU/g/day for *S. aureus* and -0.91 log CFU/g/day for *L. monocytogenes* and no shoulder periods. Regardless of bar type, both pathogens present were after enumeration on MOX and BPA following 50 d of vacuum packaged storage. Survival of these pathogens stored at 25ºC under vacuum-packaged conditions indicates further research may be needed to assess the risk of meat bars with differing a_w parameters as a controlled factor. These data provide awareness of the survival behavior of post-processing contamination of pathogens on commercially available shelf-stable meat bar snacks.

Introduction

Shelf-stable meat snacks represent a growing sector of the meat and poultry industry (*97*). Shelf-stable meat and poultry products have grown in popularity in the US, as they satisfy many consumer preferences as a snack food (*12*, *97*, *142*). There are several factors that contribute to increased demand: convenience, shelf stability, nutrient dense, and high in protein among others (*97*, *142*). Shelf-stable meat snacks are at risk for post-processing contamination of foodborne pathogens such as *Listeria. monocytogenes* and *Staphylococcus aureus*. Researchers have investigated *L. monocytogenes* and *S. aureus* on shelf-stable meat and poultry jerky products;

however, demand for convenient meat snacks has driven innovation of niche dried meat products that aren't jerky (*10*, *15*, *16*, *19*, *37*, *52*, *56*, *62*, *64*, *65*, *66*, *83*, *88*, *132*, *144*, *146*). Shelf-stable meat bars are a newer product being produced that contain significant amounts of non-meat ingredients compared to their traditional dried jerky and sausage counterparts. These non-meat ingredients are often unique in nature and might include fruits, vegetables, seeds, rice and nuts. The condition of, and ingredients in, these meat bars differ depending on brand and formulation. There is a regulatory need for validated evidence of pathogen survival behavior on currently utilized water activity parameters in meat bars.

The primary pathogen of concern in ready-to-eat (RTE) shelf-stable meat and poultry products is *L. monocytogenes* (*42*, *51*, *55*, *70*, *77*, *125*, *135*, *147*). *Listeria monocytogenes* is the public health concern in RTE meat products due to its ubiquity in meat processing environments, potential for post-processing contamination, ability to grow in refrigerated environments, and it's disease severity, including high mortality (*17*, *31*, *80*, *91*, *113*, *125*). Another ubiquitous pathogen, *S. aureus,* is a pathogen that can be introduced onto shelf-stable meat products during handling post-lethality treatment. *S. aureus* and its toxins can tolerate harsh environments such as low water activity foods, which makes it a concern in RTE shelf-stable meat products due to their extended shelf lives under ambient temperature conditions. This pathogen is referenced in USDA-FSIS jerky compliance (*136*) and other jerky research because its a_w growth limits have been used to support the a_w safe harbors for jerky production and are the standard for the production of jerky; however, these safe harbors will not necessarily prevent the survival of *S. aureus* and *L. monocytogenes*. According to the Centers for Disease Control and Prevention (CDC), *S. aureus* was involved in 13 foodborne disease outbreaks and two outbreaks were associated with *L. monocytogenes* in 2015 (*21*). Although *L. monocytogenes* was only associated

with 2 outbreaks reported in the 2015 CDC annual report, there were 19 deaths, whereas the outbreaks associated with *S. aureus* resulted in only 3 deaths (*21*). The presence of these pathogens must be addressed and controlled in shelf-stable meat products.

Currently, USDA-FSIS considers *L. monocytogenes* an adulterant in all ready-to-eat meat and poultry products (*40*, *134*). *L. monocytogenes* and *S. aureus* would only be present in dried meat products as a result of post-lethality contamination from the processing environments or employees (*134*, *136*). The USDA-FSIS requires effective thermal lethality of vegetative pathogenic bacteria; however, shelf-stable meat products must have conditions unfavorable to *S. aureus* and *L. monocytogenes* to be safe from post-processing contamination (*134*, *136*). Additionally, they require establishments to have validated evidence assessing the critical parameters needed to prevent, eliminate or reduce the presence of pathogenic bacteria from post-processing contamination (*134*, *136*). The prevention of growth has been thoroughly investigated in dried shelf-stable meat snacks. However, little research has been done assessing pathogens' ability to survive during extended storage on these products produced under the common critical parameters that were designed to prevents growth, but not necessarily presence. *Staphylococcus aureus* is most tolerant of lower a_w compared to *L. monocytogenes*; therefore, research investigating *S. aureus* in addition to *L. monocytogenes* in shelf-stable meat products is common (*66*, *136*).

There are several federal standards for the composition and condition of ready-to-eat and shelf-stable meat products (*65*). Historically, USDA-FSIS considered a meat and poultry product to be shelf-stable if it met a moisture:protein level of 0.75:1 (MPR; *125*). Recently, USDA-FSIS has been assessing shelf-stability more commonly by measuring water activity (*134*, *136*). Water activity is a more accurate and appropriate way to assess shelf stability in meat products, because

it is an indicator of available water for microbial growth (*136*). Therefore; the aforementioned a_w safe harbors, ≤ 0.85 in oxygenated environment are required to be or ≤ 0.91 in an anaerobic environment, are the critical parameters the industry uses as drying limits, but this does not necessarily control the presence of these pathogens (*136*). These limits were developed based on the growth limits for *S. aureus* under optimal conditions; however, other factors in dried meat products might contribute to inhibition of growth and survival, such as pH (*63*, *65*, *66*, *132*). It appears that the consumer today is demanding more "moist" meat snacks; therefore, many producers have chosen to produce dried meat products with $a_w \leq 0.91$ packaged in anaerobic environments to meet their demands (*36*). While intrinsic properties can be manipulated to control post-processing growth of pathogens, there also are post-processing treatments that can be applied to reduce or eliminate presence of pathogens.

There have been several research studies published investigating dried meat (whole muscle and ground) products and the survival behavior of post-processing contamination of *S. aureus* or *L. monocytogenes* during aerobic and vacuum-packaged storage (*19*, *37*, *55*, *56*, *63*, *65*, *66*, *132*, *146*). Some of these studies have assessed differing a_w and pH effects on survival of *S. aureus* and *L. monocytogenes* in shelf-stable dried meat snacks (*63*, *65*, *66*, *132*). These studies have been used as scientific support for establishments as evidence inhibition of pathogen growth and survival on shelf-stable meat products (*63*, *65*, *66*, *132*). Whole muscle and ground jerky literature may not be representative for these new shelf-stable "meat bar" snacks for the producers to draw the same conclusions about the conditions of their products in their hazard analysis.

Meat bars have commonly been dried between a_w of 0.85 and 0.91 due to consumer preferences for a more "moist" product (*36*) while still maintaining the limits for growth of post-

processing pathogen contamination. These safe harbors might be appropriate to prevent growth; however, there is no indication of the effects on the pathogens' ability to survive on these products during shelf-stable storage. It might be necessary for meat bar producers to understand the survival characteristics of *S. aureus* and *L. monocytogenes* under the conditions of commercially available meat bars. Currently, there is no literature investigating pathogen survival in ready-to-eat dried shelf-stable meat bars. Therefore, a study was conducted to evaluate the survival of inoculated populations of *L. monocytogenes* and *S. aureus* on commercially-available, beef- and poultry-based meat bars during vacuum-packaged storage.

Materials and Methods

Bacterial strains and preparation of inocula. A five-strain mixture of *Listeria monocytogenes* was used for this inoculation study. The strain identifications included *L. monocytogenes* LM 101 (serotype 4b; isolated from hard salami; Dr. Eric Johnson Food Research Institute, University of Wisconsin-Madison, Madison, WI), LM 108 (serotype 1a; isolated from hard salami; Dr. Eric Johnson Food Research Institute, University of Wisconsin-Madison, Madison, WI), LM 310 (isolated from goat cheese; Dr. Eric Johnson Food Research Institute, University of Wisconsin-Madison, Madison, WI), V7 (isolated from hard raw milk; Dr. Eric Johnson Food Research Institute, University of Wisconsin-Madison, Madison, WI), and Scott A (serotype 4b; human isolate; obtained from our laboratory's culture collection Fort Collins, CO). The first four strains were obtained from Dr. Glass at the University of Wisconsin, and previously used in dried meat snack research (*37*, *63*, *66*). A five-strain mixture of *Staphylococcus aureus* was also utilized in this study. The strains were also provided by Dr. Glass, and also used in previous dried meat snack research (*37*, *65*, *66*, *132*). These strains

included FRI 100 (isolated from cake implicated in an outbreak; Dr. Amy Wong Food Research Institute, University of Wisconsin-Madison, Madison, WI, USA), FRI 472 (isolated from turkey salad implicated in an outbreak; Dr. Amy Wong Food Research Institute, University of Wisconsin-Madison, Madison, WI) FRI 1007 (isolated from genoa salami implicated in an outbreak; Dr. Amy Wong Food Research Institute, University of Wisconsin-Madison, Madison, WI), ATCC 12600 (serotype 3; human isolate), and ATCC 25923 (serotype 3; clinical isolate).

Working cultures of the *L. monocytogenes* and *S. aureus* strains were maintained on Modified Oxford Agar (MOX; Difco, Becton Dickinson, Sparks, MD) and Baird Parker Agar (BPA; Acumedia – Neogoen; Lansing, MI), respectively. Strains were individually activated and subcultured (35°C, 24 ± 2 h) in 10 ml of tryptic soy broth (TSB; Acumedia-Neogen) for *S. aureus* or TSB supplemented with 0.6% yeast extract (TSBYE; Acumedia-Neogen) for *L. monocytogenes*. Broth cultures of the strains belonging to the same inoculum type were combined and cells harvested by centrifugation (3220 x g, 15 min, 4°C, Eppendorf model 5810R, Brinkman Instruments Inc., Hamburg, Germany), washed with 10 ml phosphate buffered saline (PBS, pH 7.4; Sigma-Aldrich, St. Louis, MO), re-centrifuged, and resuspended in PBS to the original inoculum volume to obtain a cell concentration of approximately 8 to 9 log CFU/ml.

Meat bar procurement, inoculation, and packaging. The study was repeated twice on separate start days. Three commercially available brands of meat bars, in their original packaging, were obtained for the study. Within each of the three brands brand (1, 2, 3), two different types of meat bars were selected; one was beef-based and the other turkey-based (B or T; Table 3.1). All three brands selected were similar in formulation and ingredients for each species (containing beef: fruit [cherry, peach, tomato, date, orange], nuts [walnuts], seeds [quinoa, flax], vegetables [chilies, peppers], and various spices; containing turkey: fruit [dried

cranberry, apple, orange, date, lemon], nuts [almond], seeds [quinoa, flax, chia], vegetables [squash, celery], and various spices). Bars were identified based on the brand (1, 2, 3) and meat block (beef or turkey; B or T) and were labeled as 1B, 1T, 2B, 2T, 3B, and 3T (Table 3.1). There were 120 bars for each formulation (N = 720; study repeated for two trials). Bars A and B were approximately 11.5 cm long, 4.5 cm wide, and 1.0 cm thick weighing on average 35 g. Bars C and D were approximately 10.0 cm long, 3.5 cm wide, and 1.1 cm thick weighing on average 43 g. Lastly, Bars E and F were approximately 8.0 cm long, 5.0 cm wide, and 0.9 cm thick weighing on average 35 g.

A small percentage of the meat bars were left uninoculated (n = 6) for microbiological analysis of existing background microflora at day 0 of storage. The rest of the meat bars, prior to inoculation, were aseptically removed from their original commercial packaging and placed on aluminum foil sanitized (70% ethanol) trays. Half of the bars were inoculated with *L. monocytogenes*, while the other half were inoculated with *S. aureus*. Approximately 0.2 ml aliquot (0.1 ml per side) of inoculum was spot inoculated (randomly dispersed across the surface), spread evenly with a sterile L-shaped spreader, and allowed to sit for 15 min per side (30 min) for bacterial cellular attachment. Pre-trial work was done to develop an inoculation procedure that did not affect the a_w of the meat bars following the attachment period; the pre-trial work verified that a_w did not increase with the addition of inoculum solution. The target inoculation level was approximately 6 to 7 log CFU/g.

Individual inoculated bars were aseptically placed into vacuum bags (15 × 22 cm, 3 mil std. barrier, nylon/polyethylene vacuum pouch, Koch, Kansas City, MO, USA), vacuum packaged (Hollymatic Corp., Countryside, IL, USA) and appropriately labeled for each bar and

pathogen. Vacuum packaged bars were stored in an incubator at 25ºC ± 2ºC incubator for up to 50 d.

Microbiological analysis. Samples were microbiologically analyzed on days 0, 1, 3, 5, 10, 15, 20, 30, 40 and 50 d of storage. Uninoculated bars were sampled only on day 0 to obtain initial background microflora. Bars were aseptically removed from the vacuum bag and portioned into 25 g for microbiological analysis; this same sample was used to measure pH after samples were plated on the appropriate media. The remaining portions of the meat bars were utilized for a_w analysis. The 25 g portion of meat bar was placed into a sterile 25 oz. filter Whirl-Pak bag (Nasco, Modesto, CA) then 50 ml of maximum recovery diluent (MRD, Acumedia-Neogen) was added for a 2:1 ratio (2-part diluent to 1-part sample). Bagged samples were homogenized (Stomacher 400 Circulator; Seward, Port Saint Lucie, FL) for 2 min then serially diluted in 0.1% buffered peptone water (BPW, Difco, Becton Dickinson). For enumeration of *L. monocytogenes*, 0.1 ml or 1-ml of diluted sample was surface plated on MOX for selective enumeration and tryptic soy agar supplemented with 0.6% yeast extract (TSA, Acumedia-Neogen + YE, Acumedia-Neogen) for enumeration of total aerobic plate counts. Enumeration of *S. aureus* inoculated samples were surface plated on BPA for selective enumeration and TSA for enumeration of total aerobic plate counts. Additionally, on day-0 of storage, uninoculated bars were microbially analyzed on TSA and TSAYE to determine levels of any naturally occurring microflora associated with each of the six meat bar types. Plates were incubated at 35ºC ± 2ºC for 48 h (MOX and BPA) for selective media, or 25ºC ± 2ºC for 72 h (TSA and TSAYE) for non-selective media. Following incubation, colonies were counted on appropriate dilutions, recorded, and reported as log CFU/g. The detection limit for the microbiological analysis was 0.5 log CFU/g.

Proximate, water activity, and pH analysis. Each of the six meat bar formulations were analyzed for proximate analyses that included fat (AOAC 991.36), moisture (AOAC 950.46b), protein (AOAC 992.15), percent salt as sodium chloride (AOAC 935.47) and reported as a composite result (Table 3.2). Three bars from each of the formulations for each of the repeated trials (n = 6) were composited and sent to a third-party in Denver, CO for the analyses.

All meat bars samples that were analyzed for bacterial counts, were also measured for a_w and pH. Water activity was measured using an AquaLab (model series 3, Decagon Devices Inc., Pullman, WA) water activity meter. Samples were portioned into small pieces and placed in a sample cup and covered with a lid until analysis was conducted. Calibration of the meter was verified with performance verification standards of 0.760 and 0.920 (AquaLab, Meter Group Inc., Pullman, WA) at the start of the analysis and after every 10 samples; all measurements were taken at room temperature (23ºC to 26ºC). Measurements were recorded and reported as means for each bar for the whole study (Table 3.2).

The pH measurements were taken from the same samples used in microbiological analysis (2:1 MRD to sample) following 2 min of homogenizing. The pH was obtained using a calibrated pH meter fitted with a glass electrode (Denver Instruments, Arvada, CO). Measurements were recorded and reported as means (Table 3.2).

Statistical analysis. Microbial counts were converted to log CFU/g before statistical analysis. The study was repeated for two trials, and n = 6 samples were collected for each meat bar on each storage day (0, 1, 3, 5, 10, 15, 20, 30, 40, and 50). The Mixed Procedures of SAS version 9.4 (Carry, NC, USA) was utilized to determine differences between bars within pathogen group for each sampled storage day. Differences were reported using a significance level of $\alpha = 0.05$.

Additionally, surviving log converted counts were modeled as a function of storage time (day) using the model by Baranyi et al. (*7*). Surviving pathogen counts for each bar were fitted to assess shoulder periods (the time in days where the levels of pathogen remained at the level of the initial inoculation) and inactivation rates (log CFU/g/day). Within pathogen type, differences among bars were determined using the Mixed Procedures in SAS for maximum inactivation rates and shoulder period; differences were reported using a significance level of $\alpha = 0.05$.

Results and Discussion

Chemical and physical analyses of meat bars. In the current study, all six meat bar types were analyzed for proximate percent fat, moisture, protein and salt (sodium chloride) as a composite result for each bar (Table 3.2). These analyses were done on six sample composites for each formulation. Numerically, the highest % fat was observed in beef bars 2B and turkey bars 2T, with 19.7% and 15.8%, respectively (Table 3.2). The other four bars ranged from 3.4 to 9.0% fat (Table 3.2). Percent moisture was fairly similar amongst all six bar types, ranging from 35.0 to 41.3% (Table 3.2). Similarly, % protein was also comparable for all six bar types, where the minimum was 19.9% protein for turkey bar 3T and maximum was 26.2% for turkey bar 1T (Table 3.2). Beef bar 3B and turkey bar 3T had the highest % salt, 3.0 and 3.1, respectively, compared to the other bars that ranged from 1.0 to 2.0.

Water activity was measured for all meat bars that were analyzed for microbial analysis (Table 3.2). The water activities were pooled for each bar type because there were no differences ($P \geq 0.05$) observed in water activity between each sample within bar type, during storage. Beef bar 1B and turkey bar 1T had average a_w of 0.855 and 0.861, respectively (Table 3.2). Beef bar 2B and turkey bar 2T, numerically had the highest a_w of the six bars, with an average of 0.877

and 0.891, respectively (Table 3.2). Lastly, beef bar 3B and turkey bar 3T were the only two bars with an average $a_w \leq 0.85$ (targeted value for shelf-stable products stored aerobically), averaging 0.845 and 0.835, respectively (*128*; Table 3.2).

Additionally, pH was measured for all meat bars (Table 3.2). Four out of the six bar pH values ranged between 5.21 to 5.50 and were not considered acidified. Ingham et al. (*65*) discussed that the minimum pH for *S. aureus* to produce toxins is 5.3 in some sausage products and pH of 5.1 with a wide range of %WPS was sufficient to suppress growth of *S.* aureus. Bars C and D, had average pH values of 4.41 and 4.51, which would be considered acidified and are less than 4.6 which is the common pH that inhibits microbial growth in food products (*19*). Tilkens et al. (*132*) did a study evaluating the effects of pH and water activity combination on the survival of *S. aureus* on acidified meat sticks. The relationship between pH and water activity can impact shelf stability, and there are some combinations of safe harbors that are generally recognized to inhibit pathogenic growth of meat snack, pH < 5.2 and $a_w < 0.95$ is considered shelf-stable (*16*, *132*). These safe harbors are commonly used to produce shelf-stable meat snacks, but do not necessarily prevent survival of pathogens. This combination of pH and a_w were observed in meat bars 2B and 2T; these bars have a pH well below 5 and are considered acidified. The understanding of which parameter, a_w or pH, has a greater impact on shelf-stability is not completely clear in the literature. Borneman et al. (*16*) evaluated the combination of pH, a_w, and % water phase salt (WPS) on the effect of growth inhibition of *S. aureus* (most tolerant foodborne pathogen to low a_w and high %WPS). Borneman et al. (*16*) found that all three of these parameters impacted the predictive model for *S. aureus* growth in relation to shelf-stability; however, they did not investigate the survival of these pathogens.

The bars utilized in the current study were commercially produced and purchased in their original packaging and from different commercial lots; therefore, chemical and physical properties of the meat bars were not fixed factors making it difficult to definitively make conclusions about the impact of these properties on the survival of pathogens.

Effects of storage on the microbial populations on meat bars. The level of background microflora on bars 1B, 1T, 2B, 2T, 3B and 3T were 1.6, 2.1, 1.8, 2.2, 2.5, and 0.7 log CFU/g, respectively (data not shown). Storage day was a significant effect on the microbial counts of each meat bar; all bars' pathogenic and total aerobic plate counts generally decreased over the 50-d storage period (Tables 3.3, 3.4, 3.5, and 3.6). Numerically, *S. aureus* populations on meat bars were higher at the completion of the 50th day of storage compared to the meat bars inoculated with *L. monocytogenes*. Throughout storage for both pathogens, beef bar 2B and turkey bar 2T counts were consistently lower ($P < 0.05$) compared to the other meat bars (Tables 3.3 – 3.6). This significant difference is likely attributed to the lower pH of meat bars 2B and 2T compared to the other bars. In the meat snack stick study conducted by Tilkens et al. (*132*), they observed that reducing the pH to 4.7 regardless of the water activity, appeared to provide the most control against *S. aureus*. Although, pH was not controlled specifically in the current study, the low average pH of meat bars 2B and 2T is the likely factor that affected the lower ($P < 0.05$) pathogenic populations throughout the storage period.

Inoculated meat bars with *L. monocytogenes* and *S. aureus* had higher total plate counts compared to the counts recovered on selective media (Tables 3.4 and 3.6). None of the total plate counts recovered on TSAYE or TSA had counts below the analysis detection limit at any point during 50 days of storage. Counts on non-selective media versus selective media, began to differ ($P < 0.05$) after 10 days of storage for the *L. monocytogenes* inoculated bars and after day 20 of

storage for the *S. aureus* inoculated bars (Tables 3.3 – 3.6). These differences may indicate some recovery of sublethally injured pathogenic cells and or additional background microflora recovered on the non-selective media.

Effects of storage on *Listeria monocytogenes* populations on meat bars. On storage day-0, meat bars inoculated with *L. monocytogenes* had initial counts of approximately 6.6 to 6.9 log CFU/g on MOX (Table 3.3). Within the first day of storage, beef bar 2B had lower ($P < 0.05$) *L. monocytogenes* counts compared to the other five bars; on this day, bar 2B had 4.9 log CFU/g while the other bars were all over 6 log CFU/g (Table 3.3). Additionally, by day 10, bar 2B had < 1.7 log CFU/g *L. monocytogenes* populations and 33.3% of the samples were below the analysis detection limit (< 0.5 log CFU/g) and continued to have samples below the detection limit (BDL) for the remainder of the 50-d storage (Table 3.3). Turkey bar 2T, also consistently had lower ($P < 0.05$) *L. monocytogenes* counts compared to bars 1B, 1T, 3B and 3T; however, did not have as many samples BDL compared to bar 2B (Table 3.3). Turkey bars 1T and 2T as well as beef bar 3B, displayed higher ($P < 0.05$) *L. monocytogenes* counts throughout the 50-d storage, and turkey bar 1T and beef bar 3B had the highest remaining counts at the end of storage, 1.8 log CFU/g and 1.7 log CFU/g, respectively (Table 3.3). Although bars 3B and 3T maintained higher ($P < 0.05$) *L. monocytogenes* counts throughout storage, bar 3T exhibited higher counts compared to bar 3B until day 50 (Table 3.3). Bars 1T and 3B never had *L. monocytogenes* counts below the analysis detection limit (Table 3.3).

After 50 days of storage, all meat bars had *L. monocytogenes* populations between < 0.6 log CFU/g and 1.8 log CFU/g (Table 3.3). In a different study by Ingham et al. (*66*), authors evaluated post-processing contamination of *S. aureus* and *L. monocytogenes* on commercially available beef jerky with a_w from 0.47 to 0.87. These authors generally observed lower

remaining *L. monocytogenes* populations after 28 days of anaerobic storage on the jerky products with $a_w > 0.85$ (*66*). However, Ingham et al. (*66*) also found that their highest a_w jerky (0.87) had lower remaining *L. monocytogenes* populations compared to their jerky with the lowest a_w (0.47); these authors concluded that clearly a_w was not the only factor contributing to the survival of *L. monocytogenes*. Ultimately, in the current study, bars 2B and 2T showed the greatest potential for control of *L. monocytogenes* likely due to the lower pH values.

Effects of storage on *Staphylococcus aureus* populations on meat bars. On storage day 0, meat bars inoculated with *S. aureus* had initial counts of 6.9 to 7.2 log CFU/g for bars 1B, 1T, 3B, and 3T recovered on BPA (Table 3.5). Bars 2B and 2T had lower ($P < 0.05$) initial *S. aureus* populations 5.7 and 6.7 log CFU/g; the effects of low pH were immediately observed on the *S. aureus* inoculated bars 2B and 2T (Table 3.5). This characteristic was likely observed on *S. aureus* inoculated meat bars and not as evident on the *L. monocytogenes* inoculated bars due to *L. monocytogenes* higher acid tolerance. Tilkens et al. (*132*) reported the effects of low pH (4.7) on the survival of *S. aureus* on beef snack sticks, maintaining consistently lower counts from beginning to the end of a 28-d storage period. Similar to the *L. monocytogenes* inoculated bars, bars 2B and 2T consistently had the lowest ($P < 0.05$) *S. aureus* populations (Table 3.5). Beef bar 2B exhibited the greatest potential for controlling *S. aureus* post-processing during storage; by storage day 5, *S. aureus* was reduced ($P < 0.05$) to 1.3 log CFU/g with 16.7% of the samples BDL (Table 3.5). Beef bar 2B, consistently had samples BDL between storage days 5 and 50, ultimately ending the 50-d period with 83.3% BDL, with remaining populations < 0.5 log CFU/g (Table 3.5). Turkey bar 1T had the highest ($P < 0.05$) *S. aureus* populations for the duration of the 50-d storage compared to the other bars (Table 3.5). Bar 1T did not have *S. aureus* populations below 6 log CFU/g until storage day 40 (Table 3.5). Similarly, turkey bar 3T

maintained higher counts on average compared to the other bars, not reducing to less than 6 log CFU/g until day 20 of storage (Table 3.5). Ingham et al. (*66*), in the commercially available jerky study, observed that overall, *S. aureus* had higher counts through 28 days of storage compared to *L. monocytogenes*.

In conclusion, all six meat bars had remaining *S. aureus* populations after 50 days of vacuum-packaged storage (Table 3.5). Turkey bar 1T had the highest ($P < 0.05$) remaining *S. aureus* populations of 4.5 log CFU/g, and the second highest ($P < 0.05$) remaining counts were observed on meat bars 3B and 3T, 2.1 and 1.5 log CFU/g, respectively. Bars 3B and 3T were very similar in chemical and physical properties (Table 3.1), but interestingly enough, the highest % salt (3.0 and 3.1) and the lowest a_w, even though they had some of the higher *S. aureus* populations throughout anaerobic storage. Turkey bar 1T had the highest *S. aureus* throughout the study, and also had the lowest % salt; in this example it may be possible it contributed to the longer survival of *S. aureus* (Tables 3.1 and 3.5). Overall, *S. aureus* was able to survive the longest with higher populations recovered on selective media on non-acidified meat bars compared to the *L. monocytogenes* inoculated bars. *L. monocytogenes* and *S. aureus* survived on all meat bars after 50 days of vacuum-packaged storage; pathogens were still recovered on selective media at the end of storage.

Pathogen survival on meat bars during storage: shoulder periods and inactivation rates. Shoulder periods and maximum inactivation rates were calculated from fitted inactivation curves for *L. monocytogenes* and *S. aureus* inoculated populations for each of the six meat bar types (Table 3.7 and Figure 3.1). Similar trends were observed for survival and inactivation characteristics among the meat bars that were evaluated based on the least squares mean counts within each storage day. *Staphylococcus aureus* inoculated meat bars had the longest shoulder

periods and the slowest inactivation rates compared to the *L. monocytogenes* inoculated on meat bars (Table 3.7 and Figure 3.1). The non-acidified turkey-based bars (1T and 3T) had the longest ($P < 0.05$) shoulder periods for both *L. monocytogenes* and *S. aureus* (Table 3.7).

Turkey bar 1T exhibited the longest shoulder period for both pathogens (Table 3.7). It took 9.6 days for *L. monocytogenes* counts to decrease on bar 1T, and 22.2 days for *S. aureus* start to decrease on bar 1T (Table 3.7). Bar 1T shoulder periods were numerically longer compared to turkey bar 3T, 7.3 days for *L. monocytogenes* and 21.6 days for *S. aureu*s, but statistically they did not differ ($P \geq 0.05$). This trend was also true for inactivation rates where, on bar 1T, *L. monocytogenes* inactivated -0.16 log CFU/g/day and on bar 3T -0.21 log CFU/g/day; these rates were numerically different but statistically the same (Table 3.7). The inactivation rate of *S. aureus* was numerically the slowest (-0.08 log CFU/g/day) on bar 1T compared to the rates from the other five bars.

Interestingly, all the inactivation rates were the same ($P \geq 0.05$) for *L. monocytogenes* on all the bars, excluding bar 2B which had the fastest ($P < 0.05$) rate of inactivation (-0.9 log CFU/g/day; Table 3.7). Bars 2B and 2T survival curves displayed no shoulder periods for either pathogen; however, pathogens on bar 2T inactivation rates were lower ($P < 0.05$) compared to bar C (Table 3.7). Bar 1B had the fastest maximum inactivation rate of -1.1 log CFU/g/day which declined rapidly for both pathogens in the first 10 to 15 d of storage, and then pathogen levels decreased at a slower rate causing a "tailing effect" (Table 3.7). It is clear that beef bar 1B had the greatest capability to control post-processing contamination of both *L. monocytogenes* and *S. aureus* followed by turkey bar 2T from the same brand (Figure 3.1).

In conclusion, both pathogens were detected on all six of the meat bars at the end of 50 d of vacuum-packaged storage at 25ºC. It was evident that pH had a major impact on the

inactivation of *L. monocytogenes* and *S. aureus* which was observed on bars 2B and 2T. It is worth mentioning, that there was a trend in the turkey bars within each brand tended to have higher counts, slower inactivation rates, and/or longer shoulder periods compared to the beef bar from the same brand. There was no one physical or chemical characteristic that would indicate a major difference compared to the beef bars other than the meat component. Ingham et al. (*66*) discussed that a_w, pH and the other chemical properties may not be the only factors contributing to the survival of post-processing contamination of pathogens during storage on dried meat snacks. In the current study, there may be other factors contributing to the differing trend between the beef and turkey bars such as ingredients, phytochemicals related to the ingredients, natural microflora associated with the turkey versus beef, etc. Since the meat bars utilized in this study were commercially produced, none of the physical or chemical properties were controlled as fixed factors making it difficult to make definitive conclusions on their affects of the survival of pathogens during storage. In conclusion, pathogens were able to survive extended storage (50 d) under vacuum at room temperature. Understanding the intrinsic factors that contribute to the survival of post-processing contamination of pathogens on shelf-stable meat bars needs to be further explored to better understand how to control them and produce safe product. Possible interventions need to be investigated to provide reduced lag phase and increase inactivation on these products. These data provide awareness about the survival of pathogens on commercially available shelf-stable meat bar snacks.

Table 3.1. Pooled ingredients for commercially produced beef and turkey meat bars; sorted by unique (not typical in dried meat snacks) and common ingredients in alphabetical order.

Meat Species	Non-meat Ingredients
Beef	**Unique:** Candied orange peel, celery powder, cherries, chia seeds, citric acid, crushed habanero, date paste, dried cherries, dried peaches, dried tomato, ground flax seeds, lemon juice concentrate, quinoa, rice syrup solids, tomato concentrate, vegetable glycerin, and walnuts. **Common:** Black pepper, cane sugar, chili pepper, garlic powder, ground cinnamon, ground mustard seeds, hickory smoke flavor, lactic acid, onion powder, oregano, paprika, salt, sea salt, and sugar cane.
Turkey	**Unique:** Almonds, candied orange peel, cane sugar, chia seeds, date paste, dehydrated garlic, dehydrated onion, dried apple, quinoa, dried butternut squash, dried cranberries, dried sautéed onion, ground flax seed, quinoa, rice syrup solids, and vegetable glycerin. **Common:** Black pepper, celery powder, coriander, cumin, dried parsley, dried sage, lactic acid, nutmeg, paprika, red pepper, sage, salt, sea salt, sugar, and thyme.

Table 3.2. Mean (standard deviation) water activity (a_w), pH, and compositional values for each meat bar.

Bar ID	Brand	Meat Species	a_w	pH	Fat %	Moisture %	Protein %	% Water-Phase Salt
1B	1	Beef	0.855 (0.011)	5.25 (0.10)	7.2	41.3	25.8	2.0
1T	1	Turkey	0.861 (0.014)	5.51 (0.07)	6.4	37.2	26.2	1.6
2B	2	Beef	0.877 (0.019)	4.41 (0.08)	19.7	38.8	23.1	1.8
2T	2	Turkey	0.891 (0.020)	4.54 (0.14)	15.8	40.8	24.3	1.0
3B	3	Beef	0.835 (0.025)	5.20 (0.11)	9.0	35.0	19.9	3.0
3T	3	Turkey	0.845 (0.012)	5.26 (0.13)	3.6	36.2	20.9	3.1

Table 3.3. Least squares mean (standard error) *Listeria monocytogenes* modified Oxford agar (MOX) plate counts (log CFU/g) following anaerobic storage at 25ºC obtained from inoculated meat bars.

	Meat Bar											
Storage Day	**1B**	%BDL[1]	**1T**	%BDL	**2B**	%BDL	**2T**	%BDL	**3B**	%BDL	**3T**	%BDL
0	6.9^{a} (0.1)	0	6.9^{a} (0.1)	0	6.6^{b} (0.1)	0	6.8^{ab} (0.1)	0	6.9^{a} (0.1)	0	6.9^{a} (0.1)	0
1	6.6^{a} (0.1)	0	6.7^{a} (0.1)	0	4.9^{c} (0.1)	0	6.2^{b} (0.1)	0	6.7^{a} (0.1)	0	6.6^{a} (0.1)	0
3	6.4^{a} (0.2)	0	6.6^{a} (0.2)	0	3.2^{c} (0.2)	0	5.3^{b} (0.2)	0	6.3^{a} (0.2)	0	6.5^{a} (0.2)	0
5	6.0^{a} (0.2)	0	6.6^{a} (0.2)	0	2.1^{d} (0.2)	0	4.3^{b} (0.2)	0	5.7^{b} (0.2)	0	6.3^{ab} (0.2)	0
10	4.9^{b} (0.3)	0	6.2^{a} (0.3)	0	$< 1.7^{d}$ (0.3)	33.3	3.2^{c} (0.3)	0	4.9^{b} (0.3)	0	5.9^{a} (0.3)	0
15	3.6^{b} (0.3)	0	5.8^{a} (0.3)	0	$< 0.9^{d}$ (0.3)	33.3	2.7^{c} (0.3)	0	4.2^{b} (0.3)	0	5.1^{a} (0.3)	0
20	2.1^{d} (0.2)	0	5.6^{a} (0.2)	0	$< 0.6^{e}$ (0.2)	33.3	1.3^{d} (0.2)	0	3.2^{c} (0.2)	0	4.2^{b} (0.2)	0
30	$< 0.8^{d}$ (0.3)	50.0	4.0^{a} (0.3)	0	$< 1.0^{d}$ (0.3)	33.3	$< 1.2^{d}$ (0.3)	16.7	2.4^{b} (0.3)	0	2.0^{c} (0.3)	0
40	$< 0.7^{c}$ (0.3)	66.7	3.5^{b} (0.3)	0	$< 0.9^{c}$ (0.3)	50.0	1.7^{a} (0.3)	0	1.8^{b} (0.3)	0	$< 1.3^{c}$ (0.3)	33.3
50	$< 1.0^{b}$ (0.3)	33.3	1.8^{a} (0.3)	0	$< 1.1^{b}$ (0.3)	16.7	1.6^{a} (0.3)	0	1.7^{a} (0.3)	0	$< 0.6^{b}$ (0.3)	83.3

[1] Below the analysis detection limit 0.5 log CFU/g; LSmeans with "<" indicates at least one sample was BDL.

$^{a-e}$ LSmeans with superscripts that differ within row, indicates counts are different ($P < 0.05$) at that storage time.

Table 3.4. Least squares mean (standard error) tryptic soy agar with 0.6% yeast extract (TSAYE) total plate counts (log CFU/g) following anaerobic storage at 25ºC obtained from meat bars inoculated with *Listeria monocytogenes*.

	Meat Bar											
Storage Day	**1B**	%BDL[1]	**1T**	%BDL	**2B**	%BDL	**2T**	%BDL	**3B**	%BDL	**3T**	%BDL
0	6.9^{a} (0.1)	0	6.9^{a} (0.1)	0	6.6^{b} (0.1)	0	6.6^{b} (0.1)	0	6.8^{a} (0.1)	0	6.9^{a} (0.1)	0
1	6.6^{a} (0.1)	0	6.7^{a} (0.1)	0	4.9^{c} (0.1)	0	6.1^{b} (0.1)	0	6.8^{a} (0.1)	0	6.7^{a} (0.1)	0
3	6.4^{a} (0.2)	0	6.6^{a} (0.2)	0	3.2^{c} (0.2)	0	5.4^{b} (0.2)	0	6.3^{a} (0.2)	0	6.6^{a} (0.2)	0
5	6.0^{b} (0.2)	0	6.6^{a} (0.2)	0	2.5^{d} (0.2)	0	4.3^{c} (0.2)	0	5.8^{b} (0.2)	0	6.4^{a} (0.2)	0
10	5.0^{b} (0.2)	0	6.3^{a} (0.2)	0	2.0^{d} (0.2)	0	3.1^{c} (0.2)	0	5.0^{b} (0.2)	0	5.9^{a} (0.2)	0
15	4.0^{b} (0.2)	0	5.6^{a} (0.2)	0	1.7^{d} (0.2)	0	2.9^{c} (0.2)	0	4.4^{b} (0.2)	0	5.1^{a} (0.2)	0
20	2.7^{d} (0.2)	0	5.5^{a} (0.2)	0	1.6^{e} (0.2)	0	1.8^{e} (0.2)	0	3.6^{c} (0.2)	0	4.3^{b} (0.2)	0
30	1.7^{c} (0.2)	0	4.5^{a} (0.2)	0	1.8^{c} (0.2)	0	2.1^{c} (0.2)	0	3.0^{b} (0.2)	0	2.8^{b} (0.2)	0
40	1.5^{c} (0.3)	0	3.8^{a} (0.3)	0	1.6^{bc} (0.3)	0	2.3^{b} (0.3)	0	3.2^{a} (0.3)	0	2.2^{b} (0.3)	0
50	2.1^{ab} (0.2)	0	1.9^{b} (0.2)	0	1.9^{b} (0.2)	0	2.2^{b} (0.2)	0	2.8^{a} (0.2)	0	$< 1.7^{c}$ (0.2)	16.7

[1] Below the analysis detection limit 0.5 log CFU/g; LSmeans with "<" indicates at least one sample was BDL.

$^{a-e}$ LSmeans with superscripts that differ within row, indicates counts are different ($P < 0.05$) at that storage time.

Table 3.5. Least squares mean (standard error) *Staphylococcus aureus* counts (log CFU/g) enumerated on baird parker agar (BPA) anaerobic storage at 25ºC obtained from inoculated meat bars.

	Meat Bar											
Storage Day	**1B**	%BDL[1]	**1T**	%BDL	**2B**	%BDL	**2T**	%BDL	**3B**	%BDL	**3T**	%BDL
0	7.0^{a} (0.1)	0	7.2^{a} (0.1)	0	5.7^{b} (0.1)	0	6.7^{a} (0.1)	0	6.9^{a} (0.1)	0	7.0^{a} (0.1)	0
1	6.7^{a} (0.1)	0	6.9^{a} (0.1)	0	3.6^{d} (0.1)	0	5.2^{c} (0.1)	0	6.5^{ab} (0.1)	0	6.4^{b} (0.1)	0
3	6.6^{ab} (0.1)	0	6.8^{a} (0.1)	0	2.1^{d} (0.1)	0	4.6^{c} (0.1)	0	6.6^{a} (0.1)	0	6.3^{b} (0.1)	0
5	6.5^{ab} (0.2)	0	6.8^{a} (0.2)	0	$< 1.3^{d}$ (0.2)	16.7	3.8^{c} (0.2)	0	6.5^{a} (0.2)	0	6.3^{b} (0.2)	0
10	6.1^{a} (0.2)	0	6.5^{a} (0.1)	0	$< 0.9^{c}$ (0.2)	16.7	2.9^{b} (0.2)	0	6.0^{a} (0.2)	0	6.1^{a} (0.2)	0
15	5.8^{ab} (0.3)	0	6.5^{a} (0.3)	0	$< 0.8^{d}$ (0.3)	16.7	1.7^{c} (0.3)	0	5.4^{b} (0.3	0	6.0^{a} (0.3)	0
20	4.9^{b} (0.1)	0	6.4^{a} (0.1)	0	$< 0.9^{e}$ (0.1)	33.3	$< 0.9^{e}$ (0.1)	33.3	4.4^{d} (0.1)	0	5.9^{c} (0.1)	0
30	2.2^{d} (0.3)	0	6.0^{a} (0.3)	0	$< 0.9^{e}$ (0.3)	33.3	$< 0.6^{e}$ (0.3)	66.7	3.6^{c} (0.3)	0	4.6^{b} (0.3)	0
40	$< 0.6^{d}$ (0.3)	67.7	5.5^{a} (0.3)	0	$< 0.8^{d}$ (0.3)	33.3	$< 0.6^{d}$ (0.3)	66.7	1.8^{c} (0.3)	0	3.2^{b} (0.3)	0
50	$< 0.8^{c}$ (0.4)	33.7	4.5^{a} (0.4)	0	$< 0.5^{c}$ (0.4)	83.3	$< 0.7^{c}$ (0.4)	50.0	2.1^{b} (0.4)	0	$< 1.5^{c}$ (0.4)	50.0

[1] Below the analysis detection limit 0.5 log CFU/g; LSmeans with "<" indicates at least one sample was BDL.

$^{a-e}$ LSmeans with superscripts that differ within row, indicates counts are different ($P < 0.05$) at that storage time.

Table 3.6. Least squares mean (standard error) total plate counts (log CFU/g) enumerated on tryptic soy agar (TSA) following anaerobic storage at 25ºC obtained from meat bars inoculated with *Staphylococcus aureus*.

	Meat Bar											
Storage Day	**1B**	%BDL[1]	**1T**	%BDL	**2B**	%BDL	**2T**	%BDL	**3B**	%BDL	**3T**	%BDL
0	6.6^{a} (0.1)	0	6.8^{a} (0.1)	0	5.6^{b} (0.1)	0	6.3^{c} (0.1)	0	6.6^{a} (0.1)	0	6.6^{a} (0.1)	0
1	5.9^{a} (0.1)	0	6.1^{ad} (0.1)	0	3.5^{b} (0.1)	0	4.9^{c} (0.1)	0	6.5^{d} (0.1)	0	5.7^{a} (0.1)	0
3	6.1^{a} (0.1)	0	6.2^{a} (0.1)	0	2.2^{b} (0.1)	0	4.3^{c} (0.1)	0	6.3^{a} (0.1)	0	5.9^{a} (0.1)	0
5	6.2^{a} (0.1)	0	6.5^{b} (0.1)	0	1.9^{c} (0.1)	0	3.7^{d} (0.1)	0	6.2^{a} (0.1)	0	6.0^{a} (0.1)	0
10	5.7^{a} (0.2)	0	6.2^{a} (0.2)	0	1.7^{b} (0.2)	0	2.9^{c} (0.2)	0	5.7^{a} (0.2)	0	5.8^{a} (0.2)	0
15	5.1^{a} (0.2)	0	6.0^{b} (0.2)	0	1.6^{c} (0.2)	0	2.5^{d} (0.2)	0	5.2^{a} (0.2)	0	5.6^{ab} (0.2)	0
20	4.3^{a} (0.2)	0	6.1^{b} (0.2)	0	1.7^{c} (0.2)	0	2.0^{c} (0.2)	0	4.2^{a} (0.2)	0	5.4^{d} (0.2)	0
30	2.5^{a} (0.3)	0	5.6^{b} (0.3)	0	1.8^{a} (0.3)	0	2.0^{a} (0.3)	0	3.6^{c} (0.3)	0	4.2^{c} (0.3)	0
40	1.2^{a} (0.4)	0	4.8^{b} (0.4)	0	1.6^{a} (0.4)	0	1.6^{a} (0.4)	0	2.3^{c} (0.4)	0	$< 2.1^{ac}$ (0.4)	16.7
50	1.3^{a} (0.3)	0	3.9^{b} (0.3)	0	2.0^{a} (0.3)	0	1.9^{a} (0.3)	0	2.9^{c} (0.3)	0	$< 1.6^{a}$ (0.3)	16.7

[1] Below the analysis detection limit 0.5 log CFU/g; LSmeans with "<" indicates at least one sample was BDL.

$^{a-e}$ LSmeans with superscripts that differ within row, indicates counts are different ($P < 0.05$) at that storage time.

Table 3.7. Least squares mean (± standard deviation) shoulder periods and inactivation rates fitted with the Baranyi and Roberts mathematical model (DMFit version 3.5, ComBase) for meat bars inoculated with *Listeria monocytogenes* or *Staphylococcus aureus* after anaerobic storage 25ºC.

	Listeria monocytogenes		*Staphylococcus aureus*	
Bar	Shoulder Period (days)	Inactivation Rate (log CFU/g/day)	Shoulder Period (days)	Inactivation Rate (log CFU/g/day)
1B	3.3 (2.1)	-0.28^{a} (0.06)	12.4^{b} (1.4)	-0.26^{ac} (0.03)
1T	9.6^{a} (8.0)	-0.16^{a} (0.13)	22.2^{a} (9.3)	-0.08^{a} (0.04)
2B	–	-0.91^{b} (0.46)	–	-1.20^{b} (0.49)
2T	–	-0.34^{a} (0.16)	–	-0.34^{c} (0.09)
3B	1.7^{b} (3.5)	-0.19^{a} (0.05)	5.6^{b} (6.4)	-0.16^{ac} (0.04)
3T	7.3^{ab} (2.8)	-0.21^{a} (0.04)	21.6^{a} (13.7)	-0.27^{ac} (0.17)

– indicates no shoulder period was observed, inactivation began immediately.

$^{a-c}$ LSmeans bearing different superscripts within column, differ ($P < 0.05$).

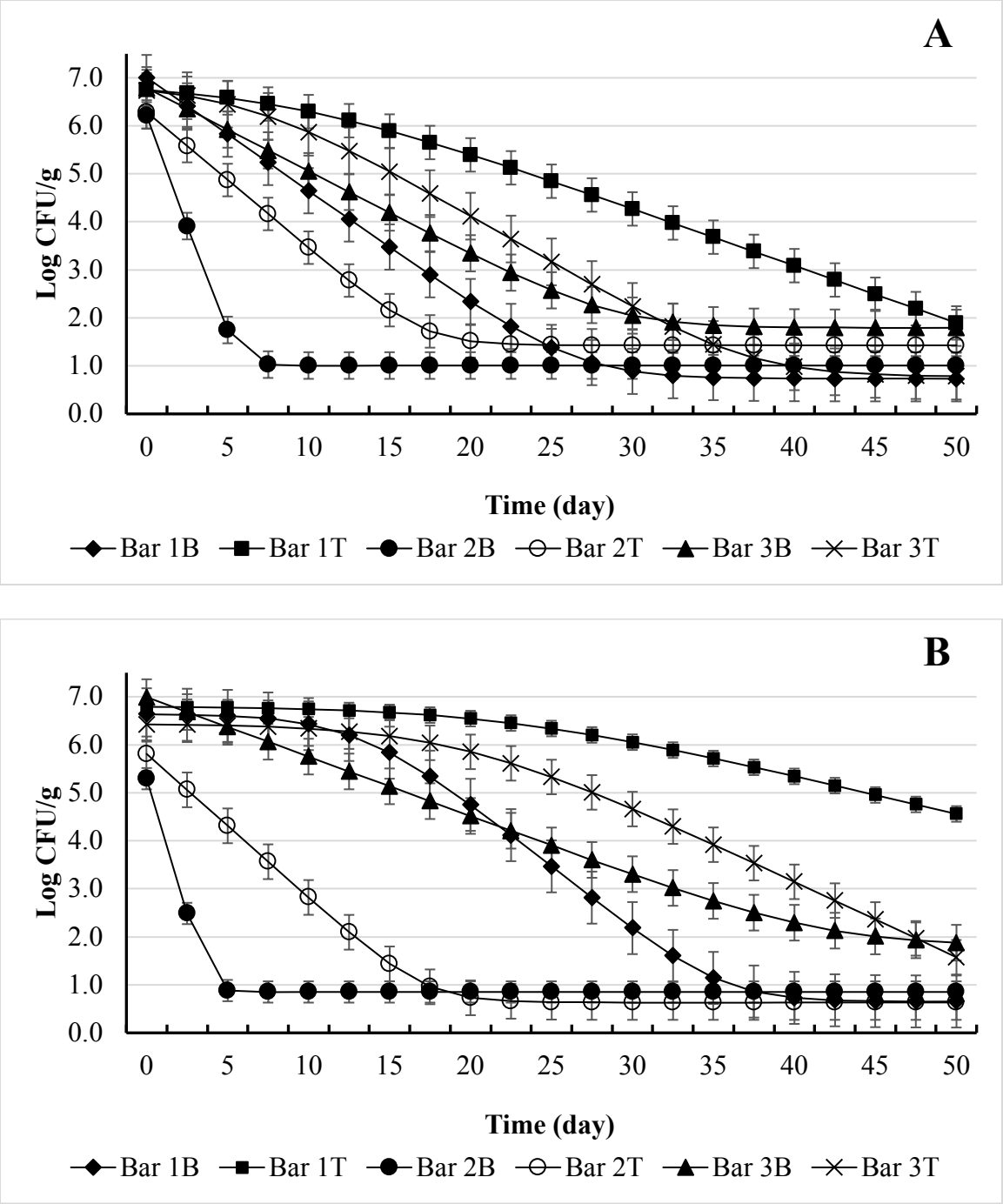

Figure 3.1. Survival curves fitted using the Baranyi mathematical model (DMFit version 3.5, ComBase) for meat bars inoculated with *Listeria monocytogenes* (**A)** or *Staphylococcus aureus* (**B**), during vacuum packed storage (25ºC). R^2 values for the **(A, B) graphs**: Bar 1B – (0.96, 0.99), 1T – (0.89, 0.84), 2B – (0.89, 0.90,) 2T – (0.91, 0.92), 3B – (0.89, 0.88), and 3T – (0.97, 0.82).

CHAPTER 4

Study II

Summary

Study II was conducted to evaluate the effects of meat bar water activity (a_w) and high-pressure processing (HPP) as a post-lethality treatment on the survival of inoculated *L. monocytogenes* populations on shelf-stable vacuum-packaged turkey-based meat bars stored at 25ºC. A five-strain mixture of *L. monocytogenes* was used in this study. The study was repeated twice on separate start days with separate cooked batches (two a_w level $\leq 0.91, \leq 0.85$) of meat bars for each trial. The study was designed as a 2 x 2 factorial, with factors of water activity ($\leq 0.91, \leq 0.85$) and treatment (control, HPP) for two different inoculation levels (3 log CFU/g, 6 log CFU/g).There were N = 240 (n = 120 each trial) bars inoculated for the $a_w \leq 0.91$ group; half were inoculated at a target level of 6 to 7 log CFU/g, while the other half were inoculated at a target of 3 to 4 log CFU/g. Additionally, bars with $a_w \leq 0.85$ were inoculated the same as those in the higher a_w group. Following inoculation, all meat bars were individually vacuum packaged. Half of the bars from each a_w group and inoculation level were labeled for HPP treatment, while the other half were labeled as "control" and were not exposed to HPP treatment. Bars were placed into foam coolers without ice and shipped over night for HPP-treatment 18 to 20 h post-inoculation. Cornell University, Department of Food Science, HPP Validation Center, treated the bars using a Hiperbaric 55 HPP machine for 180 s at 586 MPa (5ºC). Once shipped back, treated and control vacuum packaged bars were stored in an incubator (25ºC) for 40 or 50 d. The Mixed Models Procedures of SAS version 9.4 were utilized to determine differences between treatments within inoculation level on each storage day. Least squares mean differences were reported using a significance level of $\alpha = 0.05$. Surviving *L. monocytogenes* counts were modeled as a function

of storage time (day) using the model by Baranyi et al. (*7*). Surviving *L. monocytogenes* counts for each treatment were fitted to assess shoulder periods (log CFU/g/day) and inactivation rates (log CFU/g). Storage day affected ($P < 0.05$) the *L. monocytogenes* populations recovered from bars inoculated at both levels; populations tended to decrease over time. Additionally, for both inoculation levels, treatment combinations (a_w [$\leq 0.91, \leq 0.85$] and post-processing treatment [control, HPP]) differed ($P < 0.05$) in *L. monocytogenes* populations during storage. For the 6 log CFU/g inoculation level, a_w was a significant effect for shoulder period and inactivation rate of the pathogen in each of the treatment combinations during storage; there were no significant effects observed for bars inoculated at 3 log CFU/g. The HPP treatment didn't ($P \geq 0.05$) affect the survival of *L. monocytogenes* compared to the control; it only reduced ($P < 0.05$) the initial and/or end of storage counts. Initial pathogen reductions obtained with HPP ranged from 0.2 to 0.6 log CFU/g (6 log CFU/g inoculation) and 0.5 to 1.0 log CFU/g (3 log CFU/g inoculation). When inoculated to 6 log CFU/g, bars with $a_w \leq 0.91$ had longer ($P < 0.05$) shoulder periods (6.5 and 8.8 days) compared to bars dried to $a_w \leq 0.85$ (1.9, 1.8 days). Likewise, bars dried to $a_w \leq 0.91$ had slower ($P < 0.05$) pathogen inactivation rates (-0.06, -0.08 log CFU/g/day) compared to bars dried to $a_w \leq 0.85$ (-0.12, -0.10 log CFU/g/day). Regardless of treatment, *L. monocytogenes* populations were recovered from all bars following 40 or 50 d of storage at 25ºC. High pressure processing of bars with $a_w \leq 0.85$ showed the greatest potential for increased control of *L. monocytogenes* presence starting with 3 log CFU/g of post-processing contamination. The a_w impacted pathogen inactivation and surviving counts on shelf-stable meat bars. Parameters of HPP should be further investigated to better understand the most effective time and temperature to increase inactivation of *L. monocytogenes* on meat bars

Introduction

High protein diets are a nutritional fad growing in popularity in the United Sates (*12*, *36*, *142*). Protein is now being utilized as an advertising "claim" to target those consumers on these types of diets; in 2017, 39% more sales were attributed to meat products with the claim "protein" (*39*). Today's consumer is not only seeking high protein meals, but also convenient meals and snacks (*36*). Shelf-stable meat snacks are increasing in popularity, which in part could be a response to the high protein diet fads (*36*, *97*). Shelf-stable meat snacks do not need to be refrigerated, are nutrient dense and high in protein, as well as convenient, which contributes to their growing popularity (*137*). Demand for convenient meat snacks has driven innovation of niche dried meat products. Shelf-stable meat bars are a newer product being produced that contain large amounts of non-meat ingredients compared to their traditional dried jerky and sausage counterparts. These non-meat ingredients are often unique in nature and might include, fruits, vegetables, seeds, rice and nuts. The condition and ingredients of these meat bars vary depending on brand and formulation. The meat bar is often viewed as a meat based, high protein "granola" type snack.

There are several federal standards for the composition and condition of ready-to-eat and shelf-stable meat products (*65*). Historically, the U.S. Department of Agriculture, Food Safety and Inspection Service (USDA-FSIS) based shelf-stability and product standard of identity on the moisture to protein levels (MPR; *125*). For example, USDA-FSIS required shelf-stable jerky to have a MPR of 0.75:1 or less; however, more recently, USDA-FSIS has recognized that water activity and/or pH as appropriate means of assessing shelf-stability and safety (*134*, *136*). Water activity is a more accurate and appropriate way to assess shelf stability in meat products because it is an indicator of available water for microbial growth (*136*). There are safe harbors for a_w

levels for pathogen growth utilized in the meat industry; however, these safe harbors do not necessarily address survival of pathogens on shelf-stable meat products. These safe harbors consist of $a_w \leq 0.85$ if the product is stored in an oxygenated environment, or ≤ 0.91 in an anaerobic environment; these safe harbors will prevent growth of toxigenic pathogens such as *Staphylococcus aureus* and are the most common a_w levels meat snacks are being dried to (*136*). *Staphylococcus aureus* has the highest tolerance for low a_w environments and is often utilized in dried meat snack research to determine critical parameters to control post-processing contamination of pathogens such as *Listeria monocytogenes;* if it can control *S. aureus* it will control *L. monocytogenes*.

The USDA-FSIS considers *L. monocytogenes* an adulterant in ready-to-eat (RTE) meat products and has a zero tolerance for its presence in final product (*40*, *134*). To control this pathogen, a_w limits were utilized based on the growth limits for *S. aureus* under optimal conditions; however, these growth safe harbors do not address the survival of potentially present pathogens. Other factors in dried meat products might contribute to the control of pathogens such as pH (*63*, *65*, *66*, *132*). In study I, it was evident that low pH (< 4.6) was an effective intrinsic property that was able to control post-processing contamination of *L. monocytogenes* and *S. aureus* on meat bars during extended storage. However, study I did not control a_w as a factor, and therefore, was unable to make conclusions on the effect of a_w on pathogen survival on meat bars. It appears that the consumer today is demanding more "moist" meat snacks (*36*). Therefore, many producers have chosen to produce dried meat products with a_w of ≤ 0.91 packaged in anaerobic environments to meet their demands; while this level will control the growth of pathogens, it may not address the survival of pathogens during storage. Additionally, USDA-FSIS require establishments to have validated evidence assessing the critical parameters needed

to prevent, eliminate or reduce the presence of pathogenic bacteria from post-processing contamination (*134*, *136*).

Critical parameters of dried meat (whole muscle and ground) products have been extensively researched to determine the survival of *L. monocytogenes* during aerobic and anaerobic storage (*19*, *37*, *55*, *56*, *63*, *65*, *66*, *132*, *146*). Studies have assessed differing a_w and pH effects on the survival of *L. monocytogenes* on shelf-stable dried meat snacks (*63*, *65*, *66*, *132*). These studies have been used as scientific support for establishments as evidence of their critical parameters to control pathogens on shelf-stable meat products. This literature may not be appropriate for these new shelf-stable "meat bar" snacks, because the meat bars are produced differently and have a higher percentage of non-meat ingredients compared to traditional jerky. The conclusions made in a hazard analysis about traditional jerky, may not be appropriate for the meat bars; therefore, scientific evidence of pathogen risk related to meat bars is needed. Study I concluded pathogens survived on commercially produced meat bars stored under vacuum for 50 d at 25ºC. Survival after extended storage might indicate the need for a post-processing intervention to reduce shoulder periods and/or increase pathogen inactivation rates.

Interventions to control post-processing contamination in RTE meat products may include chemical antimicrobials such as acetates and lactates, natural plant based antimicrobials, packaging material with immobilized antimicrobials, and thermal pasteurization before or after packaging (*2*, *8*, *9*, *11*, *28*, *47*, *48*, *77*, *114*, *115*, *116*, *125*, *143*, *148*). There are other more novel technologies such as irradiation or high pressure processing (HPP) to control post-processing contamination of pathogens (*29*, *32*, *44*, *45*, *69*, *76*). These novel technologies are being increasingly investigated as alternatives for control as well as "clean label" options that won't add to their ingredient statement (*6*). However, the novel technologies either have a negative

consumer perception or are currently a more expensive option compared to other available interventions. Trends in consumer demands have occasionally dictated the type of interventions being used to control pathogens in meat products, with particular interest in keeping a "clean label", which is desirable to today's consumer (*6*). One of the trending intervention technologies that allows processors to provide post-processing control of pathogens while keeping a "clean label", is high pressure processing (HPP). Post-processing treatments are an effective means to reduce or eliminate pathogens; however, the most common control are final product intrinsic properties which are manipulated to control pathogens post-processing.

Furthermore, study I did not control a_w as a fixed factor making it difficult to determine the effect of a_w on the survival of pathogens. It might be necessary for meat bar producers to understand the survival characteristics of *L. monocytogenes* when they produce them under the water activity safe harbors (≤ 0.91 or ≤ 0.85) for growth. Currently, there is no literature investigating the survival of *L. monocytogenes* in ready-to-eat dried shelf-stable meat bars when dried to the different a_w levels. Therefore, a study was conducted to evaluate the effects of product water activity (a_w) and a post-processing HPP treatment on the survival of inoculated *L. monocytogenes* populations on shelf-stable vacuum-packaged meat bars stored at 25ºC.

Materials and Methods

***L. monocytogenes* strains and inoculum preparation.** A five-strain mixture of *Listeria monocytogenes* was used for this inoculation study. The strain identifications included *L. monocytogenes* LM 101 (serotype 4b; isolated from hard salami; Dr. Eric Johnson Food Research Institute, University of Wisconsin-Madison, Madison, WI), LM 108 (serotype 1a; isolated from hard salami; Dr. Eric Johnson Food Research Institute, University of Wisconsin-

Madison, Madison, WI), LM 310 (isolated from goat cheese; Dr. Eric Johnson Food Research Institute, University of Wisconsin-Madison, Madison, WI), V7 (isolated from hard raw milk; Dr. Eric Johnson Food Research Institute, University of Wisconsin-Madison, Madison, WI), and Scott A (serotype 4b; human isolate; obtained from our laboratory's culture collection Fort Collins, CO). Working cultures of *L. monocytogenes* strains were maintained on PALCAM agar (Difco, Becton Dickinson, Franklin Lakes, NJ). Strains were initially activated from frozen stock cultures that were frozen with 80% cultured tryptic soy broth with 0.6% yeast extract (TSBYE; Acumedia – Neogoen; Lansing, MI, USA) mixed with 20% glycerol and dispensed in 1 ml aliquots in cryovials, and frozen at -80ºC. Following initial activation, cultures were subcultured (35°C, 24 ± 2 h) prior to inoculum preparation. Broth cultures of the strains were combined, and cells harvested by centrifugation (3220 x g, 15 min, 4°C, Eppendorf model 5810 R, Brinkman Instruments Inc., Hamburg, Germany), washed with 10 ml phosphate buffered saline (PBS, pH 7.4; Sigma-Aldrich, St. Louis, MO), re-centrifuged, and resuspended in PBS to the original inoculum volume to obtain a cell concentration of approximately 8 to 9 log CFU/ml. The original inoculum volume was used for inoculation of the first group for a target inoculation of 6 to 7 log CFU/g. Then for the group with a target inoculation level of 3 log CFU/g, the original inoculum was serially diluted in PBS to an inoculum concentration of 5 to 6 log CFU/g.

Meat bar production, cooking, and drying. A previously formulated commercially available raw turkey-based meat bar batter was obtained from a meat processing facility. This formulation flavor profile was common, and available in several brands. The proprietary formulation included turkey as the main meat ingredient and also included a variety of fruits, vegetables, rice, seeds and spices. The batter was pre-made by the facility and delivered refrigerated (4ºC) overnight to the Meat Laboratory in the Department of Animal Sciences at

Colorado State University, Center for Meat Safety & Quality (Fort Collins, CO). Upon arrival, approximately two 22.7 kg portions were vacuum packaged and frozen (-30ºC). One week prior to the start of the inoculation study (repeated for two trials), one 22.7 g batch of raw meat batter was thawed under refrigeration (4ºC) to prepare for the extrusion of the meat bars. Meat bars were extruded and formed to weigh between 60 to 70 g and with dimensions of 11.5 cm long, 4.5 cm wide, and 1.1 cm thick. Once formed, they were placed on a mesh screen on a single oven truck in preparation for cooking. Half of the formed meat bars were designated for a cook schedule with a final drying a_w of ≤ 0.91 and the other half designated for a final target $a_w \leq 0.85$. Both batches were cooked using the same validated cooking lethality procedure with monitored oven temperature, humidity, and lethality internal product temperature before entering the drying period (Table 4.1). The final observed a_w and pH values are presented in Table 4.2. Water activity measurements were obtained using a calibrated AquaLab water activity meter (Dew Point Water Activity Meter, AquaLab 4TE, Meter Foods, Pullman, WA, USA). Twenty samples were measured for water activity from each of the two target a_w groups (n = 10 per batch per trial) and the means were reported for the observed versus the target (Table 4.2). Following the completion of cooking and drying, bars were bulk packed, single layered in a bag, vacuum packaged and stored refrigerated (4ºC) for 48 to 72 h prior to inoculation.

Inoculation and packaging. The study was repeated twice on separate start days with separate cooked batches of meat bars for each trial. There were N = 240 (n = 120 each trial) bars inoculated for the ≤ 0.91 group; half were inoculated to a target level of 6 to 7 log CFU/g, while the other half were inoculated to a target of 3 to 4 log CFU/g. Additionally, N = 240 (n = 120 for each trial) bars were inoculated for the ≤ 0.85 group; half were inoculated to a target level of 6 to 7 log CFU/g, while the other half were inoculated to a target of 3 to 4 log CFU/g. Two

inoculation levels were utilized to assess the survival of *L. monocytogenes* at a higher level of contamination, as well as, investigating the survival of *L. monocytogenes* at a lower inoculation level of approximately 3 log CFU/g. Additionally, some meat bars were left uninoculated (n = 6) for microbiological analysis of existing background microflora at day 0 of storage.

Prior to inoculation, bars were aseptically removed from their bulk packaging and placed on aluminum foiled trays sterilized with 70% ethanol. Approximately 0.1 ml of the *L. monocytogenes* inoculum was spot inoculated (randomly dispersed across the surface) on each side of the meat bar (0.2 ml total), spread evenly with a sterile L-shaped spreader, and allowed to sit for 15 min per side (30 min) for bacteria cell attachment. Pre-trial work during study I, verified that the inoculation process did not increase the a_w of the meat bars after the attachment period. The target inoculation was approximately 6 to 7 log CFU/g for half the meat bars and 3 to 4 log CFU/g for the other half.

After inoculation, individual bars were aseptically placed into vacuum bags (15 by 22 cm, 3 mil std. barrier, nylon/polyethylene vacuum pouch, Koch, Kansas City, MO), and vacuum packaged (Hollymatic Corp., Countryside, IL). Half the bars from each a_w and inoculation level were labeled and designated for high pressure processing (HPP) treatment while the other half were labeled as "control" and were not exposed to HPP treatment. All of the meat bars designated for HPP treatment and eight of the control bars were placed into foam coolers without ice and shipped (15ºC ± 5ºC) over night for treatment 18 to 20 h post-inoculation. The remaining control vacuum packaged bars were stored in an incubator at 25ºC ± 2ºC.

High pressure processing of meat bars. The inoculated bars designated for HPP treatment and the eight control bars, were shipped overnight to the HPP Validation Center in the Department of Food Science at Cornell University (Geneva, NY). Upon arrival, the meat bar

samples designated for HPP treatment were treated using a Hiperbaric 55 HPP machine (Hiperbaric, Miami, FL) for 180 s at 586 MPa (8500 lbs./in^2; 5ºC). Immediately following treatment, the collaborators at Cornell University sampled and enumerated n = 6 (n = 3 per trial) of each of the treatment combinations for the HPP-treated bars to serve as the "storage day 0" for initial *L. monocytogenes* counts (treatment combinations: $a_w \leq 0.91$ with 6 log CFU/g inoculation, ≤ 0.91 with 3 log CFU/g inoculation, ≤ 0.85 with 6 log CFU/g inoculation, and ≤ 0.85 with 3 log CFU/g). Additionally, Cornell University also sampled and enumerated n = 4 (n =2 per trial) control samples for each of the four corresponding treatment combinations for "storage day 0". Furthermore, at the same time, at Colorado State University, sampled and enumerated n = 2 (n =1 per trial) control samples for initial *L. monocytogenes* counts of "storage day 0" to determine if there were any count difference between the two locations (total of n = 6 total control samples per treatment combination sampled for day 0). After HPP treatment, the remaining bars were shipped overnight back to Colorado State University and upon arrival (36 to 48 h post-inoculation; 12 to 24 h post-treatment) were stored in the same incubator as the rest of the control samples at 25ºC for 50 d of storage.

Microbiological Analysis. Samples (n =3; repeated for two trials) were microbiologically analyzed on days 0 (at Cornell University and Colorado State University), 1, 3, 5, 10, 15, 20, 30, 40 and 50 d of storage for the bars inoculated to 6 log CFU/g. The storage days bars were sampled on for the bars inoculated to 3 log CUF/g were days 0, 1, 3, 5, 10, 15, 20, 25, 30, and 40. Uninoculated bars were sampled only on day 0 to obtain initial background aerobic populations. Bars were aseptically removed from the vacuum packages and portioned into approximately 25 g for microbiological analysis. The 25 g of meat bar was placed into a sterile 24 oz. filter Whirl-Pak bag (Nasco, Modesto, CA) then 50 ml of maximum recovery diluent

(MRD, Acumedia-Neogen) was added for a 2:1 ratio of diluent and sample. Bagged samples were homogenized (Stomacher 400 Circulator; Seward, Port Saint Lucie, FL) for 2 min then serially diluted in 0.1% buffered peptone water (BPW, Difco, Becton Dickinson). For enumeration of *L. monocytogenes*, 0.1 ml or 1 ml of diluted sample was surface plated on PALCAM for selective enumeration and tryptic soy agar supplemented with 0.6% yeast extract (TSAYE; Acumedia-Neogen) for enumeration of total aerobic populations. Plates were incubated at 35ºC ± 2ºC for 48 h (PALCAM agar) or 25ºC ± 2ºC for 72 h (TSAYE). Following incubation colonies were counted on appropriate dilutions, recorded, and reported as log CFU/g. The detection limit for the microbiological analysis was 0.5 log CFU/g. Samples with non-detectable counts were then enriched following the 2013 USDA-FSIS Microbiological Laboratory Guidelines 8.1: Isolation and Identification of *Listeria monocytogenes* from Red Meat, Poultry, Ready-To-Eat Siluriformes (Fish) and Egg Products, and Environmental Samples protocol (*140*). Following the enrichment of samples and streaking onto PALCAM agar, colonies were isolated from the PALCAM agar and confirmed as *L. monocytogenes* (AOAC 121402 Dupont™ BAX® System Real-Time PCR Assay for *L. monocytogenes*).

Proximate Analysis and pH. Each of the four treatment combinations were analyzed for proximate analyses that included fat (AOAC 991.36), moisture (AOAC 950.46b), protein (AOAC 992.15), salt as sodium chloride (AOAC 935.47). Three bars from each of the treatment combinations for each of the repeated trials (n = 6) were composited and sent to a third-party laboratory in Denver, CO for the analyses.

The pH measurements were taken from the same samples used for microbiological analysis (2:1 MRD to sample) following 2 min of homogenizing for storage day 1 only. The pH

was obtained using a calibrated pH meter fitted with a glass electrode (Denver Instruments, Arvada, CO). Measurements were recorded and reported as means (Table 4.2).

Statistical Analysis. The study was designed as a 2 x 2 factorial, with factors of water activity ($\leq 0.91, \leq 0.85$) and treatment (control, HPP) for two different inoculation levels (3 log CFU/g, 6 log CFU/g,). Inoculation level was not a factor and the data were analyzed separately within inoculation level. Microbial counts were converted to log CFU/g before statistical analysis. The study had two trials, and n = 6 samples were collected for each meat bar for each of four treatment combinations. The Mixed Procedures of SAS version 9.4 (Carry, NC, USA) was utilized to determine difference between treatments within inoculation level on each sampled storage day. Differences were reported using a significance level of $\alpha = 0.05$.

Additionally, surviving *L. monocytogenes* log converted counts were modeled as a function of storage time (day) using the model by Baranyi et al. (*7*). Surviving *L. monocytogenes* counts for each treatment were fitted to assess shoulder periods (the time in days where the levels of pathogen remained at the level of the initial inoculation) and inactivation rates (log CFU/g/day). Within pathogen type, differences among bars were determined using the Mixed Procedures in SAS for maximum inactivation rates and shoulder period; differences were reported using a significance level of $\alpha = 0.05$.

Results and Discussion

Chemical and physical properties of meat bars. Twenty bars were sampled for water activity following the cooking and drying process for each batch for both trials. The bars that were dried to a target a_w of ≤ 0.91 had observed a_w measurements of 0.903 on average (Table 4.2). The treatment group with a target a_w of ≤ 0.85 had an average observed a_w measurements of

0.838 (Table 4.2). There were no statistical differences between the pH of meat bars dried to a target $a_w \leq 0.91$ versus ≤ 0.85 or exposed to HPP or not, so the mean pH for each a_w treatment group are reported in Table 4.2. Additionally, bars were collected from each of the four treatment combinations (n = 6) and composited for proximate analysis. There were no significant differences observed between the control versus HPP-treated bars within water activity treatment; therefore, percent fat, moisture, protein, and salt were reported within water activity level only (Table 4.2). Bars with $a_w \leq 0.85$ had numerically higher percent fat, protein and salt, but lower percent moisture (Table 4.1).

Effects of storage on *Listeria monocytogenes* populations on meat bars dried to different water activities with and without HPP. Uninoculated background samples from all four treatments were enumerated on PALCAM to determine presence of background *Listeria* species prior to inoculation. No colonies were observed on PALCAM agar, indicating no presence of *Listeria* on the background samples within the analysis detection limit. The uninoculated background samples from all four treatment groups were also enumerated on TSAYE for total aerobic populations. Meat bars with a target $a_w \leq 0.91$ had 2.1 log CFU/g background microflora and meat bars with $a_w \leq 0.85$ had 2.2 log CFU/g background microflora (data not shown).

The storage day affected ($P < 0.05$) the *L. monocytogenes* populations recovered on PALCAM during the 50-d period on bars inoculated to 6 log CFU/g; populations tended to decrease over time (Table 4.3). Additionally, water activity (≤ 0.91 and ≤ 0.85) and treatment (control and HPP) were main effects ($P < 0.05$) on the meat bars inoculated with 6 log CFU/g during vacuum-packaged storage. The initial populations of the control meat bars with the target inoculation of 6 log CFU/g were, 6.1 and 6.3 log CFU/g for the bars with $a_w \leq 0.91$ and ≤ 0.85,

respectively. There was not ($P \geq 0.05$) an immediate effect of HPP treatment on the bars with $a_w \leq 0.91$ on day 0 (Table 4.3). High pressure processing reduced ($P < 0.05$) the initial *L. monocytogenes* populations obtained from meat bars with $a_w \leq 0.85$; the initial populations on the controls were 6.3 log CFU/g and after HPP, remaining populations were 5.5 log CFU/g on storage day 0 (Table 4.3). In a study by Lucore et al. (82), the researchers investigated the effects of HPP (300, 500, 700 MPa) on *L. monocytogenes* inoculated on vacuum-packaged frankfurters. In their study packages held at 500 MPa for 6 min resulted in approximately a 6-log decrease in *L. monocytogenes* and at 700 MPa resulted in the greatest inactivation of *L. monocytogenes* in the shortest period of time; however, all treatment parameters were effective at reducing inoculated populations of *L. monocytogenes* on packaged frankfurters (*86*). The time needed to greatly impact *L. monocytogenes* populations on frankfurters was 6 min at 700 MPa; it might be necessary to increase the total HPP time to largely impact *L. monocytogenes* on meat bars.

The bars inoculated to 6 log CFU/g, with a target $a_w \leq 0.91$, had control samples that were the same ($P \geq 0.05$) as the HPP samples until storage day 5; however, this difference was not consistent, but by day 50 of storage, they differed ($P < 0.05$; Table 4.3). Meat bars with a target $a_w \leq 0.85$ that were HPP-treated remained different ($P < 0.05$) from the control bars for the duration of the 50-d storage; the final control counts were higher ($P< 0.05$) compared to the HPP-treated bars (Table 4.3). None of the samples inoculated to 6 log CFU/g, from all four treatment combinations, were ever below the analysis detection limit (< 0.5 log CFU/g). At the end of the 50 days of storage, all four treatments *L. monocytogenes* was still present on the bars; however, the counts on the bars with $a_w \leq 0.91$ were higher compared to the bars with $a_w \leq 0.85$ (Table 4.3).

Similar trends were observed on meat bars that were inoculated to 3 log CFU/g (Table 4.4). Storage day was a significant effect on the *L. monocytogenes* populations; the populations tended to decrease over time. Additionally, water activity (≤ 0.91 and ≤ 0.85) and treatment (control and HPP) were main effects ($P < 0.05$) on the meat bars inoculated to 3 log CFU/g across all storage days. The control samples did not ($P \geq 0.05$) differ between the bars with $a_w \leq 0.91$ and ≤ 0.85 until storage day 20, which is when bars with $a_w \leq 0.85$ had less ($P < 0.05$) *L. monocytogenes* counts compared to the bars with $a_w \leq 0.91$ for the rest of the 40-d storage period (Table 4.4). High pressure processing had no initial affect ($P \geq 0.05$) on the *L. monocytogenes* populations on meat bars with $a_w \leq 0.91$ and ≤ 0.85 (Table 4.4). The HPP treated bars with $a_w \leq 0.85$, by day 1, were significantly different from the controls for the remainder of the 40 days of storage and maintained lower ($P < 0.05$) *L. monocytogenes* counts compared to all of the other bars in the other three treatment combinations (Table 4.4). By day 15, the HPP-treated bars with $a_w \leq 0.85$ were 33.3% BDL with *L. monocytogenes* populations of < 0.7 log CFU/g, while the corresponding control samples did not have samples BDL until storage day 25 and did not have populations < 1.0 log CFU/g until storage day 30 (Table 4.4). These data indicate that HPP treatment on meat bars with $a_w \leq 0.85$ had a significant impact on the *L. monocytogenes* populations during storage (Table 4.4). By storage days 40, *L. monocytogenes* was reduced ($P < 0.05$) to < 0.5 log CFU/g with 100.0% of the samples BDL for HPP-treated meat bars with $a_w \leq 0.85$ (Table 4.4).

Meat bars that were below the analysis detection limit were enriched and confirmed for surviving *L. monocytogenes* populations. All samples that were BDL and were enriched and grew on PALCAM agar, were confirmed for *Listeria monocytogenes.* Growth on PALCAM agar was observed for all samples that were below the detection limit, excluding one of the samples

on the 40th day of storage for meat bars initially inoculated with 3 log CFU/g, dried to $a_w \leq 0.85$ and HPP-treated. This confirms that, although samples were BDL, *L. monocytogenes* was still present after 40-d of vacuum packaged storage at room temperature on all bars, excluding the one sample.

Both inoculation levels and all four treatment combinations had higher aerobic plate counts recovered with TSAYE compared to the populations recovered on PALCAM (Tables 4.5 and 4.6). Similar trends were still observed between differing a_w, where bars with $a_w \leq 0.91$ had higher ($P < 0.05$) counts compared to bars dried to $a_w \leq 0.85$ regardless of inoculation level (Tables 4.5 and 4.6). However, almost no differences were observed between the control and HPP bars that were dried to $a_w \leq 0.91$, which could indicate that the difference observed on PALCAM agar were due to sub-lethally injured cells' inability to recover (Table 4.5). The effects of HPP on bars with $a_w \leq 0.85$ tended to be different ($P < 0.05$) compared to the corresponding controls; however, an interesting observation was that there were no differences ($P \geq 0.05$) observed at the end of the 50-d storage among any of the bars regardless of inoculation level (Tables 4.5 and 4.6). It may be necessary to investigate the potential of sublethally injured cells following HPP treatment on meat bars to better understand the efficacy of HPP against *L. monocytogenes* on these products.

These data provide evidence that HPP was not microbiologically significant against *L. monocytogenes* on meat bars dried to approximately 0.91; there was some evidence of decreased populations, but more research investigating sublethally injured cell might be necessary. High pressure processing showed the capabilities to maintain reduced populations of *L. monocytogenes* on inoculated meat bars that were dried to $a_w \leq 0.85$ compared to the control; this may be because the lower water activity provided additional lethality against potentially

sublethally injured cells, unlike the higher water activity bars. Investigation of different HPP treatment parameters, such as longer time, could provide more data that could be used in the future as a post-processing lethality treatment against surviving *L. monocytogenes* populations on meat bars.

***Listeria monocytogenes* survival and inactivation characteristics on meat bars during storage.** Shoulder periods and inactivation/death curves with calculated inactivation rates were fit for *L. monocytogenes* inoculated populations for meat bars at both inoculation levels, for all four treatments (Tables 4.7 and 4.8; Figure 4.1). For the 6 log CFU/g inoculation level, water activity was differences resulted in different ($P < 0.05$) shoulder periods and inactivation rates of *L. monocytogenes* in each of the treatment combinations during storage (Table 4.7).

The effect of HPP on bars inoculated to 6 log CFU/g did not change the survival characteristics of *L. monocytogenes* during storage, it only reduced ($P < 0.05$) the initial and/or ending counts (Table 4.7). The little effect of HPP on the inactivation characteristics of *L. monocytogenes* may be due to the lower water activity levels of the meat bars. Jofré et al. (*67*) conducted a study that evaluated the effect of HPP at 600 MPa on three convenience meat products (sliced cooked ham, sliced dry cured ham, and marinated beef) against multiple foodborne pathogens, including *L. monocytogenes*. In this study, the meat products were stored up to 120 days post treatment at 4ºC (*67*). They found that dry-cured ham with a_w of 0.918 demonstrated lower inactivation rates of *L. monocytogenes* compared to the cooked ham and beef loin (*67*). These results indicated that HPP was less effective against the lower water activity meat product in compared to the higher water activity products (*67*).

Additionally, meat bars inoculated to 6 log CFU/g, with $a_w \leq 0.91$, had longer ($P < 0.05$) shoulder periods (6.5 and 8.8 days) compared to bars dried to $a_w \leq 0.85$ (1.9 and 1.8 days; Table

4.7). Likewise, bars dried to $a_w \leq 0.91$ had slower inactivation rates (-0.06 and -0.08 log CFU/g/day) compared to bars dried to $a_w \leq 0.85$ (-0.12 and -0.10 log CFU/g/day). *Listeria monocytogenes* populations were still present at the completion of 50 days of storage for all the bars with initial inoculation level of 6 log CFU/g ranging from 3.3 and 2.5 log CFU/g for bars $a_w \leq 0.91$ and 1.8 and 1.3 for bars $a_w \leq 0.85$ (Table 4.7).

There were no significant main effects observed for water activity or HPP treatment on the bars with target inoculation of 3 log CFU/g for shoulder periods or inactivation rates (Table 4.8). There was a trend for both, but they were not significant. The only difference ($P < 0.05$) observed were the shoulder period for HPP-treated bars with $a_w \leq 0.85$ compared to the others. There was no shoulder period observed for this group; *L. monocytogenes* immediately began to inactivate at the start of the storage period (Table 4.8 and Figure 4.1). Some research suggest, that lower water activity foods tend not to respond as well to HPP treatment; however, bacteria injured by pressure are more sensitive to low water activity which will aid in inactivation which might be the reason for the largest difference for HPP-treated meat bars dried to $a_w \leq 0.85$ in the current study (*61*, *67*, *106*, *124*). Furthermore, the corresponding control meat bars had shoulder period of 4.3 days which was higher ($P < 0.05$) than the HPP-treated bars but no different ($P \geq 0.05$) compared to the bars with $a_w \leq 0.91$ (Table 4.8). There were no differences ($P \geq 0.05$) between the control and HPP from bars with $a_w \leq 0.91$ (Table 4.8). Inactivation rates were no different ($P \geq 0.05$) for all four treatment combinations; inactivation rates were -0.06, -0.07, -0.10, and -0.11 log CFU/g/day, although numerically different between a_w levels, they were not significant. It is worth mentioning that the average inactivation rates for bars inoculation levels were similar; this might suggest that the survival behavior of *L. monocytogenes* on meat bars does not change drastically based on the amount of contamination on the bar (Table 4.7 and 4.8,

Figure 4.1). High pressure processing on bars with $a_w \leq 0.85$ showed the greatest potential for increased control of surviving populations of *L. monocytogenes* starting with 3 log CFU/g of post processing contamination (Figure 4.1).

Time and temperature parameters for HPP should be further investigated to better understand the combination most effective against surviving populations of *L. monocytogenes* on meat bars. Research has shown that cell death increases with pressure, but does not necessarily follow a first-order kinetics; "tailing off" in inactivation can occur (*45*, *73*). However, 586 MPa is considered high pressure, and pressure between 30 and 50 MPa can influence gene expression and protein synthesis; it is thought to be able to interfere with replication of DNA which should have been the case with this study's treatment (*124*). It has be discussed, however, that cells subjected to prior stress, for example heat stress or cells in stationary phase, tend to be more resistant to pressure (*124*). The 18 to 20 h that the inoculated meat bars were being shipped to Cornell University for HPP treatment, might have been long enough to subject the *L. monocytogenes* to the stressful environment of the low water activity under vacuum, possibly making them more resistant to the HPP treatment. Additionally, treatment occurred at 5ºC, and temperature can play an important role in the inactivation of microbial populations when utilizing HPP (*45*). When pressure is applied at optimal growth temperatures, reduced inactivation is observed compared to higher or lower temperatures due to the fluidity of the membrane at high and low temperatures becoming more easily disrupted (*45*, *124*). Although 5ºC is considered a lower temperature for most pathogens, *L. monocytogenes* is known to still grow under these conditions, so it might be better suited to increase the chamber temperature during HPP when targeting post-processing contamination of *L. monocytogenes*.

Investigating HPP at higher temperatures and longer treatment time might aide in a more effective post-processing intervention on meat bars against *L. monocytogenes*. In general, research shows that the lower the water activity of the food product, the more protection it provides the cells from pressure, which makes the efficacy of pressure on bacteria in a low water activity environment, such as meat bars, challenging to predict (*61*, *67*, *106*, *124*). The data from the current study might suggest potential for HPP on meat bars with $a_w \leq 0.85$, because the possible sub-lethally injured *L. monocytogenes* cells post-HPP might respond better to the lower water activity. Water activity during extended storage up to 50-d proved to affect the surviving populations of *L. monocytogenes.* The data provided in this study might be useful to determine pre-shipment holding periods for production of meat bars as a post-processing intervention treatment against *L. monocytogenes.* Pre-shipment holding could be a simple intervention for meat bar producers to use that would aide in inactivation of the pathogen before entering commerce. This pre-shipment hold, might only be practical for bars dried to $a_w \leq 0.85$, because they had counts consistently lower compared to the bars dried to $a_w \leq 0.91$. Therefore, the hold period would be shorter for the dryer bars with a greater impact on reducing *L. monocytogenes*. More research investigating the risk of post-processing contamination of pathogens on meat bars is needed. There was still presence of *L. monocytogenes* after 40 and 50 days of storage under normal shelf-stable conditions with no abuse. Investigation of other post-processing interventions could provide the industry with better insight on control options for survival of pathogens post-lethality. It may also be beneficial to investigate the response of pathogens on meat bars during storage under abusive environmental temperatures and oxygenated environments to understand the magnitude of the risk involved with these products.

Table 4.1. Validated cooking lethality for meat bars dried to target a_w of either ≤ 0.91 or ≤ 0.85.

Step	Dry bulb (°F)	RH %	Wet Bulb (°F)	Time (min)/Internal Temperature
1	130	30	96.5	15
2	140	30	103	15
3	140	20	95	15
4	165	40	133	30
Instantaneous Lethality	180	55	161	Instantaneous internal temp 165°F (~ 2.5 hours)
Drying	140	20	90	Until target a_w

Table 4.2. Mean (standard deviation) water activity (a_w), pH, and compositional values of turkey-based meat bars for each target water activity group utilized in an inoculation study evaluating the survival of *Listeria monocytogenes*.

Target Water Activity	Observed a_w	pH	Fat %	Moisture %	Protein %	% Salt
≤ 0.91	0.903 (0.011)	5.51 (0.06)	6.5 (0.0)	41.3 (0.1)	23.8 (0.7)	1.2 (0.4)
≤ 0.85	0.838 (0.009)	5.48 (0.06)	7.7 (0.2)	33.3 (0.1)	25.5 (0.4)	2.1 (0.0)

Table 4.3. Least squares mean (standard error) *Listeria monocytogenes* plate counts (log CFU/g) enumerated on PALCAM agar, following either, no treatment (Control), or application of high pressure processing (HPP), during anaerobic storage (25ºC) obtained from inoculated (ca. 6 log CFU/g) meat bars (water activity: ≤ 0.91 or ≤ 0.85).

	≤ 0.91				≤ 0.85			
Storage Day	Control	%BDL[1]	HPP	%BDL	Control	%BDL	HPP	%BDL
0	6.1^{a} (0.3)	0	5.4^{ab} (0.3)	0	6.3^{a} (0.3)	0	5.5^{b} (0.3)	0
1	6.2^{a} (0.1)	0	6.0^{a} (0.1)	0	6.4^{a} (0.1)	0	5.8^{b} (0.3)	0
3	6.0^{ab} (0.1)	0	5.8^{bc} (0.1)	0	6.1^{a} (0.1)	0	5.6^{c} (0.1)	0
5	6.0^{a} (0.1)	0	5.7^{b} (0.1)	0	5.9^{a} (0.1)	0	5.4^{c} (0.1)	0
10	5.6^{a} (0.1)	0	5.3^{a} (0.1)	0	5.4^{a} (0.1)	0	4.8^{b} (0.1)	0
15	5.5^{a} (0.1)	0	5.2^{b} (0.1)	0	4.8^{c} (0.1)	0	4.2^{d} (0.1)	0
20	5.3^{a} (0.2)	0	4.8^{ab} (0.2)	0	4.3^{bc} (0.2)	0	3.9^{c} (0.2)	0
30	4.7^{a} (0.2)	0	4.1^{b} (0.2)	0	3.0^{c} (0.2)	0	2.7^{c} (0.2)	0
40	4.0^{a} (0.2)	0	3.4^{a} (0.2)	0	2.4^{b} (0.2)	0	2.0^{b} (0.2)	0
50	3.3^{a} (0.3)	0	2.5^{b} (0.3)	0	1.8^{c} (0.3)	0	1.4^{c} (0.3)	0

[1] Below the analysis detection limit 0.5 log CFU/g.

$^{a-e}$ LSmeans with superscripts that differ within row, indicates counts are different ($P < 0.05$) at that storage time.

Table 4.4. Least squares mean (standard error) *Listeria monocytogenes* plate counts (log CFU/g) enumerated on PALCAM agar, following either, no treatment (Control), or application of high pressure processing (HPP), during anaerobic storage (25ºC) obtained from inoculated (ca. 3 log CFU/g) meat bars (water activity: ≤ 0.91 or ≤ 0.85).

	≤ 0.91				≤ 0.85			
Storage Day	Control	%BDL[1]	HPP	%BDL	Control	%BDL	HPP	%BDL
0	3.0^{a} (0.2)	0	2.5^{a} (0.2)	0	3.0^{a} (0.2)	0	2.4^{a} (0.2)	0
1	3.2^{a} (0.1)	0	2.8^{b} (0.1)	0	3.2^{a} (0.1)	0	2.5^{c} (0.1)	0
3	2.9^{a} (0.1)	0	2.7^{a} (0.1)	0	2.9^{a} (0.1)	0	2.1^{b} (0.1)	0
5	3.0^{a} (0.1)	0	2.6^{b} (0.1)	0	3.0^{a} (0.1)	0	2.0^{c} (0.1)	0
10	2.7^{a} (0.1)	0	2.3^{b} (0.1)	0	2.4^{b} (0.1)	0	1.2^{c} (0.1)	0
15	2.5^{a} (0.1)	0	2.2^{a} (0.1)	0	2.1^{a} (0.1)	0	< 0.9^{b} (0.1)	33.3
20	2.2^{a} (0.2)	0	1.9^{a} (0.2)	0	1.5^{b} (0.2)	0	< 0.8^{c} (0.2)	16.7
25	1.9^{a} (0.2)	0	1.5^{a} (0.2)	0	< 1.1^{b} (0.2)	33.3	< 0.6^{c} (0.2)	50.0
30	1.8^{a} (0.1)	0	1.3^{b} (0.1)	0	< 0.6^{c} (0.1)	83.3	< 0.5^{c} (0.1)	100.0
40	1.1^{a} (0.1)	0	< 0.7^{b} (0.1)	16.7	< 0.5^{b} (0.1)	83.3	< 0.5^{b} (0.1)	100.0

[1] Below the analysis detection limit 0.5 log CFU/g; LSmeans with "<" indicates at least one sample was BDL.

$^{a-e}$ LSmeans with superscripts that differ within row, indicates counts are different ($P < 0.05$) at that storage time.

Table 4.5. Least squares mean (standard error) total plate counts (log CFU/g) enumerated on tryptic soy agar with 0.6% yeast extract (TSAYE), following either no treatment (Control) or application of high pressure processing (HPP), during anaerobic storage (25ºC) obtained from meat bars (water activity: ≤ 0.91 or ≤ 0.85) inoculated with *Listeria monocytogenes* (ca. 6 log CFU/g)[1].

	≤ 0.91		≤ 0.85	
Storage Day	Control	HPP	Control	HPP
1	6.4^{a} (0.1)	6.2^{a} (0.1)	6.4^{a} (0.1)	5.8^{b} (0.1)
3	6.2^{a} (0.1)	6.0^{a} (0.1)	6.3^{a} (0.1)	5.6^{b} (0.1)
5	6.2^{a} (0.1)	5.9^{b} (0.1)	6.1^{ab} (0.1)	5.4^{c} (0.1)
10	6.0^{a} (0.1)	5.6^{b} (0.1)	5.9^{ab} (0.1)	5.0^{c} (0.1)
15	5.8^{a} (0.1)	5.4^{b} (0.1)	5.4^{b} (0.1)	4.6^{c} (0.1)
20	5.5^{a} (0.2)	5.2^{ab} (0.2)	4.9^{b} (0.2)	4.2^{c} (0.2)
30	5.0^{a} (0.2)	4.6^{ab} (0.2)	4.1^{b} (0.2)	3.3^{c} (0.2)
40	4.6^{a} (0.1)	4.1^{a} (0.1)	3.3^{b} (0.1)	2.8^{b} (0.1)
50	4.0^{a} (0.2)	3.3^{a} (0.2)	2.6^{b} (0.2)	2.3^{b} (0.2)

[1] No samples were below the analysis detection limit of 0.5 log CFU/g.

$^{a-e}$ LSmeans with superscripts that differ within row, indicates counts are different ($P < 0.05$) at that storage time.

Table 4.6. Least squares mean (standard error) total plate counts (log CFU/g) enumerated on tryptic soy agar with 0.6% yeast extract (TSAYE), following either no treatment (Control) or application of high pressure processing (HPP), during anaerobic storage (25ºC) obtained from meat bars (water activity: ≤ 0.91 or ≤ 0.85) inoculated with *Listeria monocytogenes* (ca. 3 log CFU/g)[1].

	≤ 0.91		≤ 0.85	
Storage Day	Control	HPP	Control	HPP
1	3.4^{a} (0.1)	2.9^{b} (0.1)	3.6^{a} (0.1)	2.6^{c} (0.1)
3	3.2^{a} (0.1)	2.9^{b} (0.1)	3.2^{a} (0.1)	2.4^{c} (0.1)
5	3.3^{a} (0.1)	2.9^{b} (0.1)	3.2^{a} (0.1)	2.4^{c} (0.1)
10	2.9^{a} (0.1)	2.7^{a} (0.1)	2.8^{a} (0.1)	2.3^{b} (0.1)
15	2.8^{a} (0.1)	2.5^{a} (0.1)	2.5^{a} (0.1)	2.0^{b} (0.1)
20	2.5^{a} (0.1)	2.3^{ab} (0.1)	2.4^{ab} (0.1)	2.1^{b} (0.1)
25	2.4a (0.1)	2.2^{ab} (0.1)	2.1^{ab} (0.1)	1.9^{b} (0.1)
30	2.5^{a} (0.1)	2.1^{b} (0.1)	2.2^{b} (0.1)	2.1^{b} (0.1)
40	2.2^{a} (0.1)	1.9^{a} (0.1)	2.0^{a} (0.1)	1.8^{a} (0.1)

[1] No samples were below the analysis detection limit of 0.5 log CFU/g.

$^{a-e}$ LSmeans with superscripts that differ within row, indicates counts are different ($P < 0.05$) at that storage

Table 4.7. Least squares mean (± standard deviation) shoulder periods (days), inactivation rates (Log CFU/g/day), start and end counts (log CFU/g) fitted with the Baranyi and Roberts mathematical model (DMFit version 3.5, ComBase) from high pressure processed (HPP) meat bars or no HPP (Control) meat bars inoculated with *Listeria monocytogenes* at 6 log CFU/g, with two different water activity levels, during anaerobic storage 25ºC for 50 days.

Target Water Activity	≤ 0.91		≤ 0.85	
	Control	HPP	Control	HPP
Shoulder Period	6.5^{a} (4.8)	8.8^{a} (3.1)	1.9^{b} (2.1)	1.8^{b} (2.9)
Inactivation Rate	-0.06^{a} (0.01)	-0.08^{a} (0.01)	-0.12^{b} (0.01)	-0.10^{b} (0.01)
Start Count	6.1^{a} (0.1)	5.7^{ab} (0.1)	6.4^{a} (0.1)	5.7^{b} (0.1)
End Count	3.3^{a} (0.3)	2.5^{b} (0.3)	1.8^{c} (0.2)	1.3^{c} (0.3)

$^{a-c}$ LSmeans bearing different superscripts within column, differ ($P < 0.05$).

Table 4.8. Least squares mean (± standard deviation) shoulder periods (days), inactivation rates (Log CFU/g/day), start and end counts (log CFU/g) fitted with the Baranyi and Roberts mathematical model (DMFit version 3.5, ComBase) from high pressure processed (HPP) meat bars or no HPP (Control) meat bars inoculated with *Listeria monocytogenes* at 3 log CFU/g, with two different water activity levels, during anaerobic storage 25ºC for 50 days.

Target Water Activity	≤ 0.91		≤ 0.85	
	Control	HPP	Control	HPP
Shoulder Period	5.4[a] (5.1)	7.8[a] (4.0)	4.3[a] (2.4)	–[b]
Inactivation Rate	-0.06[a] (0.01)	-0.07[a] (0.01)	-0.10[a] (0.01)	-0.11[a] (0.01)
Start Count	3.1[a] (0.1)	2.7[a] (0.1)	3.1[a] (0.1)	2.4[a] (0.1)
End Count	1.1[a] (0.1)	< 0.5[b] (0.7)	< 0.5[b] (0.1)	< 0.5[b] (0.1)

– indicates no shoulder period observed, inactivation began immediately.

[a – c] LSmeans bearing different superscripts within column, differ ($P < 0.05$).

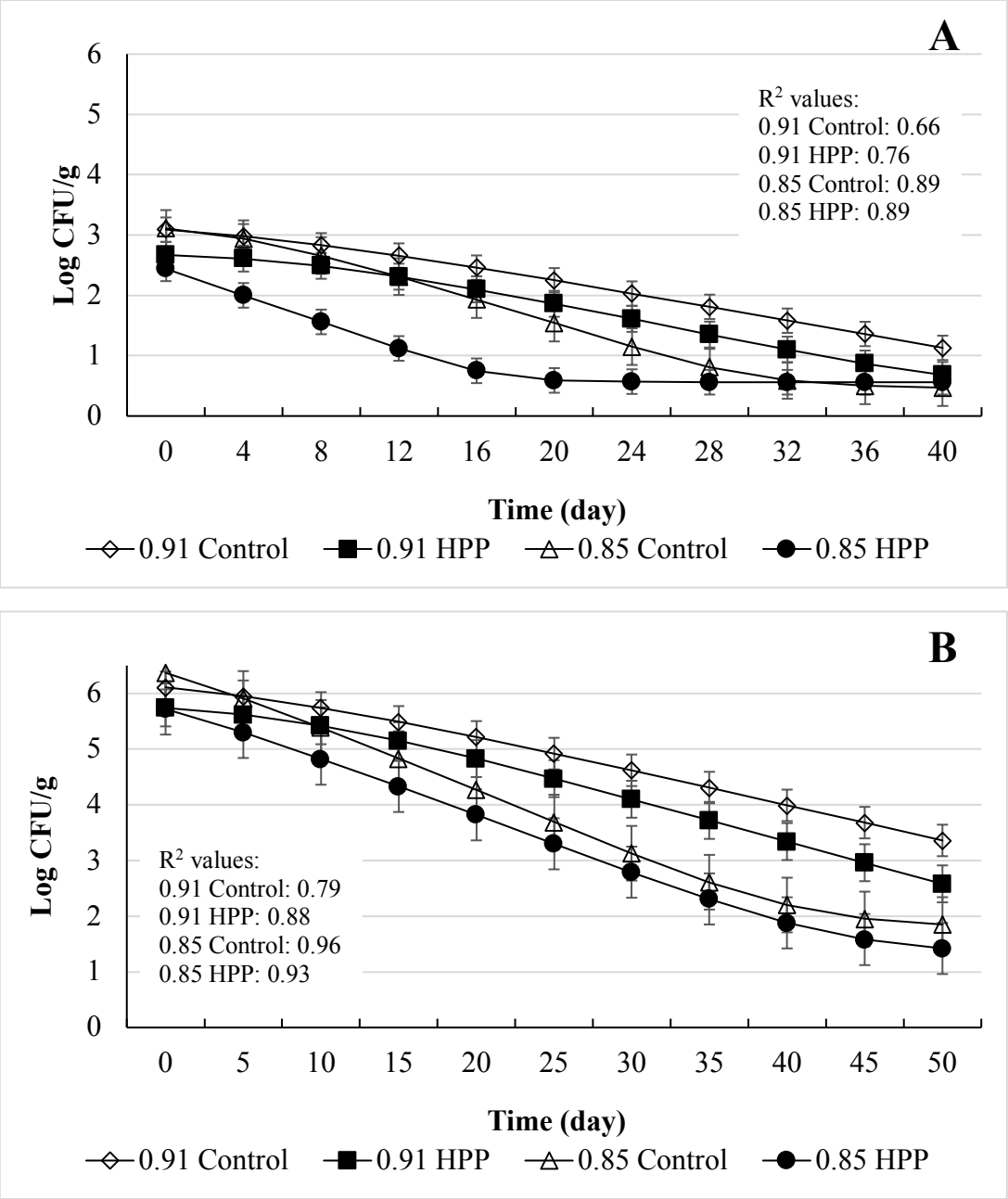

Figure 4.1. *Listeria monocytogenes* survival curves (**A:** 3 log CFU/g inoculation level; **B:** 6 log CFU/g inoculation level), fitted with the Baranyi and Roberts mathematical model (DMFit version 3.5, ComBase), for meat bars dried to two water activity levels (≤ 0.91 or ≤ 0.85) and that received (HPP) or did not receive (Control) a post-processing HPP treatment. Meat bars were stored in vacuum packages for up to 40 d or 50 d at 25ºC.

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