THESIS

SURVIVAL AND PERSISTENCE OF FOODBORNE PATHOGENS IN FOOD RESIDUES ON PACKAGING MATERIALS AND REDUCTION OF *ESCHERICHIA COLI* O157:H7 AND *SALMONELLA* IN BEEF TRIMMINGS

Submitted by

Matthew Charles Nunnelly

Department of Animal Sciences

In partial fulfillment of the requirements

For the Degree of Master of Science

Colorado State University

Fort Collins, Colorado

Spring 2012

Master's Committee:

Advisor: John N. Sofos

Dale Woerner
Patricia Kendall

ABSTRACT

SURVIVAL AND PERSISTENCE OF FOODBORNE PATHOGENS IN FOOD RESIDUES ON PACKAGING MATERIALS AND REDUCTION OF *ESCHERICHIA COLI* O157:H7 AND *SALMONELLA* IN BEEF TRIMMINGS

Foodborne pathogens continue to cause health problems for modern consumers of meat products despite efforts to control bacteria in food. New approaches to controlling pathogens and identifying sources of contamination are needed. Some of the most important foodborne pathogens that affect modern food supplies are *Salmonella* serotypes and *Escherichia coli* O157:H7, both associated with uncooked meat, and *Listeria monocytogenes*, a problematic organism for ready-to-eat foods. The objective of this thesis is to investigate survival of *E. coli* O157:H7 and *L. monocytogenes* on food packaging materials soiled with meat-based residues, and compare differences of behavior when exposed to different packaging materials and storage conditions. In addition to these investigations, a study comparing resistance of multi drug-resistant and susceptible *Salmonella* serotypes and *E. coli* O157:H7 on beef trimmings treated with decontaminating antimicrobials provides valuable information concerning the efficacy of current chemical interventions against *Salmonella* serotypes that are at the forefront of public health concerns.

To evaluate pathogen survival on contaminated food packaging materials, meat based homogenate (10% w/w) was inoculated with a multi-strain mixture of either *L. monocytogenes* or *E. coli* O157:H7 and spot-inoculated on packaging material samples,

placed in a new, empty petri dish, and stored in incubators set at either 4 or 25º C for up to 130 days. Samples were analyzed regularly until the end of the study. There were survivors of the pathogens on several soiled packaging material types even at 123 or 130 days of storage (*L. monocytogenes* or *E. coli* O157:H7, respectively).

When the decontamination of beef trimmings contaminated with multi drug-resistant and susceptible *Salmonella* was compared with *E. coli* O157:H7, there were very few statistically significant differences ($P < 0.05$) between the reduction of *Salmonella* and the response of *E. coli* O157:H7 to acidified sodium chlorite (1000ppm), peroxyacetic acid (200ppm), and sodium metasilicate (40000ppm). In addition, there were only minor differences between the reductions of antibiotic susceptible *Salmonella* and multi drug-resistant strains.

Results of these studies will aid in quantifying risks associated with contamination of food packaging materials as well as beef trimmings.

ACKNOWLEDGEMENTS

I would like to thank the Colorado State University Center for Meat Safety and Quality for the opportunity to advance my education. Special thanks go to Dr. John Sofos who continually demonstrated patience and offered guidance throughout my education. I would also like to express gratitude to Dr. Gina Geornaras for her patience, instruction, and help throughout all aspects of my work while at Colorado State University. Much appreciation is due to Dr. Jeremy Adler for his advice, help, and friendship, Aliyar Fouladkhah for his enthusiasm and expertise, and Dr. Shivani Gupta for her kindness, help, and friendship throughout my education.

I would be unable to have all the opportunities I have been blessed to have without the love and support of my parents, Mark and Lottie Nunnelly, and my brother Michael Nunnelly throughout my life. I am so grateful for the companionship of my friends and church family from Birmingham, Auburn, and now Fort Collins, as well as the Fort Collins cycling community for allowing me to stay healthy and balanced throughout my education.

Most importantly I must thank my Lord and Savior Jesus Christ for grace, mercy, guidance, and peace throughout my life. I am truly blessed.

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Chapter 1: Introduction to Thesis

Foodborne pathogens are a leading concern in the food industry, as contaminated food can lead to illness and death. In addition, presence of pathogens in the food supply has prompted development of industry guidelines and federal regulations governing food wholesomeness. In addition to the obvious public health detriment of contaminated food, there exists a steep economic loss associated with recalls as well as a loss of consumer confidence in the food supply. Three pathogens of note are *Listeria monocytogenes*, *Escherichia. coli* O157:H7, and *Salmonella* serotypes. Though pathogens generally affect consumers in rare circumstances, mortality and hospitalization are still present and problematic (Scallan et al., 2011). Countless studies have been performed to develop control systems for pathogens in food processing environments, yet contamination still occurs and pathogens persist, even in seemingly adverse environments, especially for *L. monocytogenes* (Knøchel, 2010; Riazi and Matthews, 2011). Survival under adverse conditions is not limited to *L. monocytogenes*, as *E. coli* O157:H7 has been reported to survive under similar conditions in food, food processing facilities, or the environment for extended periods of time (Uhlich et al., 2010; van Elsas et al., 2011, Wilks et al., 2005), while some *Salmonella* serotypes have been shown to resist common therapeutic antibiotics including tetracycline and streptomycin (Antunes et al., 2003).

Because of these issues, research to investigate the hardiness and survival of common foodborne pathogens is important. Building on the foundational knowledge of stress adaptation and long-term survival (Shen et al., 2011; Ingham et al., 2004; Masters et al., 1994; Allen et al., 2008; Knøchel, 2010; Tompkin, 2002) as well as on investigations on the effect of biofilm formation and food soiling on the survival of pathogens (Gram et al., 2007; Uhlich et al., 2010; Marouani-Gadri et al., 2010), it was decided that investigating the survival of *L. monocytogenes* and *E. coli* O157:H7 on soiled food packaging materials would be beneficial to our understanding of transmission of foodborne illness. During storage and retail, nearly every meat product has contact with one or more packaging materials, meaning that common materials may possibly be a source and reservoir of foodborne pathogens. With a better understanding of long-term survival of pathogens, risk assessment recommendations will have more valid scientific backing, the food industry will be better prepared to combat recalls and economic losses, and consumers will better understand the risk and danger of improper food handling and storage.

When evaluating pathogenic *Salmonella* serotypes (both antibiotic resistant and susceptible) against the well-documented behavior of *E. coli* O157:H7 contaminating beef trimmings, it is plausible that results could influence policy regarding the decontamination of beef trimmings harboring both *Salmonella* and *E. coli* O157:H7. As the presence of *E. coli* O157:H7 in ground beef and other non-intact beef products is the subject of federal policy (FDA, 9 CFR 311.33, 2011; FSIS directive 6420.2, 2004), it stands to reason that other pathogenic bacteria will become the subject of legislation in

the future. By comparing *Salmonella* serotypes to *E. coli* O157:H7, an organism that has been studied extensively (Pohlmann et al., 2007; Quilo et al., 2010; Hajmeer et al., 2004; Arthur et al., 2008), it is possible that more appropriate conclusions may be made concerning risk assessment of *Salmonella* contamination in beef trimmings.

Strains of *L. monocytogenes* have been documented in some studies to survive for at least 250 days in frozen food (Gianfranceschi and Aureli, 1996), and at least 8-10 months in a fish processing plant that was improperly cleaned (Mędrala et al., 2003), and *E. coli* O157:H7 has been found to persist for at least 9 days through cleaning treatments on food contact surfaces (Uhlich et al., 2010; Marouani-Gadri et al., 2010) and at least 10 days in fecal contamination (Semenov, 2011). In addition to this, Tompkin (2002) reports that a strain of *L. monocytogenes* survived for at least 12 years in a poultry processing plant in the United States. Clearly the bacteria have the ability to survive in a variety of conditions for a long period of time. Because of this knowledge, the objective was to investigate the behavior of the pathogens on common food packaging materials when the materials have been soiled with a contaminated food matrix. By analyzing the survival of the bacteria, readers will gain an understanding of dangers associated with contaminated and soiled food packaging materials (Semenov et al., 2011; Janssen et al., 2005).

The objective of the other study was to determine potential differences in resistance to decontaminating antimicrobials, if any, between *E. coli* O157:H7 and *Salmonella* (antibiotic susceptible and resistant serotypes). Results from this trial will show any

possible differences between five tested *Salmonella* serotypes and *E. coli* O157:H7 when inoculated on beef trimmings and treated with antimicrobials commonly used in the meat processing industry (acidified sodium chlorite, peroxyacetic acid, and sodium metasilicate).

Data obtained from these trials will allow conclusions to be made as to the behavior of three common foodborne pathogens that continue to cause problems in the meat industry. Because foodborne pathogens continue to present themselves as issues for public health and the food industry (Scallan et al., 2011), it is necessary to pursue an understanding of *L. monocytogenes*, *E. coli* O157:H7, and *Salmonella* serotypes and their behavior in food soiling matrices or beef trimmings, respectively. Benefits of an understanding of bacterial behavior are far reaching, with potential to influence everything from consumer and retail storage behavior to industry activities and federal legislation.

Chapter 2: Literature Review

I. Overview of pathogenic bacteria studied

i. *Listeria monocytogenes*

First described in the early part of the 1900s as a causative agent of human and animal illness, *L. monocytogenes* is still at the forefront of research interests today (Jay et al., 2005). The pathogen is unique in that, while incidence of infection is low, consequences of an outbreak involve higher rates of hospitalization and death than other foodborne pathogens (Scallan et al., 2011). Recent studies indicate that *L. monocytogenes* may be much more prevalent in retail settings than initially estimated (Sauders et al., 2009). Because of the danger posed by the presence of the pathogen in food, continued efforts to understand the characteristics and behavior of *L. monocytogenes* are needed.

1. Morphology and pathogenicity

Listeria monocytogenes has been defined as a species for nearly a century (Jay et al., 2005). It is a gram-positive, rod-shaped, facultatively anaerobic and motile organism. It is known to be ubiquitous, and species of the genus *Listeria* can be commonly found in environmental samples as well as areas commonly associated with processed meat products (Williams et al., 2011; WHO, 2004). *L. monocytogenes* is not a spore-forming organism, so thermal processing is typically considered a viable intervention for food. The problem, therefore, lies with consumption of food not traditionally heat treated prior to ingestion. This includes, but is not limited to, fresh produce like leafy greens and

fruits, dairy products like cheese, raw milk and ice cream, and cooked meat products like deli ham and frankfurters that can be exposed to new contamination after cooking (de Oliveira et al., 2011).

L. monocytogenes, when present in food at infectious levels, generally infects those who are at risk. This group traditionally includes the elderly, the very young, and the immunocompromised, specifically patients with HIV/AIDS or people with organ transplants, as well as fetuses of pregnant women (Jay et al., 2005). *L. monocytogenes* has the ability to traverse the placental membrane and infect a developing child, causing spontaneous abortion (WHO, 2004). Infection generally occurs in the small intestine, where the organism internalizes itself in the epithelial lining of a host and acts as an intracellular parasite, growing and multiplying until it is able to escape the cell via lysozome action. Once in the cytoplasm it quickly moves to adjacent cells where the process continues (Hamon et al., 2006).

Incidence of listeriosis can also be managed by proper education of consumers and producers alike. While producers and processors can be legally mandated to handle product and manage facilities in ways to prevent outbreaks, consumers must be approached differently. Even in the 1980s, the United States Department of Agriculture – Food Safety and Inspection Service (USDA-FSIS) began publishing information instructing consumers on proper handling of food with regards to *L. monocytogenes* (USDA, 2000). As a result of education programs, many women opt to forego deli meats

during pregnancy (Kendall et al., 2003). A marked shift in consumer behavior to prevent listeriosis is indicative of a functional education program.

2. Impact of pathogen

L. monocytogenes is of interest because infection is often associated with a high mortality rate. Although the organism is not associated with the highest number of foodborne illnesses (a title belonging to *Campylobacter* species), the mortality rate for victims of listeriosis is 16% and may be as high as 30% (Scallan et al., 2011). Therefore, it is of great interest to the food industry and public health programs to prevent the pathogen from entering the food supply. As of 2000, specific policy was enacted to target the presence of *L. monocytogenes* in the food supply. The USDA-FSIS urged the food industry to re-evaluate HACCP plans with specific regard to the threat of *L. monocytogenes* keeping in mind the goal of 0.5 cases per 100,000 people, with a goal to reduce it to 0.25 cases per 100,000 by 2010 (USDA-FSIS, 2000). USDA-FSIS currently enforces a zero-tolerance policy on foods labeled as ready-to-eat (USDA-FSIS, 2000). Though the viability of zero-tolerance policy is always a subject for debate within industry and academia, the push for such an extreme measure is highly indicative of the problem the pathogen causes for consumers.

ii. *Escherichia coli* O157:H7

Escherichia coli, especially the serotype O157:H7 has, in recent decades, become one of the most well-known, publicized, and legislated foodborne pathogens the meat industry encounters. Numerous news stories, documentary films, social practices, and legal

measures reference this pathogen either directly or indirectly, making the pathogen a household name and concern from cattle production to the dinner table. Ever since the Jack-in-the-Box *E. coli* O157:H7 outbreak of 1993, the coliform has been of special interest to the food production and foodservice industry. Due to the educational efforts of government agencies and food companies, ground meat is nearly always cooked to an internal temperature lethal to the organism (72º C or greater), even in private household settings. Finally, and possibly most importantly to the meat packing industry, *E. coli* O157:H7 has been declared an adulterant of beef, enforced with a zero-tolerance policy for any fecal contamination (FSIS directive 6420.2, 2004). Though non-pathogenic *E. coli* can be found nearly anywhere in the environment, and is common in the digestive tracts of many animals, serotype O157:H7 is of greater concern due to its pathogenicity.

1. Morphology and pathogenicity

Escherichia coli O157:H7 is a gram-negative, flagellated, rod-shaped organism. The cell wall contains the "O" antigen, while the "H" denotes the flagellar antigen present. Though of the same species as non-pathogenic *E. coli*, serotype O157:H7 can be differentiated in that it is unable to ferment sorbitol efficiently, unlike most generic *E. coli*. *E. coli* O157:H7 is specifically adapted for survival in the gastrointestinal tract of host organisms. Some strains "produce curli fimbriae that facilitate attachment of cells to surfaces" (Jay et al., 2005). This is notable in that it not only facilitates attachment of cells to host organisms, but also can aid in attachment of cells to abiotic surfaces where biofilm formation will further facilitate persistence and survival of the pathogen (Torres et al., 2005; Uhlich et al., 2010). *E. coli* O157:H7 is most well known as a causative

agent of Hemolytic Uremic Syndrome (HUS). Its virulence is due to production of non-heat stable shiga-like toxins, responsible for HUS which attack epithelial and renal cells, causing lysis, leading to bloody stool or blood in urine. The protein toxin attaches to specific receptors on these cells and disrupts protein synthesis. It is important to note that HUS is only caused when a population of shiga-toxin *E. coli* (STEC) is able to grow in a host organism, as the toxin is a protein, produced more readily at 37º C than at room temperature (Abdul-Raouf et al., 1994), though the pathogen is able to survive in a wide range of conditions including lower temperatures associated with storage of meat products.

2. Impact of pathogen

Traditionally associated with ground beef, *E. coli* O157:H7 has also been found in non-meat foods such as radish sprouts in Japan in 1996 and even hazelnuts in the Great Lakes region in 2011 (Centers for Disease Control and Prevention – OutbreakNet, 2011). As is the case with most foodborne pathogens, *E. coli* O157:H7 is of greatest concern to at-risk groups: the very young, the very old, and immuno-compromised people. People with a healthy immune system may experience gastroenteritis and diarrhea without developing HUS. Scallan et al. (2011) reported that *E. coli* O157:H7 could be responsible for over 60,000 cases of illness per year as well as being responsible for up to 20 deaths on average. In addition to the health consequences and threats posed by the pathogen, continued presence of the pathogen and resurgence in the food supply undermines consumer confidence in commercial food production and processing (Viazis and Diez-

Gonzalez, 2011). Controlling the organism and incidence of illness is beneficial from an economic , public relations, and a public health standpoint.

iii**.** *Salmonella*

Salmonella has long been known to cause foodborne illness. Though not generally referenced by species name (as is the case in *Escherichia coli*, *Campylobacter jejuni*, *Listeria monocytogenes*, etc.), different serovars have been studied in the past, most belonging to the species *enterica*, with the non-pathogenic *Salmonella* serovars belonging to the *bongori* species (Jay et al., 2005). Biologically, *Salmonella* is very similar to *E. coli* (indistinguishable from *E. coli* on nonselective media), so it is no surprise that the bacteria thrive in similar environments (Jay et al., 2005). *Salmonella* is a gram-negative facultative anaerobe that readily colonizes the intestinal tracts of host organisms, whether it be a human or animal host.

1. Morphology and pathogenicity

Also similar to *E. coli*, *Salmonella* are gram-negative rods, not spore forming, and are generally flagellated, providing motility and a wide range of antigens with which to classify the individual bacteria. Fecal-oral transmission is also one of the most common methods of infection for consumers of contaminated food, a result of improper carcass washing, poor worker hygiene, or in the case of produce, contaminated groundwater resulting in attachment of the pathogen to food products. *Salmonella* serovars are generally adapted for specific hosts, though serovars that cause typhoid fever are the most

serious threats to human health (Jay et al., 2005). Further complicating matters, the pathogen has been shown to possess antibiotic resistance to a broad spectrum of antibiotics including ampicillin, chloramphenicol, streptomycin, sulfa drugs, and tetracylines (Jay et al., 2005; Rabsch et al., 2001). Symptoms of foodborne illness stemming from *Salmonella* serotypes include diarrhea and malaise, though mortality does occur at a 0.5% rate (Scallan et al., 2011). Though effects and methods of infection of the pathogen are not identical to other *Enterobacteriaceae*, it is likely that control of the organism in the food supply will be very similar to control of other *Enterobacteriaceae*.

Salmonella infections occur when contaminated food is consumed and *Salmonella* cells colonize epithelial cells in the host intestine (Zhang et al., 2003). Aside from environmental reservoirs of the bacteria, poultry, ground beef, and pork may be sources of contamination with the pathogen (National Center for Zoonotic, Vector-Borne, and Enteric Diseases, 2009). Symptoms of salmonellosis are generally diarrhea and abdominal cramps, though more severe pain and sepsis can develop if the bacterial infection spreads outside of the intestinal lining to the blood stream or surrounding tissues (National Center for Zoonotic, Vector-Borne, and Enteric Diseases, 2009). Though salmonellosis generally is resolved by a healthy immune system in a timely fashion, multi-drug resistant strains have become problematic in treating patients suffering from salmonellosis (Arthur et al., 2008). *Salmonella* serotypes can be controlled by thermal processing and effective sanitation practices, but problems arise in ground or comminuted products, as internalized bacteria are able to survive when the product is not cooked properly (Gill et al., 2009).

2. Impact of pathogen

Salmonella serotypes have been a cause for alarm in the food industry, affecting the way consumers view food safety. In addition to causing an estimated 20,000 hospitalizations per year with nearly 400 deaths resulting from infection (Scallan et al., 2011), any outbreak of foodborne illness has the potential to cause loss of consumer trust in the food supply and skepticism of the industry's dedication to public health. *Salmonella* presence is also indicative of more sweeping problems in a food processing environment. When the pathogen is present in a food it indicates that antimicrobial hurdles have failed or are ineffective or there is a control point in food processing for which food safety plans have not accounted. If the source of infection can be traced to retail or home settings, it indicates that worker hygiene or unsanitary storage conditions may have led to cross-contamination. Because the pathogen is a serious cause of health problems and revenue loss as a result of food contamination, farther reaching factors should be noted and evaluated for overall food product safety and quality.

II. Attachment, survival, and persistence of pathogens

i. Persistence of *Listeria monocytogenes*

Research on *L. monocytogenes* in relation to the meat industry generally focuses on control and prevention of the contamination of processed products, as well as environmental persistence of the organism. Because thermal processing is generally adequate in destroying the organism, *L. monocytogenes* contamination on raw product, though not desirable by any means, is less of an issue than contamination of a product

that will not be cooked prior to eating. Despite *L. monocytogenes* not being a spore-forming organism, it is amazingly persistent in harsh environments, not only able to survive, but even possibly grow, at temperatures from 0 to 10º C (Walker, 1990). In fact, Junttila et al. (1988) reported on the ability of *L. monocytogenes* to grow within a range of 1.7 ± 0.5º C. Similarly, the organism is quite persistent in desiccated environments, simultaneously showing a high tolerance to salt (Vogel et al., 2010). These traits make *L. monocytogenes* quite problematic for the food industry. Food plants have implemented sanitation programs and manage control procedures through programs like Hazard Analysis Critical Control Points (HACCP), but unforeseen variables may arise and contamination may still occur. Sanitation programs are also not a complete guarantee that the environment is sterile. Mutation and adaptation is widely known to occur in organisms, possibly creating resistant or tolerant strains of bacteria. With regards to *L. monocytogenes*, however, cleaning programs and good hygiene practices will promote a safe and wholesome product. Great care has been taken since USDA-FSIS implemented a zero-tolerance policy and required a reassessment of HACCP plans to address the problem of *L. monocytogenes* to keep equipment clean, incoming product wholesome, and workers sanitary (USDA-FSIS, 2003; USDA-FSIS Executive Summary, 2003; USDA-FSIS, 2000).

ii. *Listeria monocytogenes* attachment on non-food surfaces

When a surface (food or non-food) is exposed to *L. monocytogenes*, pathogen cells are quick to attach. Takhistov and George (2004) reported attachment of the pathogen to an aluminum surface in as rapidly as three seconds, only increasing in a linear fashion for

the duration of their trials (a total of 300 seconds). More accumulation of cells on a surface leads to formation of complex structures – biofilms – that aid in survival of bacteria. It has also been reported that flagellar proteins aid in attachment of bacteria to surfaces (Blaschek and Wang, 2007), and it stands to reason that a flagellated pathogen, like *L. monocytogenes*, will readily attach to a surface under a variety of conditions.

After initial attachment of cells, more cells will begin to accumulate on the surface via growth and repeated contact with new sources of contamination beginning formation of a complex biofilm consisting primarily of polysaccharides, but also proteins, different species of cells, and nucleic acids (Takhistov and George, 2004, Kalmokoff et al., 2001). Accumulation and further growth not only helps anchor cells to a surface, but also serves to grow the size of the biofilm and network of cells, and protect the cells from environmental damage.

iii. *Listeria monocytogenes* survival on non-food surfaces

One avenue of food contamination that is often overlooked, however, may be contamination of the material in which the food is packaged. Reports indicate that *L. monocytogenes* can survive for many months in inactive food plants (Knøchel et al., 2010), and that the pathogen is highly capable of forming biofilms on polyvinyl chloride material (Takahashi et al., 2010). In light of this information, it is worthwhile to investigate the duration of survival of *L. monocytogenes* in food residues that may be present on common food packaging materials.

Marsh et al. (2003) investigated the structure in which *L. monocytogenes* persists on a surface. The lattice formed by extracellular material differs slightly between different strains of the pathogen, though published results confirm a complex matrix, which the same investigators hypothesize may have structures that act as channels for water, nutrients, and waste. This explanation holds merit in light of published data showing survival of *L. monocytogenes* on surfaces well after nutrients are added to the environment (Mędrala et al., 2003; Wulff et al., 2006; Vogel 2010). A complex network to optimize contact with sparse nutrients and water, and easily remove waste products will prolong the life of a cell under adverse conditions.

In addition to the physical structures that enhance survival of *L. monocytogenes* under conditions that seem less than ideal, the pathogen has been demonstrated to not only survive at refrigeration temperatures, but to grow (Walker et al., 1990). Rørvik et al. (1991) demonstrated growth of the bacteria on smoked salmon under vacuum packaged conditions at 4º C. *L. monocytogenes* populations grew from approximately 1 log CFU/ml of rinsing water to nearly 4 to 6.5 log CFU/ml of rinsing water, or approximately 3 log CFU/ml of rinsing water to over 7 log CFU/ml of rinsing water over the course of 5 weeks of storage. In this particular study, the pathogen thrived in a nutrient-rich environment despite the anaerobic and refrigerated conditions.

iv. *Listeria monocytogenes* cross-contamination

As mentioned previously, *L. monocytogenes* is able to persist in relatively inhospitable environments. It forms biofilms (Vogel et al., 2010) and can last for extended periods of

time, even up to multiple years (Sauders et al., 2009, Tompkin, 2002). The possibility of cross-contamination for longer periods of time past the initial contamination is not only plausible but also likely. Work has been done in the past to investigate and control the possibility of cross-contamination of food from workers, equipment, or other contaminated food (Tompkin et al., 1999), but contamination on a packaging material from food residue has never been investigated. Certain groups have researched the attachment of *L. monocytogenes* on surfaces similar to packaging materials and food contact surfaces, notably polyvinyl chloride and stainless steel (Lundén et al., 2006, Takahashi et al., 2010). These studies report that persistent strains of the pathogen have a very high propensity to adhere to food contact surfaces even after short contact times (1-2 h), and that initial adherence to a surface greatly impacts the ability for a contaminating bacterium to form biofim, especially if death of the cells is not immediate. This provides a foundation for the investigation, but no studies investigating the overall persistence of the organism on materials used for meat packaging exist.

Cross-contamination affecting consumers may be a result of excessive handling by a contaminated individual, storage in a contaminated display case, or leaking purge containing *L. monocytogenes* onto previously uncontaminated packages, leading to a reservoir of pathogenic bacteria and possible biofilm formation. Biofilm presence is a very real danger to the food industry, as large numbers of pathogens become concentrated near food processing or storage areas and subsequently offer opportunities for cross-contamination (Sauders et al., 2009; Kalmokoff et al., 2001) via direct contact or rinsing and cleaning. This ability is a likely mechanism by which *L. monocytogenes* persists and

continues to cross-contaminate food. These possibilities, therefore, necessitate investigation of more avenues of pathogenic harboring and cross-contamination, such as materials used in the storage, shipment, and sales of meat products.

Therefore, the purpose of the studies reported in this thesis was, in part, to evaluate the survival of *L. monocytogenes* on food packaging materials soiled with a food matrix containing the pathogen. The aim was to simulate storage in a setting typical of either a retail establishment or a household, therefore materials selected evaluated were those commonly used in food packaging. In addition, antimicrobial interventions were not applied to the samples. Ideally, it would be possible to simulate storage of a contaminated package for the entirety of the survival of the pathogen, though the pathogen survived for much longer than anticipated, meaning that only a roughly four-month window of time for the behavior of the pathogen was shown. Through extended sampling, a general idea of the survival rate of *L. monocytogenes* at different temperatures, initial populations, and inoculated on different materials was developed. The design of the experiment was such that the contamination was similar to what would occur naturally if a package holding purge contaminated with *L. monocytogenes* leaked onto an adjacent package, or if a person with hands soiled with organic material containing the pathogen touched an otherwise clean package of food.

v. Public health interest in *Escherichia coli* O157:H7

In 1993, undercooked ground beef patties contaminated with *E. coli* O157:H7 purchased from Jack in the Box restaurants caused three children to develop HUS and die soon

thereafter; a fourth child also died through person-to-person transmission. Because the pathogen has become a household name and constant concern for processors, retailers, and consumers, investigation of sources and avenues of contamination is necessary.

vi. Attachment and biofilm formation of *Escherichia coli* O157:H7

Escherichia coli O157:H7 has been known as a causative agent for illness since the early 1980s (Riley et al., 1983). The prevalence of the organism and mechanisms by which it survives, thrives, is transmitted, and infects the host, have become much more understood through continued research of the pathogen. For instance, in the meat production and processing chain, one of the most plausible ways of product contamination is from a fecal source during hide removal and evisceration of beef animals (Woerner et al., 2006). In light of this, FSIS has issued a zero-tolerance policy (Directive 6420.2) for visible fecal contamination on carcasses and slaughter facilities implement practices intended to limit exposure of product to soiled hides and gastrointestinal tracts.

Aside from the physical nature of fecal contamination on beef carcasses, *E. coli* O157:H7 is able to attach to surfaces independently of fecal mass. The O antigen has been reported to play a role in the attachment of the pathogen to food surfaces due to increased electronegativity, especially to lettuce leaves (Boyer et al., 2011). The results from the study by Boyer et al. (2011) suggest that pathogenic strains of *E. coli* (with O antigen) are more apt to adhere to food surfaces than non-pathogenic strains. Boyer et al. (2011) showed that the presence of the O157 antigen lends a slightly less negative overall charge to the bacteria, subsequently leading to greater attachment to food surfaces compared to

non-pathogenic *E. coli* (Boyer et al., 2011). *E. coli* O157:H7 is also able to survive for extended periods of time in mixed-culture biofilms (Uhlich et al., 2008). In addition, presence of a meat-based soil on food contact surfaces greatly enhances the propensity of *E. coli* O157:H7 to form biofilms that enhance growth, survival, and resistance to cleaning efforts (Skandamis et al., 2008). This information is of special importance, as food-based soil may contain a large number of non-pathogenic ubiquitous organisms, assisting *E. coli* O157:H7 in survival through conditions normally more inhospitable to survival. For these reasons, attachment and biofilm formation of *E. coli* O157:H7 should be discussed simultaneously. Food soiling matrices and non-*E. coli* bacterial biofilm production has the propensity to assist with the attachment of pathogenic bacteria on food and food-contact surfaces.

vii. Survival of *Escherichia coli* O157:H7

In addition to survival in fecal contamination, *E. coli* is able to survive in a plethora of environmental conditions. Recent outbreaks have linked pathogenic serotypes to leafy greens, and research shows that the organism is able to survive in soil and other environments less hospitable than a gastrointestinal tract (Semenov et al., 2011; van Elsas et al., 2011). Though not a spore-former, the pathogen has the ability to survive for extended periods of time without ideal growth or proliferation conditions (Møretrø et al., 2010). *E. coli* has been reported to enter a "stationary phase" during starvation or high-stress conditions, as cells are reportedly able to survive by using available energy and nutrients on maintenance of the cell membrane and DNA structure rather than growth (Hengge-Aronis, 1993). It is of special interest with regards to public health to consider

that the pathogen possesses specific pathways to preserve cell life under environmental distress.

viii. Cross-contamination with *Escherichia coli* O157:H7

Long-term survival suggests an ability for the bacteria to cross-contaminate product long after a surface has been considered clean. Bacteria can be introduced to clean food, food contact surfaces, or the environment via improper worker hygiene, contact with a previously contaminated surface, or contact with other food contaminated with pathogens. *E. coli* O157:H7 has also been found to survive cleaning and sanitizing (Marouani-Gadri et al., 2010). Most importantly, *E. coli* O157:H7 possesses an ability to attach to food and non-food surfaces (Boyer et al., 2011; Torres et al., 2005). Considering the knowledge of attachment and biofilm formation with the understanding that metabolic pathways and extracellular structures are able to preserve the pathogen for extended periods of time, it stands to reason that sporadic colonies of *E. coli* O157:H7 can serve as sources of cross-contamination.

Cassin et al. (1998) published a risk assessment of *E. coli* O157:H7 dose levels in ground beef. Using a Poisson process, the authors concluded that, at the time of the study, approximately 2.9% of packages of ground beef could be expected to contain any population of *E. coli* O157:H7. Contamination levels were predicted to be quite small (generally $\leq$ 2 log CFU/pkg), though infective dose is possibly not very high for the pathogen (as low as 1 to 100 organisms) (Paton and Paton, 1998; Cassin et al., 1998; Tuttle et al., 1999). What is important to note in the Cassin et al. study, though, is that

the only vehicle investigated for transmission of the pathogen from evisceration to commercial product was the beef itself. Since we know *E. coli* O157:H7 is able to survive on a variety of surfaces like soil, feces, stainless steel, and PVC (Torres et al., 2005; Marouani-Gadri et al., 2010; Møretrø et al., 2010; Wilks et al., 2005), we should operate under the assumption that contamination of food products can arise from food contact surfaces, food service workers, and food storage as well as the food itself.

E. coli O157:H7 is one serotype of a select group of STECs (shigatoxin producing *E. coli*) that is responsible for severe gastroenteritis and possible complications with kidney function. STEC contamination generally arises from a fecal reservoir in live animals and may affect consumers, whether from contaminated meat, produce, or water (Viazis et al., 2011). Cross-contamination occurs when a product contacts the pathogen and is not properly cleaned or processed afterwards. This is not to say that *E. coli* O157:H7 infection occurs only by eating fecal particulates, but that fecal contamination is usually the original source of the pathogen.

Since different studies confirm that *E. coli* O157:H7 is able to survive for extended periods outside of a GI tract (Torres et al., 2005; Marouani-Gadri et al., 2010; Møretrø et al., 2010; Wilks et al., 2005), it is worthwhile to consider all reservoirs of contamination. In addition to contaminated food products, food contact surfaces are also treated as potential sources of cross contamination when drafting HACCP plans or implementing sanitation standard operating procedures (SSOPs). In addition to regular antimicrobial sanitation measures in food processing facilities, HACCP plans also frequently require a

high quality starting product. This could be validated by audits of microbiological performance standards and checked with letters of guarantee.

Due to the nature of meat packing and retail, packaging materials are subject to frequent handling by employees or consumers as well as contact with other packages of product. With knowledge that, under relatively commonplace circumstances, *E. coli* O157:H7 can persist at detectable levels, the question of microbiological survival on soiled food packaging materials is one that warrants investigation. Consumer contact with food packaging materials is almost a necessity during consumption of a product, therefore simulating a scenario in which food packaging materials were contaminated with *E. coli* O157:H7 and meat exudate was devised. The goal of studies, therefore, was to investigate persistence of *E. coli* O157:H7 on soiled food packaging materials and identify factors in food storage that promote or inhibit survival of the pathogen.

III. Resistance of *Salmonella* to antimicrobials

i. *Salmonella* prevalence in food supply

Salmonella is found readily in livestock including chicken, turkey, beef animals, and swine (USDA, 2010). Fecal contamination, as is the case with most enteric pathogens, has been identified as a major source of *Salmonella* (Bacon et al., 2002). The USDA-FSIS enforces a zero-tolerance policy for visible fecal contamination on carcasses, aiming to minimize the exposure of meat products to enteric pathogens harbored in the GI tract of food animals (USDA-FSIS Directive 6420.2). Even with interventions applied to reduce microbiological loads from soiled hides, *Salmonella* serotypes continue

to be found in ground beef (Arthur et al., 2008). Even with a zero-tolerance policy for visible fecal contamination, complete sterility of food is not certain. *Salmonella* can be introduced to meat from a meat animal carcass contaminated with ruptured GI tract contents, contact with a hide soiled with fecal material, contact with contaminated equipment or workers, or contact with contaminated trimmings stored in the same container (Arthur et al., 2008; Smeltzer et al., 1980). In addition, due to the comminuted nature of ground beef, lymph material may also be present in final products, as suggested by Arthur et al. (2008).

ii. Multi-drug resistant *Salmonella* strains

Salmonella is especially of note due to a rise in antibiotic resistance among pathogenic serotypes. Though many pathogens can be controlled or remedied through therapeutic antibiotics, certain serotypes of *Salmonella* carry genetic modifications that encode for antibiotic resistance (Andrews and Bäumler, 2005). It has been suggested that extensive use of antibiotics in modern medicine and food production has led to strains that, while not necessarily more pathogenic or lethal, are more difficult to control when present in a food supply (Rabsch et al., 2001). Concern has existed as to whether these antibiotic-resistant strains are less susceptible to common food processing antimicrobials than antibiotic-sensitive strains, especially as incidence of illness as a result of these strains has increased in recent years (Zansky et al., 2002). This is unlikely, though, as the genes encoding for resistance in the pathogen are not virulence genes but rather a common genetic trait shared between clones of the same strain (Andrews and Bäumler, 2005). Regardless of the genetics of multi-drug resistance, infections with these strains are

occurring with greater frequency, and hygiene theory signals that increased use of antibiotics in the food production and processing industry could increase the amount and variety of drug-resistant strains of *Salmonella*.

iii. Threats posed by *Salmonella* serotypes

Foodborne pathogens are of great concern in the modern food industry, and *Salmonella* serovars alone are estimated to cost the United States nearly three billion dollars annually (USDA, 2010). Since 1984, the CDC has reported hundreds of outbreaks, resulting in thousands of confirmed cases of salmonellosis (Centers for Disease Control and Prevention, OutbreakNet). Also alarming is that nearly 50% of *Salmonella* strains collected from food plants are resistant to one or more antibiotics (Kiessling et al., 2002). As is the case with any possible foodborne contaminant, HACCP plans and SSOPs are in place to attempt to curtail the possibility of outbreaks. Many of the interventions in HACCP plans involve antimicrobial compounds ranging from organic acids like lactic acid to chlorine-based detergents, applied both to the food contact surfaces and the food itself. Arthur et al. (2008) explains that multi-drug resistant (MDR) strains of *Salmonella* are the most problematic pathogens in the *Salmonella* genus. Leyer and Johnson (1993) show that some *Salmonella* serotypes are able to persist in adverse environments longer when adapted to environmental stresses, such as cleaning agents, heat, or cold. Because of this, *Salmonella* serotypes that have been shown to be resistant to antibiotics may be resistant to antimicrobial interventions applied to beef trimmings in meat processing facilities.

iv. Control of *Salmonella* serovars

Commercial antimicrobials are often used for control of foodborne pathogens and other microbes on meat carcasses, primal cuts, and trimmings. Validation studies have been published detailing the effects of antimicrobials on the survival of *E. coli* O157:H7 and other bacteria on beef surfaces (Arthur et al., 2008; Gill et al., 2004; Pohlman et al., 2009; Quilo et al., 2010), specifically detailing, in addition to lactic acid, the effects of acidified sodium chlorite, peroxyacetic acid, or sodium metasilicate. With a wealth of knowledge concerning resistance or susceptibility of *E. coli* O157:H7 to these antimicrobials, it is necessary to explore any possible differences between a well-documented pathogen (*E. coli* O157:H7) and *Salmonella* serovars, which, as detailed earlier, may be responsible for over one million cases of foodborne illness per year in the United States of America (Scallan et al., 2011). Most of this work, however, involves sampling of beef tissue at a carcass level, as is the case in Arthur et al. (2008). Behavior of *E. coli* O157:H7 and *Salmonella* serotypes in beef trimmings, especially when treated with commonly used antimicrobials, is of pressing interest. Investigating any similarities or differences between the two distinct pathogens has the possibility to change how *Salmonella* is controlled during beef processing.

IV. Overview of select antimicrobials available for use in beef trim grinding operations

i. Acidified sodium chlorite

Acidified sodium chlorite (ASC) is an FDA-approved antimicrobial that consists of sodium chlorite suspended in solution with its conjugate acid (Hajmeer et al., 2004). ASC is also kosher-certified, allowing for more versatility and widespread use in different

aspects of meat production. Hajmeer et al. also reports that ASC is effective in reducing *E. coli* O157:H7 on beef briskets when applied by spraying. Using a concentration of 0.1% ASC and applied for up to 60s, *E. coli* O157:H7 populations were reduced by up to 1.6 log CFU/cm^2 of beef brisket (Hajmeer et al., 2004). These studies are limited, however, as the pathogen was only inoculated on beef brisket surfaces, not trim. Other studies have investigated the action of the intervention on beef used for trimmings, reporting effective reduction of bacteria through their application, though slight quality issues arose regarding the final product's sensory characteristics and aerobic bacteria population (Ransom et al., 2003; Quilo et al., 2009). While the antimicrobial has been demonstrated to be effective in controlling pathogenic populations on beef carcass surfaces, effective treatment of beef trim with antimicrobials has not been investigated fully.

ii. Peroxyacetic acid

Peroxyacetic acid (PAA) is an oxidizer (Rossoni and Gaylarde, 2000). It is an acidic antimicrobial that is made in solution from acetic acid and hydrogen peroxide, both of which are hostile to bacterial survival on their own. King et al. (2005) reports that PAA is effective at reducing populations of *E. coli* O157:H7 and *Salmonella* on carcass surfaces, but due to the unstable nature of the solution, the antimicrobial has an effective time span of only a few minutes (Quilo et al., 2009). The National Organic Standards Board, under the USDA, reports that acetic acid and hydrogen peroxide are the primary products of the decomposition of PAA, and is therefore available for use in food systems, as both compounds are fairly benign (National Organic Standards Board, 2009). In addition to

this, it has been demonstrated that PAA is effective in reducing *Salmonella* counts in ground beef by nearly 2 log CFU/g initially while maintaining desirable sensory characteristics (Pohlman et al., 2007). However, as previously mentioned, certain multi-drug resistant strains of *Salmonella* may have developed resistance to antimicrobials (Arthur et al., 2008). Because certain serotypes of *Salmonella* may be resistant to antimicrobials, and because the effect of the aforementioned antimicrobials is not well-documented in ground beef or beef trimmings, further investigation of the pathogen under these unknown circumstances is warranted.

iii. Sodium metasilicate

Sodium metasilicate (NMS) is a basic antimicrobial available for use in beef systems and noted for its effectiveness in controlling *E. coli* O157:H7 (Weber et al., 2004; Quilo et al., 2010; Quilo et al., 2009; Byelashov et al., 2010). Pohlman et al. (2007) reports that NMS is immediately as effective as PAA and ASC in reducing *Salmonella* populations in ground beef. As a strong alkali agent, pH of a solution may range from 11.3 to 12.7 (Weber et al, 2004). NMS is also is classified as generally recognized as safe (GRAS) by the U.S. Food and Drug Administration (FDA) (21 CFR 184.1769a).

Because the possibilities for this decontaminating antimicrobial are extensive, it is used in the meat and poultry industries (USDA-FSIS, 2009). What is not detailed, though, is whether or not there exists a difference between *E. coli* O157:H7 and multi-drug resistant and susceptible *Salmonella* serotypes with regards to sensitivity to sodium metasilicate.

Since multi-drug resistant *Salmonella* strains have been shown to be prevalent in food products available to consumers (Antunes et al., 2003), investigation is warranted to identify any pathogenic serotypes that are more resistant to common antimicrobial treatments.

Chapter 3: Survival of *Listeria monocytogenes* on common food packaging materials soiled with antimicrobial-free ham residue

Chapter Overview

Sporadic and outbreak cases of foodborne illness due to *Listeria monocytogenes* are predominantly associated with ready-to-eat foods. Contamination of such foods with the pathogen may occur during processing, distribution, food preparation activities, and from other potential sources. This study evaluated survival of *L. monocytogenes* in food residues on the outside of various materials commonly associated with food packaging. Samples (5×5 cm) of aluminum foil, butcher paper, cardboard, deli wax paper, paper grocery bags, plastic grocery bags, PVC overwrap film, and vacuum plastic bags were spot-inoculated (0.5 ml) with a ham homogenate (simulating contaminated purge) containing a 5-strain mixture of *L. monocytogenes* (4.9±0.2 log CFU/cm^2 or 2.1 ± 0.2 log CFU/cm^2). Inoculated packaging materials were stored for up to 123 days aerobically in sterile petri dishes at 4°C or 25°C, and periodically analyzed for *L. monocytogenes* (PALCAM agar) and total bacterial populations (tryptic soy agar with 0.6% yeast extract). The experiment was repeated twice with three samples analyzed at each sampling point. Even though *L. monocytogenes* populations in the ham homogenate residue gradually decreased on all packaging materials at either inoculation level during storage at 4 or 25°C, survivors (<0.2-3.2 log CFU/cm^2) were obtained on most of the tested materials with the high inoculation level even at 123 days of storage. Materials at 25°C with pathogen counts >1 log CFU/cm^2 at the end of storage included aluminum foil,

butcher paper and plastic bags (3.2±0.5, 2.3±0.5 and 1.6±0.9 log CFU/cm^2, respectively); corresponding pathogen counts on these materials stored at 4°C were 1.8±1.0, 1.3±1.2 and 2.4±1.0 log CFU/cm^2, respectively. *L. monocytogenes* counts reached non-detectable levels (<-0.1 log CFU/cm^2) on cardboard and paper bag samples on day-95 and day-123, respectively, of storage at 25°C. When *L. monocytogenes* was inoculated at a level of approximately 2 log CFU/cm^2, populations on cardboard box material, deli wax paper, and paper bag material were detectable for up to 81 days before decreasing below the detection limit. *L. monocytogenes* populations on aluminum foil only decreased to 1.9 ± 2.2 log CFU/cm^2, almost no reduction from the initial value. The 4º C temperature was less hospitable to the pathogen, with no materials harboring survivors for the full time allotted for the study. Under these conditions, populations on PVC overwrap film decreased below the detection limit by 4 days of storage, the most rapid death of the pathogen under any condition tested in the study. Even aluminum foil, plastic bag material, and vacuum pouch material, all yielded populations near the detection limit on day 123 of storage. The population on aluminum foil was 0.0 ± 0.1 log CFU/cm^2 at the end of storage, with populations on plastic bag and vacuum pouch materials at 0.1 ± 0.3 and 0.5 ± 0.5 log CFU/cm^2, respectively. Sampling was discontinued for materials that had counts below the detection limit for at least two consecutive sampling dates. The results of the study indicated that *L. monocytogenes* present in contaminated food residues may survive on the outside of used food packaging materials for long periods of time and, thus, potentially serve as a source of cross-contamination.

I. Introduction

Scallan et al. (2011) reported that *Listeria monocytogenes* is the causative agent of nearly 1,500 cases of foodborne illnesses and an estimated 255 deaths per year in the United States. In addition to the presence of this pathogen in processed foods, it is also reported that *L. monocytogenes* is able to survive under highly adverse conditions, such as near-freezing temperatures (0-1º C) and lowered water activity (0.91-0.93) (Knøchel, 2010). Though systematic approaches, like multiple antimicrobial hurdles, exist to combat the presence of pathogens in the food supply, once the product leaves a processing facility, wholesomeness is not governed by a processor's sanitation program. Because a product is not kept within the food safety parameters of the processor, possibility of post-processing contamination in retail establishments or home kitchens exist, both of which present a threat to consumer safety. Studies conducted show an alarming presence of persistent *L. monocytogenes* strains in retail establishments, namely supermarket deli operations (Sauders et al., 2009). Such persistence may be attributed partially to strain genetics, but it is also likely that biofilm formation and food soiling may be factors in the persistence of the pathogen. Biofilms are known to form readily in the presence of food soiling, and function to protect pathogenic organisms from physical removal, as well as some sanitizers (Gram et al., 2007; Riazi and Matthews, 2011).

Frequent handling of the exterior of food packaging materials may serve as a source for cross-contamination of otherwise safe food products. A general lack of education, coupled with the hardiness of *L. monocytogenes* and its ability to cross-contaminate

processed food products, necessitates research into the ability and time frame of survival under common retail case or home kitchen storage environments.

To this date, while pulse-field gel electrophoresis (PFGE) and molecular assays have been conducted to detect *L. monocytogenes* in the environment (Clark et al., 2010; Autio et al., 2003; Williams et al., 2011), very little work has been done to evaluate survival of the pathogen on soiled food packaging materials. Prior to the onset of the study, expectations were that low levels of pathogenic organisms would be able to survive for an extended period of time, though possibly injured or otherwise damaged due to desiccation and starvation conditions, since literature indicates that, under specific conditions, *L. monocytogenes* is able to survive in frozen food environments for up to 300 days (Gianfranceschi and Aureli, 1996), in meat processing plants for up to 12 years (Tompkin, 2002), and has been found in processed meats manufactured with antimicrobials for up to 11 weeks under vacuum storage (Ingham et al., 2004). This reinforces knowledge that simple control of temperature is not a foolproof means by which to control presence of the pathogen. With this information in mind, the objective of this study was to quantify the survival of *L. monocytogenes* on the outside of various food packaging materials soiled with ham purge, inoculated at different levels, and stored at two temperatures.

II. Materials and Methods

i. Strains and inoculum preparation

Five strains of the pathogen (J1-177 (human isolate, serotype 1/2b), C1-056 (human isolate, serotype 1/2a), N3-013 (food isolate, serotype 4b), N1-227 (food isolate, serotype 4b), and R2-499 (food isolate, serotype 1/2a)) were obtained with special thanks to Dr. Wiedmann (Cornell University), and individual colonies were isolated via streak plating on PALCAM agar (Becton, Dickinson & Company, Sparks, MD). A single colony from each strain was activated in 10 ml trypic soy broth (TSB; Becton, Dickinson & Company, Sparks, MD) by incubation at 30°C for 24 hours. After incubation, 100 µl of culture was pipetted into fresh TSB tubes and incubated again for 20-24 hours. Sterile "ham homogenate" was made by using 10% ham (prepared without addition of antimicrobials, Cooked Ham Lean, Water Added) and 90% distilled water. The mixture was masticated for 120 seconds at 6 strokes per second (IUL Instruments, S.A. Barcelona, Spain, Masticator Silver-Paddle Blender), subsequently strained through a cheesecloth, then autoclaved for sterility (Yang et al., 2009). Cell pellets were individually centrifuged for 15 minutes (Eppendorf 5810 R, 4° C, 4,629 x g), and the supernatant was discarded. Cell pellets were resuspended in 10 ml Phosphate Buffered Saline (pH 7.4, 0.2 g KH_2PO_4, 1.5 g $Na_2HPO_4 \bullet 7H_2O$, 8.0 g NaCl, .2 g KCl per liter dH_2O: Fisher Scientific, Fair Lawn, MO) and centrifuged for an additional 15 minutes. Finally, each strain was resuspended in 10 ml of the sterile ham homogenate (pH 6.46) and stored for 72 hours at 4°C to habituate cell cultures to an environment typical of food stored in a refrigerator or cooler.

ii. Packaging material sample preparation

Eight materials commonly associated with food packaging and handling were selected for investigation: butcher paper (Kold-Lok, Georgia-Pacific, Norwalk, CT), cardboard (Donated by Colorado Premium, Greeley, CO), deli paper (Dispens-a-wax® Deli Patty Paper, Georgia Pacific, Dixie Business, Norwalk, CT), aluminum foil (Foodservice Foil, Bakers & Chefs, Sam's West, Inc., Bentonville, AR), PVC overwrap film (Prime Source Crystal Clear PVC Wrapping Film, International Paper Co., N. Kansas City, MO), paper grocery bag (4# Kraft Bags, Duro Bag Mfg. Co., Ludlow, KY), plastic grocery bag (Carry Out Bag, Poly-America, Grand Prarie, TX), and vacuum plastic storage bag (Prime Source Vacuum Pouch, 3 mil STD barrier, International Paper Co., N. Kansas City, MO). Prior to inoculation, each material was cut into 5 cm x 5 cm samples which were placed in sterile empty petri dishes (Polystyrene Petri Dish, Fisher Scientific, Pittsburgh, PA).

iii. Inoculation

To inoculate the packaging material samples, cell culture inoculum was diluted in sterile containers of sterile ham homogenate, prepared in the same manner as described earlier. Under a biosafety cabinet, 500 µl of ham homogenate containing *L. monocytogenes* was spot-inoculated without spreading in the center of each packaging material sample, covered, and placed immediately in an incubator set at either 4 or 25º C (25º C, 13.13 ± 0.64% RH; 4º C, 37.33 ± 1.78% RH).

iv. Sampling and analysis

In order to quantify the microbial population persisting on each inoculated packaging material sample, a random selection of three samples of each material were removed from their respective incubators immediately prior to testing. An aliquot of 20 ml of Maximum Recovery Diluent (MRD; Becton, Dickinson & Company, Sparks, MD) were added to Whirl-Pak® bags (Nasco, 18oz.). Samples were aseptically removed from petri dishes, placed into bags, and masticated for 120 seconds at six strokes per second (IUL Instruments, S.A. Barcelona, Spain, Masticator Silver-Paddle Blender). Homogenized sample material was pipetted out, serial dilutions were made in 0.1% buffered peptone water (Difco, Becton and Dickinson, Franklin Lakes, NJ), and liquid was spread plated onto both selective media (PALCAM) and non-selective media (TSA+YE – 40 g Tryptic Soy Agar, Acumedia; 6 g Yeast Extract – Acumedia per 1 L distilled water: Neogen Corp., Lansing, MI). PALCAM plates were incubated at 30° C for 48 hours and colonies were counted, while TSAYE plates were incubated at 25° C for 72 hours and colonies were subsequently counted. Sampling procedures were repeated regularly, as materials were sampled weekly until 39 days of storage time, then every other week until 123 days of storage.

v. Statistical analysis

The experiment was repeated in duplicate, yielding a total of six samples of each material at each temperature and inoculation level for each sampling point. Colony counts were converted into log CFU/cm^2, and were analyzed for a replication effect using the Proc Mixed program of SAS with a Tukey-adjusted analysis of variance to separate the least

square means of the population counts for each replication (Version 9.2, SAS, Inc., Cary, NC).

Population data from both replicates were subsequently analyzed with GInaFiT, a freeware add-in for Microsoft Excel 2007 developed by Geeraerd et al. (2005). Using microbiological inactivation formulas from previous studies, including Cerf (1977), the add-in is able to fit curves to population data in Excel and generate numerical parameters for the rates of inactivation to quantify the behavior of bacterial populations. This freeware module allows a user to fit multiple different models of curves to logarithmic population data, which have been published in papers dating back to 1920. Goodness-of-fit values (R-squared) are given for each curve. A biphasic model (Cerf, 1977) consistently yielded the highest R-squared values for each curve. Biphasic models fit curves using the formula *log10(N)=log10(N0)+log10(f*exp(-kmax1*t)+(1-f)*exp(-kmax2*t))* where *log10(N0)* is the log CFU/cm^2 initial population (day 0 sampling data for each treatment), *f* is in essence the percentage of the population that is shown to behave in a manner consistent with the fit curve, *kmax1* and *kmax2* are the "specific inactivation rates of the two populations, respectively (Geeraerd et al., 2005). Therefore, *kmax1* describes the inactivation rate (1/time unit) of the cells that die initially, whereas *kmax2* describes the inactivation rate of cells that persist for a longer time. The higher the *kmax* value is, the more rapid the death during that phase of the study will be. Therefore, the values may be thought of as descriptors of the slope of the curve – larger values indicate more rapid death.

III. Results

i. General Trends

When survivor counts on PALCAM and TSAYE plates of high initial inoculation were analyzed statistically, a replication effect ($P < 0.05$) was discovered in most treatments (Appendix Tables 1 and 2). A significant ($P < 0.05$) replication effect was also present in low initial contamination counts detected on selective and non-selective media (Appendix Tables 3 and 4). Though significant replication effects were present in a range of the individual treatments, death of *L. monocytogenes* was present in both replications (Appendix Tables 5, 6, and 7). Because trends are similar, further analysis of pathogen survival may still be appropriate for risk assessment.

Significant differences ($P < 0.05$) of replication effects in most of the treatments were not entirely unexpected considering the variation in population data obtained. Differences are not surprising, considering conditions of the study and behavior of bacteria in difficult environments. Because of this inherent variation, the survivor data are presented as overall survivor trends and comparisons of fitted curves (Geeraerd et al., 2005) can be made using the GInaFiT freeware module for Microsoft Excel. Using values generated by GInaFiT, evaluation of different treatments is possible simply by comparing *kmax* values (a value expressing population persistence in a unit of 1/day), similar to the comparison published by Janssen et al. (2005). This biphasic model was selected for analysis because R-squared values were consistently the highest with regards to the data. Compared with analysis of data in later chapters of this work, the biphasic model (Cerf, 1977) was most appropriate for the data, indicated by R-squared values. In addition, data

show a pronounced biphasic decrease in population on most materials (Figures 1.1 through 1.8) where an initial decrease in population is followed by a prolonged period of persistence. Not every pathogen can be described in an identical fashion though, which is to be expected.

In addition to survival of *L. monocytogenes*, total aerobic bacterial survival followed the trends of the pathogen very closely. Because a sterile meat homogenate was used for inoculation, it was expected that bacteria recovered on each medium would be very similar. Cardboard box material was an exception, harboring nearly 3 log CFU/cm^2 of yeasts and molds (organisms able to survive in low water activity environments) which was present on the material at the onset of the study, regardless of temperature and inoculation levels. These organisms may have had an effect on survival of *L. monocytogenes*, though the pathogen was still able to persist for many days without rapid death on the material.

ii. Survival of *L. monocytogenes* on soiled food packaging material inoculated with a high initial level and stored at 25º C

When packaging material samples were inoculated at a level near 5 log CFU/cm^2 and were stored at 25º C, only cardboard box and paper bag materials reached populations consistently near the detection limit (-0.1 log CFU/cm^2) by the 95th day of storage. It is evident from the data (Figure 1.1) that for as long as 25 days of storage, cardboard box, paper bag material, and PVC overwrap film allowed survival of *L. monocytogenes*, while survival on aluminum foil, butcher paper, and plastic bag material was even greater (see

Appendix Table 5 for combined data results, and Appendix Tables 6 and 7 for individual replicate results, while Table 8 shows total aerobic plate counts and Tables 9 and 10 show individual replicates). In contrast, average counts of *L. monocytogenes* on aluminum foil under these conditions decreased by less than 2 log CFU/cm^2 for the duration of the study (Figure 1.1). The populations on the remaining soiled packaging materials decreased at a fairly similar rate throughout the study. By 67 days of storage, large changes in surviving levels of pathogens on contaminated packaging material (with the possible exception of populations inoculated on vacuum pouch material and PVC overwrap film) had stopped, and most populations appeared to be persisting or decreasing in a very consistent manner. Total aerobic bacteria counts behaved similarly (Figure 1.2). Though data will show that overall numbers of total bacteria are greater than numbers of *L. monocytogenes* (Appendix Tables 5 and 8), trends remained the same. In addition, nonselective media is able to foster growth of background contamination, possibly arising from bacteria already persisting on the packaging material.

Biphasic curve analysis of the data (Table 1.1) indicated that surviving counts on cardboard box material and paper bag material exhibited the highest *kmax1* values (1.05/day and 0.18/day, respectively), while aluminum foil exhibited the smallest *kmax1* values (0.03/day). The *kmax* refers to the inactivation rate (1/day) on a reduction curve used to describe the behavior of a pathogen, so that higher *kmax* values indicate more rapid death. In addition, 4-D reduction in counts was not reached on aluminum foil, butcher paper, cardboard box, and plastic bag material. It should be concluded, therefore, that when stored at 25º C and inoculated at a level of approximately 5 log CFU/cm^2, *L.*

monocytogenes was able to persist and survive best on soiled aluminum foil, butcher paper, and plastic bag material - evidenced by little to no initial reduction in population followed by a mild reduction in the second phase of the biphasic curve.

In contrast, the environment of cardboard box material was more lethal to *L. monocytogenes* than all other materials, as it had the highest *kmax1* value, 1.05, indicating that death was quite abrupt. Though the *kmax2* value was fairly low (0.06/day), the vast majority of *L. monocytogenes* died more rapidly on cardboard than on any other material. Based on this, it may be concluded that soiled cardboard box material and paper bag material were the least hospitable to *L. monocytogenes*, while soiled aluminum foil and butcher paper were the least lethal to the pathogen. Regardless of variations and differences observed among packaging materials and time of storage, the important point is that the pathogen was able to survive for long periods of time in meat product residues placed on several food packaging materials stored at room temperature.

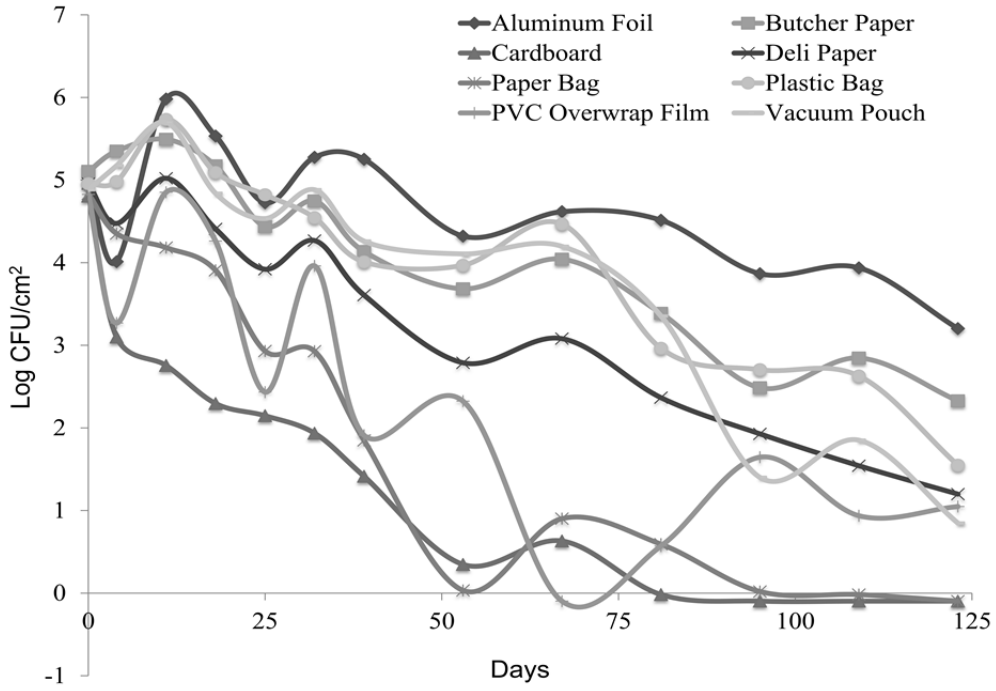

Figure 1.1 - *Listeria monocytogenes* survival (log CFU/cm^2) on food packaging materials soiled with antimicrobial-free ham homogenate inoculated with approximately 5 log CFU/cm^2 of *L. monocytogenes* and stored at 25º C for up to 123 days (Data in Appendix Table 5)

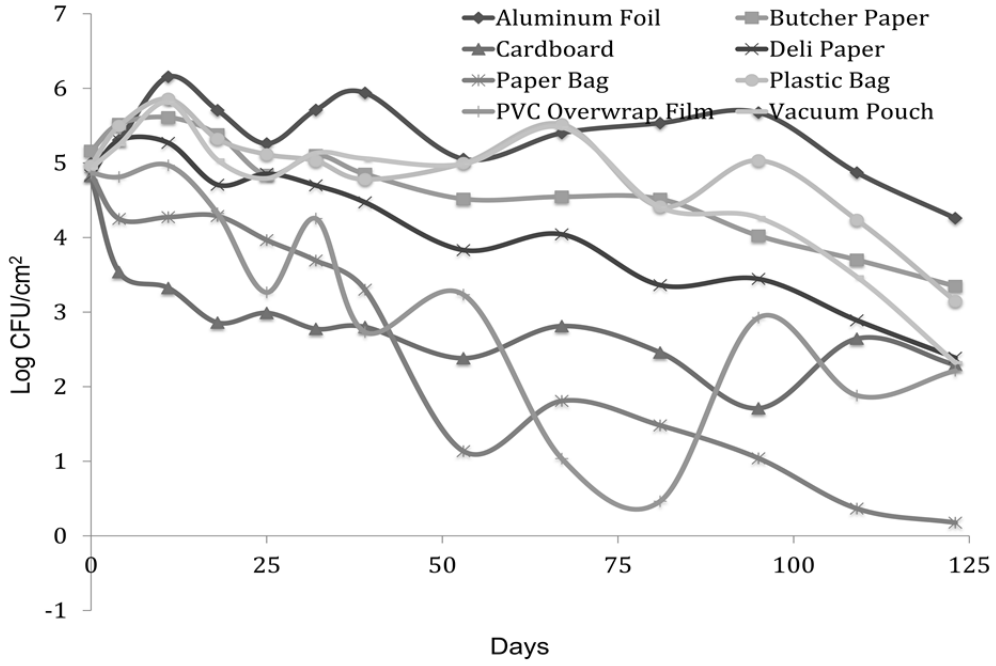

Figure 1.2 – Total aerobic bacteria survival (log CFU/cm^2) on food packaging materials soiled with antimicrobial-free ham homogenate inoculated with approximately 5 log CFU/cm^2 of *L. monocytogenes* and stored at 25º C for up to 123 days (Data in Appendix Table 8)

Table 1.1 – Inactivation parameters of *L. monocytogenes* on food packaging materials soiled with antimicrobial-free ham homogenate inoculated at a level of approximately 5 log CFU/cm^2 and stored at 25º C for up to 123 days (two replicates combined)

	R-Square	*f* (Std. Error)	*kmax1* (1/day)	*kmax2* (1/days)	4D Reduction (± days)
Aluminum foil	0.5202	0.9979 (3.1 x 10^{15})	0.03 (*)	0.03 (*)	> 123
Butcher paper	0.9269	0.9358 (1.2 x 10^{15})	0.06 (*)	0.06 (*)	> 123
Cardboard box material	0.9403	0.9935 (0.00742)	1.05 (0.42)	0.06 (0.01)	67.65
Deli paper	0.9612	0.9284 (5.9 x 10^{14})	0.7 (*)	0.07 (*)	> 123
Paper bag material	0.9613	0.9999 (0.00033)	0.18 (0.03)	0.02 (0.02)	54.12
Plastic bag material	0.8891	0.9099 (2.1 x 10^{15})	0.06 (*)	0.06 (*)	> 123
PVC overwrap film	0.7764	0.9999 (0.00071)	0.14 (0.05)	0.00 (0.06)	> 123
Vacuum pouch material	0.8809	0.8167 (1.3 x 10^{15})	0.08 (*)	0.08 (*)	114.39

* Biphasic model identified *kmax1* as equal to *kmax2*, indicating nearly linear inactivation behavior

Table 1.2 - Inactivation parameters of *L. monocytogenes* on food packaging materials soiled with antimicrobial-free ham homogenate inoculated at a level of approximately 5 log CFU/cm^2 and stored at 25º C for up to 123 days (two individual replicates)

	R-Square		*f* value		*kmax1* (1/day)		*kmax2* (1/day)		4D Reduction (± days)	
	Rep1	Rep2	Rep1	Rep2	Rep1	Rep2	Rep1	Rep2	Rep1	Rep2
Aluminum Foil	0.4237	0.4319	0.9811 ($3.1x10^{15}$)	0.9766 ($3.6x10^{15}$)	0.03 ($6.7x10^{5}$)	0.03 ($9.7x10^{5}$)	0.03 ($3.5x10^{7}$)	0.03 ($4.1x10^{7}$)	> 123	> 123
Butcher Paper	0.9269	0.8783	0.9358 ($1.2x10^{15}$)	0.9413 ($9.0x10^{14}$)	0.06 ($4.2x10^{5}$)	0.05 ($2.3x10^{5}$)	0.06 ($6.1x10^{6}$)	0.05 ($3.7x10^{6}$)	> 123	> 123
Cardboard Box Material	0.9591	0.8831	0.9967 (0.00302)	0.9999 (0.00019)	0.95 (0.030)	0.21 (0.04)	0.05 (0.01)	0.02 (0.03)	65.19	45.51
Deli Wax Paper	0.8627	0.9466	0.9987 (0.02866)	0.8479 ($7.7x10^{14}$)	0.06 (0.02)	0.09 (*)	0.00 (0.18)	0.09 (*)	> 123	103.32
Paper Bag Material	0.9576	0.9281	0.9999 (0.00014)	0.9996 (0.00148)	0.20 (0.03)	0.16 (0.04)	0.01 (0.02)	0.03 (0.04)	47.97	61.5
Plastic Bag Material	0.9379	0.7094	0.9098 ($9.1x10^{14}$)	0.9081 ($2.7x10^{15}$)	0.07 ($6.5x10^{5}$)	0.06 (*)	0.07 ($6.5x10^{6}$)	0.06 (*)	> 123	> 123
PVC Overwrap Film	0.8642	0.4632	1.0000 (0.00014)	0.9994 (0.00544)	0.16 (0.04)	0.12 (0.09)	0.00 (0.05)	0.00 (0.10)	59.04	> 123
Vacuum Pouch Material	0.8767	0.7020	0.8160 ($1.4x10^{15}$)	0.8284 ($4.0x10^{15}$)	0.09 (*)	0.07 (*)	0.09 (*)	0.07 (*)	107.01	> 123

* - GInaFit finds *kmax* values to be equal, and standard error was not able to be computed due to poor fit

iii. Survival of *L. monocytogenes* on soiled food packaging materials inoculated with a high initial level and stored at 4º C

Populations of *L. monocytogenes* (5 log CFU/cm^2) stored on soiled packaging materials at 4º C had similar counts to each other at 25 days of storage and 95 days of storage (Figure 1.3)(data in Appendix Table 11 for combined data results, and Appendix Tables 12 and 13 for individual replicate results; while Appendix Table 14 shows total aerobic plate counts and Appendix Tables 15 and 16 show data from individual replicates). No material exhibited cell reductions below the detection limit by the end of storage (123 days). However, PVC overwrap film had the least amount of survivors on day 123 of storage. *Listeria monocytogenes* survived at the highest levels on plastic bag and vacuum pouch material (Figure 1.3). Nonselective media showed more survival than selective media, but trends remained the same, and any variation may be attributed to background flora (especially in the case of cardboard box material)(Figure 1.4). At this high inoculation level, cells died more rapidly at 4º C than at 25º C.

L. monocytogenes populations stored at 4º C behaved in a manner that can be described by the biphasic model, as outlined by Cerf et al., 1977. This model once again yielded the most consistent and highest R-Squared values, save for *L. monocytogenes* on vacuum pouch material, which behaved in a more linear fashion (Table 1.3). Populations on cardboard box material and PVC overwrap film exhibited the highest *kmax1* values, while plastic bag material and butcher paper material exhibited the smallest *kmax1* values, similar to storage of identical samples at 25º C (Table 1.3). Butcher paper, deli

wax paper, paper bag material and PVC overwrap film were projected to achieve a 4-D reduction within the time of storage studied (123 days).

Compared with 25º C, every material examined was less hospitable to *L monocytogenes* at 4º C. The lowest *kmax1* value was 0.07 or 0.02 (vacuum packaging material, Table 1.4), which were similar to the values determined for vacuum packaging material stored at 25º C. No *kmax1* values were as high as those for cardboard box material stored at 25º C (Tables 1.1 and 1.3), and the general trend of pathogen populations at 4º C indicated a much more drastic initial reduction than the 25º C counterpart. Overall, more populations of *L. monocytogenes* inoculated on soiled packaging materials at 4º C decreased during the time of storage than counterparts stored at 25º C, though some cells were still able to persist for extended periods of time. It is of great importance to note that the pathogen was still very capable of surviving for extended periods of time even under these conditions, regardless of material.

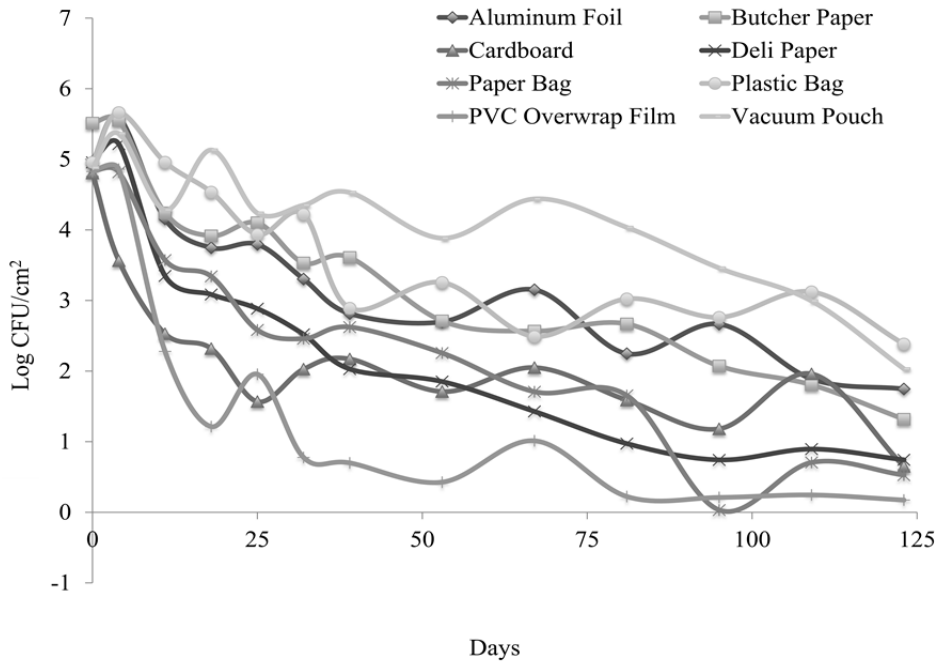

Figure 1.3 - *Listeria monocytogenes* survival (log CFU/cm^2) on food packaging materials soiled with antimicrobial-free ham homogenate inoculated with approximately 5 log CFU/cm^2 of *L. monocytogenes* and stored at 4º C for up to 123 days (Data in Appendix Table 11)

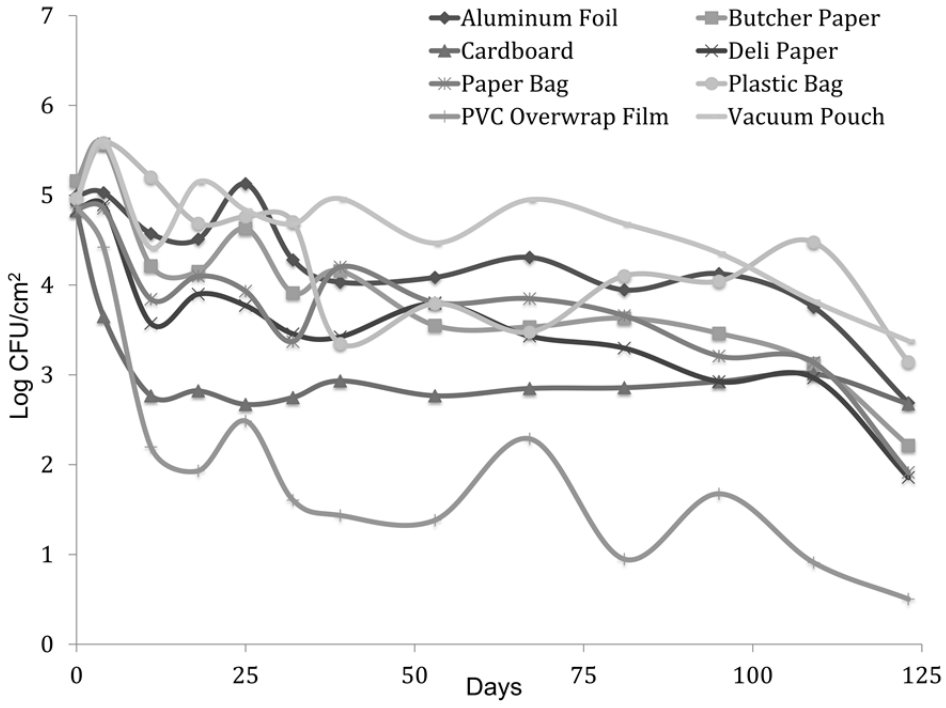

Figure 1.4 – Total aerobic bacteria survival (log CFU/cm^2) on food packaging materials soiled with antimicrobial-free ham homogenate inoculated with approximately 5 log CFU/cm^2 of *L. monocytogenes* and stored at 4º C for up to 123 days (Data in Appendix Table 14)

Table 1.3 – Inactivation parameters of *L. monocytogenes* on food packaging materials soiled with antimicrobial-free ham homogenate inoculated at a level of approximately 5 log CFU/cm^2 and stored at 4º C for up to 123 days (two replicates combined)

	R-Square	*f* (Std. Error)	*kmax1* (1/day)	*kmax2* (1/days)	4D Reduction (± days)
Aluminum foil	0.9199	0.9784 (0.02285)	0.20 (0.07)	0.03 (0.01)	> 123
Butcher paper	0.9709	0.9543 (0.02964)	0.32 (0.12)	0.06 (0.01)	109.47
Cardboard box material	0.9027	0.9958 (0.00396)	0.62 (0.26)	0.02 (0.01)	> 123
Deli paper	0.9621	0.9944 (0.00495)	0.28 (0.06)	0.05 (0.01)	88.56
Paper bag material	0.9480	0.9750 (0.02507)	0.28 (0.11)	0.06 (0.01)	93.48
Plastic bag material	0.8915	0.9970 (0.00581)	0.13 (0.03)	0.00 (0.02)	> 123
PVC overwrap film	0.9417	0.9998 (0.00025)	0.54 (0.13)	0.03 (0.01)	30.75
Vacuum pouch material	0.7848	0.9619 (*)	0.04 (*)	0.04 (*)	> 123

* - GInaFit finds *kmax* values to be equal, and standard error was not able to be computed due to poor fit

Table 1.4 – Inactivation parameters of *L. monocytogenes* on food packaging materials soiled with antimicrobial-free ham homogenate inoculated at a level of approximately 5 log CFU/cm^2 and stored at 4º C for up to 123 days (two individual replicates)

	R-Square		*f* value		*kmax1* (1/day)		*kmax2* (1/day)		4D Reduction (± days)	
	Rep1	Rep2	Rep1	Rep2	Rep1	Rep2	Rep1	Rep2	Rep1	Rep2
Aluminum Foil	0.8074	0.6822	0.9988 (0.00213)	0.9757 ($1.6x10^{15}$)	0.33 (0.12)	0.04 ($3.9x10^{5}$)	0.04 (0.02)	0.04 ($1.5x10^{7}$)	71.34	> 123
Butcher Paper	0.9636	0.8499	0.9054 (0.9060)	0.9638 (0.07293)	0.22 (0.11)	0.12 (0.06)	0.07 (0.01)	0.02 (0.02)	94.71	> 123
Cardboard Box Material	0.9023	0.8025	0.9971 (0.00336)	0.9957 (0.00512)	0.62 (0.27)	0.96 (0.41)	0.03 (0.01)	0.01 (0.01)	97.17	> 123
Deli Wax Paper	0.9504	0.9355	0.9999 (0.00009)	0.9246 (0.06352)	0.26 (0.04)	0.27 (0.16)	0.02 (0.02)	0.05 (0.01)	36.9	> 123
Paper Bag Material	0.9664	0.8763	0.9891 (0.00937)	0.9438 (0.08311)	0.30 (0.08)	0.29 (0.24)	0.06 (0.01)	0.06 (0.01)	79.95	107.01
Plastic Bag Material	0.8862	0.7519	0.9998 (0.00065)	0.9222 (0.15233)	0.16 (0.04)	0.10 (0.07)	0.00 (0.03)	0.01 (0.02)	> 123	> 123
PVC Overwrap Film	0.9681	0.7688	1.0000 (0.00003)	0.9990 (0.00219)	0.46 (0.06)	0.57 (0.32)	0.01 (0.01)	0.03 (0.02)	20.91	66.42
Vacuum Pouch Material	0.7908	0.3474	0.8590 ($2.1x10^{15}$)	0.9861 (*)	0.07 (*)	0.02 (*)	0.07 (*)	0.02 (*)	> 123	> 123

* - GInaFit finds *kmax* values to be equal, and standard error was not able to be computed due to poor fit

iv. Survival of *L. monocytogenes* on soiled food packaging materials inoculated at a low initial level and stored at 25º C

Soiled packaging materials, except for PVC overwrap film, cardboard and deli wax paper, exhibited drastic increases in population for the first part of storage (ranging from 4 to 25 days) when soiled with inoculated ham homogenate (approximately 2 log CFU/cm^2) and stored at 25º C (Figure 1.5) (see Appendix Table 17 for combined data results, and Appendix Tables 18 and 19 for individual replicate results, while Appendix Table 20 shows total aerobic plate counts and Appendix Tables 21 and 22 show individual replicates). Populations on soiled plastic bag material, butcher paper, and aluminum foil are of special note, as they grew to over 2 log CFU/cm^2 higher than the initial inoculated level within two weeks of inoculation before they began declining in number. Pathogens on many materials at this low inoculation level and 25º C environment exhibited high levels of survival, continuing this trend without much change to the end of storage (123 days). Populations on paper bag material, cardboard, and deli wax paper were below the detection limit (-0.1 log CFU/cm^2) on the 67th day of storage. Aluminum foil harbored the greatest amount of survivors on day 123 of storage at 25º C.

The pathogen was less consistent from sample to sample than when stored at other temperature and inoculation level combinations, so R-values were not as desirable as they were in other treatments, generally ranging from 0.0000 to less than 0.9 (Table 1.5). Biphasic models (Cerf et al., 1977) were still fit to the pathogenic population data for the sake of comparison. No 4-D reductions were determined for these data, as initial levels were approximately 2 log CFU/cm^2 with a detection limit of -0.1 log CFU/cm^2. Pathogen

populations exhibited the most drastic reduction on deli wax paper and cardboard, with complete elimination of counts almost immediately, indicated by high *kmax1* values of 2.46 and 0.34. Plastic bag and butcher paper materials exhibited the smallest *kmax1* values, a result of populations of *L. monocytogenes* that were able to persist for much longer periods of time, and with behavior not completely consistent with biphasic reduction.

Interestingly, compared with counterparts inoculated at a higher level, low initial *L. monocytogenes* levels on soiled paper bag material did not exhibit the most drastic decrease in population. Instead, populations decreased at a rate with a *kmax1* in the middle of the range of all materials. In addition, populations on cardboard box material exhibited an extremely rapid decrease in the first replication (*kmax1* 3.30) while reduction in the second replication (*kmax1* 0.32) was comparable to that on other materials (paper bag, PVC overwrap, and vacuum plastic bag materials) and the *kmax1* values of cardboard box material inoculated at a higher level (Table 1.6, also Table 1.2). These results suggest that survival even with low initial microbiological loads is a possibility, as *L. monocytogenes* may grow or remain viable for long periods of time on some packaging materials. Not only did populations on some materials grow, overall inactivation rates (Table 1.5) were not faster than those on high-inoculation counterparts, with low overall *kmax1* values and low *f* values. Many of these curves cannot be adequately described by a model predicting reduction, so there exists a great risk when *L. monocytogenes* contaminates select packaging materials at this temperature.

Total aerobic plate counts under these conditions followed their selective media counterparts very closely. Aside from cardboard box material, it can be gathered from this data (Figures 1.5 and 1.6) that the majority of bacteria present on packaging materials (except for that on cardboard box material) was *L. monocytogenes* which had been inoculated at the onset of storage.

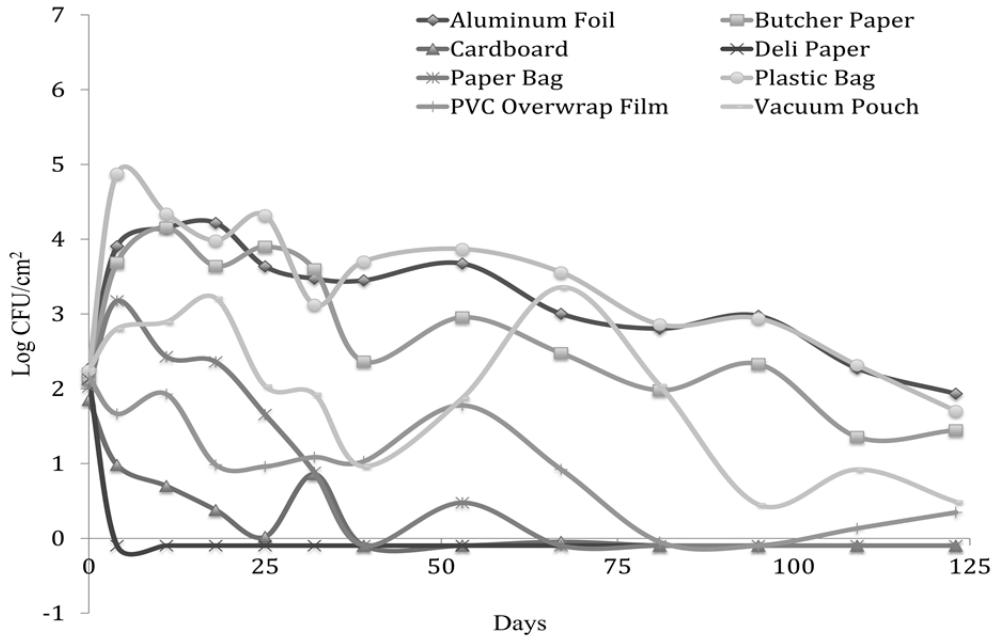

Figure 1.5 - *Listeria monocytogenes* survival (log CFU/cm^2) on food packaging materials soiled with antimicrobial-free ham homogenate inoculated with approximately 2 log CFU/cm^2 of *L. monocytogenes* and stored at 25º C for up to 123 days (Data in Appendix Table 17)

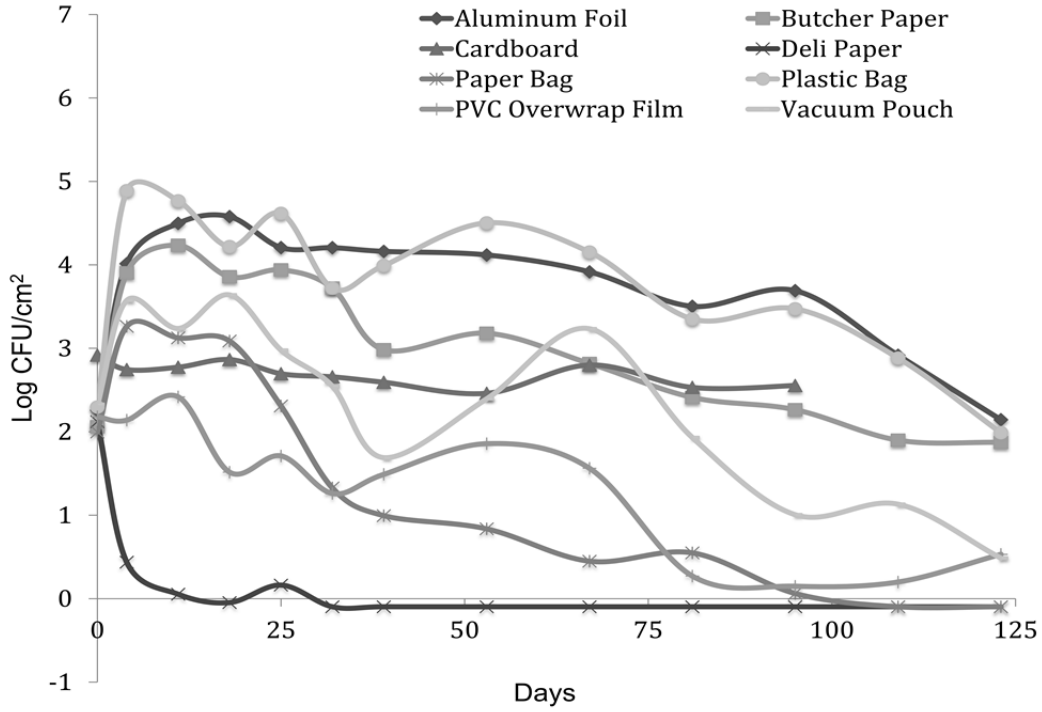

Figure 1.6 – Total aerobic bacteria survival (log CFU/cm^2) on food packaging materials soiled with antimicrobial-free ham homogenate inoculated with approximately 2 log CFU/cm^2 of *L. monocytogenes* and stored at 25º C for up to 123 days (Data in Appendix Table 20)

Table 1.5 – Inactivation parameters of *L. monocytogenes* on food packaging materials soiled with antimicrobial-free ham homogenate inoculated at a level of approximately 2 log CFU/cm^2 and stored at 25º C for up to 123 days (two replicates combined)

	R-Square	*f* (Std. Error)	*kmax1* (1/days)	*kmax2* (1/days)
Aluminum foil	0.0000	0.9743 (*)	0.00 (*)	0.00 (*)
Butcher paper†	0.0000	0.9152 (*)	0.00 (*)	0.00 (*)
Cardboard box material	0.8397	0.9548 (0.03183)	0.34 (0.17)	0.01 (0.01)
Deli paper	1.0000	0.9941 (0.00002)	2.46 (0.09)	0.00 (0.00)
Paper bag material	0.8803	0.9989 (0.00249)	0.14 (0.04)	0.00 (0.02)
Plastic bag material†	0.0000	0.9488 (†)	0.00 (*)	0.00 (*)
PVC overwrap film	0.6997	0.9866 (0.21776)	0.05 (0.04)	0.00 (0.13)
Vacuum pouch material†	0.4598	0.9565 (5.45 x 10^{15})	0.04 (5.4 x 10^5)	0.04 (1.2 x 10^7)

† Unacceptable R-Squared values and/or high standard errors indicate that the biphasic model (Cerf et al, 1977) is not an appropriate model for the behavior of *L. monocytogenes* on this packaging material
* Biphasic model identified *kmax1* as equal to *kmax2*, indicating nearly linear inactivation behavior

Table 1.6 – Inactivation parameters of *L. monocytogenes* on food packaging materials soiled with antimicrobial-free ham homogenate inoculated at a level of approximately 2 log CFU/cm^2 and stored at 25º C for up to 123 days (two individual replicates)

	R-Square		*f* value		*kmax1* (1/day)		*kmax2* (1/day)	
	Rep1	Rep2	Rep1	Rep2	Rep1	Rep2	Rep1	Rep2
Aluminum Foil	0.7170	0.0227	0.8967 (2.5x10^{15})	0.9811 (1.0x10^{15})	0.05 (*)	0.01 (*)	0.05 (*)	0.01 (*)
Butcher Paper	0.5637	0.0000	0.9805 (3.7x10^{15})	0.9266 (*)	0.04 (1.4x10^{6})	0.00 (*)	0.04 (1.4x10^{7})	0.00 (*)
Cardboard Box Material	0.7017	0.9546	0.9513 (0.05802)	0.9832 (0.00623)	3.30 (7409.01)	0.32 (0.06)	0.02 (0.01)	0.00 (0.00)
Deli Wax Paper	1.0000	1.0000	0.9930 (0.00002)	0.9950 (0.00003)	2.47 (0.09)	2.37 (0.09)	0.00 (0.00)	0.00 (0.00)
Paper Bag Material	0.9920	0.8042	0.9989 (0.00154)	0.9993 (0.00355)	0.17 (0.03)	0.11 (0.04)	0.00 (0.02)	0.00 (0.05)
Plastic Bag Material	0.6407	0.1025	0.8298 (3.1x10^{15})	0.9883 (1.0x10^{16})	0.05 (1.4x10^{6})	0.01 (*)	0.05 (7.0x10^{6})	0.01 (*)
PVC Overwrap Film	0.9250	0.4171	0.9931 (0.00468)	0.9738 (*)	0.20 (0.04)	0.04 (*)	0.00 (0.01)	0.04 (*)
Vacuum Pouch Material	0.3490	0.3518	0.9516 (5.8x10^{15})	0.9921 (0.02938)	0.04 (9.2x10^{5})	0.22 (0.24)	0.04 (1.8x10^{7})	0.01 (0.04)

* - GInaFit finds *kmax* values to be equal, and standard error was not able to be computed due to poor fit

v. Survival of *L. monocytogenes* on soiled food packaging materials inoculated at a low initial level and stored at 4º C

When inoculated at approximately 2 log CFU/cm^2, *L. monocytogenes* all survived similarly on various packaging materials. Apart from vacuum pouch and plastic bag material, counts on other packaging materials were below the detection limit on day 123 of storage (Figure 1.7) (see Appendix Table 23 for combined data results, and Appendix Tables 24 and 25 for individual replicate results, while Table 26 shows total aerobic plate counts and Appendix Tables 27 and 28 show individual replicates). In contrast to other temperature and inoculation conditions, populations stored under these conditions behaved consistently between all packaging materials, with no initial growth on any soiled material, and a steady decrease in populations of materials examined. These results are consistent with knowledge that a lower initial microbial load and low temperatures are less hospitable for the survival of bacteria.

Results from fitting a biphasic curve to population data (Table 1.7) yielded favorable R-Squared values, indicating very strong goodness-of-fit for biphasic reduction curves, and large *kmax1* values (indicating rapid death) in most cases. Once again, cardboard box material and PVC overwrap material were among the least hospitable to pathogenic survival with the highest *kmax1* values (1.14 and 2.56, respectively), though vacuum pouch material yielded *kmax1* values of 2.70 (albeit with very high standard error). Populations on butcher paper exhibited the lowest *kmax1* value (0.49). *kmax2* values ranged from 0.00 (PVC overwrap film and deli wax paper) to 0.03 (plastic bag material), indicating that the second phase of inactivation is very mild. This reinforces findings that

initial inactivation (*kmax1*) of the pathogen was the most important time for determining long-term survival of bacteria.

PVC overwrap material allowed less survival of *L. monocytogenes* at this temperature and inoculation level combination when compared to other temperature and inoculation level combinations. Cardboard box material was consistently inhospitable to *L. monocytogenes* populations, though behavior of the pathogen changed very minutely from temperature to temperature and inoculation level. All other materials experienced wide variation in *kmax* values under different storage and inoculation conditions. In general, it may be said that soiled cardboard was the least hospitable to bacterial survival, while soiled aluminum foil allowed *L. monocytogenes* to consistently persist at high levels and even grow in numbers more than any other material, regardless of storage and inoculation condition. Soiled PVC overwrap film and paper bag material were inhospitable to *L. monocytogenes* and did not foster much survival under all conditions tested.

Overall, results associated with *L. monocytogenes*, inoculated in ham homogenate and used to soil food packaging materials at a low initial level of inoculation showed that storage at 4º C was, in fact, the most lethal to the pathogen. Materials with a tendency to be inhospitable to the pathogen (high *kmax1* values) were even more inhospitable to bacterial cells under these conditions. Materials known to be more hospitable to survivors still had higher inactivation rates when compared to counterparts at higher temperatures or inoculation levels. However, the most important information is that *L. monocytogenes*

was able to survive at detectable levels for up to 123 days in adverse storage conditions on various packaging materials. Some materials (i.e., PVC overwrap film and cardboard box material) were consistently less hospitable to survival of the pathogen, though never to the point where a material could safely be deemed bacteriocidal.

Total aerobic plate counts were generally higher than counts on selective media (Figures 1.7 and 1.8). This suggests that at cold temperatures, psychotrophic bacteria better persists alongside *L. monocytogenes*. Therefore, it may not be asserted that cold temperatures are perfect for storage of packaging materials, as a wide variety of bacteria were able to persist for extended periods of time.

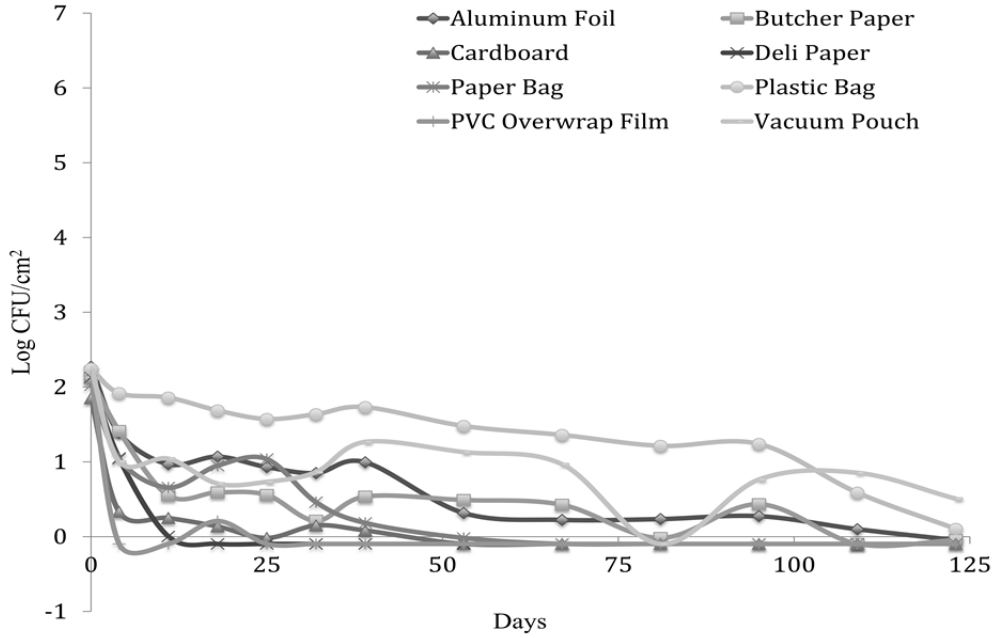

Figure 1.7 - *Listeria monocytogenes* survival (log CFU/cm^2) on food packaging materials soiled with antimicrobial-free ham homogenate inoculated with approximately 2 log CFU/cm^2 of *L. monocytogenes* and stored at 4º C for up to 123 days (Data in Appendix Table 23)

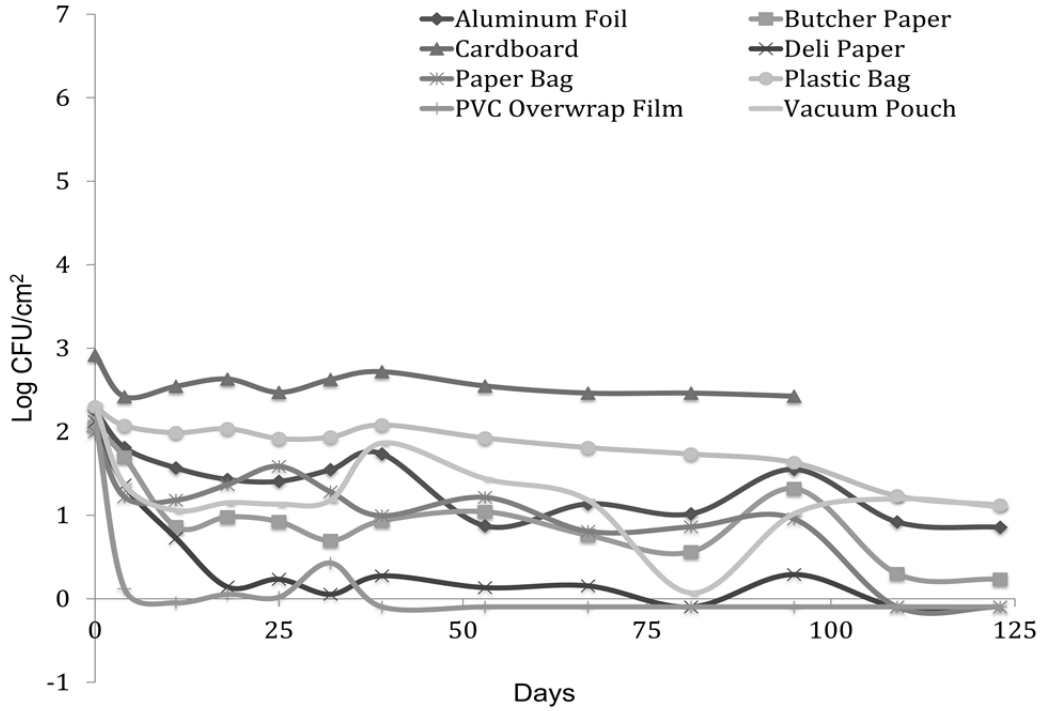

Figure 1.8 – Total aerobic bacteria survival (Log CFU/cm^2) on food packaging materials soiled with antimicrobial-free ham homogenate inoculated with approximately 2 log CFU/cm^2 of *L. monocytogenes* and stored at 4º C for up to 123 days (Data in Appendix Table 26)

Table 1.7 – Inactivation parameters of *L. monocytogenes* on food packaging materials soiled with antimicrobial-free ham homogenate inoculated at a level of approximately 2 log CFU/cm^2 and stored at 4º C for up to 123 days (two replicates combined)

	R-Square	*f* (Std. Error)	*kmax1* (1/days)	*kmax2* (1/days)
Aluminum foil	0.9508	0.9261 (0.03236)	0.68 (0.23)	0.02 (0.00)
Butcher paper	0.9348	0.9689 (0.01422)	0.49 (0.15)	0.01 (0.00)
Cardboard box material	0.9818	0.9796 (0.00445)	1.14 (0.15)	0.01 (0.00)
Deli paper	0.9998	0.9939 (0.00087)	0.63 (0.01)	0.00 (0.00)
Paper bag material	0.8735	0.9380 (0.04466)	0.76 (0.40)	0.02 (0.01)
Plastic bag material†	0.8717	0.98485 (1.1 x 10^{15})	0.03 (*)	0.03 (*)
PVC overwrap film	0.9831	0.9935 (0.00161)	2.56 (11.21)	0.00 (0.00)
Vacuum pouch material	0.6035	0.9380 (0.06517)	2.70 (812.13)	0.01 (0.01)

† Unacceptable R-Squared values and/or high standard errors indicate that the biphasic model (Cerf et al, 1977) is not an appropriate model for the behavior of *L. monocytogenes* on this packaging material
* Biphasic model identified *kmax1* as equal to *kmax2*, indicating nearly linear inactivation behavior

Table 1.8 – Inactivation parameters of *L. monocytogenes* on food packaging materials soiled with antimicrobial-free ham homogenate inoculated at a level of approximately 2 log CFU/cm^2 and stored at 4º C for up to 123 days (two individual replicates)

	R-Square		*f* value		*kmax1* (1/day)		*kmax2* (1/day)	
	Rep1	Rep2	Rep1	Rep2	Rep1	Rep2	Rep1	Rep2
Aluminum Foil	0.7803	0.9408	0.9702 (0.11417)	0.9738 (0.01319)	0.06 (0.04)	0.49 (0.16)	0.00 (0.04)	0.02 (0.00)
Butcher Paper	0.8325	0.8904	0.9257 (0.05887)	0.9896 (0.00663)	0.61 (0.38)	0.39 (0.11)	0.02 (0.01)	0.00 (0.01)
Cardboard Box Material	0.9477	0.9949	0.9783 (0.00905)	0.9797 (0.00211)	1.24 (0.40)	1.08 (0.06)	0.01 (0.00)	0.00 (0.00)
Deli Wax Paper	0.9929	0.9665	0.9920 (0.00123)	0.9971 (0.00121)	1.23 (0.08)	0.54 (0.08)	0.00 (0.00)	0.00 (0.00)
Paper Bag Material	0.8104	0.9742	0.9399 (0.05249)	0.9856 (0.00603)	2.83 (1048.10)	0.15 (0.02)	0.02 (0.01)	0.00 (0.00)
Plastic Bag Material	0.7482	0.8876	0.9845 ($1.8x10^{15}$)	0.7539 ($1.7x10^{15}$)	0.03 ($1.8x10^{5}$)	0.03 ($1.2x10^{6}$)	0.03 ($1.1x10^{7}$)	0.03 ($3.6x10^{6}$)
PVC Overwrap Film	1.0000	0.9362	0.9940 (0.00001)	0.9930 (0.00347)	2.60 (0.09)	2.74 (49.32)	0.00 (0.00)	0.00 (0.00)
Vacuum Pouch Material	0.2048	0.7195	0.9517 (9.78266)	0.9894 (0.01177)	0.02 (0.20)	1.28 (0.75)	0.00 (1.15)	0.00 (0.01)

* - GInaFit finds *kmax* values to be equal, and standard error was not able to be computed due to poor fit

vi. Discussion

Results of this study show that *L. monocytogenes*, suspended in ham homogenate and spilled onto various packaging materials, is able to survive for extended periods of time, up to 123 days, under certain conditions. This said, survival of *L. monocytogenes* varied among packaging materials as well as at different storage temperatures and inoculation levels. As suggested by the literature regarding survival of *L. monocytogenes* in adverse environments, biofilms could be an important factor concerning the survival of the pathogen in adverse environments (Knøchel, 2010; Chmielewski and Frank, 2003; Mędrala et al, 2003; Tompkin, 2002). Coupled with knowledge of the survival dynamics of *L. monocytogenes* at different temperatures (Gandhi et al., 2007) with the readiness to form biofilm systems under duress (Gandhi et al., 2007, Renier et al., 2011), it was expected that harsh conditions would promote hardiness in *L. monocytogenes* populations on contaminated materials.

L. monocytogenes on soiled cardboard box material and PVC overwrap film survived less than on all other materials under all conditions. Aluminum foil and butcher paper consistently harbored the pathogen without rapid death for much longer than any other materials within each temperature-inoculation level combination. With this information in mind, those involved with processing, storage, and retail of meat should primarily take extra precaution to prevent cross-contamination of food from occurring, and keep all packaged food at a temperature of 4º C or lower, if possible. Use of cardboard box material is desirable from a microbiological safety perspective, though problems on most

material could be avoided by preventing *L. monocytogenes* from attaching to packaging material and keeping products refrigerated.

The most probable explanation for the long-term survival of *L. monocytogenes* on various soiled packaging materials is the aptitude to form biofilms when stressed (Renier et al., 2011). Biofilms not only "shelter" the pathogens (Sofos and Geornaras, 2010), but can reorganize cells into a structure specifically suited for survival of a group of pathogens (Chmielewski and Frank, 2003). Even though biofilms are usually formed when pathogens are in a growth phase, surviving cells have been reported to form biofilms, especially when under stress (Chmielewski and Frank, 2003; Kalmokoff et al., 2001; Takhistov and George, 2004). It should also be noted that, unlike studies involving antimicrobial treatments, spot-inoculating *L. monocytogenes* on soiled food packaging materials never resulted in immediate reduction in bacterial populations, so it is possible that biofilms were quickly formed due to the presence of a food soiling matrix as well as the stress of dessication or refrigeration temperatures. In addition, studies have shown that biofilm formation is affected both by surface characteristics, and by material on which contamination occurs (Chmielewski and Frank, 2003).

The level of pathogen presence tested in the study may be higher than what might be found in environmental samples, as Chaitiemwong et al. reports a level of approximately 5 log CFU/100cm^2 on environmental conveyor belt samples (Chaitiemwong et al., 2010). Converting to the same units tested in this study, the level found by Chaitiemwong et al. was approximately 3.5 log CFU/cm^2. This means that one level of inoculation used in

this trial may be considered higher than would be expected in a food processing environment. Therefore, high initial levels of the pathogen may have also contributed to long-term survival, as Knøchel (2010) reports that *L. monocytogenes* is able to persist for extended periods of time when present in large numbers. *L. monocytogenes* is known to grow to approximately 10^9 cells per milliliter of broth, though this level is achieved in conditions tailored to the growth of the pathogen.

It is no surprise that survival was observed at both temperatures, as *L. monocytogenes* has been demonstrated to be persistent under less-than-ideal conditions (multi-year persistence – Tompkin, 2002; year-long survival, collection from freezers, etc. – Autio et al., 2003; desiccation conditions, Vogel et al., 2010), and is able to grow at temperatures below 1º C (Junttila et al., 1988). Because *L. monocytogenes* is so persistent in harsh environments, it may be concluded that a large part of variation shown in the results is due to interaction of the bacteria with various packaging materials on which it is inoculated.

Also, likely contributing to survival of the pathogen was the absence of antimicrobials in the food soiling matrix itself. The ham used to create a soiling homogenate had no antimicrobials added, and all materials used were acquired before exposure to antimicrobial interventions post-processing. This is important, as literature indicates that *L. monocytogenes* is generally susceptible to antimicrobial treatments, multiple hurdle interventions, and antimicrobials applied at some point during food processing (Chaitiemwong et al., 2010, Sofos and Geornaras, 2010), though resistance to various

antimicrobials has been reported in certain strains of the pathogen (Gandhi et al., 2007). Eliminating influence of non-ambient bacteriocidal or bacteriostatic inhibitors allowed development of a "worst-case scenario" setting for pathogen growth or survival on soiled food packaging materials.

The results from this study indicate that presence of *L. monocytogenes* on soiled food packaging may serve as a source of continued cross-contamination. Bacterial cells were consistently more persistent when stored at 25º C and died more rapidly at 4º C. Interestingly, even lower initial levels of the pathogen did not ensure rapid death of the pathogen on packaging materials. This suggests that even low doses of contamination on wholesale and retail packaging materials may present a lingering contamination problem, especially if stored at 25º C.

Since packaging materials are typically not treated with antimicrobial interventions (with the exceptions of high pressure pasteurization and irradiation), a general knowledge of the danger of contaminated packaging materials is beneficial for both industry and consumer alike. Riazi and Matthews (2011) showed that common sanitizing measures (namely sodium hypochlorite) are effective in reducing pathogenic populations by over 5 log units on a food contact surface when applied at a high concentration (512 ppm sodium hypochlorite). However, typical consumer hygienic practices related to ready-to-eat foods involve handwashing and refrigerating leftovers; rarely are packages washed and sanitized prior to handling or storage.

IV. Conclusions

In conclusion, it should be noted that the data indicated that *L. monocytogenes* was able to survive for extended periods of time in food residues deposited on common food packaging materials. Regardless of temperature of storage, initial microbiological load, or soiled material harboring the contamination, the pathogen was able to persist for at least 123 days in many cases. Care should be taken primarily to ensure that packaging is kept clean and free from food-based soil. This may be done by keeping meat processing facilities, food storage and shipping environments, those who handle meat products, and packaging free from food soil buildup and *L. monocytogenes*. With these precautions in mind, it has also been demonstrated in this study that, in case of contamination, survivors on packaging materials will be lower when stored at 4º C compared to 25º C.

It is important to stress that this pathogen is able to persist under seemingly unfavorable conditions for an extended period of time. When the general public becomes aware of the dangers posed by *L. monocytogenes*, education in methods to minimize risk of exposure to the pathogen should follow suit immediately. Consumers should be urged to keep food preparation and storage areas clean and to wash objects related to food before and after use. Knowing the dangers of *L. monocytogenes* when present on soiled packaging materials should allow consumers, retailers, and processors to more completely protect the food supply and the health and well-being of consumers.

Chapter 4: *Escherichia coli* O157:H7 survival on food packaging materials soiled with ground beef residues

Chapter Overview

Escherichia coli O157:H7 cells present in contaminated food residues, such as meat purge, may potentially be deposited on internal and external surfaces of food packaging materials, where they may attach, survive, and serve as sources of cross-contamination of other foods. This study explored behavior of *E. coli* O157:H7 in meat residues deposited on four materials commonly associated with storage and display of fresh meat (butcher paper, cardboard, PVC overwrap film, and vacuum plastic bags). Packaging material samples (5 × 5 cm) were spot-inoculated (0.5 ml) with a ground beef homogenate (10% wt/wt in distilled water) containing an eight-strain mixture of rifampicin-resistant *E. coli* O157:H7 (simulating contaminated meat residues), to achieve target inoculation levels of 4 or 6 log CFU/cm^2. Inoculated packaging materials were stored aerobically in petri dishes at 4 or 25^{o}C for up to 130 days. At regular intervals during storage, samples were analyzed (two repetitions, three samples/treatment/repetition) for surviving total microbial (tryptic soy agar) and inoculated *E. coli* O157:H7 (tryptic soy agar with 100 μg/ml rifampicin) populations. The pathogen, when placed on packaging materials via ground beef homogenate, decreased throughout storage at various rates dependent upon storage conditions and materials, though regardless of environment, pathogen survivors were obtained at the end of storage (130 days) on all tested materials. When inoculated at the low inoculum level (4 log CFU/cm^2), pathogen counts at 130 days ranged from <0.9±1.0 (cardboard) to 1.6±0.8 (butcher paper) log CFU/cm^2 on materials stored at

25°C, and <0.0±0.2 (vacuum pouch material) to <0.3±0.4 (PVC overwrap film) log CFU/cm^2 on samples stored at 4°C. For samples inoculated at 6 log CFU/cm^2, the lowest and highest pathogen counts at 130 days at 25°C were obtained on vacuum pouch material (<1.7±1.1 log CFU/cm^2) and butcher paper (1.8±0.5 log CFU/cm^2), respectively. For corresponding samples stored at 4°C, the lowest and highest pathogen counts on day 130 of storage were obtained on butcher paper (<0.3±0.6 log CFU/cm^2) and PVC overwrap film (2.4±0.2 log CFU/cm^2), respectively. The results indicated that under these conditions, *E. coli* O157:H7 was able to survive for more than four months on soiled packaging materials. Thus, contaminated meat residues deposited on packaging materials may serve as sources of cross-contamination of foods.

I. Introduction

Despite efforts to eliminate *Escherichia coli* O157:H7 from the modern food supply, the pathogen continues to be a problem for the industry and consumers. Due to publicized infections and associated deaths, consumers have lost confidence in the safety of the modern meat supply. In addition to this, recalls of products associated with the O157 serotype of *E. coli* have cost the beef industry millions of dollars, as well as lost revenue and costs associated with loss of consumer trust. In addition, up to 4,614 people may be hospitalized every year due to the pathogen (Scallan et al., 2011). As *E. coli* O157:H7 is an organism declared an adulterant by federal legislation (USDA-FSIS directive 10,010.1), its control is on the forefront of industry interest. Interventions exist to control spread of the pathogen from presence on cattle hide to carcasses ranging from

vaccinations to carcass sprays, each with varying success (Woerner et al., 2006; Carpenter et al., 2010).

However, even after focusing much effort on controlling *E. coli* O157:H7 in processing, outbreaks and contamination still occur. As evidenced by recent outbreaks of the pathogen on produce, cross-contamination is the most likely culprit of transmission of the pathogen. *E. coli* serotypes are able to attach to surfaces, both food and abiotic, and form biofilms that increase the pathogen's likelihood of survival. More alarmingly, pathogenic strains of *E. coli* such as O157:H7 possess antigens that possibly increase the ability to attach to surfaces (Torres et al., 2005). Uhlich et al. (2010) reported that survival of pathogenic bacteria is enhanced after attachment on soiled surfaces, surviving even after cleaning and sanitizing. Results of these studies suggest that risk analysis for *E. coli* O157:H7 should be expanded beyond beef carcass and trimming surfaces and food plant processing and fabrication surfaces. If the pathogen is able to cross-contaminate products due to efficient attachment and possible biofilm formation, survival of the bacteria on all materials contacting food products should be investigated.

Survival of *E. coli* O157:H7 suspended in meat homogenate has been demonstrated on food contact surfaces such as polyurethane (Marouani-Gadri et al., 2010). With knowledge of the pathogen's ability to survive on soiled food contact surfaces, researchers should consider all pathways of contamination, including the material in which food is packaged. Food that is sold is guaranteed to contact packaging materials, and consumers will most likely handle the material prior to preparation and consumption

of the product. Cross-contamination, therefore, may occur due to poor hygiene practices in household or commercial kitchens (Bloomfield et al., 2007).

It is of interest to public health and industry reputation that the survival of *E. coli* O157:H7 on a variety of soiled food packaging materials be investigated. Food packages are able to become soiled during cold storage in a processing facility, during storage or display at a retail establishment, or during storage in a consumer or service refrigerator. *E. coli* O157:H7 has been demonstrated to survive at refrigeration temperatures for up to 28 days on stainless steel and 35 days in commercial mayonnaise (Wilks et al., 2005; Weagant et al., 1994). It stands to reason, therefore, that other materials may allow survival of the pathogen, and subsequently pose a threat to consumer health. Because of the pressing issue of food safety with regards to *E. coli* O157:H7, the objective of this study was to quantify and compare survival of *E. coli* O157:H7 on various food packaging materials soiled with ground beef purge, investigating the effect on bacterial survival of different temperatures and levels of contamination.

II. Materials and Methods

i. Strain and inoculum preparation

Six rifampicin-resistant (Kaspar and Tamplin, 1993) strains of *E. coli* O157:H7 (ATCC 43895, ATCC 43888, ATCC 51658, and C1-057, C1-072, C1-109; bovine fecal isolates (Carlson et al., 2008)) were obtained and individual colonies were isolated via streak plating on TSA (Becton, Dickinson & Company, Sparks, MD) agar supplemented with 100 μg/ml rifampicin (Sigma-Aldrich Company, St. Louis, MO). A single colony from

each strain was activated in 10 ml Trypic Soy Broth (TSB; Becton, Dickinson & Company, Sparks, MD) supplemented with 100 µg/ml rifampicin (Sigma-Aldrich Company, St. Louis, MO), and incubated at 30°C for 20-24 hours. After incubation 100 µl of culture was pipetted into fresh TSB+Rif tubes and incubated again for 20-24 hours. "Ground beef homogenate" was prepared with 10% fresh ground beef (CSU Meat Science Laboratory, Fort Collins, CO) and 90% distilled, sterile water, then strained through a cheesecloth (adapted from Dourou et al. 2011). Cell pellets were individually centrifuged for 15 minutes (Eppendorf 5810 R, 4° C, 4,629 x g), then the supernatant was discarded. Cell pellets were resuspended in 10 ml Phosphate Buffered Saline (pH 7.4, PBS; Fisher Scientific, Fair Lawn, NJ) and centrifuged for an additional 15 minutes. Finally, each strain was resuspended in 10 ml of the ground beef homogenate (pH 5.47) before the inoculum was diluted to levels appropriate for inoculation (approximately 6 or 8 log CFU/ml).

ii. Packaging material sample preparation

Four materials commonly associated with food packaging and handling were selected for investigation: butcher paper (Kold-Lok, Georgia-Pacific, Norwalk, CT), cardboard box material (Donated by Colorado Premium, Greeley, CO), polyvinyl chloride (PVC) overwrap film (Prime Source Crystal Clear PVC Wrapping Film, International Paper Co., N. Kansas City, MO), and vacuum plastic bag (Prime Source Vacuum Pouch, 3 mil STD barrier, International Paper Co., N. Kansas City, MO). Prior to inoculation, each material was cut into 5 cm x 5 cm samples and placed in new, sterile petri dishes (Polystyrene Petri Dish, Fisher Scientific, Pittsburgh, PA).

iii. Inoculation

A 0.5 ml portion of the ground beef homogenate containing the *E. coli* O157:H7 inoculum was pipetted onto each individual packaging material sample without spreading, achieving an initial inoculation level of approximately 4 ("low") or 6 ("high") log CFU/cm^2. Samples were individually set in a sterile petri dish and placed in incubators set at either 4º or 25º C (25º C, 13.13 ± 0.64% RH; 4º C, 37.33 ± 1.78% RH).

iv. Sampling and analysis

For microbiological sampling, 20 ml of Maximum Recovery Diluent (MRD; Becton, Dickinson & Company, Sparks, MD) were pipetted into Whirl-Pak® bags (Nasco, 18oz.). Packaging material samples were aseptically removed from petri dishes and placed into bags. Samples were masticated for 120 seconds at six strokes per second (IUL Instruments, S.A. Barcelona, Spain, Masticator Silver-Paddle Blender). Homogenized sample was pipetted out, serial dilutions were made in 0.1% buffered peptone water (BPW; Difco, Becton, Dickinson & Company, Sparks, MD), and liquid was spread plated onto both non-selective media (TSA; Becton, Dickinson & Company, Sparks, MD) and selective media (TSA+Rif). TSA+Rif plates were incubated at 35° C for 24 hours and colonies were counted, while the TSA plates were incubated at 25° C for 72 hours and colonies were subsequently counted. Materials were sampled weekly until 39 days of storage, and then the materials were sampled every other week until 130 days of storage. Sampling was discontinued for materials that had counts below the detection limit for at

least two consecutive sampling dates. Three samples were taken at each sampling date for each material in each inoculation level.

v. Statistical analysis

To analyze the data, colony counts were converted into log CFU/cm^2 using Microsoft Excel spreadsheets, and were analyzed using the Proc Mixed program of SAS software for a Tukey-adjusted analysis of variance of the population counts for each replication (Version 9.2, SAS, Inc., Cary, NC). In analysis of the data, tests were performed to investigate the presence of a significant ($P < 0.05$) replication effect.

The population data from both replicates were subsequently analyzed with GInaFiT, a freeware add-in for Microsoft Excel 2007 developed by A.H. Geeraerd et al. (2005). Using published microbiological inactivation curves, the add-in is able to fit curves to population data in Excel and generate numerical parameters to quantify the inactivation of bacterial populations. This freeware module allows a user to fit multiple different models of curves to logarithmic population data, which have been published in papers dating back to 1920. Goodness-of-fit values (R-squared) are given for each curve. A so-called Weibull model consistently yielded very high R-squared values for each curve (Mafart et al, 2002), more so than any other modeling equation provided with the software. Weibull models fit curves using the formula $LOG10(N)=LOG10(N0)-((t/\delta)^p)$ where $N0$ refers to the initial log population of the pathogen in question, δ refers to the time (in days) for the first decimal reduction of the initial population, t is the observed time in days (using any number of days here will generate an estimate for log population

at this date t) and N is the numerical population in question, whereas p is a "shape parameter" that defines the curve as either concave or convex, useful for defining overall persistence and survival of the bacteria (Geeraerd et al., 2005). Using these parameters, evaluation of different treatments is possible simply by comparing δ and p values, similar to the comparison published by Janssen et al. (2005). The Weibull model was selected for analysis, as opposed to biphasic models (Cerf, 1977), since *E. coli* O157:H7 death did not exhibit distinctly different behaviors during persistence, and usually exhibited a more concave reduction. High R-square values reinforce the decision to use the Weibull model, rather than other models used in previous chapters.

III. Results and Discussion

i. General trends

Overall, although microbiological counts decreased over the course of the study, survivors persisted and could be a cause of concern for cross contamination. Counts on some materials decreased below the detection limit (-0.1 log CFU/cm^2) prior to the end of storage. When all samples from a material were below the detection limit for more than two consecutive sampling times, sampling was discontinued and assumed to be less than the detection limit. Significant ($P < 0.05$) replication effects were noticed in most of the treatments (Appendix Tables 29-32), though this is not unexpected considering the variation present in the data and the conditions of the study. Because of this, data are discussed as overall survivor trends and results are compared using the GInaFiT analysis and δ and p values, parameters describing time (days) for a 1-log reduction and the concavity of the Weibull curve, respectively.

Most populations on soiled materials decreased at a fairly consistent rate. Though decreases were more rapid in the early days of storage, subsequent decrease in counts for the duration of storage was very similar. Because this behavior seemed generally consistent throughout storage, a Weibull model was selected to describe survival of the bacteria. Selection of the model was validated by consistently high R-squared values generated by the curve.

Some packaging materials and storage conditions seemed to allow for more prolonged persistence of *E. coli* O157:H7 than others, though drawing conclusions from simple numerical comparison is difficult and scientifically invalid. Furthermore, simple comparison of data collected from similar sampling points is cumbersome and offers no insight to the overall behavior of the pathogen.

Using data from the GInaFiT freeware (Geeraerd et al., 2005), risk assessment is given some helpful parameters by which to quantify risk for soiled food packaging materials, namely δ values and p values. δ values describe the time (in days) that it would take for a predicted population to decrease by 1 log CFU/cm^2, while p values are shape parameters describing the concavity or linear nature of a curve. Large values (closer to 1) indicate that a curve will behave in a more linear fashion, whereas small p values indicate a highly concave curve and prolonged persistence, based on the model. Smaller δ values mean that 90% of the population of the pathogen is affected quickly by the conditions tested for

storage, but when small δ values are associated with small p values some level of the pathogen persists for a longer period of time and inactivation will occur less rapidly.

ii. Survival of *E. coli* O157:H7 on soiled food packaging materials inoculated at a high inoculation level and stored at 25º C

E. coli O157:H7 inoculated on soiled packaging materials at a level of 6 log CFU/cm^2 survived for up to 130 days when stored at 25º C. From material to material, amounts of survivors were comparable at all points throughout the study, and survivors were present on all materials at approximately 2 log CFU/cm^2 on day 130 of storage. The general trend of *E. coli* O157:H7 death and persistence on all materials was similar, though PVC overwrap film harbored the lowest mean population of survivors and cardboard harbored the highest (Figure 2.1)(see Appendix Table 33 for combined data results, and Appendix Tables 34 and 35 for individual replicate results, while Appendix Table 36 shows total aerobic plate counts and Appendix Tables 37 and 38 show individual replicates). No material consistently yielded populations at or below the detection limit, though reductions in counts did occur on all materials. In addition, counts collected from TSA plates showed a similar trend of total aerobic bacteria. Though counts were higher, background flora did not behave differently from pathogens collected on TSA+rifampicin (Figure 2.2).

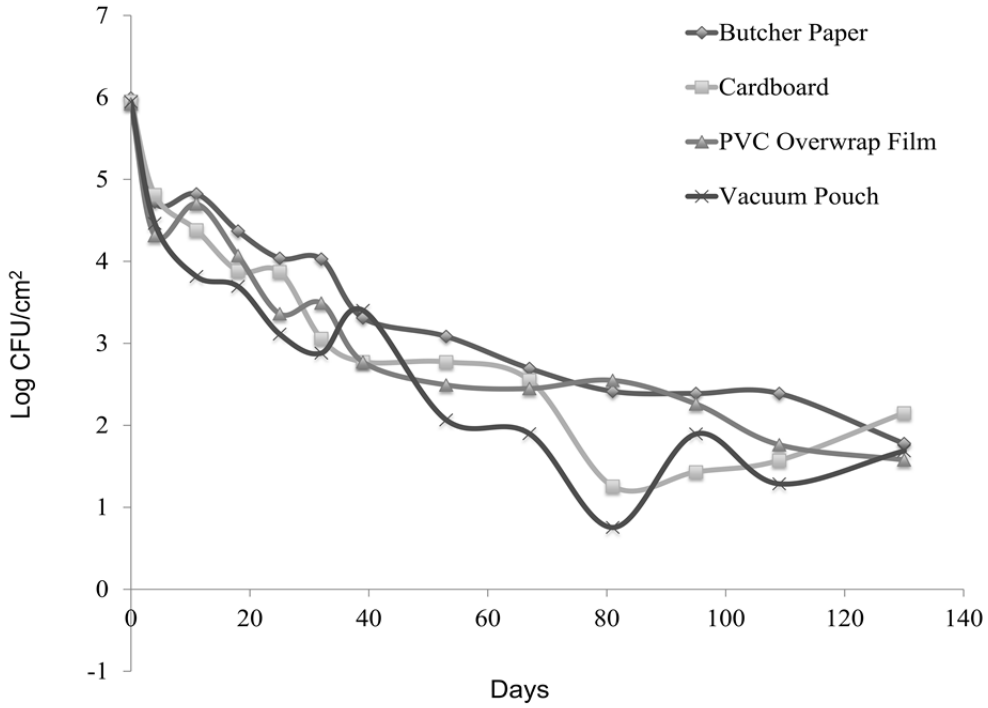

Figure 2.1 – *E. coli* O157:H7 survival (Log CFU/cm^2) on food packaging materials soiled with ground beef homogenate inoculated with approximately 6 log CFU/cm^2 of *E. coli* O157:H7 and stored at 25º C for up to 130 days (Data in Appendix Table 33)

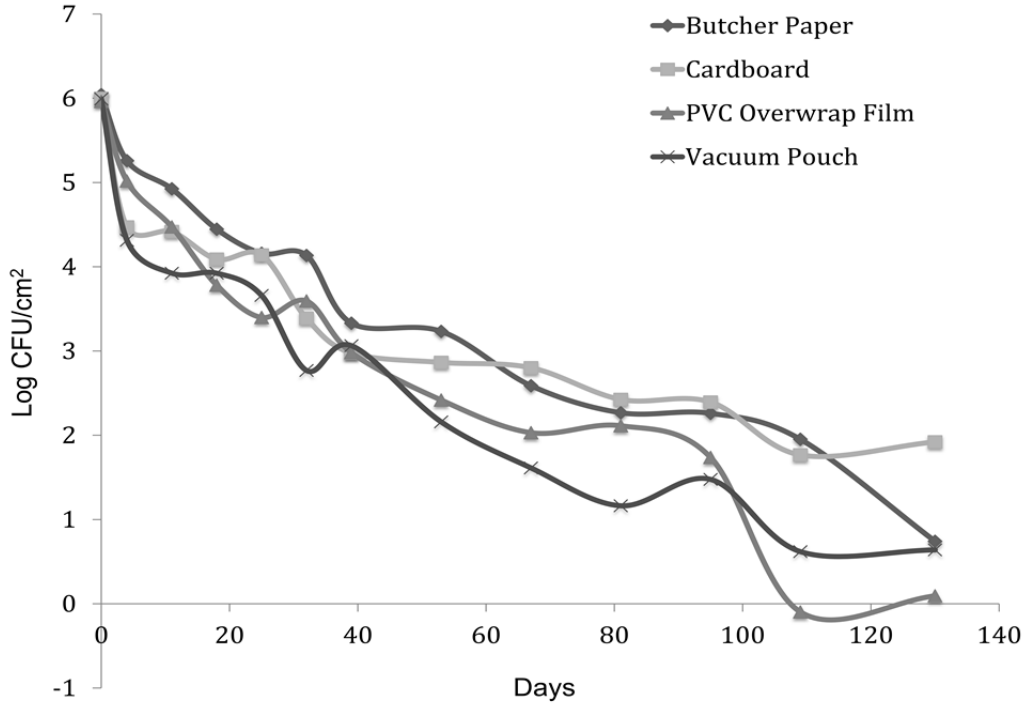

Figure 2.2 – Total aerobic bacteria survival (Log CFU/cm^2) on food packaging materials soiled with ground beef homogenate inoculated with approximately 6 log CFU/cm^2 of *E. coli* O157:H7 and stored at 25º C for up to 130 days (Data in Appendix Table 36)

The overall behavior of the pathogen on various packaging materials can be better compared based on analysis of data by the GInaFiT freeware module for Microsoft Excel, which was used to fit curves to the mean populations of each treatment (Geeraerd et al., 2005).

For the high inoculation levels of *E. coli* O157:H7 on packaging materials stored at 25º C, populations on butcher paper exhibited the highest δ value (5.45 days) (Table 2.1), whereas populations on PVC overwrap film and vacuum pouch material were estimated to have the lowest δ value (1.15 days), indicating more rapid initial population decrease. When comparing p values, butcher paper had the highest estimated value (0.45), meaning that the population decrease was more linear than on other materials, while values given to populations on PVC overwrap film and vacuum pouch material were the lowest (0.34), describing a more concave population curve.

Aside from one replicate of contamination on soiled butcher paper (Table 2.2), all populations exhibited δ values of less than 7, indicating that in less than one week after contamination populations in all treatments (packaging materials) had decreased by 1 log CFU/cm^2. Because δ values are so similar, it is beneficial to compare p parameters. From the results of this analysis, populations on butcher paper had the highest p parameter (0.45), indicating a more linear curve than the other populations (and comparatively less survival as time progressed), whereas populations on vacuum pouch material had the lowest p parameter, predicting slightly higher populations as time of storage progressed.

Additionally, analysis provides a 4-D estimation. This estimates when a 4 log CFU/cm^2 reduction in population will occur for *E. coli* O157:H7 on each material under the given storage conditions. Bacteria on vacuum plastic bags were projected to decrease by 4 log units by 72.8 days of storage, while the same bacteria on butcher paper was estimated to decrease by 4 log units on 122.2 days of storage. This parameter offers simple way of observing which material allows the pathogen to persist for a longer period of time.

Table 2.1 - GInaFiT parameters assigned to *E. coli* O157:H7 survival on food packaging materials soiled with ground beef homogenate inoculated at a level of approximately 6 log CFU/cm^2 and stored at 25º C for up to 130 days (two replicates combined)

	R-square	δ value (Std. Error) (days)	p (Std. Error)	4-D Reduction (± days)
Butcher Paper	0.9732	5.45 (2.40)	0.45 (0.05)	122.2
Cardboard Box Material	0.9200	2.12 (2.07)	0.38 (0.08)	84.5
PVC Overwrap Film	0.9516	2.42 (1.75)	0.37 (0.06)	106.6
Vacuum Pouch Material	0.9105	1.15 (1.34)	0.34 (0.07)	72.8

Table 2.2 - GInaFiT parameters assigned to *E. coli* O157:H7 survival on food packaging material soiled with ground beef homogenate when inoculated at a level of approximately 6 log CFU/cm^2 and stored at 25º C for up to 130 days (two individual replicates)

	R-Square		δ value - days		p parameter		4D Reduction (± days)	
	Rep1	Rep2	Rep1	Rep2	Rep1	Rep2	Rep1	Rep2
Butcher Paper	0.9796	0.9473	8.08 (2.77)	3.39 (2.38)	0.49 (0.05)	0.40 (0.07)	> 130	105.3
Cardboard Box Material	0.8329	0.8996	5.70 (6.72)	1.02 (1.29)	0.38 (0.12)	0.37 (0.09)	> 130	41.6
PVC Overwrap Film	0.9628	0.8295	4.57 (2.49)	0.75 (1.38)	0.44 (0.06)	0.28 (0.09)	104	105.3
Vacuum Pouch Material	0.8895	0.8611	2.02 (2.37)	0.67 (1.10)	0.36 (0.09)	0.32 (0.09)	97.5	53.3

iii. Survival of *E. coli* O157:H7 on soiled food packaging material when inoculated at a high inoculation level and stored at 4º C

Survival of *E. coli* O157:H7 on soiled food packaging material when stored at 4º C mimicked behavior at 25º C on all materials except for butcher paper, though butcher paper still harbored survivors on the 130th day of storage. Figure 2.3 shows the behavior of the pathogen on all packaging materials and shows that the general trend is rapid initial decrease with subsequent persistence (see Appendix Table 39 for results, and tables 40 and 41 for individual replicate results, while Table 42 shows total aerobic plate counts and Tables 43 and 44 show individual replicates).

Most populations do not vary much between the two temperatures, with average values of approximately 3 log CFU/cm^2 at 39 days of storage increasing in variance by day 95 of storage (Appendix Tables 33 and 39). Unlike storage at a higher temperature, *E. coli* O157:H7 on butcher paper decreased in population very rapidly. All other survivor counts were at a level of approximately 2 log CFU/cm^2 on day 130 of storage while the mean population of *E. coli* O157:H7 on butcher paper on day 130 was less than 0.3 log CFU/cm^2.

Analysis on nonselective media showed even less variation between samples (Figure 2.4). Counts were higher, though this may be attributed to background flora present in ground beef prior to inoculation.

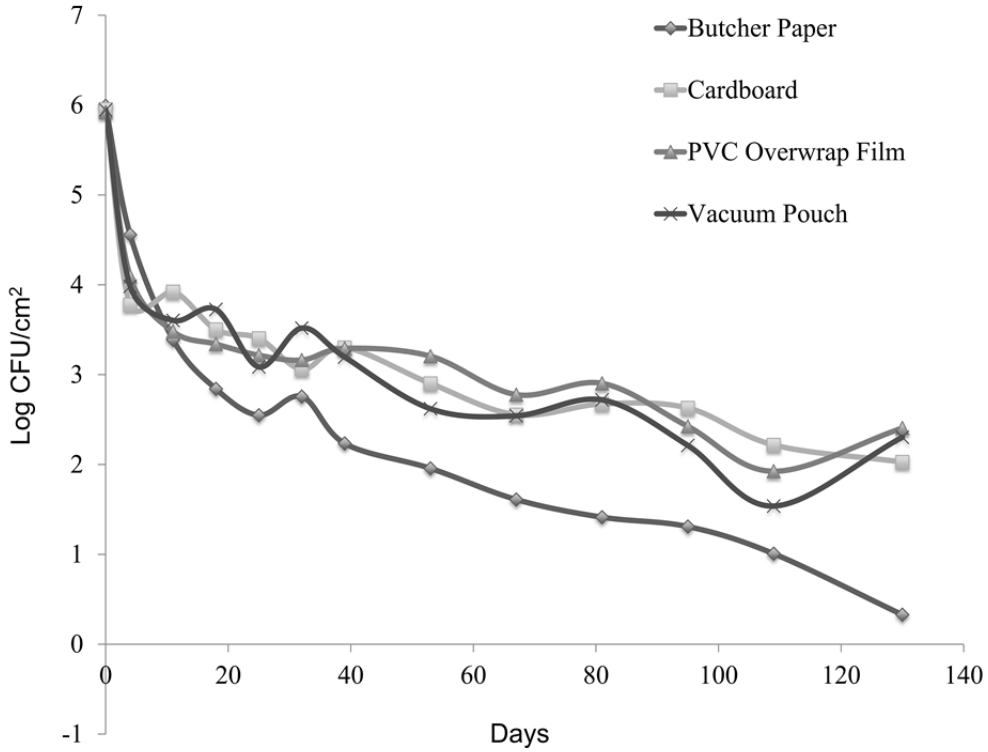

Figure 2.3 – *E. coli* O157:H7 survival (Log CFU/cm^2) on food packaging materials soiled with ground beef homogenate inoculated with approximately 6 log CFU/cm^2 of *E. coli* O157:H7 and stored at 4º C for up to 130 days (Data in Appendix Table 39)

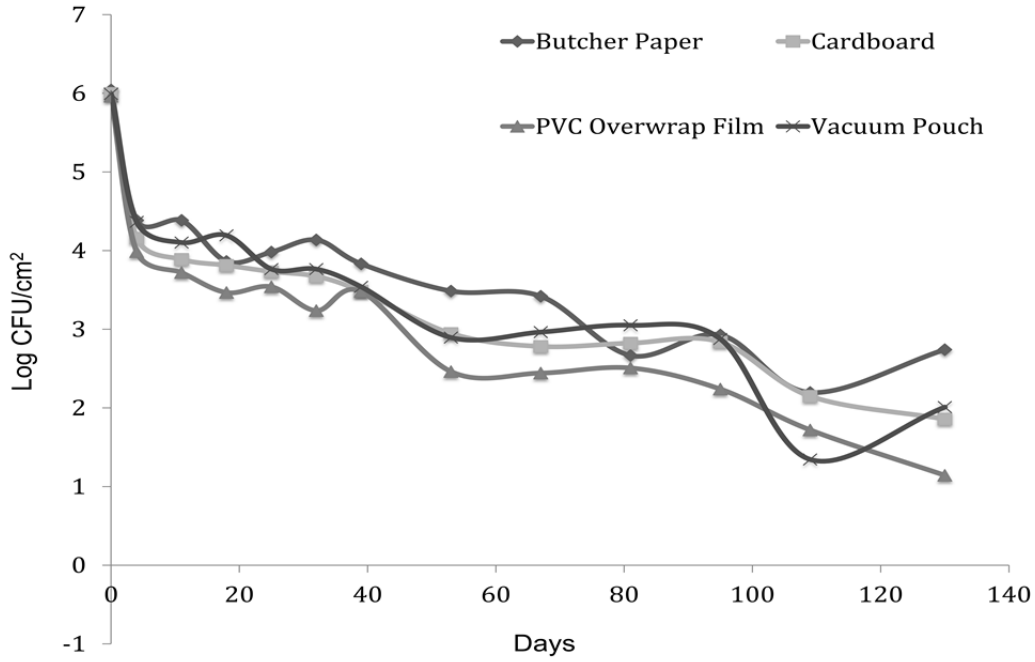

Figure 2.4 – Total aerobic bacteria survival (Log CFU/cm^2) on food packaging materials soiled with ground beef homogenate inoculated with approximately 6 log CFU/cm^2 of *E. coli* O157:H7 and stored at 4º C for up to 130 days (Data in Appendix Table 42)

At 4º C, p values were lower, as were δ values when compared to 25º C counterparts (Table 2.3). This indicates a more rapid decimal decrease in pathogenic population as well as a more pronounced concavity to the curve fit to the data by the GInaFiT module for pathogens on all materials. *E. coli* O157:H7 on butcher paper exhibited the highest p value though also exhibited the largest δ value (0.60 and 0.32, respectively), indicating that within this temperature and inoculation level, the death rate of *E. coli* O157:H7 on butcher paper was the least rapid, but the death of the bacteria was more complete than on other materials. In contrast, PVC overwrap film exhibited the lowest p value and the smallest δ value (0.16 and 0.19, respectively), indicating the most rapid initial decrease in pathogenic population, followed by a slightly greater persistence as time of storage progressed. Overall it is evident that (according to models generated), 4º C storage is initially more lethal to *E. coli* O157:H7 than 25º C ambient temperatures. While δ values in individual replicates ranged from 0.67 to 8.08 for storage at 25º C storage (Table 2.2), pathogens stored at 4º C exhibited values ranging from 0.13 to 0.80 (Table 2.4), with only one replicate showing a δ value of greater than 1 (pathogens on vacuum pouch material, 7.24 days).

Table 2.3 - GInaFiT parameters assigned to *E. coli* O157:H7 survival on food packaging materials soiled with ground beef homogenate inoculated at a level of approximately 6 log CFU/cm^2 and stored at 4º C for up to 130 days (two replicates combined)

	R-square	δ (Std. Error) (days)	p (Std. Error)	4-D Reduction (± days)
Butcher Paper	0.9835	0.60 (0.32)	0.32 (0.03)	49.4
Cardboard Box Material	0.9685	0.25 (0.23)	0.21 (0.03)	> 130
PVC Overwrap Film	0.9502	0.16 (0.21)	0.19 (0.03)	> 130
Vacuum Pouch Material	0.9312	0.43 (0.53)	0.24 (0.05)	> 130

Table 2.4 - GInaFiT parameters assigned to *E. coli* O157:H7 survival on food packaging materials soiled with ground beef homogenate inoculated at a level of approximately 6 log CFU/cm^2 and stored at 4º C for up to 130 days (two individual replicates)

	R-Square		δ value - days		p parameter		4D Reduction (± days)	
	Rep1	Rep2	Rep1	Rep2	Rep1	Rep2	Rep1	Rep2
Butcher Paper	0.9834	0.9373	0.42 (0.24)	0.80 (0.82)	0.31 (0.03)	0.32 (0.06)	39	63.7
Cardboard Box Material	0.9457	0.9231	0.36 (0.40)	0.15 (0.24)	0.23 (0.04)	0.18 (0.04)	> 130	> 130
PVC Overwrap Film	0.8727	0.9109	0.13 (0.29)	0.19 (0.32)	0.18 (0.05)	0.20 (0.05)	> 130	> 130
Vacuum Pouch Material	0.7917	0.8094	0.57 (1.26)	7.24 (9.42)	0.25 (0.09)	0.43 (0.16)	> 130	> 130

iv. Survival of *E. coli* O157:H7 on soiled food packaging materials inoculated at a low inoculation level and stored at 25º C

Soiled food packaging materials with an initial level of *E. coli* O157:H7 of approximately 4 log CFU/cm^2, had survivors even on the 130th day of storage at 25º C (Figure 2.5). Under these conditions, butcher paper material harbored the greatest number of survivors at the end of the trial, while populations on cardboard yielded the smallest counts when sampled at 130 days of storage.

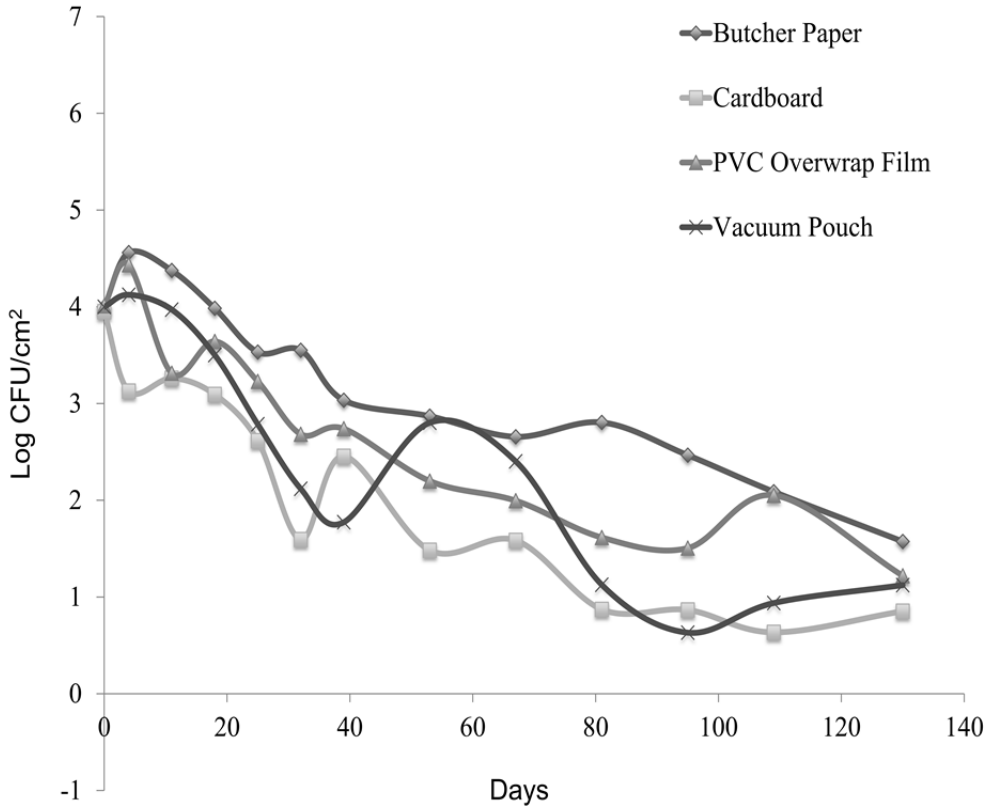

Figure 2.5 – *E. coli* O157:H7 survival (Log CFU/cm^2) on food packaging materials soiled with ground beef homogenate inoculated with approximately 4 log CFU/cm^2 of *E. coli* O157:H7 and stored at 25º C for up to 130 days (Data in Appendix Table 45)

Survivors were collected at a level near 1 log CFU/cm^2 on the last day of sampling, regardless of behavior of the pathogen during the rest of the study (see Appendix Table 45 for results, and tables 46 and 47 for individual replicate results, while Table 48 shows total aerobic plate counts and Tables 49 and 50 show individual replicates), with lowest counts found on cardboard. However, since populations were similar on many days of sampling, further analysis is warranted to discern if certain materials were less hospitable to survivors than others.

While butcher paper yielded higher counts on TSA, it must be noted that not all of the bacteria was *E. coli* O157:H7. Background flora was present, and a lower initial dose of the pathogen may have been instrumental in allowing background flora to survive in higher numbers on this material (Figure 2.6).

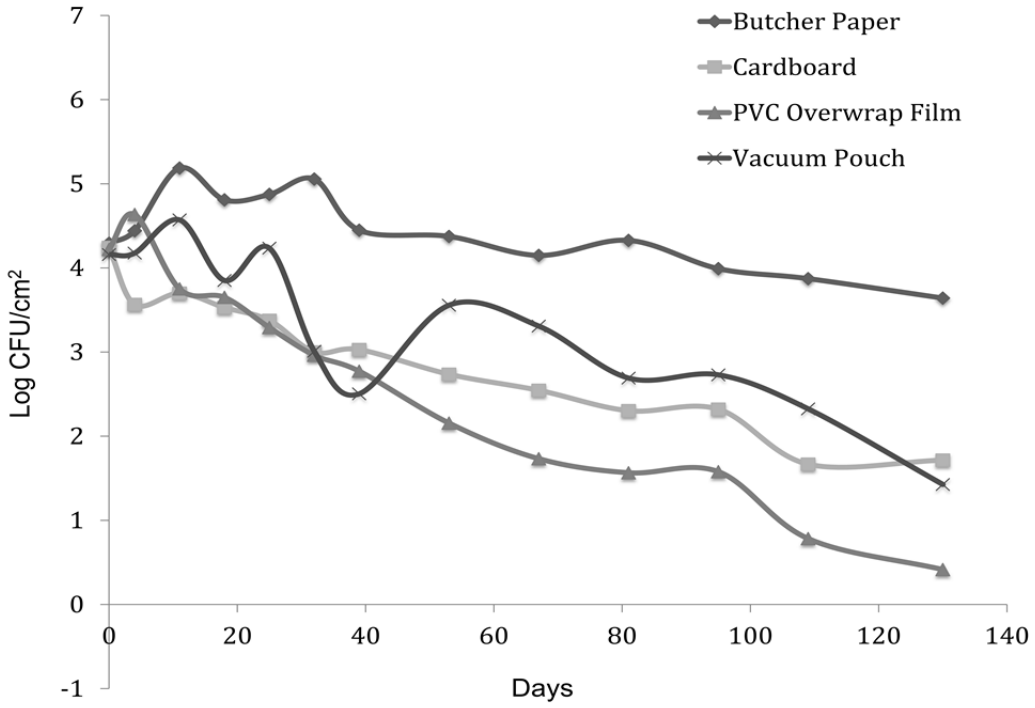

Figure 2.6 – Total aerobic bacteria survival (Log CFU/cm^2) on food packaging materials soiled with ground beef homogenate inoculated with approximately 4 log CFU/cm^2 of *E. coli* O157:H7 and stored at 25º C for up to 130 days (Data in Appendix Table 48)

E. coli O157:H7 cells inoculated on packaging materials at a lower inoculation level (approximately 4 log CFU/cm^2) seemed to mimic the behavior of the higher inoculation treatment, with steady decrease in counts over time. *E. coli* O157:H7 populations on cardboard box material exhibited the smallest δ and p values (10.9 days, 0.51, respectively), indicating a rapid initial decrease in population followed by prolonged persistence. Behavior of the bacteria on soiled butcher paper is described by the largest δ and p values (39.51 days, 0.83, respectively), describing the slowest initial decrease in population with a slightly more linear decrease in population throughout the study than the bacteria inoculated on other materials.

These results show that *E. coli* O157:H7 inoculated at approximately 4 log CFU/cm^2 and stored at 25º C will persist for more time than when inoculated at a higher level or stored at 4º C, regardless of host material. δ values were higher under these conditions than under other storage conditions, as were p values (Table 2.5, 2.6). Butcher paper, according to the Weibull model, did not reduce by 1 log unit until over 41 days of storage, while the smallest δ value describes cardboard box material (3.12 days) (Table 2.6). p parameters were higher under these conditions as well compared to other conditions, showing that death of the pathogen at 25º C followed a very linear trend among all inoculated materials.

Table 2.5 - GInaFiT parameters assigned to *E. coli* O157:H7 survival on food packaging materials soiled with ground beef homogenate inoculated at a level of approximately 4 log CFU/cm^2 and stored at 25º C for up to 130 days (two replicates combined)

	R-square	δ (Std. Error) (days)	p (Std. Error)	4-D Reduction (± days)
Butcher Paper	0.9260	39.51 (12.26)	0.83 (0.19)	> 130
Cardboard Box Material	0.9121	10.90 (7.32)	0.51 (0.12)	> 130
PVC Overwrap Film	0.9035	18.23 (10.57)	0.58 (0.14)	> 130
Vacuum Pouch Material	0.8375	14.96 (12.60)	0.60 (0.20)	> 130

Table 2.6 - GInaFiT parameters assigned to *E. coli* O157:H7 survival on food packaging materials soiled with ground beef homogenate inoculated at a level of approximately 4 log CFU/cm^2 and stored at 25º C for up to 130 days (two individual replicates)

	R-Square		δ value - days		p parameter		4D Reduction (± days)	
	Rep1	Rep2	Rep1	Rep2	Rep1	Rep2	Rep1	Rep2
Butcher Paper	0.9273	0.8849	37.32 (11.96)	41.42 (15.82)	0.82 (0.18)	0.84 (0.24)	> 130	> 130
Cardboard Box Material	0.9000	0.8279	36.64 (14.27)	3.12 (4.33)	0.80 (0.21)	0.40 (0.13)	> 130	100.1
PVC Overwrap Film	0.7659	0.9156	25.12 (21.33)	13.56 (8.21)	0.60 (0.25)	0.56 (0.13)	> 130	> 130
Vacuum Pouch Material	0.7418	0.8168	16.47 (17.93)	15.09 (13.53)	0.59 (0.27)	0.65 (0.24)	> 130	130

v. Survival of *E. coli* O157:H7 on soiled food packaging material when inoculated at a low inoculation level and stored at 4º C

Stored at 4º C, no material consistently yielded mean counts at or below the detection limit during 130 days of storage. Counts of the low inoculum level (4 log CFU/cm^2) were all below 0.5 log CFU/cm^2 on the last day of storage. PVC overwrap film harbored the highest amount of survivors and vacuum pouch material the least (Figure 2.4) (see Appendix Table 51 for results, and tables 52 and 53 for individual replicate results, while Table 54 shows total aerobic plate counts and Tables 55 and 56 show individual replicates). Decreases in the pathogen were more rapid on all packaging materials when compared with other temperature and inoculation level combinations, as is evidenced by lower δ values (time in days for a 1-log reduction). In the same fashion as other conditions of inoculation and storage, however, mean populations on the last day of storage were comparable for all materials. Aerobic bacteria counts, however, did not follow counts of *E. coli* O157:H7 (Figure 2.8). With a lower initial level of the pathogen, background flora may have been able to persist at much greater numbers. This is not entirely unexpected, as the ground beef homogenate used to soil materials was not made sterile prior to inoculation.

Under these conditions, all δ values given to *E. coli* O157:H7 populations were less than 1.00 day of storage (Table 2.7). Small δ values are indicative of extremely rapid initial population decrease, showing that low initial inoculation level, coupled with storage at 4º C is most inhospitable for *E. coli* O157:H7 on soiled packaging materials.

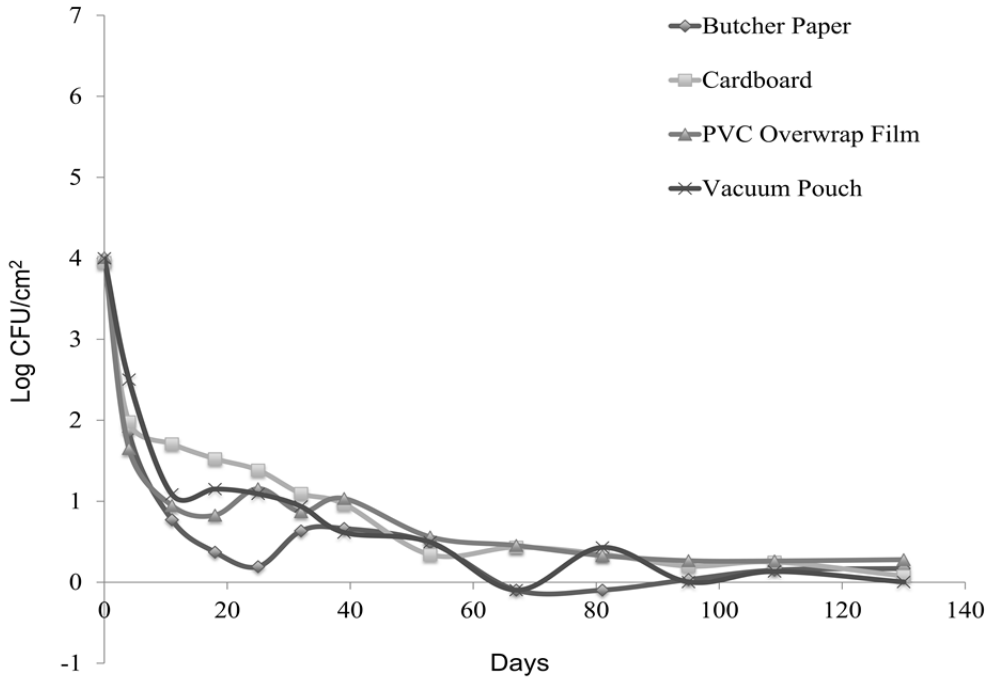

Figure 2.7 – *E. coli* O157:H7 survival (Log CFU/cm^2) on food packaging materials soiled with ground beef homogenate inoculated with approximately 4 log CFU/cm^2 of *E. coli* O157:H7 and stored at 4º C for up to 130 days (Data in Appendix Table 51)

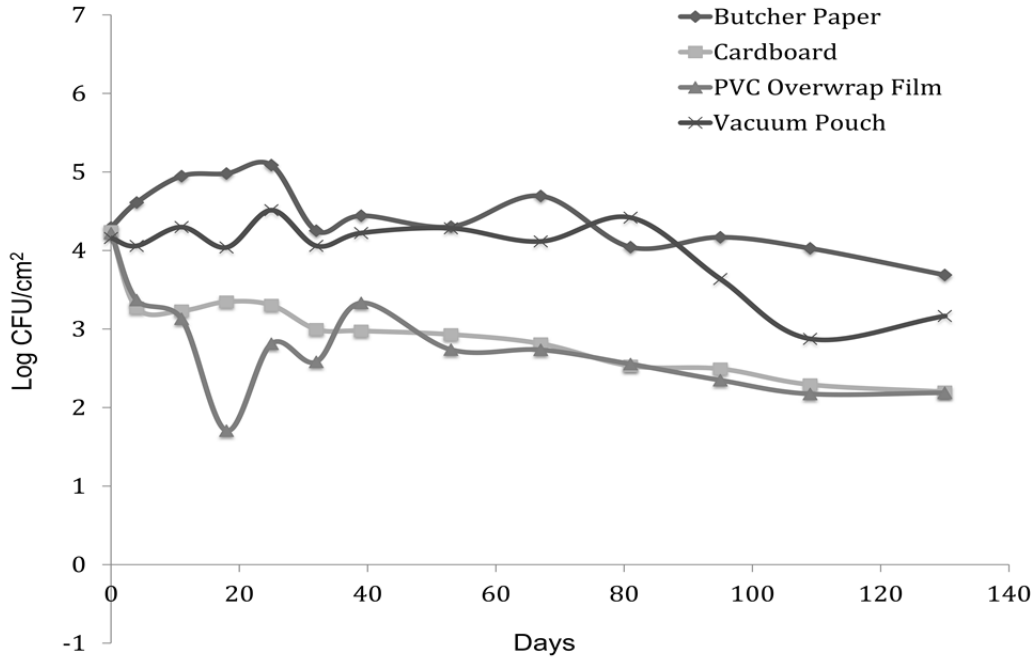

Figure 2.8 – Total aerobic bacteria survival (Log CFU/cm^2) on food packaging materials soiled with ground beef homogenate inoculated with approximately 4 log CFU/cm^2 of *E. coli* O157:H7 and stored at 4º C for up to 130 days (Data in Appendix Table 54)

p parameters were much lower under these conditions than they were under others, meaning that populations initially decrease rapidly with a portion of the population persisting for very long. In addition, δ values were the lower at this temperature/inoculation level combination than they were in any other. According to the models fit to the population data, a 1 log CFU/cm^2 decrease in population was experienced on all materials before 1 day of storage had elapsed. This is more desirable than the values at 25º C, which ranged from less than one week of storage to nearly six weeks of storage before a 1-log reduction was predicted (Table 2.5 and 2.6). *p* parameters on all materials under these conditions were lower than the parameters describing pathogen behavior when inoculated at a higher level, indicating a less rapid continued decrease in counts.

Table 2.7 - GInaFiT parameters assigned to *E. coli* O157:H7 survival on food packaging materials soiled with ground beef homogenate inoculated at a level of approximately 4 log CFU/cm^2 and stored at 4º C for up to 130 days (two replicates combined)

	R-square	δ (Std. Error) (days)	*p* (Std. Error)	4-D Reduction (± days)
Butcher Paper	0.9317	0.00 (0.00)	0.12 (0.03)	100.1
Cardboard Box Material	0.9824	0.27 (0.18)	0.22 (0.02)	> 130
PVC Overwrap Film	0.9748	0.00 (0.00)	0.12 (0.02)	> 130
Vacuum Pouch Material	0.9496	0.12 (0.15)	0.21 (0.03)	100.1

Table 2.8 - GInaFiT parameters assigned to *E. coli* O157:H7 survival on food packaging materias soiled with ground beef homogenate inoculated at a level of approximately 4 log CFU/cm^2 and stored at 4º C for up to 130 days (two individual replicates)

	R-Square		δ value (days)		*p* parameter		4D Reduction (± days)	
	Rep1	Rep2	Rep1	Rep2	Rep1	Rep2	Rep1	Rep2
Butcher Paper	0.9222	0.8528	0.00 (0.00)	0.02 (0.07)	0.10 (0.03)	0.16 (0.05)	> 130	127.4
Cardboard Box Material	0.9848	0.9360	0.47 (0.26)	0.13 (0.19)	0.26 (0.02)	0.19 (0.04)	98.8	> 130
PVC Overwrap Film	0.8712	0.9342	0.00 (0.00)	0.24 (0.33)	0.08 (0.04)	0.21 (0.04)	> 130	> 130
Vacuum Pouch Material	0.9157	0.9414	0.00 (0.01)	0.84 (0.83)	0.14 (0.04)	0.29 (0.05)	84.5	107.9

vi. Discussion

E. coli O157:H7 proved to be a hardy organism, able to survive on a variety of soiled packaging materials regardless of initial inoculation level or temperature of storage. Survival of a microorganism at 25º C in a food substrate is expected, as this temperature is well within ranges that are favorable for survival, protective biofilm formation, and rapid growth (Viazis and Diez-Gonzalez, 2011). These same researchers note that, on fresh produce (lettuce, namely), *E. coli* O157:H7 was also able to survive at 4º C. It was expected, therefore, that the pathogen would persist at some level even when stored at 4º C, though the duration of survival was unexpected.

Data from all of the treatments of this study showed that low amounts of *E. coli* O157:H7 inoculated onto various packaging materials and stored at 4º C have a poorer chance of survival compared with higher initial populations and higher temperatures. Low initial levels of the pathogen stored at 25º C survived in slightly greater numbers on day 130 of

storage compared with storage at 4º C. High levels of the pathogen stored at both 4 and 25º C survived in greater numbers proportional to starting populations than populations inoculated at approximately 4 log CFU/cm^2.

Within each temperature and inoculation level, though, it is difficult to distinguish which packaging material was least hospitable to survival of *E. coli* O157:H7, as the general trends presented in Figures 2.1-2.8 are all quite similar. Pathogen counts on all materials tested behaved similarly and did not consistently die faster on one material than on another across different temperature and inoculation combinations. Rather, the differences between δ values and p parameters seemed more dependent upon the temperature of storage and the initial level of inoculation, and not affected by packaging material.

It should be noted, however, that the homogenate used to soil the packaging materials was not sterile and contained a small level of background contamination, as is evidenced by population data on TSA plates (Appendix Tables 36-38, 42-45, 48-50, 54-56). While competition for resources may occur in a microbiological system, most aerobic plate counts corresponded very closely to populations recovered on TSA+rifampicin, suggesting that presence of background contamination had very little effect on the behavior of the pathogen regardless of initial inoculation level or storage temperature.

Another possible explanation for survival of *E. coli* O157:H7 on these materials is the probability of biofilm formation, whether the colonies were mixed-culture or not.

Multiple studies show that *E. coli* O157:H7 survival in adverse conditions is greatly enhanced by formation of biofilms (Uhlich et al., 2010; Uhlich et al., 2008; Skandamis et al., 2009). Biofilms can function to protect bacteria against adverse environments, though are generally only formed during growth phases of the bacteria (Uhlich et al., 2010). While growth of *E. coli* O157:H7 was seldom detected on soiled food packaging material in this study, rapid death occurred only when initial populations were low and stored at 4º C, suggesting the possibility that biofilm formation may occur before death, therefore protecting the bacteria to some extent.

Evidence of death of *E. coli* O157:H7 on soiled food packaging materials is not to say that the presence of the pathogen at any level is acceptable, as *E. coli* O157:H7 is considered an adulterant in any food product. In addition, results cannot be used to claim that soiled materials are bacteriocidal when exposed to a lower dose of the pathogen. Most alarming in this study, within the higher inoculation level, is the similarity between surviving populations stored at 4º and 25º C. Though a refrigerated temperature is generally held to be safer, or at least more bacteriostatic than room temperature, survival was comparable between the treatments. This furthers the notion that extreme caution and care to keep packaging materials clean must be taken to reduce the risk of cross-contamination to the consumer.

Regardless of inoculation level or temperature of storage, *E. coli* O157:H7 was able to persist for up to 130 days on various packaging materials soiled with ground beef

homogenate. *E. coli* O157:H7, even at low levels, was able to persist for extended periods of time in harsh environments. The bacteria is already regulated and included in consideration of HACCP programs, therefore additional legal measures against the pathogen on packaging materials may be unnecessary. However, Bloomfield et al. state that the infective dose for *E. coli* O157:H7 can be as low as 10 cells, and certain infections have been known to stem from doses of less than 100 total cells (2007). Because of this, extra care must be taken to keep food packaging materials clean and free from sources of contamination.

When designing food processing plants or storage facilities for meat products in retail establishments or in the home, conscious effort to protect packaging material from contamination should be a priority. Firstly, packaging should be stored in a clean, elevated area removed from food storage prior to use, free from exterior contamination. Many establishments already employ this practice, but keeping vehicles of cross-contamination away from food product would serve to limit possibilities of an outbreak, since the pathogen has been demonstrated to survive for extended periods of time, even in low doses. Secondly, when storing food in retail establishments or the home, packages that leak should be removed from the vicinity of other food packages, and areas affected by food soiling should be properly cleaned, effectively preventing otherwise unsoiled packaging materials from contamination. Lastly, any soiled packaging material or suspect sources of contamination should be properly removed from the food supply chain.

Results of this study confirmed that low initial levels of the pathogen coupled with refrigeration temperatures is most detrimental to survival of the pathogen. Though persistence of a pathogen for up to 130 days is not ideal under any circumstances, comparison between all treatments indicates that low initial levels of the pathogen, coupled with refrigeration storage, are most unfavorable to *E. coli* O157:H7.

E. coli O157:H7 can be controlled by way of antimicrobial intervention, whether it be thermal processing, antimicrobial food additives, more stringent pre-slaughter control of equipment, personnel, facilities, and animals (Viazis and Diez-Gonzalez, 2011; Woerner et al., 2006). These methods are important to implement, as van Elsas et al. (2011) show report that *E. coli* O157:H7 is able to persist in adverse environments via genetic adaptation and molecular pathways when high numbers are present. In addition, the same researchers hypothesize that when microbiological diversity is lower, *E. coli* O157:H7 has a greater chance to fill a niche within the community and utilize available resources more easily (van Elsas et al., 2011). As with any microbiological sanitation program, starting with a low microbiological load is ideal and generally leads to less chance of a pathogen reaching the food supply. This is evidenced by the results of our study, where samples inoculated with a lower initial level of *E. coli* O157:H7 and stored at 4º C had a more rapid and complete decrease in population.

IV. Conclusions

Information taken from this experiment is beneficial to develop guidelines for preventing *E. coli* O157:H7 cross contamination due to in processing, retail, and consumer environments. Because *E. coli* O157:H7 contamination on packaging and survival of the pathogen is a risk not generally regarded when developing food safety plans, information of the dangers of handling soiled packaging materials is necessary to prevent illness and outbreaks due to improper handling or a lack of education. Most importantly, this data should be considered when implementing plans to control *E. coli* O157:H7. Care should be taken to ensure that packaging material is kept clean and away from sources of raw beef to prevent soiling of new packaging material. In addition, preventing contamination of beef products, processing equipment, and workers is necessary to keep the pathogen off of food packaging materials. Finally, while these methods hopefully serve to prevent *E. coli* O157:H7 from contaminating packaging material, storage of packaged product in environments kept at 4º C or less will help to prevent growth, high amounts of survival, and subsequent cross contamination of meat products.

Chapter 5: Comparison of the efficacy of decontaminating agents against susceptible and multi-drug resistant *Salmonella* compared to *Escherichia coli* O157:H7 in beef trimmings

Chapter Overview

During hide removal and evisceration of carcasses, as well as subsequent handling of products such as trimmings, beef may become contaminated with a host of bacteria. Of specific concern is the potential presence of *Escherichia coli* O157:H7 and various *Salmonella* serotypes. For this reason, antimicrobial interventions are applied to beef trimmings in order to reduce contamination levels. The purpose of this study was to evaluate the behavior of *Salmonella* serotypes in comparison to *E. coli* O157:H7 on beef trimmings when treated with three common antimicrobials. Five different serovars of *Salmonella* (Newport – Antibiotic Susceptible, Newport – Multi-drug resistant AmpC; and Typhimurium – Antibiotic Susceptible, Typhimurium – Multi-drug resistant, and Typhimurium – Multi-Drug Resistant AmpC) and *E. coli* O157:H7 were separately inoculated on beef chuck trimmings and subsequently treated by dipping in either acidified sodium chlorite (1000ppm), peroxyacetic acid (200ppm), or sodium metasilicate (40000ppm) solutions. Samples were plated on modified MacConkey sorbitol (SMAC) agar (supplemented with 20 mg/L novobiocin and 2.5 mg/L potassium tellurite) for *E. coli* O157:H7, tryptic soy agar with 100 µg/ml rifampicin for *E. coli* O157:H7, xylose lysine deoxycholate agar for *Salmonella* serovars, and trypic soy agar for total plate counts; corresponding colony counts were obtained after 24, 48, or 72 h of incubation. The pH of samples and percent weight changes due to treatment were also measured. Log counts and pH data were analyzed with SAS using proc GLM. There was a

significant reduction ($p \leq 0.05$) of counts on samples treated with every antimicrobial intervention, but rarely a significant difference ($p \leq 0.05$) when comparing *Salmonella* serotypes to *E. coli* O157:H7; the only differences resulting from different initial inoculation levels. There also existed very few significant differences ($p \leq 0.05$) within *Salmonella* serotypes when compared to each other. Because the antimicrobials tested were as effective against various *Salmonella* serotypes as they were against *E. coli* O157:H7, it may be concluded that the tested interventions, if used in a meat processing facility against *E. coli* O157:H7 may also be considered effective for control of antibiotic susceptible and resistant *Salmonella* serotypes.

I. Introduction

Escherichia coli O157:H7 has been implicated as a cause of foodborne illness in the United States (Scallan et al., 2011). As such, presence of the pathogen is illegal in meat products (USDA-FSIS Directive 10,010.1). Evidence of fecal contamination or other gastrointestinal contents on animal carcasses is guarded against by way of a zero-tolerance policy, as pathogens are frequently harbored in fecal material (Viazis and Diez-Gonzalez, 2011). In addition to trimming visible contamination from animal carcasses, decontaminating antimicrobials are frequently applied to carcasses for the purpose of controlling *E. coli* O157:H7. Recent outbreaks, however, have not been limited to *E. coli* O157:H7, and both antibiotic susceptible and resistant *Salmonella* serotypes have been implicated as causes of foodborne illness (Scallan et al., 2011).

Salmonella is the cause of a large portion of reported foodborne illness in the United States (Scallan et al., 2011). Similar to *E. coli* O157:H7, *Salmonella* belongs to the *Enterobacteriaceae* family, and contamination of meat products generally occurs during hide removal and evisceration of meat animals. It is most problematic for foods that will not be thermally processed and may be undercooked. Similar to contamination of *E. coli* O157:H7, introduction of *Salmonella* can occur from a range of sources, whether that be fecal, cross-contamination from equipment (Smetlzer et al., 1980), or even lymph material incorporated into ground product (Arthur et al., 2008). Because of this threat, antimicrobial interventions are frequently applied to beef animal carcasses to reduce pathogens such as *E. coli* O157:H7 and *Salmonella*.

Some *Salmonella* serotypes have been shown to possess drug-resistant properties (Antunes et al., 2003). However, Bacon et al. (2003) reported that resistance or susceptibility to antibiotics has little to do with survival of *Salmonella* when challenged with decontaminating treatments commonly used in the meat industry. Because of the threat of *Salmonella* outbreaks in the food supply, it is necessary to compare the effects of decontaminating antimicrobials against antibiotic susceptible and resistant serotypes of *Salmonella* to *E. coli* O157:H7.

Selected for this study were three common antimicrobials: acidified sodium chlorite (1000ppm, 25±2 º C), peroxyacetic acid (200ppm, 25±2 º C), and sodium metasilicate (40000ppm, 25±2 º C). Acidified sodium chlorite, although acidic, functions primarily as an oxidizing agent. Peroxyacetic acid is classified as an organic acid, functioning by

altering the pH of the product to be treated. Sodium metasilicate has a very high pH and is used as an alkaline cleaner (Sharma and Beuchat, 2004). These antimicrobials have potential to be effective against *E. coli* O157:H7 when applied to whole beef carcasses prior to chilling, or applied via spray application to beef primal and subprimal cuts (Quilo et al., 2009; Pohlman et al., 2007; King et al., 2005). The objective of the study, therefore, was to evaluate whether these interventions known for reducing *E. coli* O157:H7 contamination on beef trimmings are as effective in reducing contamination of multiple drug resistant (MDR) and susceptible *Salmonella* Newport and *Salmonella* Typhimurium on beef trimmings, and to evaluate possible differences between populations of antibiotic susceptible and resistant serotypes after chemical decontamination treatments.

II. Materials and methods

i. Culture preparation

Escherichia coli O157:H7 strains used in the study include ATCC 43895, C1-057, C1-072, and C1-109. All inocula of these strains were prepared to be rifampicin-resistant according to Kaspar and Tamplin (1993). *Salmonella* Newport strains tested included antibiotic susceptible strains CVM N4505, CVM N18445, CVM N1509, and FSL S5-639. *Salmonella* Newport, multi-drug resistant, AmpC strains included CVM 22698, CVM N19852, FSL S5-436, and FSL S5-920. *Salmonella* Typhimurium, antibiotic susceptible strains included CVM N7300, CVM N15788, CVM N18534, and FSL S5-536. *Salmonella* Typhimurium, multi-drug resistant strains included CVM N6431, CVM 30662, FSL R6-215, and FSL R8-2540. Lastly, *Salmonella* Typhimurium, multi-drug

resistant, AmpC strains used included CVM N176, CVM 33831, CVM 30034, and FSL S5-786. Table 3.1 shows the sources and antibiotic resistance profiles of *Salmonella* strains.

Each *Salmonella* strain was kept on XLD agar (Xylose Lysine Deoxycholate; Acumedia, Neogen Corporation, Lansing MI) until three days prior to the experiment. One single colony was aseptically selected from the agar with a flame-sterilized, air-cooled loop and activated in 10 ml of TSB (Difco, Becton Dickinson, Sparks, MD) by incubating at 35º C for 24 hours, with the exception of the *E. coli* O157:H7 strains, which were grown on TSA+Rif agar (Acumedia, Neogen Corporation, Lansing MI, plus 5 ml Rifampicin supplement for a concentration of 100 µg/ml) and then activated in TSB+Rif broth (Difco, Becton Dickinson, Sparks, MD with 50 µl Rifampicin supplement, 100 µg/ml). After 24 h of incubation, 0.1 ml of the cultured broth was subcultured into fresh TSB (or TSB+Rif) and placed in an incubator set at 35º C for an additional 24 h.

Table 3.1: Sources and antibiotic resistance profiles of *S.* Newport and *S.* Typhimurium strains

Salmonella Serotype	Strain	Source	Antibiotic Resistance *	Phenotype: Susceptible/MDR/MDR-AmpC	Provided by
Newport	CVM N4505	Ground Turkey	S	Susceptible	Dr. Zhao
	CVM N18445	Ground Beef	S	Susceptible	Dr. Zhao
	CVM N1509	Ground Turkey	S	Susceptible	Dr. Zhao
	FSL S5-639	Human	S	Susceptible	Dr. Wiedmann
	CVM 22698	Pork Chop	AMP, CHL, STR, FIS, TET, AUG2, XNL, AXO, FOX, SXT	MDR-AmpC	Dr. Zhao
	CVM N19852	Ground Beef	AMP, CHL, STR, FIS, TET, AUG2, XNL, AXO, FOX, KAN	MDR-AmpC	Dr. Zhao
	FSL S5-436	Bovine	AMP, CHL, STR, FIS, TET, AUG2, XNL, AXO, FOX, KAN	MDR-AmpC	Dr. Wiedmann
	FSL S5-920	Bovine	AMP, CHL, STR, FIS, TET, AUG2, XNL, AXO, FOX, KAN	MDR-AmpC	Dr. Wiedmann
Typhimurium	CVM N7300	Chicken Breast	S	Susceptible	Dr. Zhao
	CVM N15788	Ground Beef	S	Susceptible	Dr. Zhao
	CVM N18534	Chicken Breast	S	Susceptible	Dr. Wiedmann
	FSL S5-536	Human	S	Susceptible	Dr. Wiedmann
	CVM N6431	Chicken Breast	AMP, CHL, STR, FIS, TET	MDR	Dr. Zhao
	CVM 30662	Chicken Breast	AMP, CHL, STR, FIS, TET	MDR	Dr. Zhao
	FSL R6-215	Human	AMP, CHL, STR, FIS, TET	MDR	Dr. Wiedmann

FSL R8-2540	Human	AMP, CHL, STR, FIS, TET	MDR	Dr. Wiedmann
CVM N176	Chicken Breast	AMP, CHL, STR, FIS, TET, AUG2, XNL, AXO, FOX	MDR-AmpC	Dr. Zhao
CVM 33831	Cattle	AMP, CHL, STR, FIS, TET, AUG2, XNL, AXO, FOX, SXT, KAN	MDR-AmpC	Dr. Zhao
CVM 30034	Ground Turkey	AMP, CHL, STR, FIS, TET, AUG2, XNL, AXO, FOX, NAL, KAN	MDR-AmpC	Dr. Zhao
FSL S5-786	Bovine	AMP, CHL, STR, FIS, TET, AUG2, XNL, AXO, FOX, KAN	MDR-AmpC	Dr. Wiedmann

* Per results of the Sensititre® antimicrobial susceptibility system CMV2AGNF panel (Trek Diagnostic Systems). Antibiotics included on the panel include ampicillin (AMP), amoxicillin/clavulanic acid (AUG2), cefoxitin (FOX), ceftiofur (XNL), ceftriaxone (AXO), chloramphenicol (CHL), ciprofloxacin (CIP), gentamicin (GEN), kanamycin (KAN), nalidixic acid (NAL), streptomycin (STR), sulfisoxazole (FIS), tetracycline (TET), trimethoprim/sulfamethoxazole (SXT)
MDR: resistant to at least ampicillin, chloramphenicol, streptomycin, sulfisoxazole, and tetracycline (ACSSuT)
MDR-AmpC: resistant to at least ACSSuT, amoxicillin-clavulanic acid and ceftiofur, and a decreased susceptibility to ceftriaxone (MIC ≥2 μg/ml) S: sensitive to all tested antibiotics

Source: Geornaras et al., 2011

The day of the experiment, all strains in a serotype were combined in centrifuge tubes and centrifuged (Eppendorf 5810 R, 4° C, 4,629 x g). The supernatant was discarded and the pellets were washed in Phosphate Buffered Saline (PBS; Fisher Scientific, Fair Lawn, NJ). The centrifugation cycle was repeated once, the supernatant discarded, and the pellets resuspended in 40 ml PBS and mixed by vortexing before serial dilutions in PBS were made to achieve an inoculum level of approximately 5-6 log CFU/ml.

ii. Beef trimmings preparation

Beef chuck rolls were obtained the week of the experiment from a local processor prior to the application of any subprimal antimicrobial intervention, and transported chilled to the Colorado State University Meat Science Laboratory. Beef chuck rolls were fabricated into 1x5x10 cm (thickness x width x length) trim samples and individual weights were recorded.

iii. Decontamination solution preparation

Depending on the day of the experiment, acidified sodium chlorite (Ecolab, St. Paul, MN, 1000ppm, 25±2 º C, pH 2.5 ± 0.0), peroxyacetic acid (Ecolab, 200ppm 25±2 º C, pH 3.8 ± 0.1), and sodium metasilicate (Danisco, New Century, KS, 40000ppm, 25±2 º C, pH 12.6 ± 0.1) were tested for efficacy. Each antimicrobial was prepared in sterile deionized water according to the manufacturer's instructions in sterile graduated cylinders less than 30 minutes prior to application of treatment to beef trimming samples and stored in sterile autoclaved containers.

iv. Inoculation of beef trimmings

In a biosafety cabinet, one side of the beef trimmings was spot-inoculated with a 100 µl cocktail of either *Salmonella* or *E. coli* O157:H7, achieving a final inoculum level of approximately 3 log CFU/cm^2. The trimmings were placed in a 4º C walk-in cooler for 10 minutes, then the opposite side was inoculated and placed back in the cooler for an additional 10 minutes. The trimmings were allowed a total of 20 minutes for attachment of the bacteria.

v. Treatment of contaminated beef trimmings

At the end of the prescribed attachment period, the trimmings were removed and either sampled or treated with a decontaminant. Untreated samples were placed in 100 ml of Dey-Engley (DE) neutralizing broth (Becton, Dickinson and Company, Sparks, MD) and masticated for 120 s in a mechanical agitator (6 strokes per second) (Masticator, IUL Instruments, Barcelona, Spain). Samples to which antimicrobial treatments were applied were treated for 30 seconds via dipping in a new Whirl-Pak bag containing 150 ml of decontaminating solution (Whirl-Pak, Modesto, CA) then allowed to drain for 60 seconds in a sterile colander suspended over a container to capture drained liquid. After treatment, the beef trimmings were placed in sterile Whirl-Pak bags and stored at 4º C for one hour before microbiological sampling to simulate time gaps in the meat industry between fabrication and processing of beef trim.

vi. Sampling of contaminated beef trimmings

Escherichia coli O157:H7 colonies were enumerated on modified MacConkey Sorbitol (SMAC) agar (mSMAC, MacConkey Sorbitol Agar, Difco, Becton and Dickinson, Sparks, MD modified with 20 mg/l novobiocin, and 2.5 mg/l Potassium Tellurite, Sigma-Aldrich, St. Louis, MO), Tryptic Soy agar (TSA) supplemented with 100 μg/ml rifampicin, and TSA agar (TSA, Acumedia, Neogen Corporation, Lansing MI). *Salmonella* colonies were enumerated on xylose lysine deoxycholate (XLD) agar, and TSA. In addition to inoculated samples, background flora was determined without treatment or inoculation on TSA, TSA+Rif, XLD, and mSMAC agar. Samples were serially diluted (10-fold) with 0.1% buffered peptone water (Difco, Becton and Dickinson, Franklin Lakes, NJ). TSA+Rif and XLD agar were incubated at 35º C for 24 hours prior to counting, mSMAC agar was incubated at 35º C for 48 hours prior to counting, and TSA agar was incubated for 72 hours at 25º C prior to counting. After incubating and counting colonies, data was entered into an Excel spreadsheet (Microsoft Excel 2008 for Mac) and converted to log CFU/cm^2.

The pH of decontaminating solutions and samples, both treated and untreated, was measured with a pH meter (UB-5 UltraBasic pH Meter, Denver Instrument, Arvada, CO). For each treatment, 24 hour pH samples were taken to evaluate the pH effect of the antimicrobials after 24 h of refrigerated storage. Values were combined and reported as mean values.

In addition, moisture uptake was calculated by weighing the samples prior to inoculation, recording the weight, and then weighing the samples after treatment and draining. Findings are expressed as a percentage increase over the untreated sample's weight.

vii. Statistical analysis of data

Three samples were analyzed per replicate, two replicate studies were conducted, for a total of six samples for each treatment. Results were analyzed in SAS (Version 9.2, SAS, Inc., Cary, NC) using the Proc MIXED procedure to compare *Salmonella* serovars to the reference organism, *E. coli* O157:H7. The student-based t-test in the proc GLM procedure was also used to compare mean counts before and after treatment of each serotype within each antimicrobial. For both analyses, *P* values smaller than 0.05 were considered to be statistically significant differences. Finally, using the Proc GLM procedure with Tukey's HSD separation of means (α 0.05), pairwise comparisons were made between all *Salmonella* serotypes and within *Salmonella* serovars, both antibiotic resistant and susceptible. *P*-values smaller than 0.05 were considered to be statistically significant.

III. Results and discussion

i. Effect of acidified sodium chlorite

Salmonella serotypes, when treated with acidified sodium chlorite (1000ppm) were not statistically different ($P \geq 0.05$) from *E. coli* O157:H7 (Figures 3.1-3.3), with the slight exception of *Salmonella* Typhimurium, multidrug resistant AmpC. However, this could

be attributed to a significantly lower ($P \geq 0.05$) starting population. It can be concluded, therefore, that while some *Salmonella* serotypes exhibit a statistically different ($P \geq 0.05$) population from *E. coli* O157:H7, when treated with ASC (1000ppm) for 30s, behavior of *Salmonella* serotypes, both antibiotic resistant and susceptible, is similar to that of *E. coli* O157:H7.

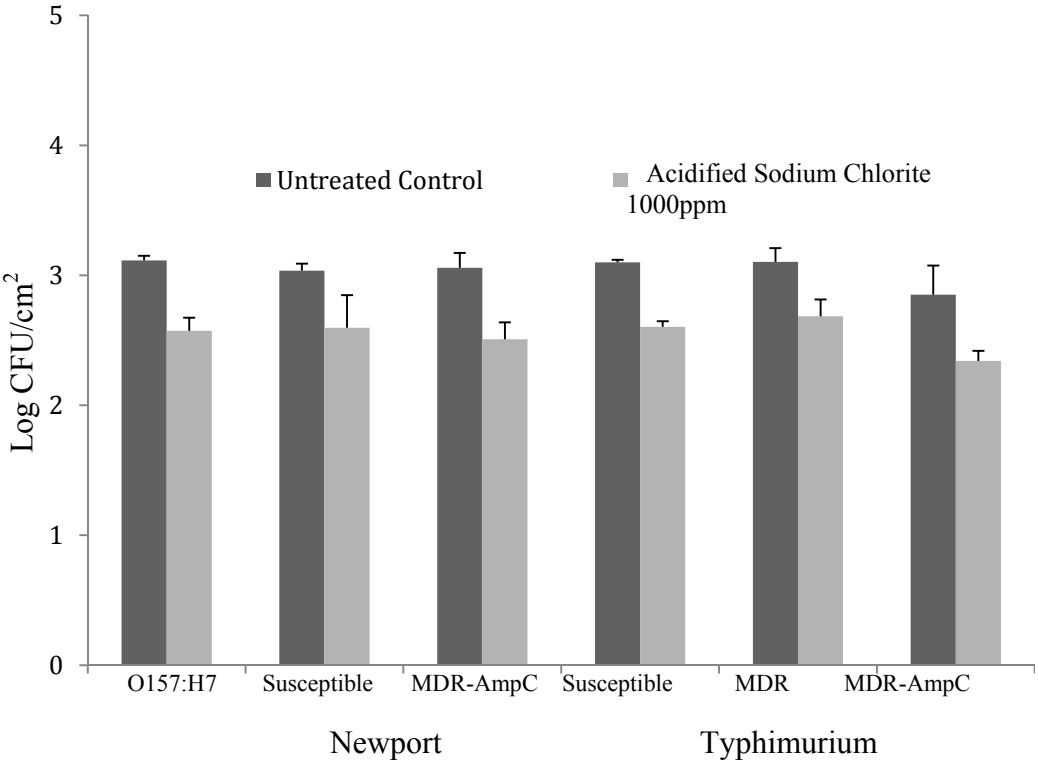

Figure 3.1 - Reduction of *Salmonella* serotypes (antibiotic susceptible, multi-drug resistant, and multi-drug resistant; AmpC) and *Escherichia coli* O157:H7 on inoculated beef trimmings when treated with acidified sodium chlorite (dipping 30s) (1000ppm) (TSA+rif/XLD) (Data in Appendix Table 57)

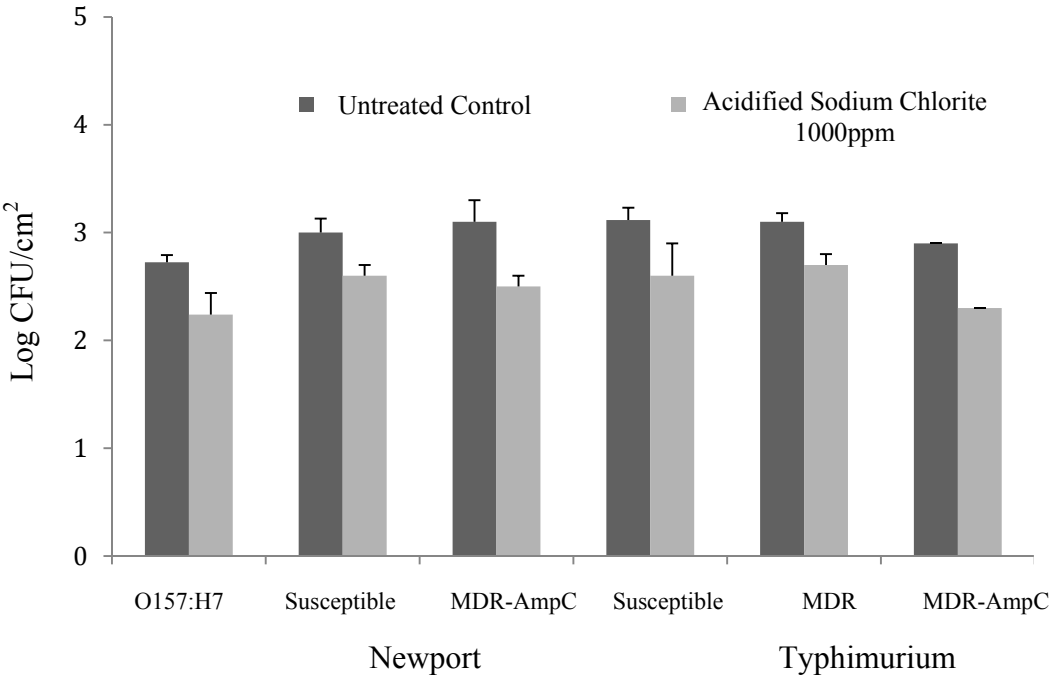

Figure 3.2 - Reduction of *Salmonella* serotypes (antibiotic susceptible, multi-drug resistant, and multi-drug resistant; AmpC) and *Escherichia coli* O157:H7 on inoculated beef trimmings when treated with acidified sodium chlorite (dipping 30s) (1000ppm) (mSMAC/XLD) (Data in Appendix Table 57)

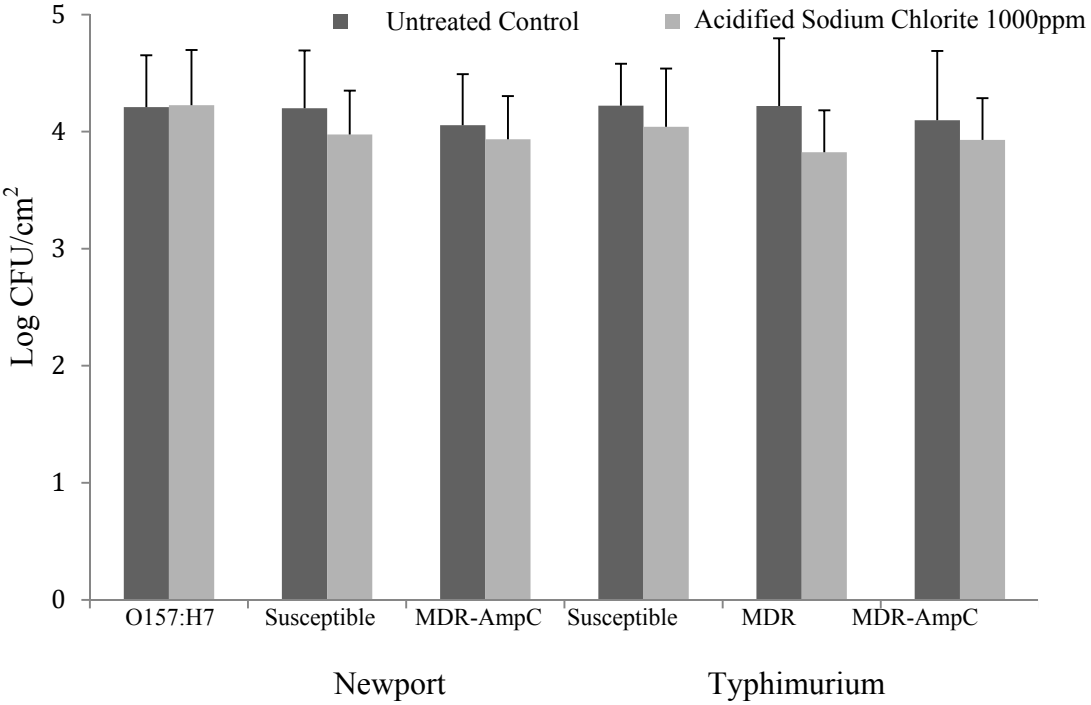

Figure 3.3 - Total aerobic plate count reductions (*E. coli* O157:H7, *Salmonella* serotypes: antibiotic susceptible, multi-drug resistant, and multi-drug resistant; AmpC) on inoculated beef trimmings when treated with acidified sodium chlorite (30s)(1000ppm) (TSA) (Data in Appendix Table 57)

In addition, when analyzed within individual serotypes, *Salmonella* serotypes exhibited minimal variation (Table 3.2). *S.* Typhimurim MDR AmpC was statistically different ($P \leq 0.05$) from all other serotypes of *Salmonella* when treated with ASC, but this may be attributed to a statistically lower ($P \leq 0.05$) population at the time of inoculation.

Within *Salmonella* populations, log reductions ranged from 0.4 (*S.* Newport, antibiotic susceptible; Typhimurium MDR) to 0.6 (*S.* Newport MDR-AmpC and Typhimurium MDR AmpC). Minimal variation in log reduction also suggests that the antibiotic resistant serotypes of the pathogen behave similarly to antibiotic susceptible serotypes.

Table 3.2: Comparisons of means (± standard deviation) of multi-drug resistant (MDR) and susceptible *Salmonella* serotypes (recovered on xylose lysine deoxycholate agar) for beef trimmings before and after treatment with acidified sodium chlorite (1000 ppm) for 30 s.

Serotype	Untreated Control	Acidified Sodium Chlorite (1000ppm)
S. Newport Susceptible	3.0 ± 0.1 ab	2.6 ± 0.1 a
S. Newport MDR-AmpC	3.1 ± 0.2 ab	2.5 ± 0.1 ab
S. Typhimurium Susceptible	3.1 ± 0.1 a	2.6 ± 0.3 a
S. Typhimurium MDR	3.1 ± 0.1 a	2.7 ± 0.1 a
S. Typhimurium MDR AmpC	2.9 ± 0.0 b	2.3 ± 0.0 b

Values (mean ± standard deviation) within each column followed by a different lowercase letter are significantly different ($P \leq 0.05$)

Table 3.3: Comparisons of means (± standard deviation) of multi-drug resistant (MDR) and susceptible *Salmonella* Typhimurium (recovered on xylose lysine deoxycholate agar) for beef trimmings before and after treatment with acidified sodium chlorite (1000 ppm) for 30 s.

Serotype	Untreated Control	Acidified Sodium Chlorite (1000ppm)
S. Typhimurium Susceptible	3.1 ± 0.1 a	2.6 ± 0.3 a
S. Typhimurium MDR	3.1 ± 0.1 a	2.7 ± 0.1 a
S. Typhimurium MDR AmpC	2.9 ± 0.0 b	2.3 ± 0.0 b

Values (mean ± standard deviation) within each column followed by a different lowercase letter are significantly different ($P \leq 0.05$)

Table 3.4: Comparisons of means (± standard deviation) of multi-drug resistant (MDR) and susceptible *Salmonella* Newport (recovered on xylose lysine deoxycholate agar) for beef trimmings before and after treatment with acidified sodium chlorite (1000 ppm) for 30 s.

Serotype	Untreated Control	Acidified Sodium Chlorite (1000ppm)
S. Newport Susceptible	3.0 ± 0.1 a	2.6 ± 0.1 a
S. Newport MDR-AmpC	3.1 ± 0.2 a	2.5 ± 0.1 a

Values (mean ± standard deviation) within each column followed by a different lowercase letter are significantly different ($P \leq 0.05$)

These results imply that, if ASC has been validated to reduce *E. coli* O157:H7 contamination on beef trimmings, it is effective in reducing populations of *Salmonella*, whether they are resistant or susceptible to antibiotics. The most important finding of this aspect of the study is that pathogens did not behave differently from species to species or serotype to serotype.

ii. Peroxyacetic acid

Reductions ($P \leq 0.05$) were experienced across all *E. coli* O157:H7 samples as well as *Salmonella* samples when treated with peroxyacetic acid (200ppm). No *Salmonella* serotypes exhibited a significantly ($P \geq 0.05$) lower count from *E. coli* O157:H7 when treated with PAA, except for Typhimurium MDR, though differences may be attributed to higher starting populations. All treated serotypes exhibited a decrease in population of 0.5 to 0.7 log CFU/cm^2 regardless of starting values, including counts on mSMAC (Figures 3.4, 3.5, and 3.6). Despite statistically different ($P \geq 0.05$) populations (before and after treatment), reductions were numerically similar. Regardless of drug resistance or susceptibility, *Salmonella* serotypes responded similarly to *E. coli* O157:H7 when treated with PAA.

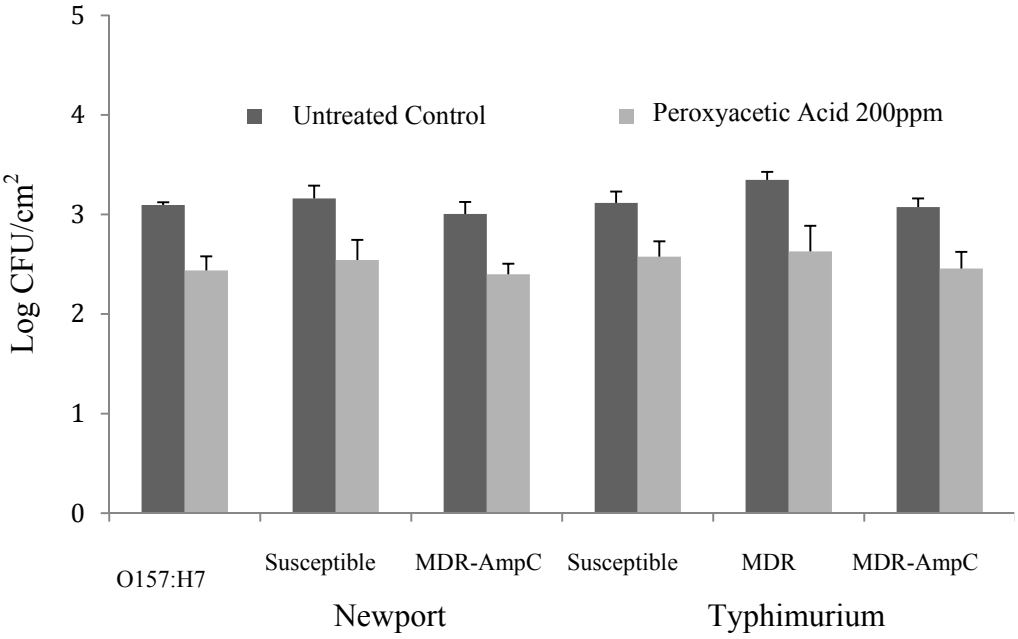

Figure 3.4 - Reduction of *Salmonella* serotypes (antibiotic susceptible, multi-drug resistant, and multi-drug resistant; AmpC) and *Escherichia coli* O157:H7 on inoculated beef trimmings when treated with peroxyacetic acid (dipping 30s) (200ppm) (TSA+rif/XLD) (Data in Appendix Table 58)

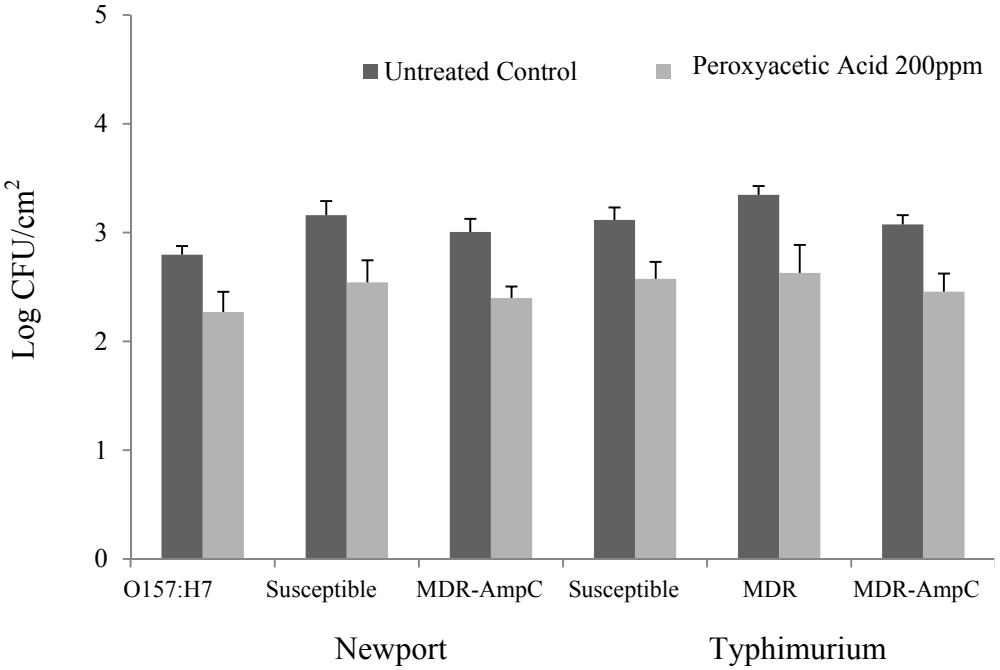

Figure 3.5 - Reduction of *Salmonella* serotypes (antibiotic susceptible, multi-drug resistant, and multi-drug resistant; AmpC) and *Escherichia coli* O157:H7 on inoculated beef trimmings when treated with peroxyacetic acid (dipping 30s) (200ppm) (mSMAC/XLD) (Data in Appendix Table 58)

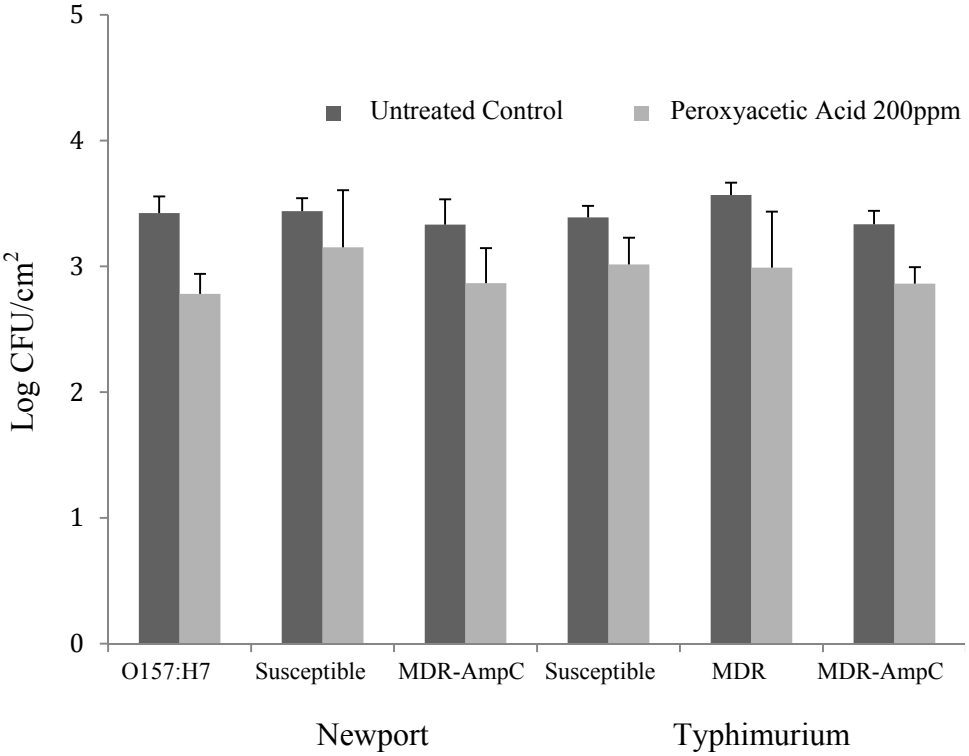

Figure 3.6 - Total aerobic plate count reductions (*E. coli* O157:H7, *Salmonella* serotypes: antibiotic susceptible, multi-drug resistant, and multi-drug resistant; AmpC) on inoculated beef trimmings when treated with peroxyacetic acid (30s)(200ppm) (TSA) (Data in Appendix Table 58)

Within individual *Salmonella* serotypes, *S.* Typhimurium MDR exhibited a significant difference ($P \leq 0.05$) from all other tested serotypes before treatment with PAA for 30 s (Table 3.5). However, after treatment, no significant difference ($P \leq 0.05$) existed between any of the serotypes. *S.* Typhimurium is not more susceptible to PAA than other *Salmonella* serotypes, since log reductions in populations were all numerically similar (Tables 3.6 and 3.7). With this information in mind, it may be said that antibiotic-resistant *Salmonella* serotypes are not different in response to PAA than antibiotic susceptible serotypes.

Each antimicrobial was applied on different days with a different control group. Therefore, while results from this study cannot be analyzed statistically to determine if reduction achieved by antimicrobials are different, numerical comparison of reductions is able to offer insight to the response of *E. coli* O157:H7 and various *Salmonella* serotypes.

Table 3.5: Comparisons of means (± standard deviation) of multi-drug resistant (MDR) and susceptible *Salmonella* serotypes (recovered on xylose lysine deoxycholate agar) for beef trimmings before and after treatment with peroxyacetic acid (200 ppm) for 30 s.

Serotype	Untreated Control	Peroxyacetic Acid (200ppm)
S. Newport Susceptible	3.2 ± 0.1 b	2.5 ± 0.2 a
S. Newport MDR-AmpC	3.0 ± 0.1 b	2.4 ± 0.1 a
S. Typhimurium Susceptible	3.1 ± 0.1 b	2.6 ± 0.2 a
S. Typhimurium MDR	3.3 ± 0.1 a	2.6 ± 0.3 a
S. Typhimurium MDR AmpC	3.1 ± 0.1 b	2.5 ± 0.2 a

Values (mean ± standard deviation) within each column followed by a different lowercase letter are significantly different ($P \leq 0.05$)

Table 3.6: Comparisons of means (± standard deviation) of multi-drug resistant (MDR) and susceptible *Salmonella* Typhimurium (recovered on xylose lysine deoxycholate agar) for beef trimmings before and after treatment with peroxyacetic acid (200 ppm) for 30 s.

Serotype	Untreated Control	Peroxyacetic Acid (200ppm)
S. Typhimurium Susceptible	3.1 ± 0.1 b	2.6 ± 0.2 a
S. Typhimurium MDR	3.3 ± 0.1 a	2.6 ± 0.1 a
S. Typhimurium MDR AmpC	3.1 ± 0.1 b	2.5 ± 0.2 a

Values (mean ± standard deviation) within each column followed by a different lowercase letter are significantly different ($P \leq 0.05$)

Table 3.7: Comparisons of means (± standard deviation) of multi-drug resistant (MDR) and susceptible *Salmonella* Newport (recovered on xylose lysine deoxycholate agar) for beef trimmings before and after treatment with peroxyacetic acid (200 ppm) for 30 s.

Serotype	Untreated Control	Peroxyacetic Acid (200ppm)
S. Newport Susceptible	3.2 ± 0.1 a	2.5 ± 0.2 a
S. Newport MDR-AmpC	3.0 ± 0.1 a	2.4 ± 0.1 a

Values (mean ± standard deviation) within each column followed by a different lowercase letter are significantly different ($P \leq 0.05$)

iii. Sodium metasilicate

No significant differences ($P \geq 0.05$) were found between counts of *E. coli* O157:H7 and *Salmonella* serotypes when enumerated before and after treatment of contaminated beef trimmings with sodium metasilicate (40000ppm) on TSA+rif and XLD agar. Untreated trimming samples inoculated with *Salmonella* Typhimurium MDR had greater (*P ≥ 0.05*) microbiological counts than samples contaminated with *E. coli* O157:H7 when evaluated using mSMAC. All trimming samples, regardless of species or serotype, exhibited significant reductions ($P \leq 0.05$) in counts ranging from 1.3-1.5 log CFU/cm^2, though no counts after treatment were significantly different (*P ≥ 0.05*) (Figures 3.7 – 3.9).

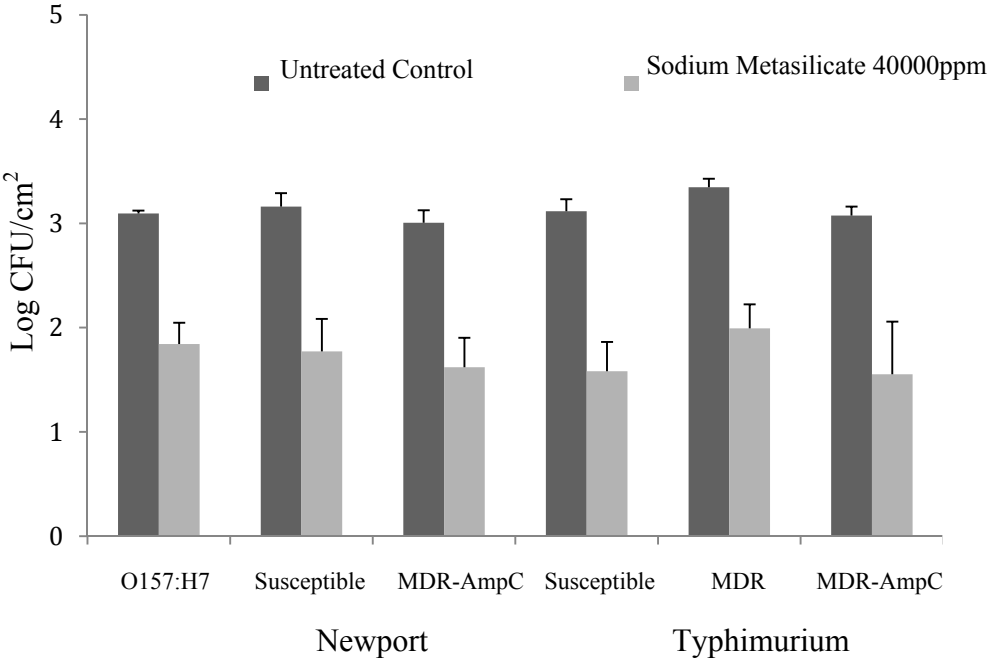

Figure 3.7 -Reduction of *Salmonella* serotypes (antibiotic susceptible, multi-drug resistant, and multi-drug resistant; AmpC) and *Escherichia coli* O157:H7 on inoculated beef trimmings when treated with sodium metasilicate (dipping 30s) (40000ppm) (TSA+rif/XLD) (Data in Appendix Table 59)

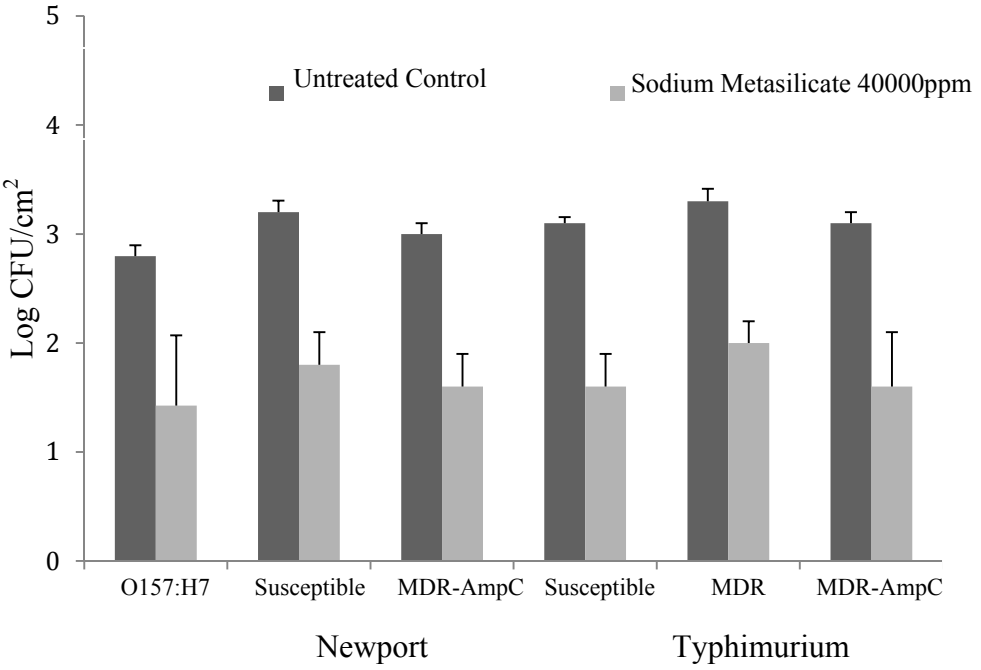

Figure 3.8 - Reduction of *Salmonella* serotypes (antibiotic susceptible, multi-drug resistant, and multi-drug resistant; AmpC) and *Escherichia coli* O157:H7 on inoculated beef trimmings when treated with sodium metasilicate (dipping 30s) (4%) (mSMAC/XLD) (Data in Appendix Table 59)

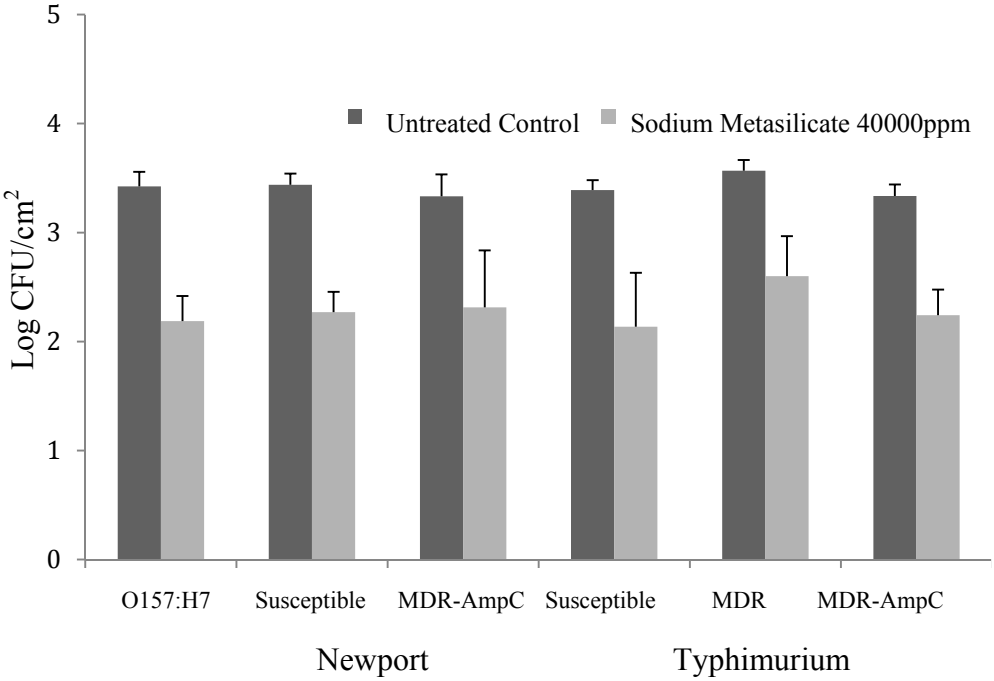

Figure 3.9 - Total aerobic plate count reductions (*E. coli* O157:H7, *Salmonella* serotypes: antibiotic susceptible, multi-drug resistant, and multi-drug resistant; AmpC) on inoculated beef trimmings when treated with sodium metasilicate (30s)(4%) (TSA) (Data in Appendix Table 59)

Though *S.* Typhimurium MDR on beef trimmings exhibited a statistically higher population ($P \geq 0.05$) than other *Salmonella* serotypes prior to treatment, no significant ($P \geq 0.05$) differences existed between serotypes after treatment with SMS for 30 s. (Table 3.8). This is not indicative of a greater susceptibility to SMS by *S.* Typhimurium MDR, but instead a function of small sample sizes. Similar amounts of reduction suggest that there is no difference in response to chemical decontaminating treatments between *Salmonella* serotypes, whether susceptible or resistant to antibiotics (Tables 3.9 and 3.10).

Table 3.8: Comparisons of means (± standard deviation) of multi-drug resistant (MDR) and susceptible *Salmonella* serotypes (recovered on xylose lysine deoxycholate agar) for beef trimmings before and after treatment with sodium metasilicate (40000ppm) for 30 s.

Serotype	Untreated Control	Sodium Metasilicate (40000ppm)
S. Newport Susceptible	3.2 ± 0.1 b	1.8 ± 0.3 a
S. Newport MDR-AmpC	3.0 ± 0.1 b	1.6 ± 0.3 a
S. Typhimurium Susceptible	3.1 ± 0.1 b	1.6 ± 0.3 a
S. Typhimurium MDR	3.3 ± 0.1 a	2.0 ± 0.2 a
S. Typhimurium MDR AmpC	3.1 ± 0.1 b	1.6 ± 0.5 a

Values (mean ± standard deviation) within each column followed by a different lowercase letter are significantly different ($P \leq 0.05$)

Table 3.9: Comparisons of means (± standard deviation) of multi-drug resistant (MDR) and susceptible *Salmonella* Typhimurium (recovered on xylose lysine deoxycholate agar) for beef trimmings before and after treatment with sodium metasilicate (40000ppm) for 30 s.

Serotype	Untreated Control	Acidified Sodium Chlorite (1000ppm)
S. Typhimurium Susceptible	3.1 ± 0.1 b	1.6 ± 0.3 a
S. Typhimurium MDR	3.3 ± 0.1 a	2.0 ± 0.2 a
S. Typhimurium MDR AmpC	3.1 ± 0.1 b	1.6 ± 0.5 a

Values (mean ± standard deviation) within each column followed by a different lowercase letter are significantly different ($P \leq 0.05$)

Table 3.10: Comparisons of means (± standard deviation) of multi-drug resistant (MDR) and susceptible *Salmonella* Newport (recovered on xylose lysine deoxycholate agar) for beef trimmings before and after treatment with sodium metasilicate (40000ppm) for 30 s.

Serotype	Untreated Control	Acidified Sodium Chlorite (1000ppm)
S. Newport Susceptible	3.2 ± 0.1 a	1.8 ± 0.3 a
S. Newport MDR-AmpC	3.0 ± 0.1 a	1.6 ± 0.3 a

Values (mean ± standard deviation) within each column followed by a different lowercase letter are significantly different ($P \leq 0.05$)

Sodium metasilicate caused the greatest numerical reduction in population of any decontaminating agent tested. High numerical reduction is obviously a desirable trait for an antimicrobial, especially when it is demonstrated that the solution is as effective against antibiotic resistant strains of *Salmonella* as it is against antibiotic susceptible strains of the same bacteria, as well as *E. coli* O157:H7, though use of SMS may not be appropriate for all applications.

iv. pH

pH values between untreated and treated samples and between samples taken at 0 h and 24 h of storage at 4º C were not statistically greater or lower ($P < 0.05$). Values for beef trimmings left untreated ranged from 5.79 to 6.23 (0 h, 24 h of storage, respectively) (untreated). When trimmings were treated with ASC, pH values changed from 5.75 at 0 h to 5.63 by 24 h of storage at 4º C (Figure 3.10). Trimmings treated with PAA changed from 5.98 to 5.42 from 0 to 24 h, respectively (Figure 3.11) while values for untreated controls changed from 6.04 (0 h) to 5.44 (24 h). Samples used to test SMS ranged from 6.04 to 5.44 (untreated controls) while treated samples yielded values of 8.66 (0 h) and then 6.52 (24 h) (Figure 3.12). Processors may worry that an antimicrobial treatment may cause quality defects in a product over a period of time in which the beef trimmings are stored, especially if the treatments cause drastic changes in pH. These results clearly demonstrate that, under these conditions, only SMS causes a drastic increase in pH, with levels returning close to neutral by 24h of storage at 4º C.

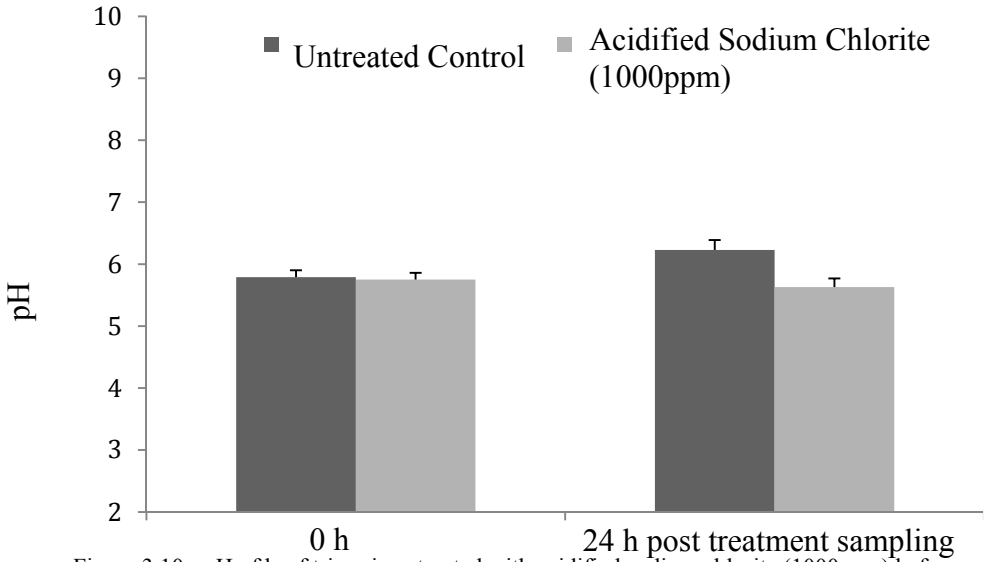

Figure 3.10 - pH of beef trimmings treated with acidified sodium chlorite (1000ppm) before and after treatment (dipping, 30s) and after 24h (Data in Appendix Table 60)

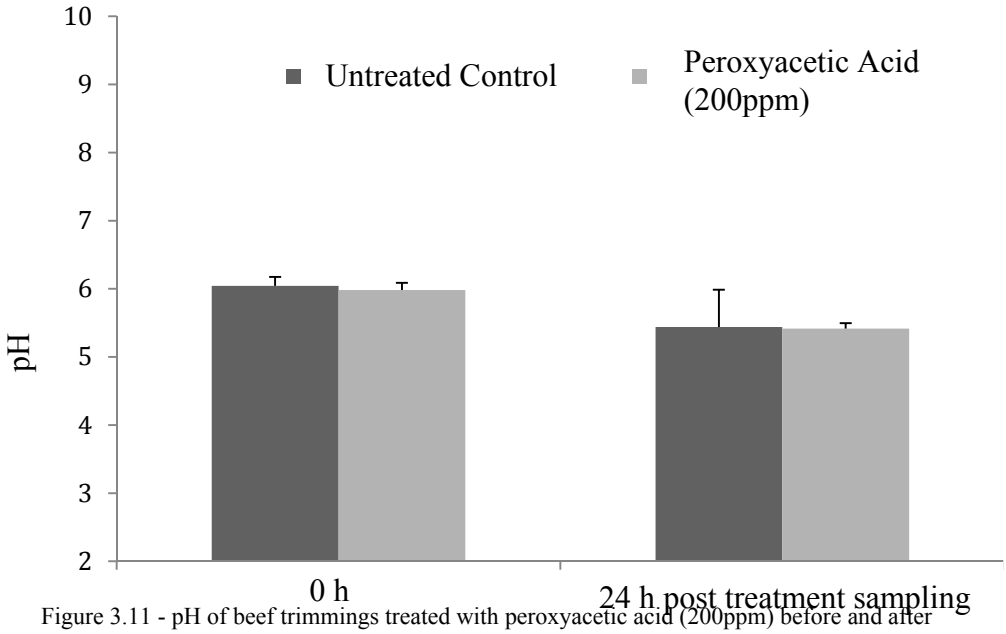

Figure 3.11 - pH of beef trimmings treated with peroxyacetic acid (200ppm) before and after treatment (dipping, 30s) and after 24h (Data in Appendix C, Table 61)

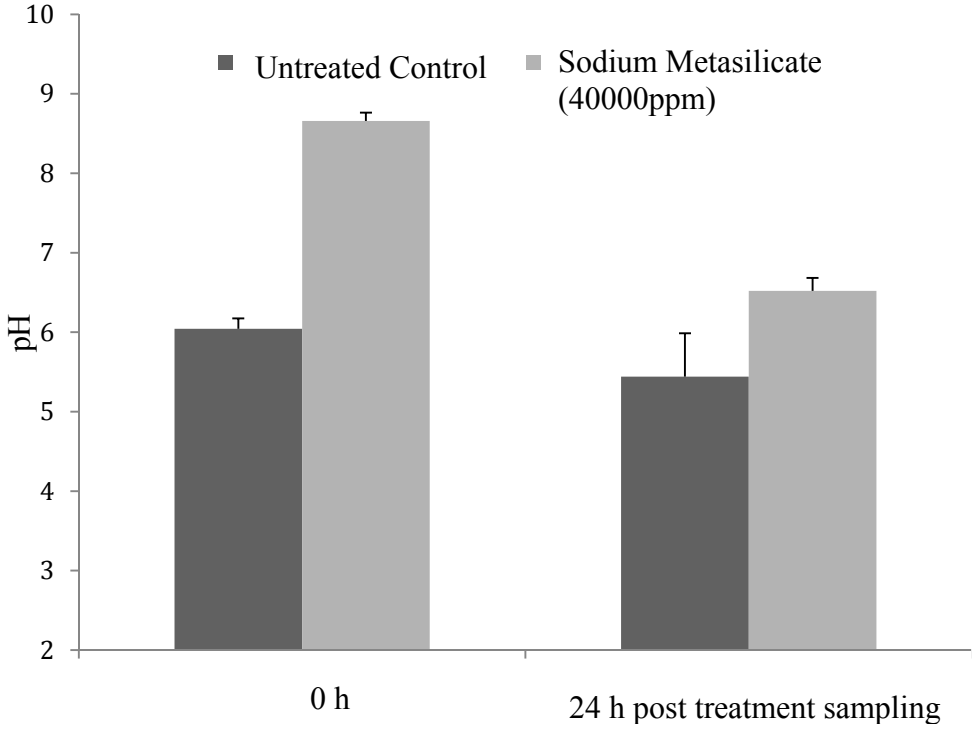

Figure 3.12 - pH of beef trimmings treated with sodium metasilicate (4%) before and after treatment (dipping, 30s) and after 24h (Data in Appendix Table 62)

v. Percent weight change

Sodium metasilicate consistently caused greater moisture retention of beef trimming samples than the other two antimicrobials tested, with values ranging from 4.7 to 6.1% overall moisture uptake, as opposed to 2.3 – 4.4% and 1.8 – 4.0% increase for acidfied sodium chlorite and peroxyacetic acid , respectively (Table 3.11). This information may be necessary to consider by processors, as percent weight added by water is controlled by the USDA (9 CFR 381, 441). The final rule states that no water may be added to raw meat other than what is necessary to meet food safety requirements. Thusly, knowledge of the amount of water retained by beef trimmings treated with these decontaminating solutions is necessary to prevent recourse from the sale of meat with unnecessary water weight added.

Table 3.11 - Mean weight change (percentage of original weight, ± standard deviation) of beef trimmings treated with Acidified Sodium Chlorite (1000ppm), Peroxyacetic Acid (200ppm), or Sodium Metasilicate (40000ppm) for 30 s.

Serotype	Peroxyacetic Acid	Sodium Metasilicate	Acidified Sodium Chlorite
	Weight Change (%)	Weight Change (%)	Weight Change (%)
Escherichia coli O157:H7	3.43 ± 1.79	6.12 ± 1.52	2.85 ± 0.84
Salmonella Newport – Susceptible	3.99 ± 1.51	5.50 ± 1.48	3.85 ± 1.95
Salmonella Newport – MDR-AmpC	3.10 ± 1.09	4.89 ± 0.37	4.37 ±1.12
Salmonella Typhiumurim – Susceptible	2.21 ± 1.78	4.67 ± 1.03	3.30 ± 0.71
Salmonella Typhiumurim – MDR	2.78 ± 0.97	5.42 ± 1.41	2.25 ± 0.91
Salmonella Typhiumurim – MDR-AmpC	1.81 ± 0.40	5.30 ± 1.42	2.89 ± 1.03

IV. Conclusions

The goal of these studies was to compare the resistance to antimicrobials of *Salmonella* serotypes (antibiotic susceptible and resistant) to a well-studied pathogenic model (*E. coli* O157:H7). All decontaminating treatments were effective in reducing both resistant and susceptible *Salmonella* as well as *E. coli* O157:H7, with only very small differences observed in total reduction of each pathogen tested. Sodium metasilicate caused the greatest reduction in pathogenic population of all the solutions tested, with ASC and PAA reducing the pathogens in similar numbers. In addition to microbiological parameters, changes in pH values and weight added due to the treatments were minimal. As mentioned previously, statistical differences between serotypes in treatment with ASC and PAA are most likely due to differing starting population values and a subsequently small standard deviation. Reductions were similar numerically, and all antimicrobial treatments caused a statistically significant ($P \leq 0.05$) decrease in population. Though sodium metasilicate exhibited more drastic reductions in bacterial populations in this trial, different methods of application may yield different results. Moreover, an alkaline cleaner may not be appropriate for every setting, just as an oxidizer or organic acid may not be appropriate for a certain application. Numerically, the reductions in populations of *E. coli* O157:H7 and assorted *Salmonella* serotypes in response to PAA were similar to that caused by treatment with ASC. As mentioned previously, peroxyacetic acid has different characteristics than ASC, one being primarily an oxidizing agent while PAA is an organic acid. Therefore, processors may decide that one agent is more suitable to apply to their products than the other. With the results of this study in mind, processors may use whichever decontaminating agent is appropriate with confidence.

Interventions implemented for control of *E. coli* O157:H7 should be considered effective for control of a variety of *Salmonella* serotypes as well. This study investigated the efficacy of antimicrobials against *E. coli* O157:H7 and *Salmonella* serotypes through dipping, though other methods of application have been proven effective. In considering antimicrobial interventions for *Salmonella* serotypes, no change is necessary to control for the pathogen if measures are already in place to combat the presence of *E. coli* O157:H7. If industry regulations and legislation is considered to control *Salmonella* spp. on beef trimmings, data from this study may be useful to the industry in combating the presence of these pathogenic bacteria in beef trimmings.

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Appendix:

Tables of persistence and survival of *Listeria monocytogenes* on food packaging soiled with antimicrobial-free ham homogenate and stored for up to 123 days;

Tables of persistence and survival of *Escherichia coli* O157:H7 on food packaging soiled with ground beef homogenate and stored for up to 130 days;

Tables of comparison of decontaminating agents against *Escherichia coli* O157:H7, susceptible and multi-drug resistant *Salmonella* in beef trimming

Table A.1: Summary of statistical analysis results showing which soiled materials inoculated with *Listeria monocytogenes* (inoculated at 5 log CFU/cm^2 and stored up to 123 days at 25º C) exhibited a replication effect

Packaging Material	*P* Value (PALCAM)	*P* Value (TSA+YE)
Aluminum Foil	0.0671	0.0546
Butcher Paper	0.2298	0.0096*
Cardboard Box Material	0.0317*	<0.0001*
Deli Wax Paper	<0.0001*	<0.0001*
Paper Bag Material	<0.0001*	<0.0001*
Plastic Bag Material	0.0018*	0.7879
PVC Overwrap Film	0.0004*	0.0066*
Vacuum Pouch Material	0.0005*	0.8889

* Effects are significant if $P < 0.05$

Table A.2: Summary of statistical analysis results showing which soiled materials inoculated with *Listeria monocytogenes* (inoculated at 5 log CFU/cm^2 and stored up to 123 days at 4º C) exhibited a replication effect

Packaging Material	*P* Value (PALCAM)	*P* Value (TSA+YE)
Aluminum Foil	<0.0001*	<0.0001*
Butcher Paper	<0.0001*	<0.0001*
Cardboard Box Material	<0.0001*	0.0521
Deli Wax Paper	<0.0001*	<0.0001*
Paper Bag Material	<0.0001*	0.0013*
Plastic Bag Material	<0.0001*	<0.0001*
PVC Overwrap Film	<0.0001*	<0.0001*
Vacuum Pouch Material	<0.0001*	0.0046*

* Effects are significant if $P < 0.05$

Table A.3: Summary of statistical analysis results showing which soiled materials inoculated with *Listeria monocytogenes* (inoculated at 2 log CFU/cm^2 and stored up to 123 days at 25º C) exhibited a replication effect

Packaging Material	*P* Value (PALCAM)	*P* Value (TSA+YE)
Aluminum Foil	<0.0001*	<0.0001*
Butcher Paper	0.0072*	0.0275*
Cardboard Box Material	0.0109*	0.0018*
Deli Wax Paper	0.0060*	0.1050
Paper Bag Material	0.0003*	<0.0001*
Plastic Bag Material	<0.0001*	<0.0001*
PVC Overwrap Film	<0.0001*	<0.0001*
Vacuum Pouch Material	0.0057*	0.0114*

* Effects are significant if $P < 0.05$

Table A.4: Summary of statistical analysis results showing which soiled materials inoculated with *Listeria monocytogenes* (inoculated at 2 log CFU/cm^2 and stored up to 123 days at 4º C) exhibited a replication effect

Packaging Material	*P* Value (PALCAM)	*P* Value (TSA+YE)
Aluminum Foil	0.1799	0.4843
Butcher Paper	0.0008*	0.0039*
Cardboard Box Material	<0.0001*	0.2631
Deli Wax Paper	<0.0001*	0.2402
Paper Bag Material	0.7266	0.0432*
Plastic Bag Material	<0.0001*	<0.0001*
PVC Overwrap Film	0.2803	0.1913
Vacuum Pouch Material	<0.0001*	<0.0001*

* Effects are significant if $P < 0.05$

Table A.5: Populations of *Listeria monocytogenes* (n = 6) on soiled food packaging surfaces when inoculated at a level of approximately 5 log CFU/cm^2 and stored at 25º C – Combined Means (Figure 1.1)

	Materials							
Day of Storage	Butcher Paper	Cardboard	Deli Wax Paper	Aluminum Foil	PVC Overwrap Film	Paper Bag	Plastic Bag	Vacuum Pouch
0	5.1 ± 0.1	4.8 ± 0.1	5.0 ± 0.2	5.0 ± 0.1	4.8 ± 0.1	4.8 ± 0.3	5.0 ± 0.1	4.9 ± 0.1
4	5.4 ± 0.2	3.1 ± 0.2	4.5 ± 0.3	4.0 ± 0.5	3.3 ± 0.3	4.4 ± 0.4	5.0 ± 0.4	5.2 ± 0.5
11	5.5 ± 0.3	2.8 ± 0.4	5.0 ± 0.4	6.0 ± 0.5	4.9 ± 1.0	4.2 ± 0.4	5.7 ± 0.7	5.7 ± 0.4
18	5.2 ± 0.5	2.3 ± 0.3	4.4 ± 0.1	5.5 ± 0.2	4.3 ± 0.8	3.9 ± 0.3	5.1 ± 0.4	4.8 ± 0.8
25	4.4 ± 0.4	2.1 ± 0.8	3.9 ± 0.7	4.7 ± 0.6	2.4 ± 1.0	2.9 ± 1.0	4.8 ± 0.1	4.5 ± 0.7
32	4.7 ± 0.2	1.9 ± 0.8	4.3 ±0.4	5.3 ± 0.5	4.0 ± 0.3	2.9 ± 1.1	4.5 ± 0.2	4.9 ± 0.5
39	4.1 ± 0.2	1.4 ± 0.6	3.6 ± 0.2	5.3 ± 0.3	1.9 ± 1.3	<1.8 ± 1.4	4.0 ± 0.5	4.3 ± 0.7
53	3.7 ± 0.2	<0.3 ± 0.5	2.8 ± 0.3	4.3 ± 0.9	2.3 ± 2.0	<0.0 ± 0.3	4.0 ± 0.4	4.1 ± 1.7
67	4.0 ± 0.3	<0.6 ± 0.4	3.1 ± 0.6	4.6 ± 0.7	-0.1 ± 0.0	<0.9 ± 0.7	4.5 ± 0.6	4.2 ± 1.4
81	3.4 ± 0.3	<-0.0 ± 0.2	2.4 ± 0.6	4.5 ± 0.4	<0.6 ± 1.0	<0.6 ± 0.7	3.0 ± 0.5	3.4 ± 0.6
95	2.5 ± 1.0	<-0.1	1.9 ± 0.9	3.9 ± 1.0	1.6 ± 1.0	<0.0 ± 0.3	2.7 ± 1.0	1.4 ± 0.5
109	2.8 ± 0.3	<-0.1	1.5 ± 0.4	3.9 ± 0.6	<0.9 ± 1.6	<-0.0 ± 0.2	2.6 ± 0.7	1.9 ± 1.3
123	2.3 ± 0.5	<-0.1	<1.2 ± 1.4	3.2 ± 0.5	<1.0 ± 1.1	<-0.1	1.5 ± 0.9	<0.8 ± 0.8

Table A.6: Populations of *Listeria monocytogenes* (n = 3) on soiled food packaging surfaces when inoculated at a level of approximately 5 log CFU/cm^2 and stored at 25º C First Replicate

	Materials							
Day of Storage	Butcher Paper	Cardboard	Deli Paper	Foil	Overwrap	Paper Bag	Plastic Bag	Vacuum Pouch
0	5.1 ± 0.0	4.8 ± 0.1	5.1 ± 0.1	5.0 ± 0.1	4.9 ± 0.7	4.7 ± 0.3	5.0 ± 0.1	4.8 ± 0.1
4	5.5 ± 0.2	3.2 ± 0.2	4.3 ± 0.3	3.7 ± 0.4	3.5 ± 0.1	4.3 ± 0.5	5.2 ± 0.5	5.3 ± 0.6
11	5.3 ± 0.1	2.5 ± 0.2	4.7 ± 0.1	5.6 ± 0.4	4.0 ± 0.8	4.0 ± 0.5	5.1 ± 0.4	5.4 ± 0.1
18	5.2 ± 0.8	2.1 ± 0.3	4.4 ± 0.1	5.4 ± 0.1	3.9 ± 0.2	3.7 ± 0.1	4.8 ± 0.2	4.5 ± 1.0
25	4.8 ± 0.2	1.5 ± 0.1	4.5 ± 0.3	5.1 ± 0.6	3.5 ± 0.4	2.1 ± 0.8	4.7 ± 0.1	4.6 ± 0.3
32	4.7 ± 0.3	1.9 ± 0.3	4.6 ± 0.2	5.6 ± 0.2	3.8 ± 0.1	2.5 ±1.5	4.8 ± 0.1	4.9 ± 0.6
39	4.2 ± 0.2	1.1 ± 0.3	3.7 ± 0.3	5.4 ± 0.2	3.1 ± 0.1	0.7 ± 1.1	4.1 ± 0.4	4.2 ± 0.9
53	3.7 ± 0.3	0.8 ± 0.1	2.9 ± 0.3	3.8 ± 1.0	0.6 ± 0.5	< -0.1 ± 0.0	3.7 ± 0.2	2.8 ± 1.4
67	4.0 ± 0.3	0.7 ± 0.1	3.6 ± 0.2	4.3 ± 1.0	<0.1 ± 0.0	0.7 ± 1.0	4.0 ± 0.2	3.3 ± 1.4
81	3.2 ± 0.4	<-0.1 ± 0.0	2.9 ± 0.2	4.7 ± 0.3	<0.6 ± 1.3	<0.1 ± 0.3	3.1 ± 0.2	3.7 ± 0.1
95	1.7 ± 0.5	<-0.1	2.7 ± 0.2	<3.3 ± 1.2	<1.0 ± 1.1	<-0.1 ± 0.0	1.9 ± 0.6	<1.2 ± 0.5
109	2.7 ± 0.6	<-0.1	1.8 ± 0.5	3.5 ± 0.4	<-0.1 ± 0.0	<-0.1 ± 0.0	2.1 ± 0.8	0.7 ± 0.8
123	2.7 ± 0.1	<-0.1	2.5 ± 0.2	3.6 ± 0.2	<0.2 ± 0.6	<-0.1	2.0 ± 0.9	<1.0 ± 1.2

Table A.7: Populations of *Listeria monocytogenes* (n = 3) on soiled food packaging surfaces when inoculated at a level of approximately 5 log CFU/cm^2 and stored at 25º C – Second Replicate

	Materials							
Day of Storage	Butcher Paper	Cardboard	Deli Paper	Foil	Overwrap	Paper Bag	Plastic Bag	Vacuum Pouch
0	5.2 ± 0.1	4.8 ± 0.1	4.9 ± 0.1	4.9 ± 0.1	4.7 ± 0.1	5.0 ± 0.0	4.9 ± 0.0	4.9 ± 0.0
4	5.2 ± 0.2	3.0 ± 0.2	4.7 ± 0.3	4.3 ± 0.4	3.1 ± 0.2	4.4 ± 0.3	4.8 ± 0.1	5.0 ± 0.3
11	5.7 ± 0.2	3.0 ± 0.3	5.4 ± 0.1	6.3 ± 0.1	5.7 ± 0.1	4.3 ± 0.2	6.3 ± 0.1	6.0 ± 0.3
18	5.1 ± 0.2	2.5 ± 0.2	4.4 ± 0.1	5.7 ± 0.2	4.7 ± 1.1	4.1 ± 0.1	5.4 ± 0.4	5.1 ± 0.6
25	4.1 ± 0.1	2.8 ± 0.1	3.3 ± 0.4	4.3 ± 0.1	2.3 ± 0.3	3.7 ± 0.4	4.9 ± 0.1	4.5 ± 1.0
32	4.8 ± 0.1	2.0 ± 1.2	4.0 ± 0.2	5.0 ± 0.6	4.2 ± 0.2	3.4 ± 0.4	4.3 ± 0.1	4.9 ± 0.6
39	4.1 ± 0.0	1.7 ± 0.7	3.6 ± 0.1	5.1 ± 0.2	<0.9 ± 0.2	3.0 ± 0.1	4.0 ± 0.6	4.4 ± 0.7
53	3.7 ± 0.2	<-0.1 ± 0.0	2.7 ± 0.3	4.9 ± 0.2	4.1 ± 0.1	<0.2 ± 0.	4.3 ± 0.4	5.5 ± 0.2
67	4.1 ± 0.4	<0.6 ± 0.0	2.5 ± 0.3	5.0 ± 0.1	<-0.1 ± 0.0	1.1 ± 0.1	5.0 ± 0.2	5.2 ± 0.1
81	3.5 ± 0.3	<0.1 ± 0.3	1.9 ± 0.1	4.3 ± 0.5	<0.5 ± 0.8	1.1 ± 0.4	2.9 ± 0.7	3.1 ± 0.8
95	3.3 ± 0.4	<-0.1	1.2 ± 0.4	4.4 ± 0.2	2.3 ± 0.3	0.1 ± 0.4	3.5 ± 0.6	1.6 ± 0.5
109	3.0 ± 0.3	<-0.1	1.3 ± 0.3	4.4 ± 0.3	2.0 ± 1.8	<0.1 ± 0.3	3.1 ± 0.1	3.0 ± 0.0
123	2.1 ± 0.5	<-0.1	<-0.1 ± 0.0	2.8 ± 0.1	1.9 ± 0.7	<-0.1	1.1 ± 0.8	0.7 ± 0.2

Table A.8: Total aerobic plate count (n = 6) on various soiled food packaging materials when inoculated at a level of approximately 5 log CFU/cm^2 and stored at 25º C – Combined Means (Figure 1.2)

	Materials							
Day of Storage	Butcher Paper	Cardboard	Deli Wax Paper	Aluminum Foil	PVC Overwrap Film	Paper Bag	Plastic Bag	Vacuum Pouch
0	5.2 ± 0.1	4.8 ± 0.1	4.8 ± 0.3	5.0 ± 0.2	4.9 ± 0.1	4.8 ± 0.2	5.0 ± 0.1	4.9 ± 0.1
4	5.5 ± 0.5	3.5 ± 0.4	5.3 ± 0.5	5.3 ± 0.7	4.8 ± 0.4	4.2 ± 0.9	5.5 ± 0.6	5.2 ± 0.6
11	5.6 ± 0.2	3.3 ± 0.4	5.3 ± 0.1	6.2 ± 0.4	5.0 ± 0.9	4.3 ± 0.2	5.9 ± 0.6	5.8 ± 0.3
18	5.4 ± 0.5	2.9 ± 0.2	4.7 ± 0.2	5.7 ± 0.3	4.3 ± 0.5	4.3 ± 0.4	5.3 ± 0.5	5.0 ± 0.9
25	4.8 ± 0.5	3.0 ± 0.2	4.9 ± 0.4	5.3 ± 0.6	3.3 ± 0.7	4.0 ± 0.4	5.1 ± 0.1	4.8 ± 1.1
32	5.1 ± 0.1	2.8 ± 0.4	4.7 ± 0.2	5.7 ± 0.5	4.3 ± 0.1	3.7 ± 0.6	5.0 ± 0.3	5.1 ± 0.5
39	4.9 ± 0.1	2.8 ± 0.2	4.5 ± 0.2	5.9 ± 0.2	<2.7 ± 1.7	3.3 ± 0.9	4.8 ± 0.5	5.1 ± 0.4
53	4.5 ± 0.2	2.4 ± 0.2	3.8 ± 0.2	5.1 ± 1.0	3.2 ± 1.6	<1.1 ± 1.4	5.0 ± 0.3	5.0 ± 0.9
67	4.5 ± 0.3	2.8 ± 0.2	4.0 ± 0.4	5.4 ± 0.7	1.0 ± 1.1	1.8 ± 0.8	5.5 ± 0.3	5.5 ± 0.3
81	4.5 ± 0.3	2.5 ± 0.2	3.4 ± 0.4	5.5 ± 0.4	<0.5 ± 0.9	1.5 ± 0.7	4.4 ± 0.3	4.4 ± 0.6
95	4.0 ± 0.3	1.7 ± 0.9	3.4 ± 0.8	5.7 ± 0.2	<2.9 ± 0.6	<1.0 ± 1.1	5.0± 0.5	4.3 ± 0.4
109	3.7 ± 0.3	2.6 ± 0.3	2.9 ± 0.7	4.9 ± 0.4	1.9 ± 1.1	<0.4 ± 0.5	4.2 ± 0.5	3.5 ± 0.8
123	3.3 ± 0.6	2.3 ± 0.2	2.4 ± 1.1	4.3 ± 0.9	2.2 ± 0.6	<0.2 ± 0.4	3.1 ± 0.7	2.3 ± 1.5

Table A.9: Total aerobic plate count (n = 3) on various soiled food packaging materials when inoculated at a level of approximately 5 log CFU/cm^2 and stored at 25º C – First Replicate

Day of Storage	Materials							
	Butcher Paper	Cardboard	Deli Paper	Foil	Overwrap	Paper Bag	Plastic Bag	Vacuum Pouch
0	5.2 ± 0.1	4.8 ± 0.6	4.8 ± 0.4	5.1 ± 0.1	4.9 ± 0.1	4.7 ± 0.3	5.0 ± 0.1	4.9 ± 0.1
4	5.9 ± 0.5	3.8 ± 0.4	5.7 ± 0.4	5.9 ± 0.4	5.1 ± 0.3	4.8 ± 0.6	6.0 ± 0.3	5.4 ± 0.8
11	5.5 ± 0.1	3.6 ± 0.2	5.2 ± 0.1	6.0 ± 0.5	4.3 ± 0.7	4.2 ± 0.2	5.4 ± 0.4	5.6 ± 0.1
18	5.4 ± 0.7	2.7 ± 0.3	4.6 ± 0.2	5.5 ± 0.1	4.1 ± 0.2	4.0 ± 0.2	5.0 ± 0.2	4.7 ± 0.9
25	5.3 ± 0.1	2.9 ± 0.2	5.0 ± 0.3	5.8 ± 0.3	4.0 ± 0.1	3.8 ± 0.5	5.1 ± 0.2	5.3 ± 0.4
32	5.0 ± 0.1	3.0 ± 0.1	4.9 ± 0.2	6.1 ± 0.1	4.2 ± 0.2	3.5 ± 0.8	5.3 ± 0.2	5.0 ± 0.6
39	4.8 ± 0.1	2.9 ± 0.2	4.6 ± 0.1	6.0 ± 0.2	3.8 ± 0.2	2.5 ± 0.5	4.9 ± 0.4	5.0 ± 0.5
53	4.6 ± 0.2	2.6 ± 0.1	3.8 ± 0.3	4.4 ± 1.0	1.9 ± 0.6	0.0 ± 0.1	4.7 ± 0.1	4.4 ± 1.0
67	4.4 ± 0.2	2.9 ± 0.1	4.4 ± 0.1	5.2 ± 1.1	<0.7 ± 1.3	1.2 ± 1.2	5.3 ± 0.2	5.2 ± 0.1
81	4.7 ± 0.3	2.6 ± 0.1	3.7 ± 0.3	5.7 ± 0.3	<0.0± 0.2	0.9 ± 0.7	4.6 ± 0.2	4.9 ± 0.2
95	3.9 ± 0.2	2.5 ± 0.2	4.1 ± 0.2	5.7 ± 0.1	2.4 ± 0.1	<0.0± 0.2	4.9 ± 0.5	4.6 ± 0.3
109	3.7 ± 0.3	2.9 ± 0.2	3.5 ± 0.1	4.6 ± 0.4	1.3 ± 0.8	<0.4 ± 0.5	4.0 ± 0.4	3.2 ± 1.0
123	3.9 ± 0.1	2.5 ± 0.2	3.5 ± 0.3	5.1 ± 0.1	2.0 ± 0.2	<0.3 ± 0.6	3.7 ± 0.3	2.9 ± 2.0

Table A.10: Total aerobic plate count (n = 3) on various soiled food packaging materials when inoculated at a level of approximately 5 log CFU/cm^2 and stored at 25º C – Second Replicate

	Materials							
Day of Storage	Butcher Paper	Cardboard	Deli Paper	Foil	Overwrap	Paper Bag	Plastic Bag	Vacuum Pouch
0	5.2 ± 0.1	4.8 ± 0.1	4.9 ± 0.1	4.9 ± 0.2	4.8 ± 0.2	5.0 ± 0.1	4.9 ± 0.1	5.0 ± 0.0
4	5.2 ± 0.2	3.2 ± 0.2	4.9 ± 0.2	4.8 ± 0.2	4.5 ± 0.3	3.4 ± 0.3	5.0 ± 0.1	5.1 ± 0.4
11	5.7 ± 0.2	3.0 ± 0.3	5.4 ± 0.1	6.3 ± 0.1	5.7 ± 0.1	4.3 ± 0.2	6.3 ± 0.1	6.0 ± 0.3
18	5.4 ± 0.2	3.0 ± 0.1	4.9 ± 0.0	6.0 ± 0.1	4.6 ± 0.8	4.6 ± 0.3	5.7 ± 0.4	5.4 ± 0.8
25	4.4 ± 0.1	3.1 ± 0.2	4.8 ± 0.5	4.7 ± 0.1	2.8 ± 0.4	4.1 ± 0.4	5.1 ± 0.1	4.3 ± 1.4
32	5.2 ± 0.1	2.6 ± 0.6	4.5 ± 0.2	5.2 ± 0.3	4.3 ± 0.1	3.9 ± 0.4	4.8 ± 0.3	5.3 ± 0.6
39	4.9 ± 0.1	2.8 ± 0.2	4.4 ± 0.2	5.8 ± 0.3	<1.7 ± 1.9	4.1 ± 0.3	4.7 ± 0.6	5.1 ± 0.3
53	4.4 ± 0.2	2.2 ± 0.1	3.8 ± 0.2	5.7 ± 0.3	4.6 ± 0.3	2.4 ± 0.3	5.3 ± 0.1	5.6 ± 0.4
67	4.7 ± 0.3	2.7 ± 0.2	3.7 ± 0.1	5.6 ± 0.1	1.6 ± 0.3	2.2 ± 0.1	5.8 ± 0.1	5.7 ± 0.2
81	4.3 ± 0.1	2.3 ± 0.1	3.1 ± 0.1	5.4 ± 0.4	1.2 ± 1.4	2.0 ± 0.2	4.2 ± 0.1	3.9 ± 0.3
95	4.1 ± 0.4	<0.9 ± 0.9	2.8 ± 0.5	5.7 ± 0.4	3.3 ± 0.4	2.1 ± 0.2	5.2 ± 0.4	4.0 ± 0.2
109	3.7 ± 0.4	2.4 ± 0.1	2.3 ± 0.4	5.1 ± 0.4	2.8 ± 0.6	<0.3 ± 0.5	4.5 ± 0.5	3.8 ± 0.7
123	3.0 ± 0.6	2.1 ± 0.1	1.3 ± 0.4	3.5 ± 0.4	2.5 ± 0.8	<0.1 ± 0.2	2.6 ± 0.6	<1.8 ± 0.8

Table A.11: Populations of *Listeria monocytogenes* (n = 6) on soiled food packaging surfaces when inoculated at a level of approximately 5 log CFU/cm^2 and stored at 25º C – Combined Means (Figure 1.3)

	Materials							
Day of Storage	Butcher Paper	Cardboard	Deli Wax Paper	Aluminum Foil	PVC Overwrap Film	Paper Bag	Plastic Bag	Vacuum Pouch
0	5.1 ± 0.1	4.8 ± 0.1	5.0 ± 0.2	5.0 ± 0.1	4.8 ± 0.1	4.8 ± 0.3	5.0 ± 0.1	4.9 ± 0.1
4	5.56 ± 0.4	3.6 ± 0.4	5.2 ± 0.4	5.6 ± 0.4	4.9 ± 0.6	4.8 ± 0.6	5.7 ± 0.4	5.4 ± 0.6
11	4.2 ± 0.4	2.5 ± 0.4	3.4 ± 0.6	4.2 ± 0.3	2.3 ± 0.4	3.6 ± 0.4	5.0 ± 0.2	4.3 ± 0.7
18	3.9 ± 0.2	2.3 ± 0.3	3.1 ± 0.5	3.8 ± 0.6	1.2 ± 0.6	3.3 ± 0.7	4.5 ± 0.7	5.1 ± 0.4
25	4.1 ± 0.8	1.6 ± 0.3	3.1 ± 1.2	3.8 ± 1.9	2.0 ± 1.8	2.6 ± 0.7	3.9 ± 1.1	4.2 ± 0.7
32	3.5 ± 0.1	2.0 ± 0.4	2.5 ± 0.5	3.3 ± 1.2	0.8 ± 0.9	2.5 ± 0.5	4.2 ± 0.5	4.4 ± 0.8
39	3.6 ± 0.4	2.2 ± 0.2	2.0 ± 1.1	2.8 ± 1.5	<0.7 ± 0.9	2.6 ± 0.6	2.9 ± 0.9	4.5 ± 0.5
53	2.7 ± 0.4	1.7 ± 0.5	1.9 ± 1.3	2.7 ± 1.2	<0.4 ± 0.6	2.3 ± 1.0	3.3 ± 1.0	3.9 ± 0.8
67	2.6 ± 0.8	2.1 ± 0.9	<1.4 ± 1.5	3.2 ± 0.6	<1.0 ± 1.2	1.7 ± 1.0	2.5 ± 1.7	4.4 ± 0.7
81	2.7 ± 0.2	1.6 ± 0.5	<1.0 ± 1.2	<2.3 ± 1.9	<0.2 ± 0.6	1.7 ± 0.6	3.0 ± 1.4	4.0 ± 0.7
95	2.1 ± 1.0	<1.2 ± 1.1	<0.7 ± 1.0	2.7 ± 1.3	<0.2 ± 0.5	<0.0 ± 0.2	2.8 ± 1.1	<3.5 ± 1.4
109	1.8 ± 1.0	2.0 ± 0.6	<0.9 ± 1.1	<1.9 ± 1.8	<0.3 ± 0.5	<0.7 ± 1.1	3.1 ± 0.6	3.0 ± 1.0
123	1.3 ± 1.2	<0.7 ± 0.9	<0.7 ± 0.9	1.8 ± 1.0	<0.2 ± 0.3	<0.5 ± 0.8	2.4 ± 1.0	<2.0 ± 2.3

Table A.12: Populations of *Listeria monocytogenes* (n = 3) on soiled food packaging surfaces when inoculated at a level of approximately 5 log CFU/cm^2 and stored at 4º C – First Replicate

	Materials							
Day of Storage	Butcher Paper	Cardboard	Deli Paper	Foil	Overwrap	Paper Bag	Plastic Bag	Vacuum Pouch
0	5.1 ± 0.0	4.8 ± 0.1	5.1 ± 0.1	5.0 ± 0.1	4.9 ± 0.7	4.7 ± 0.3	5.0 ± 0.1	4.8 ± 0.1
4	5.5 ± 0.6	3.9 ± 0.1	5.4 ± 0.5	5.5 ± 0.4	5.0 ± 0.3	4.5 ± 0.6	5.6 ± 0.5	5.1 ± 0.7
11	4.5 ± 0.2	2.2 ± 0.4	2.9 ± 0.0	4.2 ± 0.3	2.2 ± 0.5	3.4 ± 0.4	5.0 ± 0.2	4.4 ± 0.1
18	3.9 ± 0.4	2.1 ± 0.2	2.7 ± 0.1	3.3 ± 0.4	1.4 ± 0.7	2.8 ± 0.5	4.3 ± 0.9	5.1 ± 0.6
25	3.4 ± 0.2	1.8 ± 0.4	1.8 ± 0.3	1.7 ± 0.3	0.3 ± 0.4	2.0 ± 0.5	3.0 ± 0.7	4.1 ± 0.8
32	3.6 ± 0.2	1.8 ± 0.2	2.0 ± 0.3	2.2 ± 0.5	0.0 ± 0.2	2.3 ± 0.5	3.8 ± 0.3	4.1 ± 0.8
39	3.3 ± 0.2	2.1 ± 0.1	1.0 ± 0.2	1.8 ± 0.8	<0.3 ± 0.8	2.1 ± 0.0	2.6 ± 1.1	4.2 ± 0.6
53	2.5 ± 0.4	1.4 ± 0.2	0.6 ± 0.3	1.9 ± 0.3	0.5 ± 0.3	1.4 ± 0.0	2.5 ± 0.8	3.5 ± 0.7
67	2.0 ± 0.7	1.4 ± 0.9	0.1 ± 0.3	2.9 ± 0.1	<-0.1	0.9 ± 0.5	1.0 ± 0.8	3.8 ± 0.3
81	2.5 ± 0.0	1.4 ± 0.2	<-0.1	<0.7 ± 1.4	<-0.1	1.3 ± 0.3	1.9 ± 1.1	3.9 ± 0.5
95	1.4 ± 1.0	<0.2 ± 0.4	<-0.1	1.6 ± 1.1	<-0.1	<-0.1 ± 0.0	1.8 ± 0.3	<2.3 ± 0.9
109	0.9 ± 0.0	1.7 ± 0.7	<-0.1	<0.3 ± 0.4	<-0.1	<-0.1	2.7 ± 0.1	2.3 ± 0.9
123	0.3 ± 0.4	<-0.1 ± 0.0	<-0.1	0.9 ± 0.1	<-0.1	0.1 ± 0.3	1.5 ± 0.1	<-0.1 ± 0.0

Table A.13: Populations of *Listeria monocytogenes* (n = 3) on soiled food packaging surfaces when inoculated at a level of approximately 5 log CFU/cm^2 and stored at 4º C – Second Replicate

	Materials							
Day of Storage	Butcher Paper	Cardboard	Deli Paper	Foil	Overwrap	Paper Bag	Plastic Bag	Vacuum Pouch
0	5.2 ± 0.1	4.8 ± 0.1	4.9 ± 0.1	4.9 ± 0.1	4.7 ± 0.1	5.0 ± 0.0	4.9 ± 0.0	4.9 ± 0.0
4	5.6 ± 0.4	3.2 ± 0.2	5.1 ± 0.1	5.7 ± 0.3	4.8 ± 0.8	5.1 ± 0.6	5.7 ± 0.5	5.7 ± 0.1
11	4.0 ± 0.3	2.8 ± 0.2	3.8 ± 0.5	4.1 ± 0.4	2.4 ± 0.4	3.7 ± 0.4	5.0 ± 0.1	4.1 ± 0.2
18	3.9 ± 0.1	2.5 ± 0.3	3.5 ± 0.4	4.2 ± 0.1	1.0 ± 0.3	3.9 ± 0.4	4.8 ± 0.1	5.2 ± 0.2
25	4.8 ± 0.5	1.4 ± 0.2	4.0 ± 0.4	5.2 ± 0.1	3.6 ± 0.1	3.2 ± 0.3	4.8 ± 0.1	4.4 ± 0.7
32	3.5 ± 0.1	2.3 ± 0.5	3.0 ± 0.1	4.4 ± 0.3	1.6 ± 0.2	2.6 ± 0.5	4.7 ± 0.1	4.6 ± 0.9
39	3.9 ± 0.3	2.3 ± 0.2	3.1 ± 0.1	4.3 ± 0.0	<1.1 ± 1.0	3.1 ± 0.1	3.3 ± 0.2	4.8 ± 0.1
53	2.9 ± 0.1	2.1 ± 0.4	3.1 ± 0.2	3.5 ± 1.3	<0.4 ± 0.8	3.1 ± 0.1	4.1 ± 0.3	4.3 ± 0.8
67	3.2 ± 0.1	2.7 ± 0.2	2.8 ± 0.2	3.4 ± 0.9	2.1 ± 0.2	2.5 ± 0.4	4.0 ± 0.6	5.0 ± 0.1
81	2.8 ± 0.2	1.8 ± 0.6	2.0 ± 0.3	3.8 ± 0.1	<0.5 ± 0.7	2.1 ± 0.4	4.2 ± 0.1	4.2 ± 0.9
95	2.8 ± 0.1	2.1 ± 0.1	1.6 ± 0.5	3.7 ± 0.1	<0.5 ± 0.5	<0.2 ± 0.2	3.7 ± 0.6	4.6 ± 0.2
109	2.7 ± 0.0	2.7 ± 0.2	1.9 ± 0.3	3.5 ± 0.1	0.6 ± 0.5	1.5 ± 1.0	3.6 ± 0.4	3.7 ± 0.7
123	2.4 ± 0.3	1.4 ± 0.5	1.6 ± 0.2	2.6 ± 0.4	0.4 ± 0.3	<1.0 ± 1.0	3.3 ± 0.3	4.2 ± 0.2

Table A.14: Total aerobic plate count (n = 6) on various soiled food packaging materials when inoculated at a level of approximately 5 log CFU/cm^2 and stored at 4º C – Combined Means (Figure 1.4)

	Materials							
Day of Storage	Butcher Paper	Cardboard	Deli Wax Paper	Aluminum Foil	PVC Overwrap Film	Paper Bag	Plastic Bag	Vacuum Pouch
0	5.2 ± 0.1	4.8 ± 0.1	4.8 ± 0.3	5.0 ± 0.2	4.9 ± 0.1	4.9 ± 0.2	5.0 ± 0.1	4.9 ± 0.1
4	5.6 ± 0.4	3.7 ± 0.9	4.9 ± 0.5	5.0 ± 1.0	4.2 ± 0.9	4.0 ± 0.8	5.6 ± 0.6	5.6 ± 0.5
11	4.2 ± 0.4	2.8 ± 0.3	3.6 ± 0.4	4.6 ± 0.6	2.2 ± 0.6	3.8 ± 0.4	5.2 ± 0.3	4.4 ± 0.8
18	4.2 ± 0.4	2.8 ± 0.3	3.9 ± 0.5	4.5 ± 0.4	1.9 ± 0.4	4.1 ± 0.5	4.7 ± 0.7	5.2 ± 0.4
25	4.6 ± 0.8	2.7 ± 0.2	3.8 ± 0.7	5.1 ± 0.9	2.5 ± 1.0	3.9 ± 0.3	4.8 ± 1.0	4.8 ± 0.6
32	3.9 ± 0.1	2.8 ± 0.3	3.5 ± 0.6	4.23 ± 0.4	1.6 ± 0.9	3.4 ± 0.4	4.7 ± 0.2	4.7 ± 0.7
39	4.2 ± 0.2	2.9 ± 0.1	3.4 ± 0.8	4.0 ± 0.7	1.4 ± 1.2	4.2 ± 0.1	3.4 ± 0.9	5.0 ± 0.4
53	3.6 ± 0.2	2.8 ± 0.1	3.8 ± 0.5	4.1 ± 0.7	1.4 ± 0.8	3.8 ± 0.6	3.8 ± 0.9	4.5 ± 0.7
67	3.5 ± 0.6	2.9 ± 0.2	3.4 ± 0.7	4.3 ± 0.5	2.3 ± 0.7	3.9 ± 0.6	3.5 ± 1.3	5.0 ± 0.3
81	3.6 ± 0.2	2.9 ± 0.3	3.3 ± 0.5	4.0 ± 0.7	<1.0 ± 0.7	3.7 ± 0.3	4.1 ± 0.8	4.7 ± 0.7
95	3.5 ± 0.4	2.9 ± 0.2	2.9 ± 1.0	4.1 ± 0.5	1.7 ± 0.3	3.2 ± 0.3	4.0 ± 0.5	4.4 ± 0.8
109	3.1 ± 0.5	3.0 ± 0.2	3.0 ± 0.6	3.8 ± 0.7	0.9 ± 0.7	3.1 ± 0.4	4.5 ± 0.2	3.8± 0.9
123	2.2 ±1.5	2.7 ± 0.2	1.9 ± 1.9	2.7 ± 1.6	<0.5 ± 0.7	1.9 ± 0.9	3.1 ± 1.3	3.4 ± 1.6

Table A.15: Total aerobic plate count (n = 3) on various soiled food packaging materials when inoculated at a level of approximately 5 log CFU/cm^2 and stored at 4º C – First Replicate

Day of Storage	Materials							
	Butcher Paper	Cardboard	Deli Paper	Foil	Overwrap	Paper Bag	Plastic Bag	Vacuum Pouch
0	5.2 ± 0.1	4.8 ± 0.6	4.8 ± 0.4	5.1 ± 0.1	4.9 ± 0.1	4.7 ± 0.3	5.0 ± 0.1	4.9 ± 0.1
4	5.3 ± 0.1	3.3 ± 0.4	4.5 ± 0.3	4.2 ± 0.4	3.7 ± 0.2	4.3 ± 0.6	5.2 ± 0.5	5.4 ± 0.6
11	4.6 ± 0.3	2.7 ± 0.4	3.4 ± 0.3	5.0 ± 0.1	1.9 ± 1.0	4.0 ± 0.3	5.5 ± 0.1	4.7 ± 0.1
18	4.0 ± 0.5	2.7 ± 0.1	3.4 ± 0.0	4.3 ± 0.5	2.0 ± 0.6	3.9 ± 0.6	4.5 ± 1.1	5.1 ± 0.6
25	3.9 ± 0.2	2.9 ± 0.2	3.2 ± 0.5	4.2 ± 0.2	1.6 ± 0.2	4.1 ± 0.3	4.1 ± 1.1	4.9 ± 0.7
32	3.9 ± 0.2	2.6 ± 0.1	2.9 ± 0.3	4.0 ± 0.3	0.8 ± 0.3	3.6 ± 0.4	4.6 ± 0.2	4.7 ± 0.7
39	4.1 ± 0.2	2.9 ± 0.1	2.7 ± 0.1	3.9 ± 0.3	1.1 ± 0.7	4.1 ± 0.0	3.1 ± 0.2	4.9 ± 0.5
53	3.4 ± 0.3	2.6 ± 0.0	3.4 ± 0.3	4.0 ± 0.1	1.3 ± 0.6	3.3 ± 0.2	3.0 ± 0.4	4.3 ± 0.8
67	3.1 ± 0.4	2.7 ± 0.1	2.8 ± 0.2	4.2 ± 0.2	1.7 ± 0.4	3.8 ± 0.1	2.5 ± 0.9	4.8 ± 0.3
81	3.4 ± 0.1	2.9 ± 0.3	2.9 ± 0.1	3.4 ± 0.7	0.9 ± 0.4	3.6 ± 0.3	3.5 ± 0.6	4.9 ± 0.5
95	3.2 ± 0.2	2.8 ± 0.1	2.1 ± 0.7	3.7 ± 0.4	1.5 ± 0.1	3.3 ± 0.4	3.7 ± 0.0	3.8 ± 0.8
109	2.7 ± 0.1	2.9 ± 0.2	2.5 ± 0.3	3.2 ± 0.6	0.8 ± 0.5	3.0 ± 0.4	4.5 ± 0.3	3.5 ± 1.2
123	0.8 ± 0.3	2.7 ± 0.3	0.1 ± 0.2	<1.3 ± 0.3	<-0.1 ± 0.0	1.1 ± 0.2	1.9 ± 0.4	1.9 ± 0.3

Table A.16: Total aerobic plate count (n = 3) on various soiled food packaging materials when inoculated at a level of approximately 5 log CFU/cm^2 and stored at 4º C – Second Replicate

	Materials							
Day of Storage	Butcher Paper	Cardboard	Deli Paper	Foil	Overwrap	Paper Bag	Plastic Bag	Vacuum Pouch
0	5.2 ± 0.1	4.8 ± 0.1	4.9 ± 0.1	4.9 ± 0.2	4.8 ± 0.2	5.0 ± 0.1	4.9 ± 0.1	5.0 ± 0.0
4	5.8 ± 0.4	4.0 ± 1.2	5.3 ± 0.1	5.9 ± 0.2	5.2 ± 0.7	5.4 ± 0.6	6.0 ± 0.5	5.9 ± 0.2
11	4.0 ± 0.3	2.8 ± 0.2	3.8 ± 0.5	4.1 ± 0.4	2.4 ± 0.4	3.7 ± 0.4	5.0 ± 0.1	4.1 ± 1.1
18	4.4 ± 0.1	2.9 ± 0.4	4.3 ± 0.2	4.7 ± 0.0	1.9 ± 0.1	4.4 ± 0.5	4.9 ± 0.1	5.2 ± 0.2
25	5.3 ± 0.2	2.5 ± 0.1	4.4 ± 0.1	5.8 ± 0.2	3.4 ± 0.2	3.8 ± 0.2	5.4 ± 0.2	4.8± 0.7
32	3.9 ± 0.1	2.9 ± 0.2	4.0 ± 0.1	4.6 ± 0.2	2.4 ± 0.4	3.1 ± 0.4	4.8 ± 0.1	4.7 ± 0.8
39	4.2 ± 0.3	2.9 ± 0.0	4.1 ± 0.1	4.2 ± 1.0	1.8 ± 1.6	4.3 ± 0.0	3.6 ± 1.3	5.0 ± 0.1
53	3.7 ± 0.0	2.9 ± 0.0	4.3 ± 0.2	4.2 ± 1.0	1.5 ± 1.1	4.3 ± 0.2	4.6 ± 0.3	4.6 ± 0.7
67	4.0 ± 0.1	3.0 ± 0.2	4.0 ± 0.1	4.4 ± 0.7	2.9 ± 0.2	3.9 ± 0.2	4.5 ± 0.7	5.2 ± 0.0
81	3.8 ± 0.1	2.8 ± 0.3	3.7 ± 0.2	4.5 ± 0.1	<1.0 ± 1.0	3.8 ± 0.3	4.7 ± 0.2	4.5 ± 0.8
95	3.8 ± 0.3	3.1 ± 0.1	3.7 ± 0.3	4.6 ± 0.1	1.9 ± 0.3	3.1 ± 0.1	4.4 ± 0.5	4.9 ± 0.2
109	3.6 ± 0.1	3.1 ± 0.2	3.4 ± 0.2	4.3 ± 0.1	1.0 ± 1.0	3.2 ± 0.5	4.5 ± 0.2	4.2 ± 0.5
123	3.6 ± 0.1	2.7 ± 0.2	3.6 ± 0.1	4.1 ± 0.3	1.1 ± 0.5	2.5 ± 0.6	4.4 ± 0.0	4.9 ± 0.3

Table A.17: Populations of *Listeria monocytogenes* (n = 6) on soiled food packaging surfaces when inoculated at a level of approximately 2 log CFU/cm^2 and stored at 25º C – Combined Means (Figure 1.5)

	Materials							
Day of Storage	Butcher Paper	Cardboard	Deli Wax Paper	Aluminum Foil	PVC Overwrap Film	Paper Bag	Plastic Bag	Vacuum Pouch
0	2.1 ± 0.1	1.9 ± 0.2	2.1 ± 0.1	2.3 ± 0.2	2.2 ± 0.0	2.0 ± 0.2	2.2 ± 0.2	2.3 ± 0.2
4	3.7 ± 1.5	1.0 ± 0.6	-0.1 ± 0.0	3.9 ± 0.7	1.7 ± 0.6	3.2 ± 0.8	4.9 ± 1.0	2.8 ± 1.0
11	4.2 ± 0.1	0.7 ± 0.5	-0.1 ± 0.0	4.2 ± 0.6	1.9 ± 1.1	2.4 ± 0.9	4.3 ± 1.5	2.9 ± 1.8
18	3.6 ± 0.5	0.4 ± 0.3	-0.1 ± 0.0	4.2 ± 0.9	1.0 ± 1.4	2.4 ± 0.8	4.0 ± 0.7	3.2 ± 1.1
25	3.9 ± 0.5	0.0 ± 0.3	-0.1 ± 0.0	3.6 ± 0.9	1.0 ± 1.4	1.7 ± 0.8	4.3 ± 0.7	2.0 ± 1.1
32	3.6 ± 1.1	0.9 ± 1.0	-0.1 ± 0.0	3.5 ± 1.4	1.1 ± 1.8	<0.9 ± 0.9	3.1 ± 0.9	1.6 ± 1.3
39	2.4 ± 0.7	-0.1 ± 0.0	-0.1 ± 0.0	3.4 ± 1.6	1.0 ± 1.5	<-0.1 ± 0.0	3.7 ± 1.1	1.0 ± 1.7
53	3.0 ± 0.6	-0.1 ± 0.0	-0.1 ± 0.0	3.7 ± 0.9	1.8 ± 2.1	<0.5 ± 0.7	3.9 ± 0.9	1.9 ± 1.7
67	2.5 ± 0.5	-0.1 ± 0.0	-0.1 ± 0.0	3.0 ± 1.4	0.9 ± 1.2	<-0.1 ± 0.0	3.6 ± 0.9	3.4 ± 0.7
81	2.0 ±0.7	-0.1 ± 0.0	-0.1 ± 0.0	2.8 ± 1.8	0.0 ± 0.1	<-0.1 ± 0.0	2.9 ± 1.6	2.0 ± 0.9
95	2.3 ± 0.6	-0.1 ± 0.0	-0.1 ± 0.0	3.0 ±1.4	-0.1 ± 0.0	<-0.1	2.9 ± 1.8	0.5 ± 1.0
109	1.4 ± 0.8	-0.1 ± 0.0	-0.1 ± 0.0	2.3 ± 1.4	0.1 ± 0.6	<-0.1	2.3 ± 1.3	0.9 ± 1.6
123	1.4 ± 0.3	-0.1 ± 0.0	-0.1 ± 0.0	1.9 ± 2.2	0.3 ± 0.7	<-0.1	1.7 ± 2.1	0.5 ± 1.0

Table A.18: Populations of *Listeria monocytogenes* (n = 3) on soiled food packaging surfaces when inoculated at a level of approximately 2 log CFU/cm^2 and stored at 25º C – First Replicate

Day of Storage	Materials							
	Butcher Paper	Cardboard	Deli Paper	Foil	Overwrap	Paper Bag	Plastic Bag	Vacuum Bag
0	2.1 ± 0.0	2.1 ± 0.1	2.1 ± 0.03	2.1 ± 0.0	2.1 ± 0.1	2.0 ± 0.05	2.1 ± 0.0	2.1 ± 0.2
4	3.0 ± 2.1	0.5 ± 0.3	<-0.1	3.1 ± 0.8	1.3 ± 1.8	3.1 ± 0.40	4.5 ± 0.1	1.8 ± 1.7
11	4.0 ± 0.1	1.1 ± 0.1	<-0.1	3.6 ± 0.6	1.7 ± 0.4	2.3 ± 0.94	3.6 ± 1.1	2.9 ± 0.9
18	4.0 ± 0.5	0.8 ± 0.3	<-0.1 ± 0.0	3.7 ± 0.5	<0.6 ± 1.1	1.7 ± 0.63	2.9 ± 1.3	4.7 ± 0.1
25	3.7 ± 0.2	<0.1 ± 0.4	<-0.1	2.9 ± 0.4	<-0.1	1.0 ± 0.48	4.0 ± 0.5	2.7 ± 1.0
32	<3.1 ± 1.3	1.4 ± 1.1	Discontinued	2.4 ± 1.2	<-0.1	<0.5 ± 0.57	2.5 ± 0.8	2.4 ± 0.2
39	2.0 ± 0.8	<-0.1	Discontinued	2.1 ± 0.9	<-0.1	<-0.1 ± 0.0	3.0 ± 1.2	<2.0 ± 1.9
53	2.8 ± 0.9	<-0.1	Discontinued	2.8 ± 0.1	<-0.1	<-0.1	3.0 ± 0.2	3.3 ± 0.2
67	2.3 ± 0.6	0.0 ± 0.2	Discontinued	1.8 ± 0.8	0.0 ± 0.2	<-0.1 ± 0.0	2.7 ± 0.3	2.8 ± 0.3
81	2.5 ± 0.5	<-0.1	Discontinued	<1.3 ± 1.2	<0.0 ± 0.2	<-0.1 ± 0.0	1.6 ± 0.8	1.8 ± 0.3
95	2.0 ± 0.4	<-0.1	Discontinued	1.7 ± 0.2	<-0.10	<-0.10	<1.5 ± 1.4	<0.8 ± 1.2
109	0.7 ± 0.5	Discontinued	Discontinued	1.1 ± 1.1	<-0.10	Discontinued	1.6 ± 1.6	1.9 ± 1.8
123	1.1 ± 0.1	Discontinued	Discontinued	<-0.1 ± 0.0	<-0.10	Discontinued	<-0.1 ± 0.0	<0.1 ± 0.4

Table A.19: Populations of *Listeria monocytogenes* (n = 3) on soiled food packaging surfaces when inoculated at a level of approximately 2 log CFU/cm^2 and stored at 25º C – Second Replicate

Day of Storage	Materials							
	Butcher Paper	Cardboard	Deli Paper	Foil	Overwrap	Paper Bag	Plastic Bag	Vacuum Bag
0	2.1 ± 0.1	1.6 ± 0.1	2.2 ± 0.1	2.4 ± 0.1	2.2 ± 0.0	2.0 ± 0.3	2.4 ± 0.2	2.4 ± 0.1
4	4.4 ± 0.5	1.5 ± 0.4	<-0.1 ± 0.0	4.7 ± 0.2	2.0 ± 0.3	3.2 ± 0.4	5.2 ± 0.1	4.3 ± 0.7
11	4.3 ± 0.1	<0.3 ± 0.8	<-0.1 ± 0.0	4.7 ± 0.2	2.2 ± 0.7	2.5 ± 0.9	5.1 ± 0.1	2.9 ± 1.3
18	3.3 ± 0.4	<0.0 ± 0.2	<-0.1 ± 0.0	4.7 ± 0.2	1.4 ± 1.0	3.1 ± 0.2	5.1 ± 0.1	<1.7 ± 0.9
25	4.1 ± 0.7	<-0.1 ± 0.0	<-0.1 ± 0.0	4.4 ± 0.3	2.0 ± 1.3	2.4 ± 1.4	4.6 ± 0.9	1.4 ± 0.9
32	4.1 ± 0.8	<0.3 ± 0.4	<-0.1 ± 0.0	4.5 ± 0.3	<2.3 ± 2.1	1.2 ± 1.2	3.7 ± 0.4	1.3 ± 1.9
39	2.8 ± 0.5	<-0.1 ± 0.0	Discontinued	4.8 ± 0.2	2.2 ± 0.4	<-0.1 ± 0.0	4.4 ± 0.1	<-0.1 ± 0.0
53	3.2 ± 0.4	<-0.1 ± 0.0	Discontinued	4.2 ± 0.6	3.7 ± 0.3	1.1 ± 0.6	4.0 ± 0.2	<0.5 ± 1.0
67	2.7 ± 0.2	<-0.1 ± 0.0	Discontinued	4.2 ± 0.6	1.8 ± 1.1	<-0.1 ± 0.0	4.4 ± 0.4	4.0 ± 0.3
81	1.5 ± 0.4	<-0.1 ± 0.0	Discontinued	4.3 ± 0.1	<-0.1	<-0.1	4.1 ± 0.9	2.3 ± 1.4
95	2.8 ± 0.5	<-0.1 ± 0.0	Discontinued	4.2 ± 0.1	<-0.1 ± 0.0	<-0.1	4.4 ± 0.2	<-0.1
109	2.0 ± 0.3	Discontinued	Discontinued	3.4 ± 0.1	<0.4 ± 0.8	Discontinued	3.0 ± 0.5	<-0.1 ± 0.0
123	1.7 ± 0.1	Discontinued	Discontinued	4.0 ± 0.1	<0.8 ± 0.8	Discontinued	3.5 ± 1.1	<0.8 ± 1.4

Table A.20: Total aerobic plate count (n = 6) on various soiled food packaging materials when inoculated at a level of approximately 2 log CFU/cm^2 and stored at 25º C – Combined Means (Figure 1.6)

	Materials							
Day of Storage	Butcher Paper	Cardboard	Deli Wax Paper	Aluminum Foil	PVC Overwrap Film	Paper Bag	Plastic Bag	Vacuum Pouch
0	2.1 ± 0.1	2.9 ± 0.2	2.1 ± 0.1	2.3 ± 0.2	2.2 ± 0.1	2.0 ± 0.2	2.3 ± 0.1	2.3 ± 0.2
4	2.9 ± 1.4	2.7 ± 0.2	0.4 ± 0.2	4.0 ± 0.5	2.1 ± 0.8	3.3 ± 0.7	4.9 ± 0.7	3.6 ± 1.1
11	4.2 ± 0.2	2.8 ± 0.2	0.1 ± 0.1	4.5 ± 0.4	2.4 ± 0.8	3.1 ± 0.6	4.8 ± 1.5	3.2 ± 1.4
18	3.9 ± 0.5	2.9 ± 0.4	0.0 ± 0.3	4.6 ± 0.6	1.5 ± 1.1	3.1 ± 0.7	4.2 ± 0.6	3.6 ± 0.8
25	3.9 ± 0.4	2.7 ±0.4	0.2 ± 0.3	4.2 ± 0.6	1.7 ± 1.1	2.3 ± 0.7	4.6 ± 0.6	3.0 ± 0.8
32	3.7 ± 1.3	2.7 ± 0.1	-0.1 ± 0.0	4.2 ± 0.8	1.3 ± 2.0	1.3 ± 0.5	3.7 ± 1.1	2.5 ± 1.3
39	3.0 ± 0.8	2.6 ± 0.3	-0.1 ± 0.0	4.2 ± 0.9	1.5 ± 1.6	1.0 ± 1.2	4.0 ± 0.9	1.7 ± 1.1
53	3.2 ± 0.4	2.5 ± 0.4	-0.1 ± 0.0	4.1 ± 0.6	1.9 ± 2.1	0.8 ± 0.9	4.5 ± 0.6	2.4 ± 1.9
67	2.8 ± 0.3	2.8 ± 0.3	-0.1 ± 0.0	3.9 ± 0.7	1.6 ± 1.2	0.4 ± 0.8	4.2 ± 0.7	3.2 ± 0.9
81	2.4 ± 0.6	2.5 ± 0.4	-0.1 ± 0.0	3.5 ± 1.5	0.3 ± 0.6	0.5 ± 0.4	3.4 ± 1.3	1.9 ± 0.6
95	2.3 ± 0.4	2.6 ± 0.2	-0.1 ± 0.0	3.7 ± 1.0	0.1 ± 0.4	0.1 ± 0.4	3.5 ± 1.5	1.0 ± 1.4
109	1.9 ± 0.8	Discontinued	-0.1 ± 0.0	2.9 ± 1.5	0.2 ± 0.4	-0.1 ± 0.0	2.9 ± 1.4	1.1 ± 1.7
123	1.9 ± 0.5	Discontinued	-0.1 ± 0.0	2.1 ± 2.5	0.5 ± 1.0	-0.1 ± 0.0	2.0 ± 2.1	0.5 ± 0.9

Table A.21: Total aerobic plate count (n = 3) on various soiled food packaging materials when inoculated at a level of approximately 2 log CFU/cm^2 and stored at 25º C – First Replicate

Day of Storage	Materials							
	Butcher Paper	Cardboard	Deli Paper	Foil	Overwrap	Paper Bag	Plastic Bag	Vacuum Bag
0	2.1 ± 0.1	2.8 ± 0.1	2.1 ± 0.1	2.1 ± 0.1	2.1 ± 0.1	2.0 ± 0.1	2.2 ± 0.0	2.1 ± 0.0
4	3.3 ± 1.9	2.5 ± 0.1	0.3 ± 0.3	3.3 ± 0.6	1.7 ± 2.0	3.2 ± 0.1	4.5 ± 0.1	3.0 ± 0.4
11	4.1 ± 0.0	2.9 ± 0.2	<0.0 ± 0.2	4.1 ± 0.1	1.8 ± 0.5	2.6 ± 0.7	4.3 ± 0.7	3.2 ± 0.7
18	4.2 ± 0.3	3.0 ± 0.1	<0.0 ± 0.2	4.2 ± 0.3	1.2 ± 0.9	2.6 ± 0.4	3.2 ± 1.6	4.8 ± 0.1
25	3.7 ± 0.2	2.9 ± 0.4	<0.1 ± 0.3	3.8 ± 0.5	1.0 ± 1.2	2.1 ± 0.5	4.3 ± 0.2	3.3 ± 1.1
32	3.2 ± 1.6	2.7 ± 0.0	Discontinued	3.6 ± 0.5	<0.0 ± 0.2	1.0 ± 0.5	3.1 ± 1.3	2.8 ± 0.3
39	2.6 ± 0.8	2.6 ± 0.2	Discontinued	3.4 ± 0.2	<0.1 ± 0.4	<-0.1 ± 0.0	3.4 ± 0.9	1.7 ± 1.6
53	3.0 ± 0.3	2.7 ± 0.4	Discontinued	3.6 ± 0.3	<-0.1 ± 0.0	<0.1 ± 0.3	4.0 ± 0.4	4.2 ± 0.6
67	2.8 ± 0.4	3.1 ± 0.1	Discontinued	3.4 ± 0.4	0.5 ± 0.5	<-0.1 ± 0.0	3.6 ± 0.2	2.6 ± 0.9
81	2.9 ± 0.4	2.8 ± 0.4	Discontinued	2.5 ± 1.6	0.6 ± 0.6	0.6 ± 0.3	2.5 ± 1.1	2.3 ± 0.1
95	2.3 ± 0.5	2.6 ± 0.3	Discontinued	2.8 ± 0.3	<-0.1 ± 0.0	<0.2 ± 0.5	2.3 ± 1.2	1.5 ± 1.7
109	1.2 ± 0.3	Discontinued	Discontinued	1.6 ± 0.6	<-0.1 ± 0.0	Discontinued	2.1 ± 1.8	2.4 ± 1.7
123	1.5 ± 0.6	Discontinued	Discontinued	<-0.1 ± 0.0	<-0.1 ± 0.0	Discontinued	<0.2 ± 0.5	<0.5 ± 1.1

Table A.22: Total aerobic plate count (n = 3) on various soiled food packaging materials when inoculated at a level of approximately 2 log CFU/cm^2 and stored at 25º C – Second Replicate

Day of Storage	Materials							
	Butcher Paper	Cardboard	Deli Paper	Foil	Overwrap	Paper Bag	Plastic Bag	Vacuum Bag
0	2.0 ± 0.0	3.1 ± 0.1	2.2 ± 0.1	2.4 ± 0.1	2.3 ± 0.1	2.0 ± 0.3	2.3 ± 0.1	2.4 ± 0.1
4	4.5 ± 0.4	3.0 ± 0.3	0.6 ± 0.3	4.8 ± 0.1	2.8 ± 0.2	3.4 ± 0.1	5.2 ± 0.2	4.2 ± 0.0
11	4.4 ± 0.2	2.7 ± 0.1	<0.1 ± 0.2	4.9 ± 0.1	3.0 ± 0.6	3.6 ± 0.3	5.3 ± 0.1	3.2 ± 1.7
18	3.5 ± 0.4	2.8 ± 0.1	<-0.1 ± 0.0	4.9 ± 0.1	1.9 ± 0.7	3.5 ± 0.1	5.2 ± 0.1	2.5 ± 0.8
25	4.2 ± 0.4	2.5 ± 0.2	<0.3 ± 0.3	4.7 ± 0.3	2.4 ± 0.0	2.6 ± 0.9	4.9 ± 0.8	2.6 ± 0.3
32	4.3 ± 0.6	2.6 ± 0.2	<-0.1 ± 0.0	4.8 ± 0.4	<2.5 ± 2.3	1.8 ± 0.1	4.4 ± 0.2	2.2 ± 2.4
39	3.4 ± 0.6	2.5 ± 0.4	Discontinued	5.0 ± 0.1	2.8 ± 0.8	2.1 ± 0.2	4.6 ± 0.6	1.7 ± 0.2
53	3.3 ± 0.5	2.3 ± 0.1	Discontinued	4.6 ± 0.4	3.8 ± 0.2	1.6 ± 0.5	5.0 ± 0.1	<1.2 ± 1.2
67	2.9 ± 0.2	2.5 ± 0.1	Discontinued	4.4 ± 0.6	2.6 ± 0.6	1.0 ± 0.8	4.7 ± 0.4	3.8 ± 0.2
81	2.0 ± 0.4	2.3 ± 0.3	Discontinued	4.5 ± 0.1	<-0.1 ± 0.0	0.5 ± 0.6	4.2 ± 0.9	<1.5 ± 0.6
95	2.2 ± 0.0	2.5 ± 0.1	Discontinued	4.6 ± 0.2	<0.4 ± 0.4	<-0.1 ± 0.0	4.7 ± 0.3	0.3 ± 0.6
109	2.6 ± 0.1	Discontinued	Discontinued	4.3 ± 0.4	0.5 ± 0.4	Discontinued	2.7 ± 0.5	<-0.1 ± 0.0
123	2.2 ± 0.0	Discontinued	Discontinued	4.4 ± 0.1	<1.2 ± 1.2	Discontinued	3.8 ± 1.1	0.4 ± 0.7

Table A.23: Populations of *Listeria monocytogenes* (n = 6) on soiled food packaging surfaces when inoculated at a level of approximately 2 log CFU/cm^2 and stored at 4º C – Combined Means (Figure 1.7)

	Materials							
Day of Storage	Butcher Paper	Cardboard	Deli Wax Paper	Aluminum Foil	PVC Overwrap Film	Paper Bag	Plastic Bag	Vacuum Pouch
0	2.1 ± 0.1	1.9 ± 0.2	2.1 ± 0.1	2.3 ± 0.2	2.2 ± 0.0	2.0 ± 0.2	2.2 ± 0.2	2.3 ± 0.2
4	1.4 ± 0.4	0.3 ± 0.4	1.1 ± 0.9	1.4 ± 0.6	-0.1 ± 0.0	1.0 ± 0.4	1.9 ± 0.2	<1.0 ± 1.0
11	0.6 ± 0.4	<0.2 ± 0.4	0.0 ± 0.2	1.0 ± 0.3	-0.1 ± 0.0	<0.7 ± 0.5	1.9 ± 0.3	<1.0 ± 1.2
18	0.6 ± 0.3	0.1 ± 0.4	<-0.1 ± 0.1	1.1 ± 0.2	0.2 ± 0.7	0.9 ± 0.3	1.7 ± 0.1	<0.7 ± 0.8
25	0.6 ± 0.5	<0.0 ± 0.2	<-0.1 ± 0.1	0.9 ± 0.3	<-0.1 ± 0.1	1.0 ± 0.5	1.6 ± 0.4	<0.7 ± 1.0
32	<0.2 ± 0.5	0.1 ± 0.3	<-0.10	0.8 ± 0.6	<-0.1 ± 0.1	0.4 ± 0.5	1.6 ± 0.2	<0.9 ± 0.8
39	0.5 ± 0.6	<0.1 ± 0.2	<-0.10	1.0 ± 0.2	<-0.1 ± 0.1	<0.2 ± 0.3	1.7 ± 0.2	<1.3 ± 1.1
53	0.5 ± 0.5	<-0.1 ± 0.1	<-0.10	<0.3 ± 0.5	<-0.1 ± 0.1	<0.0 ± 0.2	1.5 ± 0.3	<1.1 ± 1.0
67	0.4 ± 0.7	<-0.1 ± 0.1	<-0.10	0.2 ± 0.4	<-0.1 ± 0.1	<-0.1 ± 0.1	1.4 ± 0.4	<1.0 ± 1.1
81	<-0.1 ±0.2	<-0.1 ± 0.1	<-0.10	<0.2 ± 0.4	<-0.1 ± 0.1	<-0.1 ± 0.1	1.2 ± 0.2	<-0.1 ± 0.0
95	0.4 ± 0.5	<-0.1 ± 0.1	<-0.10	<0.3 ± 0.4	<-0.1 ± 0.1	<-0.1 ± 0.1	1.2 ± 0.7	<0.8 ± 0.8
109	<-0.1 ± 0.1	<-0.1 ± 0.1	<-0.10	<0.1 ± 0.3	<-0.1 ± 0.1	<-0.1 ± 0.1	0.6 ± 0.6	<0.9 ± 1.0
123	<-0.1 ± 0.1	<-0.1 ± 0.1	<-0.10	<0.0 ± 0.1	<-0.1 ± 0.1	<-0.1 ± 0.1	<0.1 ± 0.3	<0.5 ± 0.5

Table A.24: Populations of *Listeria monocytogenes* (n = 3) on soiled food packaging surfaces when inoculated at a level of approximately 2 log CFU/cm^2 and stored at 4º C – First Replicate

Day of Storage	Materials							
	Butcher Paper	Cardboard	Deli Paper	Foil	Overwrap	Paper Bag	Plastic Bag	Vacuum Bag
0	2.1 ± 0.0	2.1 ± 0.1	2.1 ± 0.0	2.1 ± 0.0	2.1 ± 0.1	2.0 ± 0.1	2.1 ± 0.0	2.1 ± 0.1
4	1.3 ± 0.6	0.5 ± 0.2	0.2 ± 0.3	1.1 ± 0.7	<-0.1 ± 0.0	0.7 ± 0.5	2.1 ± 0.0	1.4 ± 0.7
11	0.7 ± 0.1	0.6 ± 0.1	<0.1 ± 0.3	1.2 ± 0.1	<-0.1 ± 0.0	<0.2 ± 0.4	2.2 ± 0.1	2.2 ± 0.1
18	0.6 ± 0.3	0.4 ± 0.4	<-0.1 ± 0.0	1.2 ± 0.0	<-0.10	1.0 ± 0.2	1.7 ± 0.1	1.0 ± 0.8
25	0.9 ± 0.2	0.1 ± 0.3	<-0.10	1.1 ± 0.1	<-0.10	1.2 ± 0.3	1.9 ± 0.2	1.3 ± 1.2
32	<0.5 ± 0.5	<0.3 ± 0.4	<-0.10	1.3 ± 0.2	<-0.10	0.6 ± 0.7	1.7 ± 0.2	1.3 ± 0.9
39	1.1 ± 0.1	0.3 ± 0.1	<-0.10	1.1 ± 0.2	<-0.10	<0.3 ± 0.3	1.9 ± 0.0	1.9 ± 0.3
53	0.6 ± 0.3	<-0.1 ± 0.0	<-0.10	<0.1 ± 0.4	<-0.10	<0.1 ± 0.4	1.7 ± 0.2	1.8 ± 0.2
67	0.9 ± 0.7	<-0.1 ± 0.0	<-0.10	0.0 ± 0.2	Discontinued	<-0.1 ± 0.0	1.7 ± 0.1	1.9 ± 0.1
81	<-0.1 ± 0.0	<-0.1 ± 0.0	<-0.10	<0.5 ± 0.5	Discontinued	<-0.1 ± 0.0	1.4 ± 0.1	<-0.1 ± 0.0
95	0.3 ± 0.4	<-0.1 ± 0.0	<-0.10	<0.3 ± 0.4	Discontinued	<-0.1 ± 0.0	1.5 ± 0.1	<1.0 ± 1.0
109	<-0.1 ± 0.0	Discontinued	Discontinued	0.3 ± 0.4	Discontinued	Discontinued	1.1 ± 0.2	1.7 ± 0.3
123	<-0.1 ± 0.0	Discontinued	Discontinued	<-0.10	Discontinued	Discontinued	<0.1 ± 0.3	0.7 ± 0.2

Table A.25: Populations of *Listeria monocytogenes* (n = 3) on soiled food packaging surfaces when inoculated at a level of approximately 2 log CFU/cm^2 and stored at 4º C – Second Replicate

Day of Storage	Materials							
	Butcher Paper	Cardboard	Deli Paper	Foil	Overwrap	Paper Bag	Plastic Bag	Vacuum Bag
0	2.1 ± 0.1	1.6 ± 0.1	2.2 ± 0.1	2.4 ± 0.1	2.2 ± 0.0	2.0 ± 0.28	2.4 ± 0.2	2.4 ± 0.1
4	1.5 ± 0.2	<0.2 ± 0.5	1.9 ± 0.1	1.7 ± 0.4	<-0.1 ± 0.0	1.3 ± 0.10	1.8 ± 0.1	<0.6 ± 1.3
11	0.4 ± 0.5	<-0.1	<-0.1 ± 0.0	0.7 ± 0.1	<-0.1 ± 0.0	1.1 ± 0.03	1.6 ± 0.0	<-0.1 ± 0.0
18	0.5 ± 0.5	<-0.1 ± 0.0	<-0.1 ± 0.0	0.9 ± 0.1	<-0.1 ± 0.0	0.9 ± 0.44	1.7 ± 0.0	<0.4 ± 0.8
25	0.2 ± 0.2	<-0.1	<-0.1 ± 0.0	0.7 ± 0.4	<-0.1 ± 0.0	0.9 ± 0.64	1.3 ± 0.4	<0.2 ± 0.5
32	<-0.1 ± 0.0	<0.0 ± 0.2	<-0.10	0.4 ± 0.5	<-0.1 ± 0.0	0.3 ± 0.43	1.5 ± 0.1	<0.5 ± 0.5
39	<0.0 ± 0.2	<-0.1	<-0.10	0.9 ± 0.3	<-0.1 ± 0.0	0.1 ± 0.28	1.5 ± 0.1	<1.3 ± 1.2
53	0.4 ± 0.8	<-0.1 ± 0.0	<-0.10	0.5 ± 0.5	<-0.1 ± 0.0	<-0.1 ± 0.0	1.3 ± 0.1	0.5 ± 1.0
67	<-0.1 ± 0.0	<-0.1	<-0.10	<0.4 ± 0.5	Discontinued	<-0.1	1.1 ± 0.1	<0.0 ± 0.2
81	<0.1 ± 0.3	<-0.1 ± 0.0	<-0.10	0.0 ± 0.2	Discontinued	<-0.1	1.0 ± 0.1	<-0.1
95	<0.5 ± 0.6	<-0.1	<-0.10	0.3 ± 0.4	Discontinued	<-0.1 ± 0.0	<0.9 ± 0.9	<0.6 ± 0.7
109	<-0.1	Discontinued	Discontinued	<-0.1 ± 0.0	Discontinued	Discontinued	<0.1 ± 0.4	<0.0 ± 0.2
123	<0.0 ± 0.2	Discontinued	Discontinued	<0.0 ± 0.2	Discontinued	Discontinued	0.1 ± 0.4	<0.3 ± 0.8

Table A.26: Total aerobic plate count (n = 6) on various soiled food packaging materials when inoculated at a level of approximately 2 log CFU/cm^2 and stored at 4º C – Combined Means (Figure 1.8)

	Materials							
Day of Storage	Butcher Paper	Cardboard	Deli Wax Paper	Aluminum Foil	PVC Overwrap Film	Paper Bag	Plastic Bag	Vacuum Pouch
0	2.1 ± 0.1	2.9 ± 0.2	2.1 ± 0.1	2.3 ± 0.2	2.2 ± 0.1	2.0 ± 0.2	2.3 ± 0.1	2.3 ± 0.2
4	1.7 ± 0.3	2.4 ± 0.1	1.4 ± 0.8	1.8 ± 0.3	0.1 ± 0.3	1.2 ± 0.3	2.1 ± 0.2	1.4 ± 0.7
11	0.9 ± 0.4	2.5 ± 0.2	0.7 ± 0.1	1.6 ± 0.4	0.0 ± 0.1	1.2 ± 0.5	2.0 ± 0.1	1.1 ± 1.3
18	1.0 ± 0.3	2.6 ± 0.2	0.1 ± 0.3	1.4 ± 0.5	<0.1 ± 0.2	1.4 ± 0.5	2.0 ± 0.1	1.1 ± 0.9
25	0.9 ± 0.4	2.5 ± 0.1	0.2 ± 0.4	1.4 ± 0.6	<0.0 ± 0.3	1.6 ± 0.4	1.9 ± 0.3	1.1 ± 0.9
32	0.7 ± 0.3	2.6 ± 0.2	0.1 ± 0.3	1.5 ± 0.2	0.4 ± 0.7	1.3 ± 0.3	1.9 ± 0.1	1.2 ± 0.7
39	0.9 ± 0.4	2.7 ± 0.1	0.3 ± 0.3	1.7 ± 0.1	-0.1 ± 0.0	1.0 ± 0.6	2.1 ± 0.1	1.9 ± 0.7
53	1.0 ± 0.4	2.5 ± 0.2	0.1 ± 0.3	0.9 ± 0.8	-0.1 ± 0.0	1.2 ± 0.6	1.9 ± 0.1	1.4 ± 0.9
67	0.8 ± 0.7	2.5 ± 0.1	0.2 ± 0.2	1.1 ± 0.6	-0.1 ± 0.0	0.8 ± 0.5	1.8 ± 0.3	1.2 ± 1.1
81	0.6 ± 0.3	2.5 ± 0.3	-0.1 ± 0.0	1.0 ± 0.6	-0.1 ± 0.0	0.9 ± 0.2	1.7 ± 0.1	0.1 ± 0.3
95	1.3 ± 0.5	2.4 ± 0.2	0.3 ± 0.4	1.5 ± 0.2	-0.1 ± 0.0	1.0 ± 0.2	1.6 ± 0.4	1.0 ± 0.8
109	0.3 ± 0.5	Discontinued	-0.1 ± 0.0	0.9 ± 0.4	-0.1 ± 0.0	-0.1 ± 0.0	1.2 ± 0.5	1.2 ± 1.1
123	0.2 ± 0.4	Discontinued	-0.1 ± 0.0	0.9 ± 0.3	-0.1 ± 0.0	-0.1 ± 0.0	1.1 ± 0.4	1.1 ± 0.7

Table A.27: Total aerobic plate count (n = 3) on various soiled food packaging materials when inoculated at a level of approximately 2 log CFU/cm^2 and stored at 4º C – First Replicate

Day of Storage	Materials							
	Butcher Paper	Cardboard	Deli Paper	Foil	Overwrap	Paper Bag	Plastic Bag	Vacuum Bag
0	2.1 ± 0.1	2.8 ± 0.1	2.1 ± 0.1	2.1 ± 0.1	2.1 ± 0.1	1.9 ± 0.1	2.2 ± 0.0	2.1 ± 0.0
4	1.7 ± 0.5	2.4 ± 0.1	0.7 ± 0.5	1.7 ± 0.3	0.1 ± 0.4	1.0 ± 0.3	2.2 ± 0.1	1.6 ± 0.6
11	1.2 ± 0.1	2.6 ± 0.1	0.7 ± 0.2	1.8 ± 0.1	<0.0 ± 0.2	0.8 ± 0.3	2.1 ± 0.1	2.2 ± 0.0
18	0.9 ± 0.2	2.5 ± 0.1	0.2 ± 0.3	1.7 ± 0.1	<-0.1	1.7 ± 0.1	2.1 ± 0.1	1.5 ± 0.8
25	1.3 ± 0.3	2.4 ± 0.0	<0.4 ± 0.6	1.8 ± 0.0	<-0.1	1.9 ± 0.1	2.1 ± 0.1	1.6 ± 0.9
32	0.9 ± 0.1	2.6 ± 0.1	<0.1 ± 0.4	1.7 ± 0.1	0.3 ± 0.6	1.4 ± 0.7	1.9 ± 0.2	0.7 ± 1.5
39	1.2 ± 0.2	2.7 ± 0.1	0.4 ± 0.2	1.8 ± 0.1	<-0.1	<0.9 ± 0.9	2.2 ± 0.0	2.1 ± 0.2
53	1.2 ± 0.1	2.7 ± 0.1	0.3 ± 0.3	0.2 ± 0.5	<-0.1	1.7 ± 0.1	2.0 ± 0.1	2.0 ± 0.2
67	1.2 ± 0.5	2.5 ± 0.1	0.1 ± 0.2	0.8 ± 0.8	Discontinued	1.2 ± 0.2	1.8 ± 0.5	2.1 ± 0.1
81	0.7 ± 0.2	2.4 ± 0.3	<-0.1 ± 0.0	0.9 ± 0.9	Discontinued	1.0 ± 0.1	1.8 ± 0.2	0.2 ± 0.3
95	1.0 ± 0.1	2.4 ± 0.3	0.1 ± 0.4	1.5 ± 0.2	Discontinued	0.9 ± 0.1	1.9 ± 0.1	<1.0 ± 1.0
109	0.4 ± 0.6	Discontinued	Discontinued	1.3 ± 0.2	Discontinued	Discontinued	1.6 ± 0.2	2.1 ± 0.2
123	<0.3 ± 0.3	Discontinued	Discontinued	0.6 ± 0.2	Discontinued	Discontinued	1.1 ± 0.6	1.4 ± 0.1

Table A.28: Total aerobic plate count (n = 3)on various soiled food packaging materials when inoculated at a level of approximately 2 log CFU/cm^2 and stored at 4º C – Second Replicate

Day of Storage	Materials							
	Butcher Paper	Cardboard	Deli Paper	Foil	Overwrap	Paper Bag	Plastic Bag	Vacuum Bag
0	2.0 ± 0.0	3.1 ± 0.1	2.2 ± 0.1	2.4 ± 0.1	2.3 ± 0.1	2.0 ± 0.3	2.3 ± 0.1	2.4 ± 0.1
4	1.7 ± 0.2	2.4 ± 0.1	2.1 ± 0.1	1.9 ± 0.2	<0.1 ± 0.2	1.4 ± 0.1	2.0 ± 0.1	1.1 ± 0.9
11	0.6 ± 0.4	2.5 ± 0.2	0.7 ± 0.1	1.3 ± 0.6	<-0.1 ± 0.0	1.6 ± 0.1	1.9 ± 0.1	<-0.1 ± 0.0
18	1.1 ± 0.4	2.8 ± 0.1	0.1 ± 0.3	1.2 ± 0.7	0.2 ± 0.0	1.0 ± 0.6	1.9 ± 0.1	<0.8 ± 0.9
25	0.6 ± 0.2	2.5 ± 0.1	<0.1 ± 0.2	1.0 ± 0.7	<0.1 ± 0.4	1.3 ± 0.3	1.7 ± 0.2	<0.6 ± 0.6
32	0.5 ± 0.3	2.7 ± 0.4	<0.0 ± 0.2	1.4 ± 0.1	0.6 ± 0.8	1.1 ± 0.2	1.9 ± 0.1	0.9 ± 0.6
39	0.7 ± 0.4	2.8 ± 0.1	0.2 ± 0.5	1.7 ± 0.1	<-0.1 ± 0.0	1.1 ± 0.4	1.9 ± 0.0	1.6 ± 1.1
53	0.9 ± 0.5	2.4 ± 0.2	0.0 ± 0.2	1.6 ± 0.1	<-0.1 ± 0.0	0.7 ± 0.5	1.8 ± 0.0	0.8 ± 1.0
67	0.3 ± 0.4	2.5 ± 0.1	<0.2 ± 0.3	1.5 ± 0.1	Discontinued	<0.5 ± 0.5	1.8 ± 0.1	<0.2 ± 0.3
81	<0.4 ± 0.4	2.5 ± 0.3	<-0.1 ± 0.0	1.1 ± 0.1	Discontinued	0.7 ± 0.1	1.7 ± 0.1	<-0.1 ± 0.0
95	1.6 ± 0.6	2.5 ± 0.2	<0.4 ± 0.5	1.6 ± 0.0	Discontinued	1.0 ± 0.2	1.3 ± 0.5	1.0 ± 0.7
109	0.2 ± 0.5	Discontinued	Discontinued	0.5 ± 0.2	Discontinued	Discontinued	0.8 ± 0.2	<0.3 ± 0.6
123	0.2 ± 0.5	Discontinued	Discontinued	1.1 ± 0.2	Discontinued	Discontinued	1.1 ± 0.1	<0.8 ± 0.9

Table A.29: Summary of statistical analysis results showing which soiled materials inoculated with *Escherichia coli* O157:H7 (inoculated at 6 log CFU/cm^2 and stored up to 130 days at 25º C) exhibited a replication effect

Packaging Material	*P* Value (TSA+Rifampicin)	*P* Value (TSA)
Butcher Paper	<0.0001*	<0.0001*
Cardboard Box Material	<0.0001*	<0.0001*
PVC Overwrap Film	0.0103*	0.0102*
Vacuum Pouch Material	0.0206*	0.1983

* Effects are significant if $P < 0.05$

Table A.30: Summary of statistical analysis results showing which soiled materials inoculated with *Escherichia coli* O157:H7 (inoculated at 6 log CFU/cm^2 and stored up to 130 days at 4º C) exhibited a replication effect

Packaging Material	*P* Value (TSA+Rifampicin)	*P* Value (TSA)
Butcher Paper	<0.0001*	<0.0001*
Cardboard Box Material	0.0002*	0.0001*
PVC Overwrap Film	0.0269*	0.1170
Vacuum Pouch Material	0.7422	0.0006*

* Effects are significant if $P < 0.05$

Table A.31: Summary of statistical analysis results showing which soiled materials inoculated with *Escherichia coli* O157:H7 (inoculated at 4 log CFU/cm^2 and stored up to 130 days at 25º C) exhibited a replication effect

Packaging Material	*P* Value (TSA+Rifampicin)	*P* Value (TSA)
Butcher Paper	0.1162	0.0024*
Cardboard Box Material	<0.0001*	<0.0001*
PVC Overwrap Film	<0.0001*	<0.0001*
Vacuum Pouch Material	0.0004	<0.0001*

* Effects are significant if $P < 0.05$

Table A.32: Summary of statistical analysis results showing which soiled materials inoculated with *Escherichia coli* O157:H7 (inoculated at 4 log CFU/cm^2 and stored up to 130 days at 4º C) exhibited a replication effect

Packaging Material	*P* Value (TSA+Rifampicin)	*P* Value (TSA)
Butcher Paper	0.0043*	<0.0001*
Cardboard Box Material	0.4333	0.1543
PVC Overwrap Film	<0.0001*	<0.0001*
Vacuum Pouch Material	0.0021*	0.6265

* Effects are significant if $P < 0.05$

Table A.33: *Escherichia coli* O157:H7 (n = 6) survival on soiled food packaging materials when inoculated at a level of approximately 6 log CFU/cm^2 and stored at 25º C (TSA + Rifampicin) – Combined Means (Figure 2.1)

	Materials			
Day of Storage	Butcher Paper	Cardboard	Overwrap	Vacuum Pouch
0	6.0 ± 0.2	6.0 ± 0.1	5.9 ± 0.1	6.0 ± 0.2
4	4.7 ± 0.6	4.8 ± 0.6	4.3 ± 1.2	4.5 ± 0.5
11	4.8 ± 0.2	4.4 ± 0.8	4.7 ± 0.3	3.8 ± 0.4
18	4.4 ± 0.2	3.9 ± 1.3	4.1 ± 0.4	3.7 ± 0.9
25	4.0 ± 0.3	3.9 ± 0.9	3.4 ± 0.7	3.1 ± 1.4
32	4.0 ± 0.3	3.0 ± 1.5	3.5 ± 0.5	2.9 ± 0.4
39	3.3 ± 0.3	2.8 ± 0.7	2.8 ± 0.7	3.4 ± 0.7
53	3.1 ± 0.4	2.8 ± 0.7	2.5 ± 0.5	<2.1 ± 1.2
67	2.7 ± 0.4	2.6 ± 1.4	2.5 ± 0.3	1.9 ± 0.8
81	2.4 ± 0.4	<1.3 ± 1.6	2.6 ± 0.4	<0.8 ± 0.9
95	2.4 ± 0.6	<1.4 ± 1.7	2.3 ± 0.8	2.0 ± 0.7
109	2.4 ± 0.5	<1.6 ± 1.7	1.8 ± 0.9	1.3 ± 0.5
130	1.8 ± 0.5	<2.2 ± 1.3	1.6 ± 0.8	<1.7 ± 1.1

Table A.34: *Escherichia coli* O157:H7 (n = 3)survival on soiled food packaging materials when inoculated at a level of approximately 6 log CFU/cm^2 and stored at 25º C (TSA + Rifampicin) – First Replicate

	Materials			
Day of Storage	Butcher Paper	Cardboard	Overwrap	Vacuum Bag
0	5.9 ± 0.1	5.9 ± 0.0	5.9 ± 0.1	5.8 ± 0.0
4	5.2 ± 0.2	5.1 ± 0.1	5.1 ± 0.3	4.7 ± 0.6
11	4.9 ± 0.2	5.0 ± 0.2	4.6 ± 0.1	4.0 ± 0.4
18	4.5 ± 0.2	4.9 ± 0.3	4.4 ± 0.4	3.2 ± 0.9
25	4.3 ± 0.2	4.7 ± 0.3	3.8 ± 0.1	3.7 ± 0.6
32	4.3 ± 0.1	4.2 ± 0.5	3.9 ± 0.2	3.1 ± 0.3
39	3.5 ± 0.1	2.9 ± 0.6	3.1 ± 0.3	3.7 ± 0.9
53	3.4 ± 0.3	3.3 ± 0.4	2.6 ± 0.8	2.8 ± 0.5
67	3.0 ± 0.1	3.7 ± 0.2	2.5 ± 0.2	2.2 ± 0.9
81	2.6 ± 0.4	2.6 ± 0.8	2.3 ± 0.4	1.1 ± 1.0
95	2.4 ± 0.1	3.0 ± 0.4	1.8 ± 0.9	1.8 ± 0.7
109	2.6 ± 0.7	3.1 ± 0.4	2.3 ± 0.4	1.6 ± 0.5
130	2.2 ± 0.2	3.1 ± 0.0	1.7 ± 0.2	2.1 ± 0.2

Table A.35: *Escherichia coli* O157:H7 (n = 3)survival on soiled food packaging materials when inoculated at a level of approximately 6 log CFU/cm^2 and stored at 25º C (TSA + Rifampicin) – Second Replicate

	Materials			
Day of Storage	Butcher Paper	Cardboard	Overwrap	Vacuum Bag
0	6.1 ± 0.2	6.0 ± 0.1	6.0 ± 0.1	6.1 ± 0.1
4	4.3 ±0.4	4.6 ± 0.8	3.5 ± 1.3	4.2 ± 0.3
11	4.7 ± 0.1	3.8 ± 0.7	4.8 ± 0.4	3.7 ± 0.3
18	4.2 ± 0.0	2.9 ± 1.2	3.8 ± 0.0	4.2 ± 0.6
25	3.8 ± 0.1	3.1 ± 0.4	3.0 ± 0.9	2.5 ± 1.8
32	3.8 ± 0.2	1.2 ± 1.3	3.1 ± 0.2	2.7 ± 0.4
39	3.1 ± 0.1	2.6 ± 0.8	2.5 ± 0.9	3.2 ± 0.4
53	2.8 ± 0.3	2.2 ± 0.4	2.4 ± 0.4	1.3 ± 1.3
67	2.4 ± 0.1	1.4 ± 0.9	2.4 ± 0.3	1.6 ± 0.7
81	2.2 ± 0.3	<-0.1 ± 0.0	2.8 ± 0.2	<0.1 ± 0.9
95	2.4 ± 1.0	<-0.10	2.7 ± 0.4	2.0 ± 0.7
109	2.2 ± 0.3	<0.0 ± 0.2	1.3 ± 1.0	1.0 ± 0.5
130	1.3 ± 0.1	<1.2 ± 1.1	1.4 ± 1.1	1.3 ± 1.6

Table A.36: Total aerobic plate count (n = 6) on soiled food packaging materials when inoculated at a level of approximately 6 log CFU/cm^2 and stored at 25º C (TSA) – Combined Means (Figure 2.2)

	Materials			
Day of Storage	Butcher Paper	Cardboard	Overwrap	Vacuum Pouch
0	6.0 ± 0.2	6.0 ± 0.2	6.0 ± 0.2	6.0 ± 0.2
4	5.3 ± 0.2	4.5 ± 0.7	5.0 ± 0.3	4.3 ± 0.8
11	4.9 ± 0.3	4.4 ± 0.8	4.5 ± 0.3	3.9 ± 0.4
18	4.5 ± 0.3	4.1 ± 1.2	3.8 ± 0.7	3.9 ± 0.7
25	4.2 ± 0.3	4.1 ± 0.7	3.4 ± 0.6	3.7 ± 1.4
32	4.1 ± 0.4	3.4 ± 1.2	3.6 ± 0.5	2.8 ± 0.6
39	3.3 ± 0.4	3.0 ± 0.4	3.0 ± 0.7	3.1 ± 0.8
53	3.2 ± 0.5	2.9 ± 0.6	2.4 ± 0.5	2.2 ± 1.4
67	2.6 ± 0.4	2.8 ± 0.7	2.0 ± 0.3	<1.6 ± 1.1
81	2.3 ± 0.5	2.4 ± 0.6	2.1 ± 0.1	<1.2 ± 1.0
95	2.3 ± 0.6	2.4 ± 0.9	1.7 ± 0.4	1.5 ± 0.3
109	2.0 ± 0.4	1.8 ± 0.6	<-0.1 ± 0.0	0.6 ± 0.6
130	0.7 ± 0.5	1.9 ± 0.3	<0.1 ± 0.3	<0.6 ± 1.0

Table A.37: Total aerobic plate count (n = 3) on soiled food packaging materials when inoculated at a level of approximately 6 log CFU/cm^2 and stored at 25º C (TSA) – First Replicate

	Materials			
Day of Storage	Butcher Paper	Cardboard	Overwrap	Vacuum Bag
0	6.0 ± 0.0	5.9 ± 0.0	5.9 ± 0.2	5.9 ± 0.2
4	5.4 ± 0.1	5.1 ± 0.0	5.2 ± 0.2	4.6 ± 0.6
11	5.1 ± 0.2	5.0 ± 0.2	4.5 ± 0.2	4.1 ± 0.5
18	4.7 ± 0.2	5.0 ± 0.2	4.3 ± 0.3	3.6 ± 0.6
25	4.4 ± 0.2	4.7 ± 0.4	3.7 ± 0.1	4.4 ± 0.4
32	4.5 ± 0.1	4.3 ± 0.4	4.0 ± 0.1	3.2 ± 0.3
39	3.7 ± 0.0	3.1 ± 0.4	3.1 ± 0.4	3.0 ± 1.5
53	3.6 ± 0.4	3.3 ± 0.2	2.7 ± 0.4	3.1 ± 0.9
67	2.9 ± 0.2	3.5 ± 0.2	2.0 ± 0.2	2.1 ± 0.9
81	2.5 ± 0.5	2.8 ± 0.5	2.1 ± 0.1	1.2 ± 1.1
95	2.2 ± 0.1	3.1 ± 0.4	1.8 ± 0.6	0.9 ± 0.1
109	2.0 ± 0.4	2.3 ± 0.1	<-0.10	0.7 ± 0.5
130	1.0 ± 0.4	1.8 ± 0.4	<-0.10	0.1 ± 0.2

Table A.38: Total aerobic plate count (n = 3) on soiled food packaging materials when inoculated at a level of approximately 6 log CFU/cm^2 and stored at 25º C (TSA) – Second Replicate

	Materials			
Day of Storage	Butcher Paper	Cardboard	Overwrap	Vacuum Bag
0	6.1 ± 0.2	6.1 ± 0.1	6.0 ± 0.1	6.1 ± 0.1
4	5.2 ± 0.1	3.9 ± 0.3	4.8 ± 0.1	4.0 ± 1.0
11	4.8 ± 0.3	3.9 ± 0.7	4.4 ± 0.4	3.8 ± 0.4
18	4.2 ± 0.1	3.2 ± 1.0	3.3 ± 0.5	4.3 ± 0.6
25	3.9 ± 0.2	3.5 ± 0.1	3.1 ± 0.8	2.9 ± 1.8
32	3.8 ± 0.1	2.5 ± 0.8	3.2 ± 0.3	2.3 ± 0.4
39	3.0 ± 0.2	2.9 ± 0.5	2.9 ± 1.0	3.1 ± 0.4
53	2.9 ± 0.2	2.4 ± 0.2	2.2 ± 0.5	1.3 ± 1.3
67	2.3 ± 0.1	2.3 ± 0.2	2.0 ± 0.5	<1.1 ± 1.2
81	2.0 ± 0.3	2.0 ± 0.3	2.1 ± 0.2	<1.1 ± 1.2
95	2.5 ± 0.8	1.7 ± 0.6	1.7 ± 0.3	1.8 ± 1.7
109	1.9 ± 0.4	1.2 ± 0.4	<-0.1 ± 0.0	0.5 ± 0.8
130	0.5 ± 0.5	2.1 ± 0.1	0.23 ± 0.4	<1.0 ± 1.2

Table A.39: *Escherichia coli* O157:H7 (n = 6) survival on soiled food packaging materials when inoculated at a level of approximately 6 log CFU/cm^2 and stored at 4º C (TSA + Rifampicin) – Combined Means (Figure 2.3)

	Materials			
Day of Storage	Butcher Paper	Cardboard	Overwrap	Vacuum Pouch
0	6.0 ± 0.2	6.0 ± 0.1	5.9 ± 0.1	6.0 ± 0.2
4	4.6 ± 0.8	3.8 ± 0.3	4.1 ± 0.8	4.0 ± 0.8
11	3.4 ± 0.4	3.9 ± 0.1	3.5 ± 0.4	3.6 ± 0.9
18	2.8 ± 0.3	3.5 ± 0.4	3.3 ± 0.3	3.7 ± 0.3
25	2.6 ± 0.1	3.4 ± 0.3	3.2 ± 0.4	3.1 ± 0.4
32	2.8 ± 0.4	3.1 ± 0.7	3.2 ± 0.8	3.5 ± 0.7
39	2.2 ± 0.7	3.3 ± 0.3	3.3 ± 0.1	3.2 ± 0.4
53	2.0 ± 0.7	2.9 ± 0.2	3.2 ± 0.2	2.6 ± 0.5
67	1.6 ± 0.9	2.6 ± 0.1	2.8 ± 0.3	2.5 ± 0.3
81	1.4 ± 0.8	2.7 ± 0.5	2.9 ± 0.4	2.7 ± 0.3
95	1.3 ± 0.7	2.6 ± 0.5	2.4 ± 0.5	2.2 ± 0.6
109	1.0 ± 0.7	2.2 ± 0.5	1.9 ± 0.4	1.5 ± 0.9
130	<0.3 ± 0.6	2.0 ± 0.4	2.4 ± 0.2	2.3 ± 0.6

Table A.40: *Escherichia coli* O157:H7 (n = 3) survival on soiled food packaging materials when inoculated at a level of approximately 6 log CFU/cm^2 and stored at 4º C (TSA + Rifampicin) – First Replicate

	Materials			
Day of Storage	Butcher Paper	Cardboard	Overwrap	Vacuum Bag
0	5.9 ± 0.1	5.9 ± 0.0	5.9 ± 0.1	5.8 ± 0.0
4	3.9 ± 0.2	3.9 ± 0.1	3.5 ± 0.6	4.1 ± 0.8
11	3.0 ± 0.2	3.9 ± 0.1	3.3 ± 0.1	3.1 ± 0.6
18	2.6 ± 0.2	3.6 ± 0.2	3.3 ± 0.4	3.7 ± 0.3
25	2.5 ± 0.1	3.2 ± 0.3	3.4 ± 0.2	2.9 ± 0.6
32	2.5 ± 0.3	2.5 ± 0.3	3.6 ± 0.5	4.0 ± 0.4
39	2.0 ± 0.9	3.1 ±0.1	3.3 ± 0.0	3.3 ± 0.4
53	1.5 ± 0.1	2.8 ± 0.2	3.2 ± 0.1	2.5 ± 0.3
67	0.7 ± 0.3	2.6 ± 0.1	2.7 ± 0.2	2.3 ± 0.1
81	0.7 ± 0.4	2.3 ± 0.5	2.5 ± 0.3	2.0 ± 0.1
95	0.8 ± 0.3	2.6 ± 0.2	2.3 ± 0.2	2.5 ± 0.7
109	<0.6 ± 0.7	1.9 ± 0.3	1.6 ± 0.2	<0.9 ± 0.9
130	<-0.1 ± 0.0	1.8 ± 0.3	2.4 ± 0.2	2.5 ± 0.5

Table A.41: *Escherichia coli* O157:H7 (n = 3) survival on soiled food packaging materials when inoculated at a level of approximately 6 log CFU/cm^2 and stored at 4º C (TSA + Rifampicin) – Second Replicate

	Materials			
Day of Storage	Butcher Paper	Cardboard	Overwrap	Vacuum Bag
0	6.1 ± 0.2	6.0 ± 0.1	6.0 ± 0.1	6.1 ± 0.1
4	5.2 ± 0.2	3.7 ± 0.3	4.7 ± 0.4	3.9 ± 0.9
11	3.8 ± 0.1	3.9 ± 0.1	3.6 ± 0.6	4.1 ± 0.9
18	3.0 ± 0.2	3.4 ± 0.6	3.4 ± 0.3	3.8 ± 0.3
25	2.6 ± 0.1	3.6 ± 0.2	3.1 ± 0.6	3.2 ± 0.1
32	3.0 ± 0.1	3.6 ± 0.2	2.8 ± 0.8	3.1 ± 0.5
39	2.5 ± 0.3	3.5 ± 0.2	3.2 ± 0.1	3.1 ± 0.4
53	2.5 ± 0.6	3.0 ± 0.2	3.2 ± 0.3	2.7 ± 0.6
67	2.2 ± 0.6	2.5 ± 0.2	2.9 ± 0.4	2.8 ± 0.1
81	2.1 ± 0.2	3.0 ± 0.2	3.3 ± 0.0	3.4 ± 0.2
95	2.1 ± 0.3	2.6 ± 0.8	2.5 ± 0.7	1.9 ± 0.5
109	1.4 ± 0.4	2.6 ± 0.3	2.3 ± 0.2	1.0 ± 1.1
130	0.8 ± 0.5	2.2 ± 0.3	2.4 ± 0.2	2.1 ± 0.7

Table A.42: Total aerobic plate count (n = 6) on soiled food packaging materials when inoculated at a level of approximately 6 log CFU/cm^2 and stored at 4º C (TSA) – Combined Means (Figure 2.4)

	Materials			
Day of Storage	Butcher Paper	Cardboard	Overwrap	Vacuum Pouch
0	6.0 ± 0.2	6.0 ± 0.2	6.0 ± 0.2	6.0 ± 0.2
4	4.4 ± 0.2	4.2 ± 0.1	4.0 ± 0.8	4.4 ± 0.6
11	4.4 ± 0.2	3.9 ± 0.3	3.7 ± 0.4	4.1 ± 0.8
18	3.7 ± 0.4	3.8 ± 0.1	3.5 ± 0.4	4.2 ± 0.4
25	4.0 ± 0.8	3.7 ± 0.2	3.5 ± 0.4	3.8 ± 0.7
32	4.1 ± 0.7	3.7 ± 0.2	3.2 ± 0.8	3.8 ± 0.5
39	3.8 ± 0.8	3.5 ± 0.2	3.5 ± 0.2	3.5 ± 0.4
53	3.5 ± 0.8	3.0 ± 0.2	2.5 ± 0.7	2.9 ± 0.6
67	3.4 ± 0.8	2.8 ± 0.2	2.4 ± 0.4	3.0 ± 0.6
81	2.7 ± 0.5	2.8 ± 0.2	2.5 ± 0.4	3.1 ± 0.7
95	2.9 ± 0.6	2.8 ± 0.3	2.2 ± 0.5	2.9 ± 0.6
109	2.2 ± 0.7	2.2 ± 0.3	1.7 ± 0.6	1.3 ± 0.6
130	2.7 ± 1.1	1.9 ± 0.2	1.1 ± 0.5	2.0 ± 0.2

Table A.43: Total aerobic plate count (n = 3) on soiled food packaging materials when inoculated at a level of approximately 6 log CFU/cm^2 and stored at 4º C (TSA) – First Replicate

	Materials			
Day of Storage	Butcher Paper	Cardboard	Overwrap	Vacuum Bag
0	6.0 ± 0.0	5.9 ± 0.0	5.9 ± 0.2	5.9 ± 0.2
4	4.3 ± 0.3	4.1 ± 0.2	3.9 ± 0.9	4.4 ± 0.9
11	3.9 ± 0.3	3.6 ± 0.2	3.4 ± 0.2	3.6 ± 0.0
18	3.3 ± 0.1	3.8 ± 0.1	3.5 ± 0.5	3.9 ± 0.3
25	3.5 ± 0.2	3.6 ± 0.2	3.6 ± 0.1	3.4 ± 0.4
32	3.1 ± 0.2	3.7 ± 0.2	3.7 ± 0.4	4.0 ± 0.4
39	2.8 ± 0.2	3.3 ± 0.0	3.6 ± 0.0	3.5 ± 0.5
53	2.7 ± 0.1	2.9 ± 0.2	3.1 ± 0.2	2.6 ± 0.4
67	3.1 ± 0.5	2.8 ± 0.1	2.5 ± 0.1	2.5 ± 0.1
81	2.5 ± 0.4	2.7 ± 0.3	2.2 ± 0.2	2.5 ± 0.3
95	2.5 ± 0.3	2.9 ± 0.2	2.3 ± 0.2	3.0 ± 0.7
109	1.7 ± 0.4	2.0 ± 0.2	1.9 ± 0.8	1.2 ± 0.2
130	1.7 ± 0.1	1.9 ± 0.3	1.5 ± 0.4	1.9 ± 0.2

Table A.44: Total aerobic plate count (n = 3) on soiled food packaging materials when inoculated at a level of approximately 6 log CFU/cm^2 and stored at 4º C (TSA) – Second Replicate

	Materials			
Day of Storage	Butcher Paper	Cardboard	Overwrap	Vacuum Bag
0	6.1 ± 0.2	6.1 ± 0.1	6. ± 0.1	6.1 ± 0.1
4	4.4 ± 0.1	4.2 ± 0.0	4.0 ± 1.0	4.4 ± 0.2
11	3.8 ± 0.6	4.3 ± 0.1	4.1 ± 0.1	4.9 ± 0.2
18	4.7 ± 0.2	3.8 ± 0.1	3.4 ± 0.4	4.5 ± 0.3
25	4.7 ± 0.4	3.9 ± 0.1	3.5 ± 0.6	4.4 ± 0.7
32	4.6 ± 0.1	3.7 ± 0.2	2.7 ± 0.9	3.5 ± 0.5
39	4.2 ± 0.4	3.6 ± 0.2	3.4 ± 0.3	3.6 ± 0.5
53	4.1 ± 0.5	3.0 ± 0.1	2.0 ± 0.5	3.1 ± 0.7
67	4.0 ± 0.7	2.8 ± 0.2	2.4 ± 0.7	3.5 ± 0.1
81	2.9 ± 0.5	2.9 ± 0.2	2.8 ± 0.2	3.7 ± 0.2
95	3.4 ± 0.4	2.8 ± 0.4	2.2 ± 0.8	2.7 ± 0.4
109	2.7 ± 0.6	2.3 ± 0.3	1.5 ± 0.3	1.6 ± 0.9
130	3.8 ± 0.2	1.9 ± 0.1	0.8 ± 0.2	2.2 ± 0.0

Table A.45: *Escherichia coli* O157:H7 (n = 6) survival on soiled food packaging materials when inoculated at a level of approximately 4 log CFU/cm^2 and stored at 25º C (TSA + Rifampicin) – Combined Means (See Figure 2.5)

	Materials			
Day of Storage	Butcher Paper	Cardboard	Overwrap	Vacuum Pouch
0	4.0 ± 0.1	4.0 ± 0.2	4.0 ± 0.2	4.0 ± 0.1
4	4.6 ± 0.1	3.1 ± 0.9	4.4 ± 0.2	4.1 ± 0.8
11	4.4 ± 0.2	3.3 ± 0.5	3.3 ± 0.5	4.0 ± 0.7
18	4.0 ± 0.2	3.1 ± 0.4	3.6 ± 0.5	3.5 ± 0.8
25	3.5 ± 0.3	2.6 ± 1.0	3.2 ± 0.6	2.8 ± 0.7
32	3.6 ± 0.4	1.6 ± 1.5	2.7 ± 1.0	2.1 ± 1.0
39	3.0 ± 0.3	2.5 ± 1.1	2.7 ± 0.7	<1.8 ± 1.4
53	2.9 ± 0.3	<1.5 ± 1.7	2.2 ± 0.7	2.8 ± 0.7
67	2.7 ± 0.6	<1.6 ± 1.3	2.0 ± 0.2	2.4 ± 0.9
81	2.8 ± 0.4	<0.9 ± 1.1	<1.6 ± 1.1	<1.1 ± 1.1
95	2.5 ± 0.3	<0.9 ± 0.9	<1.5 ± 0.9	<0.6 ± 1.0
109	2.1 ± 0.6	<0.6 ± 0.8	<2.1 ± 1.2	0.9 ± 1.0
130	1.6 ± 0.8	<0.9 ± 1.0	<1.2 ± 1.1	1.1 ± 1.3

Table A.46: Escherichia coli O157:H7 (n = 3) survival on soiled food packaging materials when inoculated at a level of approximately 4 log CFU/cm2 and stored at 25º C (TSA + Rifampicin) – First Replicate

	Materials			
Day of Storage	Butcher Paper	Cardboard	Overwrap	Vacuum Bag
0	4.1 ± 0.1	4.1 ± 0.1	4.1 ± 0.1	4.1 ± 0.0
4	4.6 ± 0.1	3.9 ± 0.1	4.6 ± 0.2	4.6 ± 0.5
11	4.5 ± 0.2	3.6 ± 0.0	3.5 ± 0.5	4.2 ± 0.8
18	4.1 ± 0.2	3.3 ± 0.2	4.0 ± 0.4	3.8 ± 0.7
25	3.8 ± 0.2	3.5 ± 0.4	3.8 ± 0.2	2.8 ± 0.9
32	3.9 ± 0.2	3.0 ± 0.2	3.5 ± 0.5	2.8 ± 0.5
39	3.1 ± 0.1	3.4 ± 0.3	3.2 ± 0.1	2.6 ± 0.5
53	2.8 ± 0.3	3.1 ± 0.1	2.5 ± 0.7	3.2 ± 0.4
67	2.8 ± 0.8	2.7 ± 0.2	2.2 ± 0.2	2.7 ± 0.4
81	2.6 ± 0.4	1.8 ± 0.5	<1.6 ± 1.5	<0.9 ± 1.7
95	2.4 ± 0.4	1.5 ± 0.3	2.1 ± 0.4	<1.1 ± 1.1
109	2.2 ± 0.6	1.4 ± 0.3	2.9 ± 0.1	1.2 ± 1.3
130	1.7 ± 0.9	1.8 ± 0.2	2.0 ± 0.4	2.2 ± 0.7

Table A.47: *Escherichia coli* O157:H7 (n = 3) survival on soiled food packaging materials when inoculated at a level of approximately 4 log CFU/cm^2 and stored at 25º C (TSA + Rifampicin) – Second Replicate

	Materials			
Day of Storage	Butcher Paper	Cardboard	Overwrap	Vacuum Bag
0	3.9 ± 0.1	3.8 ± 0.1	3.9 ± 0.1	3.9 ± 0.1
4	4.5 ± 0.1	2.3 ± 0.4	4.3 ± 0.1	3.7 ± 0.8
11	4.3 ± 0.2	2.9 ± 0.3	3.1 ± 0.6	3.7 ± 0.5
18	3.9 ± 0.1	2.9 ± 0.4	3.3 ± 0.3	3.2 ± 1.0
25	3.2 ± 0.3	1.7 ± 0.4	2.7 ± 0.3	2.8 ± 0.7
32	3.3 ± 0.4	0.2 ± 0.4	1.9 ± 0.4	1.4 ± 0.9
39	3.0 ± 0.4	1.6 ± 0.4	2.2 ± 0.8	<1.2 ± 1.6
53	3.0 ± 0.2	<-0.1 ± 0.0	1.9 ± 0.7	2.5 ± 0.8
67	2.5 ± 0.4	<0.4 ± 0.5	1.8 ± 0.1	2.2 ± 1.3
81	3.0 ± 0.3	<-0.1	1.6 ± 0.9	1.4 ± 0.5
95	2.6 ± 0.2	<-0.1	<1.0 ± 0.9	<-0.1 ± 0.0
109	2.0 ± 0.7	<-0.1	<1.3 ± 1.3	<0.7 ± 0.9
130	1.4 ± 0.9	<-0.1	<0.5 ± 1.0	<0.0 ± 0.2

Table A.48: Total aerobic plate count (n = 6) on soiled food packaging materials when inoculated at a level of approximately 4 log CFU/cm^2 and stored at 25º C (TSA) – Combined Means (Figure 2.6)

	Materials			
Day of Storage	Butcher Paper	Cardboard	Overwrap	Vacuum Pouch
0	4.3 ± 0.3	4.2 ± 0.2	4.2 ± 0.1	4.2 ± 0.2
4	4.4 ± 0.5	3.6 ± 0.6	4.6 ± 0.0	4.2 ± 0.5
11	5.2 ± 0.2	3.7 ± 0.3	3.8 ± 0.4	4.6 ± 0.6
18	4.8 ± 0.2	3.5 ± 0.2	3.7 ± 0.4	3.9 ± 0.8
25	4.9 ± 0.2	3.4 ± 0.5	3.3 ± 0.6	4.2 ± 0.6
32	5.1 ± 0.3	3.0 ± 0.4	3.0 ± 0.8	3.0 ± 0.7
39	4.5 ± 0.3	3.0 ± 0.7	2.8 ± 0.7	<2.5 ± 1.9
53	4.4 ± 0.1	2.7 ± 0.6	2.2 ± 0.6	3.6 ± 0.6
67	4.2 ± 0.2	2.5 ± 0.4	1.7 ± 0.3	3.3 ± 0.9
81	4.3 ± 0.3	2.3 ± 0.4	1.6 ± 0.9	2.7 ± 0.8
95	4.0 ± 0.3	2.3 ± 0.5	1.6 ± 0.7	2.7 ± 1.0
109	3.9 ± 0.4	1.7 ± 0.5	0.8 ± 0.9	2.3 ± 1.1
130	3.6 ± 0.7	1.7 ± 0.2	<0.4 ± 0.5	<1.4 ± 1.4

Table A.49: Total aerobic plate count (n = 3) on soiled food packaging materials when inoculated at a level of approximately 4 log CFU/cm^2 and stored at 25º C (TSA) – First Replicate

	Materials			
Day of Storage	Butcher Paper	Cardboard	Overwrap	Vacuum Bag
0	4.3 ± 0.1	4.3 ± 0.1	4.3 ± 0.1	4.2 ± 0.1
4	4.1 ± 0.4	4.2 ± 0.0	4.7 ± 0.1	4.5 ± 0.1
11	5.2 ± 0.1	4.0 ± 0.1	3.9 ± 0.5	5.0 ± 0.6
18	4.8 ± 0.2	3.5 ± 0.3	3.8 ± 0.3	4.2 ± 0.9
25	5.0 ± 0.2	3.7 ± 0.3	3.7 ± 0.2	4.6 ± 0.1
32	5.3 ± 0.1	3.3 ± 0.1	3.7 ± 0.4	3.6 ± 0.2
39	4.5 ± 0.5	3.6 ± 0.1	3.2 ± 0.1	3.8 ± 0.8
53	4.4 ± 0.2	3.3 ± 0.1	2.5 ± 0.3	4.1 ± 0.0
67	4.2 ± 0.2	2.8 ± 0.3	1.9 ± 0.3	3.8 ± 0.6
81	4.5 ± 0.1	2.3 ± 0.5	1.5 ± 1.2	2.6 ± 1.1
95	4.2 ± 0.3	2.1 ± 0.2	1.7 ± 0.5	3.4 ± 0.7
109	4.2 ± 0.1	1.9 ± 0.4	1.6 ± 0.1	3.0 ± 0.7
130	4.2 ± 0.2	1.8 ± 0.3	0.8 ± 0.1	2.4 ± 0.1

Table A.50: Total aerobic plate count (n = 3) on soiled food packaging materials when inoculated at a level of approximately 4 log CFU/cm^2 and stored at 25º C (TSA) – Second Replicate

	Materials			
Day of Storage	Butcher Paper	Cardboard	Overwrap	Vacuum Bag
0	4.3 ± 0.4	4.2 ± 0.3	4.1 ± 0.0	4.2 ± 0.1
4	4.9 ± 0.1	3.2 ± 0.4	4.6 ± 0.0	3.9 ± 0.5
11	5.2 ± 0.3	3.4 ± 0.2	3.6 ± 0.3	4.2 ± 0.1
18	5.0 ± 0.2	3.1 ± 0.5	3.5 ± 0.4	3.3 ± 0.2
25	4.8 ± 0.1	2.7 ± 0.3	2.9 ± 0.4	3.9 ± 0.6
32	4.7 ± 0.1	2.5 ± 0.5	2.3 ± 0.2	2.5 ± 0.5
39	4.4 ± 0.2	2.2 ± 0.4	2.3 ± 0.7	<1.2 ± 1.9
53	4.4 ± 0.1	2.2 ± 0.4	1.8 ± 0.6	3.2 ± 0.5
67	4.1 ± 0.2	2.3 ± 0.3	1.6 ± 0.3	2.6 ± 0.8
81	4.2 ± 0.3	2.3 ± 0.3	1.6 ± 0.7	2.8 ± 0.5
95	3.8 ± 0.3	3.6 ± 0.7	1.4 ± 0.9	1.8 ± 0.0
109	3.5 ± 0.2	1.4 ± 0.5	0.0 ± 0.2	1.6 ± 1.1
130	3.1 ± 0.7	1.7 ± 0.1	<0.0 ± 0.2	<-0.1 ± 0.0

Table A.51: *Escherichia coli* O157:H7 (n = 6) survival on soiled food packaging materials when inoculated at a level of approximately 4 log CFU/cm^2 and stored at 4º C (TSA + Rifampicin) – Combined Means (Figure 2.7)

	Materials			
Day of Storage	Butcher Paper	Cardboard	Overwrap	Vacuum Pouch
0	4.0 ± 0.1	4.0 ± 0.2	4.0 ± 0.2	4.0 ± 0.1
4	1.9 ± 0.4	2.0 ± 0.3	1.7 ± 0.7	2.5 ± 1.2
11	<0.8 ± 0.6	1.7 ± 0.1	1.0 ± 1.1	1.1 ± 0.7
18	0.4 ± 0.4	1.5 ± 0.1	<0.8 ± 0.8	1.2 ± 0.7
25	<0.2 ± 0.2	1.4 ± 0.3	1.2 ± 0.6	1.1 ± 0.5
32	<0.6 ± 1.0	1.1 ± 0.4	0.9 ± 0.7	0.9 ± 0.2
39	0.7 ± 0.6	1.0 ± 0.3	1.0 ± 0.5	<0.6 ± 0.6
53	<0.5 ± 0.6	<0.3 ± 0.4	0.6 ± 0.5	0.5 ± 0.6
67	<-0.1	0.4 ± 0.3	0.5 ± 0.3	<-0.1 ± 0.0
81	<-0.1	<0.4 ± 0.6	<0.3 ± 0.3	<0.4 ± 0.5
95	<0.0 ± 0.3	<0.2 ± 0.3	<0.3 ± 0.4	<0.0 ± 0.3
109	<0.2 ± 0.6	<0.3 ± 0.4	<0.3 ± 0.3	<0.1 ± 0.3
130	<0.2 ± 0.7	<0.1 ± 0.4	<0.3 ± 0.4	<0.0 ± 0.2

Table A.52: *Escherichia coli* O157:H7 (n = 3) survival on soiled food packaging materials when inoculated at a level of approximately 4 log CFU/cm^2 and stored at 4º C (TSA + Rifampicin) – First Replicate

	Materials			
Day of Storage	Butcher Paper	Cardboard	Overwrap	Vacuum Bag
0	4.1 ± 0.1	4.1 ± 0.1	4.1 ± 0.1	4.1 ± 0.0
4	1.5 ± 0.3	2.1 ± 0.1	1.5 ± 0.9	2.1 ± 1.6
11	<0.6 ± 0.9	1.7 ± 0.0	0.0 ± 0.2	0.5 ± 0.0
18	0.2 ± 0.2	1.6 ± 0.0	<0.2 ± 0.2	0.6 ± 0.3
25	0.3 ± 0.1	1.5 ± 0.3	0.6 ± 0.1	1.1 ± 0.3
32	<-0.1 ± 0.0	1.1 ± 0.2	0.9 ± 0.2	0.8 ± 0.1
39	0.6 ± 0.3	1.1 ± 0.3	0.7 ± 0.5	<0.2 ± 0.5
53	<0.0 ± 0.3	0.7 ± 0.3	0.8 ± 0.6	0.4 ± 0.5
67	<-0.1	0.3 ± 0.4	0.3 ± 0.1	<-0.1 ± 0.0
81	<-0.1	<0.1 ± 0.4	<0.1 ± 0.1	<0.1 ± 0.2
95	<-0.1	<-0.1 ± 0.0	<-0.1 ± 0.0	<-0.1
109	<-0.1	<-0.1	<0.3 ± 0.3	<0.3 ± 0.4
130	<0.4 ± 0.9	<-0.1	0.3 ± 0.4	0.1 ± 0.2

Table A.53: *Escherichia coli* O157:H7 (n = 3) survival on soiled food packaging materials when inoculated at a level of approximately 4 log CFU/cm^2 and stored at 4º C (TSA + Rifampicin) – Second Replicate

	Materials			
Day of Storage	Butcher Paper	Cardboard	Overwrap	Vacuum Bag
0	3.9 ± 0.1	3.8 ± 0.1	3.9 ± 0.1	3.9 ± 0.1
4	2.2 ± 0.2	1.9 ± 0.4	1.8 ± 0.5	2.9 ± 0.8
11	1.0 ± 0.3	1.7 ± 0.2	1.9 ± 0.2	1.7 ± 0.3
18	0.6 ± 0.4	1.5 ± 0.1	1.5 ± 0.5	1.7 ± 0.5
25	<0.1 ± 0.3	1.3 ± 0.2	1.7 ± 0.1	1.1 ± 0.7
32	1.4 ± 1.0	1.1 ± 0.5	0.9 ± 1.0	1.1 ± 0.2
39	0.8 ± 0.9	0.8 ± 0.2	1.4 ± 0.1	1.1 ± 0.1
53	1.0 ± 0.5	<0.0 ± 0.2	0.3 ± 0.4	<0.6 ± 0.9
67	<-0.1	0.6 ± 0.2	0.6 ± 0.4	<-0.1 ± 0.1
81	<-0.1	0.6 ± 0.8	0.5 ± 0.2	0.8 ± 0.4
95	<0.2 ± 0.5	0.5 ± 0.1	0.6 ± 0.2	<0.1 ± 0.4
109	<0.4 ± 0.9	0.6 ± 0.4	<0.3 ± 0.4	<0.0 ± 0.2
130	<-0.1 ± 0.0	<0.3 ± 0.6	<0.2 ± 0.6	<-0.1 ± 0.0

Table A.54: Total aerobic plate count (n = 6) on soiled food packaging materials when inoculated at a level of approximately 4 log CFU/cm^2 and stored at 4º C (TSA) – Combined Means (Figure 2.8)

	Materials			
Day of Storage	Butcher Paper	Cardboard	Overwrap	Vacuum Pouch
0	4.3 ± 0.3	4.2 ± 0.2	4.2 ± 0.1	4.2 ± 0.2
4	4.6 ± 0.4	3.3 ± 0.3	3.4 ± 0.6	4.1 ± 0.1
11	5.0 ± 0.3	3.2 ± 0.3	3.1 ± 1.3	4.3 ± 0.4
18	5.0 ± 0.7	3.4 ± 0.1	1.7 ± 0.5	4.0 ± 0.8
25	5.1 ± 0.7	3.3 ± 0.2	2.8 ± 1.5	4.5 ± 0.2
32	4.3 ± 0.3	3.0 ± 0.3	2.6 ± 0.6	4.1 ± 1.2
39	4.4 ± 0.7	3.0 ± 0.3	3.3 ± 0.7	4.2 ± 0.3
53	4.3 ± 0.7	2.9 ± 0.2	2.7 ± 0.4	4.3 ± 0.4
67	4.7 ± 0.5	2.8 ± 0.2	2.7 ± 0.3	4.1 ± 0.6
81	4.0 ± 0.5	2.5 ± 0.2	2.6 ± 0.4	4.4 ± 0.3
95	4.2 ± 0.9	2.5 ± 0.2	2.4 ± 0.3	2.6 ± 0.6
109	4.0 ± 0.6	2.3 ± 0.2	2.2 ± 0.6	2.9 ± 1.5
130	3.7 ± 0.3	2.2 ± 0.5	2.2 ± 0.4	3.2 ± 0.5

Table A.55: Total aerobic plate count (n = 3) on soiled food packaging materials when inoculated at a level of approximately 4 log CFU/cm^2 and stored at 4º C (TSA) – First Replicate

	Materials			
Day of Storage	Butcher Paper	Cardboard	Overwrap	Vacuum Bag
0	4.3 ± 0.1	4.3 ± 0.1	4.3 ± 0.1	4.2 ± 0.1
4	4.3 ± 0.3	3.1 ± 0.2	3.3 ± 0.7	4.1 ± 0.0
11	5.1 ± 0.3	3.3 ± 0.4	4.0 ± 0.6	4.6 ± 0.0
18	4.5 ± 0.6	3.4 ± 0.1	1.2 ± 0.1	3.6 ± 1.2
25	4.5 ± 0.6	3.3 ± 0.2	1.5 ± 0.6	4.6 ± 0.2
32	4.1 ± 0.3	3.0 ± 0.2	2.6 ± 0.2	3.8 ± 1.6
39	3.9 ± 0.1	2.8 ± 0.1	2.8 ± 0.5	4.0 ± 0.1
53	3.7 ± 0.2	2.8 ± 0.3	2.5 ± 0.1	4.6 ± 0.5
67	4.3 ± 0.1	2.7 ± 0.2	2.6 ± 0.3	4.4 ± 0.8
81	3.6 ± 0.2	2.6 ± 0.2	2.3 ± 0.1	4.6 ± 0.1
95	3.4 ± 0.4	2.5 ± 0.1	2.1 ± 0.0	3.5 ± 0.7
109	3.7 ± 0.3	2.1 ± 0.1	2.0 ± 0.2	3.5 ± 1.5
130	3.5 ± 0.4	2.3 ± 0.3	2.1 ± 0.4	3.6 ± 0.0

Table A.56: Total aerobic plate count (n = 3) on soiled food packaging materials when inoculated at a level of approximately 4 log CFU/cm^2 and stored at 4º C (TSA) – Second Replicate

	Materials			
Day of Storage	Butcher Paper	Cardboard	Overwrap	Vacuum Bag
0	4.3 ± 0.4	4.2 ± 0.3	4.1 ± 0.0	4.2 ± 0.1
4	4.9 ± 0.2	3.5 ± 0.4	3.5 ± 0.7	4.0 ± 0.1
11	5.1 ± 0.3	3.3 ± 0.4	4.0 ± 0.6	4.6 ± 0.0
18	5.5 ± 0.1	3.3 ± 0.2	2.0 ± 0.4	4.3 ± 0.3
25	5.7 ± 0.2	3.4 ± 0.2	4.1 ± 0.4	4.4 ± 0.1
32	4.5 ± 0.2	3.0 ± 0.3	2.6 ± 0.9	4.5 ± 0.1
39	5.0 ± 0.3	3.2 ± 0.2	3.9 ± 0.3	4.4 ± 0.3
53	4.9 ± 0.2	3.0 ± 0.2	3.0 ± 0.4	4.0 ± 0.1
67	5.1 ± 0.4	2.9 ± 0.1	2.8 ± 0.3	3.9 ± 0.1
81	4.5 ± 0.2	2.5 ± 0.2	2.8 ± 0.4	4.1 ± 0.3
95	5.0 ± 0.4	2.5 ± 0.4	2.6 ± 0.0	3.8 ± 0.6
109	4.4 ± 0.7	2.5 ± 0.2	2.4 ± 0.8	2.3 ± 1.5
130	2.8 ± 0.3	2.1 ± 0.7	2.3 ± 0.4	2.8 ± 0.2

Table A.57: Mean (± standard deviation; log CFU/cm^2) populations of rifampicin-resistant *Escherichia coli* O157:H7 (recovered on tryptic soy agar supplemented with 100 µg/mL rifampicin; TSArif, or modified sorbitol MacConkey agar; mSMAC) *Salmonella* serotypes (recovered on xylose lysine deoxycholate agar; XLD) and total bacteria (recovered on tryptic soy agar; TSA) for beef trimmings before and after treatment with Acidified Sodium Chlorite (1000ppm) for 30 s.

Serotype	TSA + Rifampicin (100 µg/mL) (*E. coli* O157:H7) or XLD agar (*Salmonella* sp.)		Modified SMAC agar (*E. coli* O157:H7) or XLD agar (*Salmonella* sp.)		TSA (Total Bacterial Population)	
	Untreated Control	Acidified Sodium Chlorite (1000ppm)	Untreated Control	Acidified Sodium Chlorite (1000ppm)	Untreated Control	Acidified Sodium Chlorite (1000ppm)
E. coli O157:H7	3.1 ± 0.0 A	2.6 ± 0.1 B	2.7 ± 0.1 A	2.2 ± 0.2 B	4.2 ± 0.4 A	4.2 ± 0.5 A
S. Newport Susc.	3.0 ± 0.1 *A	2.6 ± 0.1 *B	3.0 ± 0.1 A	2.6 ± 0.1 B	4.2 ± 0.5 *A	4.0 ± 0.4 *A
S. Newport MDR-AmpC	3.1 ± 0.2 *A	2.5 ± 0.1 *B	3.1 ± 0.2 A	2.5 ± 0.1 B	4.1 ± 0.4 *A	3.9 ± 0.4 *A
S. Typhimurium Susc.	3.1 ± 0.1 *A	2.6 ± 0.3 *B	3.1 ± 0.1 A	2.6 ± 0.3 B	4.2 ± 0.4 *A	4.0 ± 0.5 *A
S. Typhimurium MDR	3.1 ± 0.1 *A	2.7 ± 0.1 *B	3.1 ± 0.1 A	2.7 ± 0.1 B	4.2 ± 0.6 *A	3.8 ± 0.4 B
S. Typhimurium MDR-AmpC	2.9 ± 0.0 A	2.3 ± 0.0 B	2.9 ± 0.0 *A	2.3 ± 0.0 *B	4.1 ± 0.6 *A	3.9 ± 0.4 *A

Values (mean ± SD) within each row followed by different uppercase letters are significantly ($P \leq 0.05$) different.
Values (mean ± SD) within each column followed by * are not significantly ($P \geq 0.05$) different with *Escherichia coli* O157:H7

Table A.58: Mean (± standard deviation; log CFU/cm^2) populations of rifampicin-resistant *Escherichia coli* O157:H7 (recovered on tryptic soy agar supplemented with 100 µg/mL rifampicin; TSArif, or modified sorbitol MacConkey agar; mSMAC) *Salmonella* serotypes (recovered on xylose lysine deoxycholate agar; XLD) and total bacteria (recovered on tryptic soy agar; TSA) for beef trimmings before and after treatment with Peroxyacetic Acid (200ppm) for 30 s.

Serotype	TSA + Rifampicin (100 µg/mL) (*E. coli* O157:H7) or XLD agar (*Salmonella* sp.)		Modified SMAC agar (*E. coli* O157:H7) or XLD agar (*Salmonella* sp.)		TSA (Total Bacterial Population)	
	Untreated Control	Peroxyacetic Acid (200ppm)	Untreated Control	Peroxyacetic Acid (200ppm)	Untreated Control	Peroxyacetic Acid (200ppm)
E. coli O157:H7	3.1 ± 0.0 A	2.4 ± 0.1 B	2.8 ± 0.1 A	2.3 ± 0.2 B	3.4 ± 0.1 A	2.8 ± 0.2 B
S. Newport Susc.	3.2 ± 0.1 *A	2.5 ± 0.2 *B	3.2 ± 0.1 A	2.5 ± 0.2 B	3.4 ± 0.1 *A	3.2 ± 0.5 B
S. Newport MDR-AmpC	3.0 ± 0.1 *A	2.4 ± 0.1 *B	3.0 ± 0.1 A	2.4 ± 0.1 *B	3.3 ± 0.2 *A	2.9 ± 0.3 *B
S. Typhimurium Susc.	3.1± 0.1 *A	2.6 ± 0.2 *B	3.1± 0.1 A	2.6 ± 0.2 B	3.4 ± 0.1 *A	3.0 ± 0.2 *B
S. Typhimurium MDR	3.3 ± 0.1 A	2.6 ± 0.3 B	3.3 ± 0.1 A	2.6 ± 0.3 B	3.6 ± 0.1 *A	3.0 ± 0.4 *B
S. Typhimurium MDR-AmpC	3.1 ± 0.1 *A	2.5 ± 0.2 *B	3.1 ± 0.1 A	2.5 ± 0.2 B	3.3 ± 0.1 *A	2.9 ± 0.1 *B

Values (mean ± SD) within each row followed by different uppercase letters are significantly ($P \leq 0.05$) different.
Values (mean ± SD) within each column followed by * are not significantly ($P \geq 0.05$) different with *Escherichia coli* O157:H7

Table A.59: Mean (± standard deviation; log CFU/cm^2) populations of rifampicin-resistant *Escherichia coli* O157:H7 (recovered on tryptic soy agar supplemented with 100 µg/mL rifampicin; TSArif, or modified sorbitol MacConkey agar; mSMAC) *Salmonella* serotypes (recovered on xylose lysine deoxycholate agar; XLD) and total bacteria (recovered on tryptic soy agar; TSA) for beef trimmings before and after treatment with Sodium Metasilicate (40000ppm) for 30 s.

Serotype	TSA + Rifampicin (100 µg/mL) (*E. coli* O157:H7) or XLD agar (*Salmonella* sp.)		Modified SMAC agar (*E. coli* O157:H7) or XLD agar (*Salmonella* sp.)		TSA (Total Bacterial Population)	
	Untreated Control	Sodium Metasilicate (40000ppm)	Untreated Control	Sodium Metasilicate (40000ppm)	Untreated Control	Sodium Metasilicate (40000ppm)
E. coli O157:H7	3.1 ± 0.0 A	1.8 ± 0.2 B	2.8 ± 0.1 A	1.4 ± 0.6 B	3.4 ± 0.1 A	2.2 ± 0.2 B
S. Newport Susc.	3.2 ± 0.1 *A	1.8 ± 0.3 *B	3.2 ± 0.1 *A	1.8 ± 0.3 *B	3.4 ± 0.1 *A	2.3 ± 0.2 *B
S. Newport MDR-AmpC	3.0 ± 0.1 *A	1.6 ± 0.3 *B	3.0 ± 0.1 *A	1.6 ± 0.3 *B	3.3 ± 0.2 *A	2.3 ± 0.5 *B
S. Typhimurium Susc.	3.1± 0.1 *A	1.6 ± 0.3 *B	3.1± 0.1 *A	1.6 ± 0.3 *B	3.4 ± 0.1 *A	2.1 ± 0.5 *B
S. Typhimurium MDR	3.3 ± 0.1 *A	2.0 ± 0.2 *B	3.3 ± 0.1 A	2.0 ± 0.2 B	3.6 ± 0.1 *A	2.6 ± 0.4 B
S. Typhimurium MDR-AmpC	3.1 ± 0.1 *A	1.6 ± 0.5 *B	3.1 ± 0.1 *A	1.6 ± 0.5 *B	3.3 ± 0.1 *A	2.2 ± 0.2 *B

Values (mean ± SD) within each row followed by different uppercase letters are significantly ($P \leq 0.05$) different.Values (mean ± SD) within each column followed by * are not significantly ($P \geq 0.05$) different with *Escherichia coli* O157:H7

Table A.60: pH values of beef trimmings treated with Acidified Sodium Chlorite (1000ppm) for 30s

Treatment	0 h pH	24 h pH
Untreated Control	5.79 ± 0.11 aB	6.23 ± 0.11 aA
Treatment with Acidified Sodium Chlorite (1000ppm)	5.75 ± 0.16 aA	5.63 ± 0.14 bA

Within a column, values lacking a common lowercase letter are significantly different ($p < 0.05$). Within a row, values lacking a common uppercase letter are significantly different ($P \leq 0.05$).

Table A.61: pH values of beef trimmings treated with Peroxyacetic Acid (1000ppm) for 30s

Treatment	0 h pH	24 h pH
Untreated Control	6.04 ± 0.13 aA	5.44 ± 0.11 aB
Treatment with Peroxyacetic Acid (200ppm)	5.98 ± 0.14 aA	5.42 ± 0.08 aB

Within a column, values lacking a common lowercase letter are significantly different ($p < 0.05$). Within a row, values lacking a common uppercase letter are significantly different ($P \leq 0.05$).

Table A.62: pH values of beef trimmings treated with Sodium Metasilicate (40000ppm) for 30s

Treatment	0 h pH	24 h pH
Untreated Control	6.04 ± 0.13 bA	5.44 ± 0.11 bB
Treatment with Sodium Metasilicate (40000ppm)	8.66 ± 0.55 aA	6.52 ± 0.16 aB

Within a column, values lacking a common lowercase letter are significantly different ($p < 0.05$). Within a row, values lacking a common uppercase letter are significantly different ($P \leq 0.05$)