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Inactivation of *Escherichia coli* O157:H7 and Shiga Toxin Producing *E. coli* (STEC) Throughout Beef Summer Sausage Production and the use of High Pressure Processing as an Alternative Intervention to Thermal Processing

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INACTIVATION OF *Escherichia coli* O157:H7
AND SHIGA TOXIN PRODUCING *E. coli* (STEC)
THROUGHOUT BEEF SUMMER SAUSAGE PRODUCTION
AND THE USE OF HIGH PRESSURE PROCESSING
AS AN ALTERNATIVE INTERVENTION TO THERMAL PROCESSING

By

Eric Layne Oliver

A THESIS

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and Harshavardhan Thippareddi

Lincoln, Nebraska

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Inactivation of *Escherichia coli* O157:H7 and Shiga Toxin Producing *E. coli* (STEC)
Throughout Beef Summer Sausage Production and the Use of High Pressure Processing
as an Alternative Intervention to Thermal Processing

Eric Layne Oliver, M.S.

University of Nebraska, 2015

Advisors: Bing Wang and Harshavardhan Thippareddi

Escherichia coli O157:H7 and six additional serogroups of Shiga Toxin-producing *E. coli* (STEC-7) have been implicated in foodborne illness outbreaks related to fermented sausage products and are considered adulterants by the USDA-FSIS. The objective of this study was to evaluate high pressure processing (HPP) as an alternative to thermal treatment to reduce STEC-7 meeting the USDA-FSIS performance standards (5.0-log reduction). A rifampicin-resistant, non-pathogenic *E. coli* (surrogate) cocktail, was used in manufacturing testing. Sausages were fermented at ~42.2°C, slowly increased to 54.4°C internal temperature, cold showered, and chilled for 6 hours. Sampling occurred post-fermentation (PF; pH 5.0), internal temperature 48.9°C (I-48.9°C), 54.4°C (I-54.4°C), and post chilling (PC) for high fat (HF-17%) and low fat (LF-8%) sausage. Samples were HPP treated at 586 MPa for 1 sec, 1, 2, 3, 4 and 5 mins. Reductions of 1.2, 1.3, 3.1 and 4.2 log CFU/g were achieved at PF, I-48.9°C, I-54.4°C and PC, respectively. HPP resulted in additional reductions of 3.2 and >3.4 log CFU/g, at 1 and 2 mins,

respectively (PC, LF). HPP (1s) of LF summer sausage that was heated to 54.4°C (no hold), resulted in >6.6 log CFU/g reduction. Additionally, HPP was evaluated with three *E. coli* species cocktails which were inoculated onto pre-processed sausage including *E. coli* surrogates, *E. coli* O157:H7, and STEC. Cocktails were evaluated for HPP treatment at 586 MPa at 1s, 2, 4, and 6 mins. Reductions of 3.5, 5.0, 5.3, and 5.3 log CFU/g (STEC-cocktail) and 3.4, 5.2, 5.2, and 5.3 log CFU/g (O157:H7-cocktail) were obtained in LF sausage after 1 sec, 2, 4, and 6 mins. Surrogate reductions in LF sausage of 4.3 and ≥ 5.5 log CFU/g were obtained after 1 second and 2 minutes. HPP can be utilized as an alternative non-thermal lethality treatment in beef summer sausage to meet USDA-FSIS performance standards for *E. coli* O157:H7 and STEC.

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Dedication

This thesis is dedicated to my parents, Layne and Teri Oliver,
for always being there for me and teaching me
the value of life, family, and friendship.

I love you both!

Acknowledgments

It is my honor to be able to acknowledge all of the individuals who helped me along my path in coming back to school, mentoring me, and helping me to achieve my goals and obtain a Master's Degree. This thesis would not have been possible without their encouragement and involvement.

I would like to thank my beautiful wife for always standing by my side and sacrificing every day to allow me to pursue my dream of obtaining a higher degree. She is my strength and my light and I will never be able to repay her sacrifice. I would not have made it this far in my education without her support. Madelynn H. Oliver I will always love you.

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THESIS INTRODUCTION

Escherichia coli (*E. coli*) is one of the most important foodborne pathogens in the food and health industries, is recognized worldwide by individuals of all ages, is considered one of the biggest problems in food safety that our world faces today, and yet it is only 2-6 µm in length (Holt et al. 2000). It is amazing to think that something so small could cause so much harm and grief, and yet it does. As human beings we inherently value life and the preservation of life. Because of this, it is important to study *Escherichia coli* so that we might better understand the organism, how it functions, and why it causes disease in humans, in order to guide us to possible solutions in controlling this potentially deadly foodborne pathogen and treatments for those individuals whom have become ill.

Throughout this thesis we will discuss many aspects of *E. coli*, look at past research, evaluate the effectiveness of the manufacturing process of summer sausages products on controlling *E. coli*, and look at high pressure processing as an alternative intervention for the inactivation of *E. coli* and the preservation of the quality of sausage products. Below is an overview of each chapter and what is discussed within this thesis.

This thesis will begin with a literature review of *Escherichia coli*. The review will focus on many different aspects of this potentially deadly foodborne pathogen including the general properties of the organism, classification and serotyping (such as it is gram negative and is a facultative anaerobe) to help us understand what *E. coli* is and how it functions in nature. The epidemiology of Shiga Toxin Producing *Escherichia coli*

(STEC) will be reviewed including; common reservoirs, food products affected by its presence, virulence factors associated with infection and the pathogenicity of the organism, the diseases it causes and prophylaxis (treatment), and finally outbreaks that have occurred as a result of STEC contamination of food products.

Additionally this chapter will focus in on the manufacturing of fermented sausage products and how to control *E. coli* during the production stages as well as the various control measures currently in place and how they are utilized via Hurdle Technology to control foodborne pathogens. Thermal processing, one of the many hurdles used in production of sausage products, will be discussed due to the important role it plays in the inactivation of *E. coli* in beef products. High pressure processing (HPP) also will be discussed including what it is, what products it is currently used for, how it impacts quality of those products, and how it could be used in fermented beef summer sausage production. Finally the literature review will conclude with a review of the regulatory bodies that control the production of fermented beef summer sausages, the regulations that are in place for manufacturing sausage products, and the federal regulations that have been passed due to outbreaks of *Escherichia coli*.

After the literature review, the research that was performed for this thesis will be presented and discussed. First, we will take a look at the inactivation of *Escherichia coli* at various stages throughout the manufacturing of fermented beef summer sausages.

Testing was performed to evaluate how much (log reduction) *E. coli* is inactivated during a standard sausage production protocol without the use of thermal processing as an

intervention. This study will tell us if the standard manufacturing protocol is sufficient, on its own, to meet the United States Department of Agriculture (USDA) and Food Safety Inspection Service (FSIS) regulations for the reduction of *E. coli* in sausage products of 5.0 logs. As part of this study, high pressure processing at 586 MPa (industry standard 600 MPa) was then performed on the samples at various dwell times to determine if HPP can be utilized as a suitable alternative intervention to thermal processing while still achieving the USDA-FSIS regulations. This portion of the study is important because many fermented sausages manufacturers, outside the United States, do not use a thermal processing step during production. So, in order to make fermented sausages safe for consumption finding alternative methods for the lethality of foodborne pathogens that still maintain the quality attributes of the products may be necessary.

The final chapter of the thesis contains research that was performed on the effectiveness of high pressure processing on the inactivation of *Escherichia coli* species (surrogate and pathogenic) in fermented beef summer sausage without the stressors of other interventions from the manufacturing process. Beef summer sausage was inoculated post manufacturing and subjected to HPP of 586 MPa (industry standard 600 MPa) at various dwell times to properly evaluate the organisms in a none stressed form. This study will tell us the true effectiveness of HPP at inactivating *E. coli* allowing us to determine if it could be used as a sole intervention in controlling foodborne pathogens, such as *E. coli*, what level of control is obtainable, and if other interventions are necessary in combination with HPP to achieve appropriate lethality levels.

In conclusion, *Escherichia coli* is still a major concern in the food industry and research, such as the research performed in this thesis, is necessary for helping researchers, food industry employees, and consumers become aware of the issues surrounding foodborne disease and what is being done to help control outbreaks in the future. We must continue to learn about and perform research on *E. coli* and other foodborne pathogens so that we might drive the food industry, and potentially policy, in the right direction for reducing the risk of foodborne disease and making food safer for everyone to enjoy.

CHAPTER 1

LITERATURE REVIEW

“Enterohemorrhagic *Escherichia coli*”

INTRODUCTION TO *Escherichia coli*

Imagine it is a beautiful afternoon and you and your family are at the park having a picnic. As part of this picnic the main course is hamburgers which have been previously prepared but were accidentally undercooked not reaching 71.1°C in the middle of each hamburger patty. You eat your meal and go throughout the rest of the day feeling just fine and enjoying the beautiful weather. As the week progresses you start to feel ill and by the third or fourth day you have stomach pains, cramps and bloody diarrhea. It's time to see the doctor because you have an *Escherichia coli* (*E. coli*) infection!

The scenario above is all too common as foodborne pathogens begin to emerge, reemerge, and adapt to new environments. It is necessary to understand where human pathogens reside and how they are transferred to the human host in order to better understand foodborne pathogens, control/minimize outbreaks, and protect the human population from exposure to the potentially deadly infections and intoxications that these pathogens can cause.

Escherichia coli O157:H7 is one of the major foodborne pathogens currently known to scientists that was recognized as a foodborne disease causing agent in 1982 (Holt et al. 2000) and then was declared an adulterant in ground beef by the United States Department of Agriculture Food Safety Inspection Service (USDA-FSIS) in 1994. *E. coli* O157:H7, and six other Shiga Toxin Producing *Escherichia Coli* (STEC), O26:H11, O45:H2, O103:H2, O111:H-, O121:H19, and O145:NM (constituting the “Big Seven”),

have been implicated in many foodborne illness outbreaks that are endemic, epidemic and have the potential to be pandemic due to the current world trade system and the distribution of food globally by food manufacturers. These outbreaks have resulted in many illnesses, including death, over the past 30+ years and account for an estimated >73,000 illnesses from *E. coli* O157:H7 and >36,000 illnesses from (Todd 1989; Rangel et al., 2005; Mead et al., 1999) STEC per year which leads to an estimated 223 million dollars combined (Todd 1989) in medical costs in the United States of America (USA) alone.

Several outbreaks have occurred involving fermented sausage products (containing beef) including an outbreak in 1998 involving Genoa Salami in Ontario Canada which produced 36 confirmed infections with O157:H7 (Williams et al., 2000) and an outbreak in Adelaide Australia in 1995 resulting in 23 cases of confirmed hemolytic uremic syndrome (HUS) from *E. coli* O111:NM in individuals who had consumed uncooked fermented sausages (Ross and Shadbolt 2000; CDC 1995a). Additionally an outbreak was reported in Sweden in 2002 consisting of 39 cases of *E. coli* O157:H7 in which 30 of the 39 cases were directly linked to fermented sausage products and 12 individuals developed hemolytic uremic syndrome (Sartz et al., 2008).

Escherichia coli is no longer a localized issue but has truly developed into a worldwide problem over the last 30+ years leading to the need for increased knowledge of the organism and for increased or better controls/interventions to help prevent *E. coli* from entering the food system or to eradicate it from food products, such as fermented

sausages, to make them safe for consumption. Current interventions that are utilized in the United States of America and abroad during the production of fermented beef sausage products to control foodborne pathogens include; thermal processing, pH adjustment, addition of salts and spices, and water activity adjustment (Incze 1998; Comi et al., 2005; USDA 1999a). None of these interventions are adequate to control *E. coli* or other foodborne pathogens on their own so they must be used in combination to help eradicate the pathogens from the sausage products.

Even though combining processing hurdles (usually two or more) (Incze 1998; USDA 1999a) helps to control *Escherichia coli* during the production of fermented beef sausage products there are still reports of outbreaks as mentioned previously. This leads us to look at alternative solutions or interventions that would help to control/eradicate foodborne pathogens from the food source but still maintain the product quality that is desirable. One of these alternative interventions is high pressure processing (HPP) which will be discussed later on and is a focus of the research performed in this thesis.

In order to provide the safest food products to the general population and help consumers to understand the importance of foodborne pathogens it is important to evaluate all aspects of foodborne pathogens to better understand what they are, where they come from, the diseases they cause and how to treat those diseases, how to prevent and control these pathogens during food production, and what is being done on a regulatory level to protect us now and in the future. The following sections of this review will discuss these topics and provide important knowledge and background in support of the experiments

that have been performed looking at the inactivation of *E. coli* O157:H7 and other STEC in beef summer sausage and the use of alternative interventions in order to control the growth of these potentially deadly foodborne pathogens.

GENERAL PROPERTIES OF *E. coli*

Classification

Scientific Name:	<i>Escherichia Coli</i>
Original Name:	<i>Bacillus coli communis</i> Escherich
Discovered By:	Dr. Theodore Escherich – German Bacteriologist
Important Serotypes:	O26:H11, O45:H2, O103:H2, O111:H, O121:H19, O145:NM and O157:H7
Phylogeny:	Kingdom Eubacteria, Domain Bacteria, Phylum Proteobacteria, Class Gammaproteobacteria, Order Enterobacteriales, Family Enterobacteriaceae, Genus Escherichia, Species <i>E. coli</i>

(NCBI 2015; Montville and Matthews 2008)

Overview of *E. coli*

Escherichia coli (*E. coli*) is a gram negative organism in the genus *Escherichia*. Each *E. coli* organism is comprised of a single straight rod shaped cell that is approximately 1-2 μm wide and 2-6 μm long and can occur in nature as an individual cell or in groups of two or more cells each being an individual organism (Holt et al. 2000; Berg 2004). *E. coli* species are non-spore forming and are considered to be facultative anaerobes which are chemoorganotrophic allowing them to utilize both respiratory and fermentative metabolism making it possible for them to survive and perform metabolic functions in both aerobic and anaerobic environments (Holt et al. 2000; Murray, Rosenthal, and Pfaller 2013). Additionally, the species is motile (although some are non-motile) with flagella extending from the organism in a peritrichous arrangement (flagella protruding in all directions), allowing them to move throughout various substrates including the human host (Holt et al. 2000; Murray, Rosenthal, and Pfaller 2013; Berg 2004).

Escherichia coli species can ferment glucose and lactose producing acids and gases (hydrogen and carbon dioxide) when incubated at temperatures of 35-37°C. *E. coli*'s biochemical properties include mannitol + with gas (usually), indole +, methyl red +, Voges-Proskauer, Oxidase, H₂S, and Citrate negative, and does not hydrolyze urea making it unable to utilize it as a sole source of nitrogen (Holt et al. 2000; Murray, Rosenthal, and Pfaller 2013). Finally, *E. coli* can survive in many different environments and can perform mixed acid fermentations in true anaerobic conditions giving lactate, succinate, ethanol, acetate, and carbon dioxide as by products (Holt et al. 2000; Clark 1989; Blankschien, Clomburg, and Gonzalez 2010).

Species of *E. coli* are ubiquitous and are able to survive (for at least short periods of time) in soil, water, fruits and vegetables, like melons and sprouts, and also can reside within the intestines of warm blooded animals such as cattle and *Homo sapiens* (Murray, Rosenthal, and Pfaller 2013; Montville and Matthews 2008; Rangel et al., 2005). It is transmittable from the environment to humans (feces, water, etc.), animals to humans (goats, cattle, deer, sheep, dogs, cats, etc.), and from humans to humans through various pathways depending on the specific serogroups present making it very difficult to control (Murray, Rosenthal, and Pfaller 2013; Montville and Matthews 2008; Clements et al., 2012). There are many serogroups of *E. coli* that cause disease in the human host making it a major human pathogen and a force as a foodborne pathogen causing disease throughout the world. These serogroups will be discussed in the following section.

Serotyping and Virotypes

Since there are so many different strains of *Escherichia coli* it is important to be able to distinguish them from one another, grouping them in order to better understand how they function and what actually causes the pathogenic strains to cause disease in human beings. One way to do this grouping is through serotyping the different strains. A serotype (serovar) is a variation between individual species of a bacteria that utilizes the cell surface antigens of the organisms to epidemiologically classify them into groups and subgroups (Merriam-Webster 2015b; Murray, Rosenthal, and Pfaller 2013). Organism that have been grouped together due to their common surface antigens are referred to as serogroups within a particular species. Serotyping of similar organisms allows scientists

to better understand the relationship between molecular attributes of the organisms and how those attributes play a role in the functional properties of the organism and whether they are pathogenic or not.

Escherichia coli contains many serogroups with greater than 167 O antigens, 74 K antigens, and 53 H antigens. Most of these serogroups are completely harmless and of no threat to humans with only a small percentage considered to be pathogenic (Montville and Matthews 2008; Nguyen and Sperandio 2012; Holt et al. 2000; Orskov and Orskov 1992). Serogroups of *E. coli* are classified by three separate levels of identification. First the serotype looks at the “O” polysaccharide which is the outermost somatic saccharide on the heat stable Lipopolysaccharide (LPS) cell wall antigen. The LPS contains the “O” polysaccharide, a core polysaccharide, and Lipid A which is responsible for endo-toxic activity. These components make utilizing the LPS and “O” polysaccharide important to serotyping/classifying the different *E. coli* strains. Additionally, the “H” proteins located in the flagella are considered and utilized to further segregate the different strains. The “K” antigens are located in the capsule and are not used for the serotyping of the different strains, but are necessary to understand and recognize as they can interfere with the detection of the “O” antigens via agglutination testing (Murray, Rosenthal, and Pfaller 2013; Holt et al. 2000; Diamant et al., 2004).

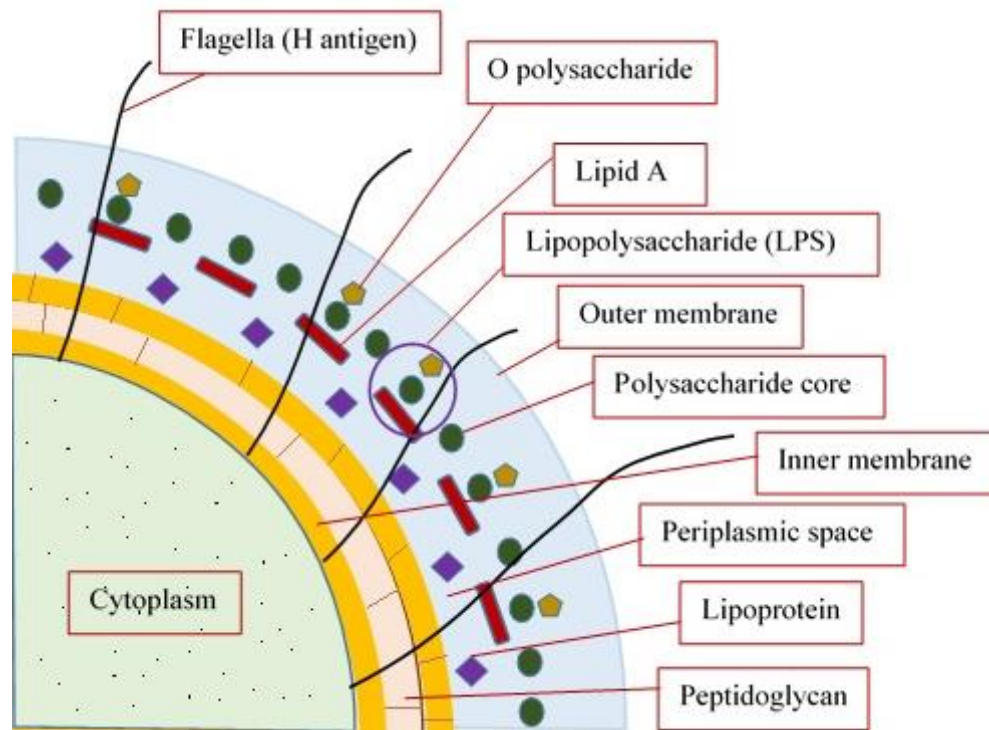


Figure 1.1: Cell surface of gram negative bacteria. Important cell components are displayed including Flagella (H antigen) and LPS (O antigen). The components of LPS are also shown in the purple circle including O polysaccharide, polysaccharide core and Lipid A (endotoxin). Picture adapted from Murray, Rosenthal, and Pfaller 2013. “Medical Microbiology.” Seventh Edition.

Along with serotyping the *Escherichia coli* strains by their “O” antigens and “H” flagella to better group them and understand their differences, there also is a classification of separation that has been established called viotypes. Although all of these viotypes or groups/classes of *E. coli* are similar in that they all cause intestinal disease and diarrhea, they are distinct from one another in their pathogenicity. The different viotype groups include Enterotoxigenic *Escherichia Coli* (ETEC), Enteroinvasive *Escherichia coli* (EIEC), Enterohemorrhagic *Escherichia coli* (EHEC), Enteropathogenic *Escherichia coli* (EPEC), Enteroaggregative *Escherichia coli* (EAEC) and Diffusely Adherent

Escherichia coli (DAEC) (Murray, Rosenthal, and Pfaller 2013; Chaudhuri and Henderson 2012; Diamant et al., 2004; Clements et al., 2012). Each one of these virotypes utilizes a different mode of attacking the human host whether it be for infection or intoxication. Of these different virotypes, EHEC are most commonly associated with foodborne illness (Nguyen and Sperandio 2012) with the likes of *E. coli* O157:H7 and the other shiga toxin producing *E. coli* as the prominent members of the enterohemorrhagic variety of *Escherichia coli*.

Enterohemorrhagic *E. coli* cause disease through infection and intoxication of the host and are most commonly associated with disease in developed countries (Murray, Rosenthal, and Pfaller 2013). EHEC cause disease ranging from mild diarrhea to hemorrhagic colitis consisting of abdominal pains and in some cases bloody diarrhea with some infected individuals developing hemolytic uremic syndrome consisting of renal failure and thrombocytopenia (Nguyen and Sperandio 2012; Murray, Rosenthal, and Pfaller 2013). For infection to occur ingestion of less than 100 organisms is required (Murray, Rosenthal, and Pfaller 2013), and after infection intoxication can occur from the release of shiga toxins into the blood stream (Tran et al., 2014). In some cases it has been reported that the infectious dose for EHEC can be as low as 10 (10-700) organisms (Nguyen and Sperandio 2012; Schmid-Hempel and Frank 2007; Tuttle et al., 1999; FDA 2015a) making EHEC extremely effective at infecting the host once it has entered the intestinal tract. The way in which EHEC infect the human host, the virulence factors associated with EHEC, and the diseases that are caused by EHEC will be discussed in the following sections.

EPIDEMIOLOGY OF *Escherichia coli*

Epidemiology: Branch of medical science that looks at the incidence, distribution, and control of disease as well as the presence and absence of disease or pathogens in a population (Merriam-Webster 2015a).

Certain strains of *Escherichia coli* are pathogenic to human beings, are able to survive in a multitude of environments, even if it is just for short periods of time, and cause infections and/or intoxications (Murray, Rosenthal, and Pfaller 2013; Montville and Matthews 2008; Rangel et al., 2005). *E. coli* are the most common gram negative bacteria associated with the intestinal tract of human beings, but are typically harmless. Although infections can occur endogenously from those species, most infections or intoxications associated with *E. coli* are derived from pathogenic strains that are taken in exogenously (Murray, Rosenthal, and Pfaller 2013). From the previous section we gained a better understanding of what the foodborne pathogen *Escherichia coli* is and many of its attributes. Now that we have that better understanding let's now take a look at where pathogenic *E. coli* comes from, what types of products could be effected by its presence, and how it infects human beings. Additionally, we will look at the diseases caused by pathogenic *E. coli*, how to treat and control those diseases, outbreaks of disease that have occurred, and finally the devastating effects those outbreaks have had on the food industry.

Environmental Sources and Reservoirs

Escherichia coli has adapted over time to become an extremely effective human pathogen. But, in order for it to be effective at invading the human host, it must be able to survive in the environment, within animal hosts, and then eventually within the human host. *E. coli* is widely distributed in nature and is commonly found in feces of animals, especially cattle, and in contaminated waste water (Montville and Matthews 2008; Rangel et al., 2005; Kaper and O'Brien 1998; Hancock et al., 1998). With *Escherichia coli*'s capacity to spread through fecal contamination it has allowed the introduction of this potentially deadly foodborne pathogen too many new environmental mediums in which it has adapted to and thrived increasing its overall presence in food sources.

Species of *E. coli* (specifically EHEC) are most commonly associated with cattle as their main reservoir with research showing that *E. coli* (*E. coli* O157:H7) resides predominately in the Recto-Anal Junction (RAJ) (Nguyen and Sperandio 2012) and is spread through fecal routes (Lim et al., 2007; Rangel et al., 2005; Hancock et al., 1998). Despite this, other reservoirs such as sheep, swine, deer, goats, dogs, and cats also have been reported with sheep having surprisingly high numbers of carriers (Montville and Matthews 2008; Kaper and O'Brien 1998). In Germany, EHEC strains were isolated from 66.6% of sheep tested as compared to just 21.1% of cattle, and it also was noted that the increased spread of *Escherichia coli* species to food crops could be due to the spread of feces by feral animals (Montville and Matthews 2008). In a study in the northwestern United States near feedlots and dairy farms it was seen that cattle had a prevalence of *E. coli* O157:H7 at a rate of 6.1% in their fecal samples while dogs in the area had a rate

3.1% in their fecal samples (Hancock et al., 1998). The movement of *E. coli* to new reservoirs poses a major problem to the food industry because as it spreads and reaches out finding sanctuary in new environments controlling this deadly pathogen will become more and more of a dream than a reality.

Food Products Affected by *E. coli* Contamination

Once thought only to be a major concern in beef and beef products, *E. coli* has extended its reach globally invading new environments and solidifying itself as a major foodborne pathogen not just in beef and beef products but in a wide variety of other foods. These products include drinking water (including ice), raw milk, pasteurized milk, cheeses, fruit juices (including apple cider), sprouts, salads, and a variety of fruit and vegetable products (Montville and Matthews 2008; Kaper and O'Brien 1998; Rangel et al., 2005). Additionally, fermented beef or pork based products such as beef sausage and salamis also have been adulterated by *Escherichia coli* despite the many processing steps taken to ensure their safety for consumption (Sartz et al., 2008; CDC 1995b; CDC 1995a; Williams et al., 2000).

Although beef and beef products are considered to be the main transmitter of pathogenic *Escherichia coli* as they are implicated in transmission ~41% of time for outbreaks, produce and produce products are not far behind transmitting *E. coli* in approximately 21% of reported food outbreaks associated with pathogenic *E. coli* strains including *E. coli* O157:H7 and non-O157:H7 shiga toxin producing *E. coli* (Montville and Matthews

2008). Since pathogenic *E. coli* can survive in all of these mediums it then can be transmitted to the human host directly from the food product itself infecting the human host and potentially causing infection or intoxication.

Pathogenicity, Illnesses Caused, and Virulence Factors

Since we know that Enterohemorrhagic *Escherichia coli* (EHEC) can be transmitted to humans from many sources including animals, food products, water, and even other humans (Montville and Matthews 2008; Kaper and O'Brien 1998), it becomes important to understand how *E. coli* infects the human host, what diseases it causes, and what the symptoms of those diseases are. *Escherichia coli* may enter into the human host through several different pathways but almost all of those pathways rely on ingestion of the organism. It accesses the body's digestive system through fecal oral contamination, fecally contaminated food or water, or from contaminated food products including raw or undercooked products (Murray, Rosenthal, and Pfaller 2013; Montville and Matthews 2008).

Once the pathogenic *E. coli* (specifically EHEC) enters the digestive system of the human host it enters into and begins to colonize the large intestine beginning what would be a 2-12 day incubation (typically 3-4 days in most patients) period before full infection occurs. EHEC can cause many diseases with varying levels of symptoms ranging from mild diarrhea up to and including hemorrhagic colitis and hemolytic uremic syndrome (Murray, Rosenthal, and Pfaller 2013). The symptoms of a complex EHEC infection start

out with watery diarrhea combined with severe abdominal pains within the first couple of days (1-4 days) followed by bloody diarrhea and continued severe abdominal cramping and pains which can last up to 10 days (Murray, Rosenthal, and Pfaller 2013; Montville and Matthews 2008; Kaper and O'Brien 1998). Vomiting also is seen in approximately half of patients, but it is important to note that a fever is not typical of an EHEC infection (Murray, Rosenthal, and Pfaller 2013).

In some cases infected individuals may develop a more severe disease known as hemolytic uremic syndrome (HUS) which is a medical disorder consisting of acute renal failure (kidneys stop filtering blood), thrombocytopenia (platelet deficiency in blood), and microangiopathic hemolytic anemia (loss of red blood cells) (Murray, Rosenthal, and Pfaller 2013; Montville and Matthews 2008). This occurs mostly in children under the age of 10 (Murray, Rosenthal, and Pfaller 2013), but also can be promoted in immunocompromised individuals and in some cases healthy individuals but at much lower percentages of infection. Additionally some patients experience other symptoms including seizures, strokes, and other issues associated with the central nervous system (Kaper and O'Brien 1998). Even though we are aware of the diseases and symptoms caused by EHEC the pathogenicity of the organism is not fully understood, and the routes of adherence and transmission within the human host are still not recognized and have to be studied to fully understand how EHEC causes infection (Montville and Matthews 2008; Karch 2001).

Despite not fully understanding how Enterohemorrhagic *E. coli* (EHEC) infect the human host, we do understand and recognize several of the virulence factors (proteins and genes) that are present in EHEC and what their purpose is for pathogenesis (Table 1.1). All of the members of the EHEC group that are currently known produce the cytotoxins called shiga toxins including *stx1* and *stx2* which inhibit protein synthesis (Montville and Matthews 2008; Kaper and O'Brien 1998; Murray, Rosenthal, and Pfaller 2013; Karch 2001). These shiga toxins are directly linked to the development of HUS resulting from the damage of endothelial cells which in turn leads to the platelet activation and eventually acute renal failure within the infected individual. Shiga toxins also cause issues within the cells such as an increased response of the inflammatory cytokines within the cellular system (Murray, Rosenthal, and Pfaller 2013; Karch 2001).

EHEC also have virulence factors that cause lesions in the intestines. These lesions called attaching-and-effacing (A/E) lesions occur after the outer membrane adhesion protein *intimin* allows for the attachment of the EHEC to the intestinal cells giving it access to the cell and causing the loss of microvilli which causes an increase in F actin (which is important for many cellular processes) in the cytoplasm (Phillips et al., 2000; Montville and Matthews 2008; Kaper, Nataro, and Mobley 2004; Kaper and O'Brien 1998). It also is important to know that the same virulence factors that cause A/E lesions in humans as cause A/E lesions in cattle indicating that the pathway for attaching and effacing is similar between the two species and that the organism is not host specific in its utilization of those pathways (Phillips et al., 2000).

Table 1.1: Enterohemorrhagic *E. coli* (EHEC) Virulence Factors and Function

Genetic Locust	Protein Description	Gene(s)	Function
Chromosome (locus of enterocyte effacement)	Intimin	<i>eae</i>	Adherence
	Tir	<i>tir</i>	Intimin receptor
	Secretion proteins	<i>espA, espB, espD</i>	Induces signal transduction
Phage	Type III secretion System	<i>escC, escD, escF, escJ, escN, escR, escS, escT, escU, escV, sepQ, sepZ</i>	Apparatus for extracellular protein secretion
	Stx	<i>stx1, stx2, stx2c, stx2d</i>	Inhibits protein synthesis
	EHEC hemolysin	EHEC- <i>hlyA</i>	Disrupts cell membrane permeability
Plasmid	Catalase-peroxidase	<i>katP</i>	

Table includes the virulence factors associated with EHEC, the genes that encode those factors, and the function that is believed the virulence factor plays a role in. Stx are considered the most important virulence factors which also play a role in the development of HUS. Source: Table was acquired from Montville and Mathews 2008, Food Microbiology, An Introduction, Chapter 9 Enterohemorrhagic E. coli, pg. 136, Table 9.3 Proteins and Genes Involved in Pathogenesis of EHEC. Permission for use given by ASM Press (See Appendix A).

Prophylaxis

Although EHEC can cause many different symptoms that are not desirable by humans like diarrhea, vomiting, and bloody diarrhea, the correct form of treatment is “wait it out.” The Mayo Clinic stated on their website that “For illness caused by *E. coli*, no current treatments can cure the infection, relieve symptoms or prevent complications. For most people, treatment includes: rest and fluids to prevent dehydration” (Mayo 2015). This course of treatment is recommended for multiple reasons. First, *E. coli* serotypes are

becoming less susceptible to antibiotics showing resistance to streptomycin and tetracyclines (Tadesse et al., 2012; Montville and Matthews 2008) as well as many other gram negative associated drug options. If antibiotics are used when antibiotic resistant bacteria are present it can create an environment that is actually more favorable to the pathogenic antibiotic resistant organism by decreasing the numbers of commensal bacteria in the intestinal tract that are competing for space and survival (Tadesse et al., 2012; Aarestrup, Wegener, and Collignon 2008).

Some patients that have been treated with antibiotics for severe *E. coli* infections did not see reduced symptoms or a reduction in the amount of time the symptoms presented themselves making the use of the antibiotics irrelevant to the treatment of the disease (Kaper and O'Brien 1998; Riley et al., 1983). For instance, an outbreak in Washington State, USA with data available for 80 patients in which 37 were treated with some form of antibiotics to help prevent hemolytic uremic syndrome (HUS). Of these patients 11 developed HUS, the overall period in which symptoms were seen did not decrease and in some cases the duration of bloody diarrhea actually increased (Kaper and O'Brien 1998; Ostroff, Kobayashi, and Lewis 1989).

If the pathogenic *E. coli* species are deactivated by the use of antibiotics or other non-immune response associated methods within the intestinal tract they might release toxins such as *stx1* and *stx2* into the blood stream that had been contained in the organism (Wong et al., 2000). This release could increase the onset of systems and also increase the likelihood of developing more severe forms of disease such as hemolytic uremic

syndrome (Tran et al., 2014; Stahl et al., 2015; Brabban et al., 2004; Wong et al., 2000). While studying an outbreak in Sakai City, Japan, Wong et al., 2000 showed that children treated with an antibiotic had a relative risk (17.7%) of developing hemolytic uremic syndrome (HUS) and concluded that children receiving antibiotics were more likely (depending on antibiotic used) to develop HUS than children who were not treated. This is why antibiotics are not recommended to treat infections from STEC.

Although there are many other treatment options that have been tried to help reduce and control the symptoms of an *E. coli* infection including antidiarrheal agents, anti-motility agents (Kaper and O'Brien 1998), and even studies looking at the use of bacteriophage as a control measure to reduce *E. coli* populations and remove them from the intestinal tract (Tanji et al., 2005), the best method for reducing symptoms and recovering from an infection from pathogenic *E. coli* is still rest and the intake of plenty of fluids (Kaper and O'Brien 1998; Tarr 1995).

So, what do we do to stop *E. coli* from making us sick and causing disease? First we must understand that some individuals are more at risk for infection from foodborne disease than others. These at risk individuals include children (especially newborns), older adults, immunocompromised or sick individuals, and pregnant women (Brabban et al., 2004; Kaper, Nataro, and Mobley 2004; CDC 2014; CDC 2015) or individuals participating in risky behavior (Rangel et al., 2005). The Center for Disease Control (CDC) website recommends hand washing as the main deterrent from foodborne illnesses including *E. coli* infections and indicates that handwashing should occur after using the bathroom,

changing diapers, after contact with animals, and before preparing or eating food, to name a few (CDC 2015; CDC 2014). The CDC also mentions that hand sanitizers can be used if no soap or water is available but that “alcohol-based hand sanitizer...can quickly reduce the number of germs on hands...but they are not a substitute for washing with soap and running water” (CDC 2014). In fact, handwashing stations were proposed by the Center of Disease Control in 2003 to be mandatory at all petting zoos and open farms (Brabban et al., 2004) and then were updated in 2011 to include other outdoor arenas and the handling of amphibians (NASPHV 2011).

Handwashing is a good control measure to help prevent the spread of *E. coli* and EHEC, but there are other measures that can be taken to help eradicate the organism if it is present in food. These measures include properly cooking meat that has been ground or tenderized to at least 71.1°C internal temperature (especially ground beef), washing all cooking utensils, cutting boards and kitchen areas to prevent cross contamination of pathogens onto other food products, avoiding the swallowing of potentially contaminated water (while swimming or performing other water activities), and avoiding the consumption of raw milk, raw meats, unwashed sprouts, undercooked sausages, and unpasteurized milk and fruit juices (CDC 2014; CDC 2015; Brabban et al., 2004; NASPHV 2011; Kaper, Nataro, and Mobley 2004; HHS 2015). In order to ensure that we are safe from infection of pathogenic *E. coli*, such as EHEC, we must take responsibility for our own actions and do what we can to keep ourselves, our loved ones, and other individuals free from infection by following proper cooking and cleaning procedures and by avoiding risky products that might lead to disease.

Outbreaks Associated With STEC in Food Products

Escherichia coli has been implicated in many outbreaks over the past 30+ years after contaminating a wide variety of products. These outbreaks have resulted in many illnesses, including death, and account for an estimated >109,000 cases of *E. coli* O157:H7 and STEC infections combined (Todd 1989; Rangel et al., 2005; Mead et al., 1999) per year leading to an estimated 223 million dollars combined (Todd 1989) in medical costs in the United States of America alone.

Several outbreaks have occurred involving fermented sausage products (containing beef) including one of the first recorded outbreaks involving dry fermented sausages in 1993 in the western United States in which 23 individuals became ill (Burros 1995), an outbreak in 1998 involving Genoa Salami in Ontario Canada which produced 36 confirmed cases of O157:H7 infection (Williams et al., 2000), and an outbreak in Adelaide Australia in 1995 resulting in 23 cases of confirmed hemolytic uremic syndrome (HUS) from *E. coli* O111:NM in individuals who had consumed uncooked fermented sausages (Ross and Shadbolt 2000; CDC 1995a). Additionally an outbreak was reported in Sweden in 2002 consisting of 39 cases of *E. coli* O157:H7 infection in which 30 of the 39 cases were directly linked to fermented sausage products and 12 individuals developed hemolytic uremic syndrome (Sartz et al., 2008).

The most important outbreak of *E. coli* O157:H7 occurred in undercooked ground beef patties that were distributed from a fast food chain in 1993 resulting in 500 laboratory confirmed infections and 4 deaths (CDC 1993; Rangel et al., 2005; Bell et al., 1997). This

outbreak led to regulations declaring *E. coli* O157:H7 an adulterant in ground beef by the United States Department of Agriculture Food Safety Inspection Service in 1994.

Many other products have been implicated in outbreaks involving pathogenic *E. coli*. The Center for Disease Control website report shows that a range of products from spinach (2006), pizza (2007), cookie dough (2009), cheese (2010), and even hazelnuts (2011) have been implicated in outbreaks of *E. coli* O157:H7 (CDC 2015). In 2010 there was an outbreak of shiga toxin producing *E. coli* O26 associated with sprouts at a fast food chain in which 29 cases, of which 7 individuals were hospitalized, of infection were confirmed across 11 states (CDC 2015). Between April and August of 2015 www.foodsafetynews.com reported several incidences involving *E. coli* including an outbreak in Canada involving 24 cases with 5 hospitalizations, outbreaks in Indiana, USA involving 6 cases including 1 death, and 9 confirmed cases of *E. coli* O157:H7 infection in an outbreak in Washington, USA (FSN and FSN 2015a; FSN and FSN 2015b; FSN and Siegner 2015). In the outbreaks in Canada and in Indiana the source of the outbreak is still unknown while the case in Washington was linked to Mexican food served at farmers markets from food trucks (all cases are still ongoing) (FSN and FSN 2015a; FSN and FSN 2015b; FSN and Siegner 2015).

Despite control measures in place to help eradicate *Escherichia coli* from our food products it still continues to cause outbreaks and create headlines in the news. It is important to continue to study *E. coli* and to evaluate the manufacturing processes that are used to create our food products to ensure that interventions are in place to properly

control pathogenic organisms and to see if new interventions may be necessary to help prevent future problems.

CONTROLLING FOODBORNE PATHOGENS DURING MANUFACTURING PROCESS OF SUMMER SAUSAGES

Escherichia coli has become a major problem in all food industries due to its advancement into new mediums. It is no longer just a beef issue but is now an issue in almost all types of food products because it can survive not just in animal hosts but also in contaminated water and on the vegetables/leafy greens (Xicohtencatl-Cortes et al., 2009; Montville and Matthews 2008; Rangel et al., 2005; Kaper and O'Brien 1998). Many products have been implicated in outbreaks of various shiga toxin producing *E. coli* strains including ground beef, beef products such as fermented beef sausages, sprouts, and others. Due to this continued problem it has become more important than ever to evaluate the manufacturing process of food products and determine if the interventions being used to control deadly foodborne pathogens such as *E. coli* are adequate and if other methods of control are better or in some cases necessary to make the products safe for consumption.

Sausage Manufacturing Process and Process Controls

Production processes for each type of food product are different, hence they have different interventions that are applied for control of pathogens at various stages

throughout manufacturing. The manufacturing process for fermented semi-dry beef summer sausages is no exception. Beef summer sausages are produced utilizing many control points (CPs), critical control points (CCPs) which may be a part of the hazard analysis critical control points (HACCP) plan, and follow regulations set forth by the United States Department of Agriculture and Food Safety Inspection Service (USDA-FSIS) for the release of product for consumption in the United States of America (USDA 2015). These control points or process control measures include many different approaches for controlling foodborne pathogens.

Typically, control measures that are used in the production of fermented beef sausage products to control foodborne pathogens, such as *E. coli*, include steps during processing such as; keeping the meat at temperatures below 4.4°C during production, addition of salts and nitrates to the product formulations, addition of spices that inhibit growth of organisms, use of competing cultures and starter cultures, lowering of the pH of the product to ≤ 5.3 (typically below 5.0), thermal processing (cooking at lower than lethality) of the sausage at specified times and temperatures, adjusting the water activity levels to ≤ 0.93 through drying procedures, and then chilling the product to control growth of any surviving organisms (Barbuti and Parolari 2002; USDA 1999a; Incze 1998; Domowe 2015).

All equipment used for the manufacturing of sausage products should be properly cleaned/sanitized between each use and in-between each different product type to reduce cross contamination (CFR 2011; USDA 1999d) as equipment could be a source of

unwanted pathogens (Barbuti and Parolari 2002). The addition of salts, nitrates, and other spices help to control growth of certain organisms (USDA 1999a), and the addition of competing cultures of organism or starter cultures that facilitate fermentation of the product help to create an unfavorable environment (Leroy, Verluyten, and De Vuyst 2006). Fermentation is the process of reducing the pH (acidity) levels of the beef summer sausage to create a hostile living environment for foodborne pathogens with the pH level of the sausage reaching levels of ≤ 5.3 (most beef summer sausages) (USDA 1999a; Incze 1998; Domowe 2015). Some summer sausages are cooked but not to the lethality levels recommended by the USDA of 71.1°C. Instead beef summer sausages are typically only thermally processed (cooked) to 58.3°C internal temperature (or less) (Domowe 2015).

Other process controls that might be used during beef summer sausage production include adjustment of water activity levels (a_w) and chilling of the product to reduce the growth rates of the *Escherichia coli* or other pathogens that may still be present (USDA 1999a). Not all of these measures are taken during all summer sausage production, but usually many of them are utilized in combination to help produce a product that is presumably safe for consumption.

Hurdle Technology and Fermented Sausages

Fermented sausage manufacturing is slightly unique in that there are no true lethality steps during the manufacturing process. None of the interventions that are utilized for

controlling the growth of pathogenic organisms mentioned in the previous section are adequate to control *E. coli* or other foodborne pathogens on their own (at the levels applied) so they must be used in combination to help eradicate the pathogens from the sausage products. The process of relying on multiple interventions during production for the control of pathogenic organisms is referred to as the “Hurdle Effect” or Hurdle Technology (Leistner 2000). Hurdle Technology has been proven to be effective in controlling pathogenic organisms in foods like fermented sausage products (Shalini and Singh 2014; Leistner 2000; Barbuti and Parolari 2002) which are considered some of the safest shelf stable products available, and is approved of by the USDA-FSIS as an acceptable methodology (USDA 2012b; USDA 1999a).

Even though combining many interventions (usually two or more) helps to control *Escherichia coli* during the production of fermented beef sausage products there are still reports of outbreaks as mentioned previously. This could be due to manufacturers not utilizing all of the “Hurdles” or underutilizing the hurdles/interventions that they have incorporated, or due to contamination events within the manufacturing process itself. One such example is the manufacturing of fermented sausages in Northeast Italy where it is common for them to only lower the pH level to between 5.6 and 5.7 but then lower the water activity level to 0.91-0.92. Although the water activity is at adequate levels to control foodborne pathogen growth, the pH level is much too high allowing for the growth of those pathogens essentially negating the Hurdle Technology being used (Comi et al., 2005; USDA 2012b). The typical pH level required to control *Escherichia coli* is approximately 4.5 but it has been known to survive at lower levels (Small et al., 1994;

USDA 1999a). The reason they produce sausages with the high pH levels despite the risk is to help control the loss of flavor and texture that the producer of the sausage is looking for (Comi et al., 2005).

The lack of a true lethality step could be what is leading to outbreaks still occurring due to the consumption of fermented beef sausage products despite the use of so many interventions to control *E. coli* and other pathogens. Thermal processing (cooking) to lethality temperatures (internal temperatures of 71.1°C) would be a logical solution to this growing issue but it can potentially alter the flavor, texture, and other attributes of the beef sausage that make it desirable even when utilized at lower temperatures such as 53°C for manufacturing purposes (Ferrini et al., 2014; Domowe 2015).

Thermal Processing

Of the many hurdles or interventions that are utilized in the manufacturing process of beef summer sausages, thermal processing is likely the most effective method of pathogen control (Domowe 2015). Thermal processing has been shown to be extremely effective in the inactivation of *E. coli* when used correctly (Domowe 2015; USDA 2012b; Stringer, George, and Peck 2000; USDA 1999a). Despite the fact that sausage manufacturers do not fully cook the sausage to meet lethality requirements for *Escherichia coli* in beef products, the thermal processing step is still very important to the control of *E. coli* and other foodborne pathogens that might be present, but is not always used (Comi et al., 2005; Domowe 2015).

Many manufacturers that utilize a thermal processing step in their production system typically use temperatures between 48.9-60.0°C and cook the sausage anywhere from 1-3 hours based on the guidelines set forth by the USDA-FSIS (USDA 1999a; USDA 1999b). Data has shown that when summer sausages are cooked at 54.4°C for 60 minutes in combination with other hurdles that *E. coli* is reduced to undetectable levels (Calicioglu et al., 1997). Once again, thermal processing is effective in controlling foodborne pathogens, but is not used at full lethality levels during the manufacturing of summer sausage products due to the loss of the flavor, texture, and other attributes that sausage enthusiasts enjoy.

High Pressure Processing

Since outbreaks of *Escherichia coli* are still occurring in a multitude of food products including beef summer sausages, it is necessary to look at additional interventions or alternative interventions to help ensure that these products are safe for consumption. One such intervention is high pressure processing (HPP). High pressure processing (Pascalization), also referred to as high hydrostatic pressure (Hugas, Garriga, and Monfort 2002) and/or ultra-high pressure (UHP) processing (Smelt 1998; NFL 2013; Farkas and Hoover 2000), is a cold pasteurization technique that utilizes isostatic pressure to neutralize the growth of bacteria, denature proteins, and at the same time preserve the quality of the food product with little to no effect on the color, flavor or texture of the products being processed (Hiperberic 2014; Norton and Sun 2008; Farkas and Hoover 2000; Oey et al., 2008; Matser et al., 2004; NFL 2013).

High pressure processing works by utilizing a suitable isostatic medium, such as water, and pressurizing the fluid within the machine using pumps (Hugas, Garriga, and Monfort 2002; Farkas and Hoover 2000; Hiperberic 2014; USDA 2012a). The fluid can be heated or kept cold with processing temperatures ranging from 0°C to 100°C. Pressure that is applied to the food products is uniformly distributed and controlled with a potential range for processing being from 100-800 megapascals (MPa) with the industry typically using between 300-600 MPa (~43,500-87,000 psi) (Hugas, Garriga, and Monfort 2002; Oey et al., 2008; Farkas and Hoover 2000; Hiperberic 2014; NFL 2013).

The amount of time each product is subjected to the desired pressure also can be adjusted ranging from milliseconds up to 20 minutes (Farkas and Hoover 2000). Products (solids) can be placed directly into the vessels that are then inserted into the HPP machine for processing, but this is not typical. Most products, solids and liquids, are prepackaged before being subjected to the pressure treatments and then placed into the vessels to receive pressure treatments at the various dwell times (Farkas and Hoover 2000; Hiperberic 2014).

Products currently using HPP and Quality

Although it is not as popular of a lethality treatment, HPP has been and is currently being utilized showing potential within the food industries as an additional intervention to help ensure the safety of many different types of food products (Norton and Sun 2008). There have been many studies that have shown that HPP is effective in eradicating foodborne

pathogens such as *E. coli* and at the same time maintaining the quality of the food product. Testing has been done on various types of products to ensure the quality is maintained such as: navel oranges, meats (ham, ground beef, etc.), fruit juice, and purees (apple, plum, strawberry) (Bull et al., 2004; Cheftel and Culioli 1997; Garriga et al., 2004; Jiang et al., 2015; Hsu et al., 2015; Deliza et al., 2005; García-Parra et al., 2014; Hsu et al., 2014; Landl et al., 2010). Additionally other products such as oysters, fruits and vegetable, and even fermented sausages have been studied to understand how HPP effects the quality of products (He et al., 2002; Butz et al., 2003; Hiperberic 2014) and determine if HPP is more capable of maintaining quality then thermal processing.

However, some varying results have been seen as to whether or not HPP effects the quality of the product, but it is important to understand that there are many parameters involved and some of the testing results that are available may not account for all parameters. Butz et al. (2003) concluded that high pressure processing did not have a significant effect (in most cases) on the beneficial substances in fruits and vegetables such as anti-oxidant factors, sugars, carotenoids, etc. within fruit and vegetable products such as carrots, tomatoes, strawberries, and apples. But, in another study it was seen that the ascorbic acid, vitamin C, and total phenolic content were unchanged in apple puree at 400 MPa but at higher processing levels such as 600 MPa these compounds were effected negatively (Landl et al., 2010). In orange juices it has been shown that pectin methyl esterase (PME is an enzyme that facilitates cell wall modification) is reduced significantly by HPP at 600 MPa for 1 minute, but that other quality parameters such as color, browning index, acid content, and viscosity as well as the concentrations of

ascorbic acid and β -carotene did not change (Bull et al., 2004) which is in contrast to the apple puree product.

High pressure processing also has been evaluated on meat and meat products to determine if the quality of the meat is affected. At lower temperature (1-3°C) the product quality of meat (beef) and meat products seems to be unchanged, but at higher temperatures (>3°C) the heated medium (water or other suitable fluid starts to affect the color causing a loss of redness (starts to turn brown) visually and a decrease in the tenderness of the meat with increased purge (Lowder, Waite-Cusic, and Mireles DeWitt 2014). Based on these findings for product quality it is very apparent that the quality of the product, after high pressure processing, is directly associated with the individual product itself, the products attributes, and the processing parameters including MPa level and the dwell time. If used appropriately high pressure processing could be utilized as a suitable additional or alternative solution for maintaining the safety of food products and for maintain the quality of the products being processed.

The majority of studies that are available agree that HPP is effective at controlling *E. coli*. For instance, it has been found that shiga toxin producing *E. coli* including O157:H7 are reduced by greater than 5 logs in ground beef when subjected to HPP at just 480 MPa for 15 minutes (Hsu et al., 2015). In fruit products such as strawberry puree it has been shown that *E. coli* O157:H7 and non-O157:H7 STEC can be controlled using HPP giving ~6 logs of reduction at just 350 MPa for 5 minutes of dwell time (Hsu et al., 2014). Additionally, it has been shown in fermented sausages (beef Norwegian and genoa

salami) that several logs of reduction of *E. coli* (2.7 logs to >5.8 logs depending on sausage type and recipe) can be achieved when utilizing high pressure processing as an additional intervention in combination with other processing hurdles (Omer et al., 2010; Porto-Fett et al., 2010).

Despite the effectiveness of HPP on inactivating foodborne pathogens, there have been studies that have raised a concern about the development of barotolerance (resistance to high pressure) in *E. coli* species with one study showing that mutant strains could in fact survive high pressure processing when utilizing lower megapascal levels to induce tolerance, (Hauben et al., 1997) but these mutants were not subjected to the additional hurdles utilized such as pH adjustment, in fermented sausage production. Most of the research available shows, that if used appropriately at the correct amount of pressure and dwell time, that high pressure processing could be used to help control foodborne pathogens making products safer for consumption and potentially increasing the shelf life of the products.

Potential uses for HPP in Fermented Sausage Products

Since we know that high pressure processing is a viable option as an additional intervention in controlling foodborne pathogens (Omer et al., 2010; Hayman et al., 2004; Yuste et al., 2000) and also does not affect product quality if used appropriately, then it could be used for the production of fermented semi-dry or dry beef summer sausages (Mor-Mur and Yuste 2003). As mentioned previously many hurdles are utilized in the

production of beef summer sausages to make sure they are safe for consumption, but outbreaks are still occurring. HPP could be a good option for helping to improve the sausage production process without sacrificing the quality of the product that would be lost with increased thermal processing (Oey et al., 2008; Matser et al., 2004). If utilized as an additional processing step or hurdle it could be done on whole logs before the drying phase of production, or could be utilized on finalized, sliced, packaged product before it goes to retail for sale to restaurants for use in food products.

High pressure processing could also help to increase the shelf life of sausage products by not only reducing pathogens but also reducing levels of spoilage organisms (Smelt 1998; Hiperberic 2014) making the product more shelf-stable allowing for export to markets worldwide. Because high pressure processing is effective it could allow for the reduction of salts and other additives within the sausage recipes allowing for new product formulations. Additionally, high pressure processing has the potential to replace thermal processing as the main lethality step within the production process of sausages products while maintaining the quality attributes (Hiperberic 2014) of the sausage that are desirable if it can be consistently shown that *E. coli* and other pathogens are reduced by sufficient levels to meet USDA-FSIS guidelines.

Stressors and Stress Response of *E. coli*

Escherichia coli is resilient being able to survive in multiple environments and in food sources even when subjected to interventions or hurdles. *E. coli* reacts when stressors

(interventions) are applied to the organism during production of products such as fermented beef summer sausages when salts are added, pH is lowered during fermentation or when the products are heated. For instance, when *E. coli* is subjected to heat as a stressor it responds by generating heat shock proteins (HSPs) such as IbpA and IbpB which protect heat denatured proteins from irreversibly aggregating (Kuczynska-Wisnik et al., 2002) allowing the organism to function and survive within the new hostile environment. HSPs including groES and groEL (for protein folding), grpE, dnaK, dnaJ and others work together within the cellular system and play an important role in protein-protein interactions within the bacterium acting as chaperons and assisting in proper protein conformation, prevention of unwanted aggregation of proteins, repair of proteins, DNA replication, nucleotide exchange factors and potentially as thermos-sensors (Harrison 2003; Storz and Hengge-Aronis 2000). Within *E. coli* there are several genes that regulate heat shock responses and the release of the heat shock proteins including RopH (σ_{32}) which is used in transcription of heat shock genes, RpoN (σ_{54}) which is responsible for nitrogen metabolism and RpoS (σ_{38}) which helps with the actual stress response (Storz and Hengge-Aronis 2000).

Additionally *E. coli* also responds to other stressors including the cold temperatures during production, lowered acidity levels (pH) during fermentation, lowered water activity levels during drying, etc. (Chung, Bang, and Drake 2006). Like with heat stress response, *E. coli* produces cold shock proteins (CSPs) to allow it to remain functional at colder temperatures and continue to survive (Chung, Bang, and Drake 2006). Similarly, when *Escherichia coli* is subjected to high pressure (HPP) many of the same shock

proteins are upregulated that are seen in heat shock and especially cold shock responses (Welch et al., 1993). According to research done by Welch et al. (1993) high pressure will elicit a similar response in shock protein regulation as to cold shock response and these two stressors (high pressure and cold shock) are the only interventions that inhibit the growth of the pathogen but do not reduce the synthesis of upregulated proteins.

These attributes of *Escherichia coli* are the reason it is important to focus on controlling the growth of the organisms and performing research to find the best interventions possible for eradicating *Escherichia coli* during the production process of beef summer sausages to reduce the risk of infection to consumers. With its ability to adapt via stress response combined with the reduced levels of control for each hurdle during production of fermented beef sausage products it is plausible that *E. coli* could survive and lead to continued outbreaks in our current world market.

REGULATIONS / ALTERNATIVE SOLUTIONS

Regulatory Body, Current USDA-FSIS Production Regulations, Alternative Solutions

In the United States of America (USA), the production of fermented dry and semi-dry sausage products such as beef summer sausages is regulated by the United States Department of Agriculture and Food Safety Inspection Service (USDA-FSIS). This regulatory body proposed guidance and implementation of regulations in 2001 for production of processed meat and poultry products including fermented dry and semi-dry

sausages that require a minimum 5.0 log reduction in *E.coli* and declaring final product to be adulterated if tested positive for *E. coli* O157:H7 in 2001 (USDA 1999c; USDA 2001b). Furthermore, in 2011, USDA-FSIS declared six other shiga toxin producing *E. coli* (O26, O45, O103, O111, O121, and O145) as adulterants (USDA 2011), for certain beef products sold in the United States.

In the United States many fermented sausage producers use thermal processing in conjunction with other processing hurdles (pH and water activity adjustment, etc.) in order to meet USDA-FSIS guidelines for required 5.0 log reductions of *E. coli* and 6.5 log reductions of Salmonella (USDA 2001b) within fermented meat products. Additional regulations include standards of identity that must be met in order to ensure the product was made correctly, moisture to protein ratio requirements, cooking temperatures, drying times, etc. (USDA 1999a; CFR 2012a). USDA-FSIS released Appendix A for the use in regulating the production process of meat and poultry products which sets performance standards for lethality based on cook temperatures and time requirements (USDA 1999b). This is important for beef summer sausage production in the USA as it allows manufacturers to cook at lower temperatures by increasing the cook time to still meet the 5.0 log reduction requirements. For example a beef summer sausage producer can cook at 54.4°C for a minimum of 112 minutes to meet the requirement (USDA 1999b; USDA 1999a) instead of fully cooking the product to 71.1°C and damaging the organoleptic properties that they are trying to maintain for quality purposes. These products are considered to be ready-to-eat (RTE) products by the USDA-FSIS and must also meet requirements for being RTE in 9 CFR 430 (CFR 2012b; USDA 2014).

Many regulations are in place in order to help ensure that the food products being manufactured are safe for consumption but that does not mean that new processes or procedures do not need to be pursued and that research should not be done in order to improve the current production processes and possibly alter the regulations that are currently in place. As mentioned previously thermal processing (cooking) is very effective in helping to control *Escherichia coli* and other foodborne pathogens at the manufacturing level, and the regulations reflect this fact, but thermal processing also affects the organoleptic qualities and attributes of many products including beef summer sausages. Alternate technologies that use reduced heat, no heat, or other methods have also been investigated with some success. One such technique is electron beam radiation (e-beam). One study showed that e-beam radiation was effective at controlling pathogen growth while maintaining the sensory properties (appearance, odor, taste) of the fermented sausage showing no difference in these qualities between irradiated and non-irradiated samples (Cabeza et al., 2009). Although radiation is an effective tool in controlling pathogens, is approved for use by the FDA (FDA 2015b), and also maintains the quality of the product, it is not widely used due to consumer concerns (Wilcock et al., 2004; NFID 2015).

Alternatively high pressure processing (HPP) is starting to be used more frequently, is approved by the USDA-FSIS for use with food products, and may be used without prior approval by the USDA-FSIS for products containing meat, poultry or eggs (USDA 2012a). It has been shown in many food products to be effective in controlling foodborne pathogens and also for maintaining quality of the products since it uses pressure rather

than heat for the lethality step (USDA 2012a; Norton and Sun 2008). Evaluating these techniques along with re-evaluating the current processes that are in place will lead to improved methods being developed and the potential for new processing procedures to be utilized that not only control foodborne pathogens but also help to improve the quality of the products that we consume.

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CHAPTER 1 APPENDIX A:

LICENSE FOR TABLE 1.1: ENTEROHEMORRHAGIC *E. COLI* (EHEC)

VIRULENCE FACTORS AND FUNCTION

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CHAPTER 2

INACTIVATION OF *Escherichia coli* THROUGHOUT FERMENTED BEEF SUMMER SAUSAGE PRODUCTION

AND

USE OF HIGH PRESSURE PROCESSING AS AN ALTERNATIVE TO THERMAL PROCESSING

INTRODUCTION

Enterohemorrhagic *Escherichia coli* (EHEC) including *E. coli* O157:H7 and the other Shiga Toxin Producing *E. coli* (STEC), O26:H11, O45:H2, O103:H2, O111:H-, O121:H19, and O145:NM (constituting the “Big Seven”) have been implicated in many outbreaks in the United States of America (USA) and worldwide over the past 20 to 30+ years. *E. coli* O157:H7 was first recognized as a disease causing agent in 1982 (Rangel et al., 2005) and then was declared an adulterant in ground beef by the United States Department of Agriculture Food Safety Inspection Service (USDA-FSIS) in 1994. Additionally, in 2011 the USDA-FSIS also declared the other STEC adulterants in certain beef products (USDA 2011). Outbreaks involving *Escherichia coli* have occurred in many different food products including fermented sausages worldwide. These outbreaks have occurred in the USA, Australia, Canada, and Sweden (Burros 1995; CDC 1995b; Williams et al., 2000; CDC 1995a; Sartz et al., 2008) all resulting in confirmed infection from shiga toxin producing *E. coli* species, and in many cases with patients being hospitalized with hemolytic uremic syndrome (HUS).

Research has been done to better understand the inactivation of *E. coli* O157:H7 and the STECs in sausage products looking at the various stages of manufacturing including batter, fermentation, thermal processing, drying, and cold storage (Calicioglu et al., 1997; Hwang et al., 2009; Calicioglu et al., 2002; Faith et al., 1997; Faith et al., 1998; Porto-Fett et al., 2008; Porto-Fett et al., 2010). In the United States many fermented sausage producers use post-fermentation thermal processing in order to meet USDA-FSIS guidelines for required 5.0 log reduction of *E. coli* and 6.5 log reductions of *Salmonella*

spp. (USDA 2001b; USDA 2001a) within fermented meat products, including beef summer sausages. But, this process is susceptible to damaging the organoleptic properties (flavor, color, texture) of the sausage and is considered to be undesirable by many sausage enthusiasts (Ferrini et al., 2014; Domowe 2015). In order to avoid loss of the quality attributes of the fermented sausage products while still maintaining the safety of the product and meeting the USDA-FSIS guidelines for pathogen reduction it becomes important to look at alternative technologies that might be used in place of thermal processing such as high pressure processing (HPP).

High pressure processing, or high hydrostatic pressure, is not a new technology but is one that is increasing in use in the food industry and has been studied for application in many products including apple puree, orange juice, fruits and vegetables, and even meat products (Landl et al., 2010; Linton, McClements, and Patterson 1999; Oey et al., 2008; Cheftel and Culioli 1997). HPP is the process of subjecting prepackaged (some products treated before packaging process) food products to isostatic pressures ranging from 300-600 megapascals (MPa) (~43,500 to 87,000 psi) or higher (Hugas, Garriga, and Monfort 2002; Oey et al., 2008; Farkas and Hoover 2000) to achieve USDA-FSIS lethality standards. Many studies have been done on the effects of the quality of the food products as well as the effectiveness of the HPP on the inactivation of *E. coli* and other foodborne pathogens, but not very many studies have been done specifically on fermented sausage products.

A few studies have been performed looking at low acid sausage at 300 MPa with ripening (Marcos, Aymerich, and Garriga 2005) and slightly fermented Mediterranean style sausages at 400 MPa followed by ripening (Garriga et al., 2005) but, both of these studies focused primarily on *Listeria monocytogenes* and *Salmonella* and did not include *Escherichia coli* in their investigations. One study, looking at the effects of HPP on microbial load, specifically for lactic acid bacteria, noted that Enterobacteriaceae (including *E. coli*) levels were reduced below the detectable limit of $<1.00 \log \text{ CFU/g}$ ($>3.0 \log$ reduction from control) when subjected to high isostatic pressures of 260 MPa for 390 seconds or 400 MPa for 154 seconds (Alfaia et. Al, 2015). Another study, however, focused on the inactivation of *E. coli* in Genoa Salami at 483 MPa / 600 MPa but the samples were subjected to extended fermentation times (exceeding 900 degree hours) and were dried prior to final HPP testing, with the process samples yielding additional reductions of *E. coli* from 4.7 to $>5.8 \log$ s post HPP (Porto-Fett et al., 2010) so it is hard to tell if the results are comparable to a standard processing procedure.

The long-term goal of this research project is to give a better understanding of how *Escherichia coli* can be controlled within the production process of fermented beef summer sausages and, through the use of HPP as an alternative intervention, improve the quality and safety of fermented sausage products. To achieve this long-term goal two primary objectives were explored within this research project.

1. The first objective was to re-evaluate the effectiveness of the manufacturing process of fermented semi-dry beef summer sausage (as a model sausage) to determine the

levels of log reduction of *E. coli* obtained without thermal processing. Testing was done to see if the manufacturing process “by itself” without the use of a thermal processing (cook) step is adequate in the reduction of *Escherichia coli* species (surrogates) to achieve the lethality regulations of a 5.0 log reduction of *E. coli* species within fermented dry and semi-dry sausage products set forth by the United States Department of Agriculture and Food Safety Inspection Service (USDA 2001a; USDA 2001b). No additional bacteriocins or additives were used and a standard production protocol was followed to best mirror industry practices.

2. The second objective was to evaluate the use of high pressure processing as an alternative intervention to thermal processing (cooking step) to determine if HPP could be utilized as a suitable alternative intervention to thermal processing in achieving the USDA-FSIS lethality requirements of a 5.0 log reduction of *E. coli* in fermented sausage products while also maintaining product quality. Fermented beef summer sausages from the manufacturing process evaluation above (sausage was subjected to stressors of production process) were packaged and subjected to HPP at 586 MPa (14 MPa below the standard of 600 MPa) at various dwell times to determine what the minimum dwell time at 586 MPa would be effective at reducing *Escherichia coli* species in combination with the manufacturing process to achieve the lethality standards for fermented dry and semi-dry sausage products.

Note: HPP processing was performed below the standard of 600 MPa at 586 MPa to ensure that if a manufacturer were to utilize our results that they would be able to continue to use the standard of 600 MPa and still meet the lethality requirements with room for error in processing. If 600 MPa were used for HPP testing then the data would require the manufacturer to always have to meet or exceed 600 MPa to ensure they are exceeding the lethality requirements.

MATERIALS AND METHODS

Bacterial Cultures and Inoculum Preparation

Five strains of Rifampicin (Rif) resistant non-pathogenic STEC were used as a surrogate cocktail for testing the production and HPP as an alternative intervention experiments of fermented beef summer sausage. *Escherichia coli* strains chosen for testing were Rifampicin resistant strains BAA-1427 P1, BAA-1428 P3, BAA-1429 P8, BAA-1430 P14, and BAA-1431 P68 acquired from Dr. Gary Acuff at Texas A&M University Center for Food Safety. All of these cultures were tested by Kansas State University to verify that they did not contain virulence factors with all strains testing negative for factors *eae*, *Stx1* and *Stx2* via Assurance Generic Detection System (GDS). Strains were individually reactivated from -80°C freezer stock (20% glycerol in tryptic soy broth) by aseptically removing one cryo-protectant bead of each strain and placing it in 9 ml of Tryptic Soy Broth (TSB, Becton Dickinson and Company, Sparks, MD, USA) and incubating at 35°C for 24 hours. Inoculum was prepared by transferring 1 ml of reactivated organism to 200 ml of TSB containing 1% dextrose and 0.1 g per liter Rifampicin (Sigma-Aldrich, St. Louis, MO, USA). The Rif stock was prepared by adding 0.1 g Rifampicin (Rif) with 5 ml methanol and then dissolving mixture for 1 minute followed by sterile filtering – Rif

stock solution was added to 200 ml TSB at 0.1 g per liter or ~5 ml of stock solution into 1 liter. Inoculum bottles (1 for each strain) were incubated at ~35°C for 24 hours. After incubation ~10 ml of each culture strain was transferred into a tube to create a cocktail, and then the culture cocktail was centrifuged (Beckman Centrifuge 404543, Beckman Coulter, Pasadena, CA, USA) at 5,514 x g (6,000 rpm) for 15 minutes at 4°C. The supernatant was removed and pellets were suspended in 50 ml of sterile 0.1% peptone water. Each individual strain was then mixed together to create the inoculum added to the sausage batter. Inoculum was made fresh for each production process and used within ~6 hours of preparation.

Preparation of Summer Sausage Batter and Production Samples

Meat, fat, and ingredients were supplied by the Kansas State University (KSU) Meat Lab for production of the beef summer sausage batter. Sausage batter was produced by the KSU Meat Lab to our specifications and provided already ground/blended for our use. Meat for the sausage batter was initially ground (pre-break) at 1/2 inch (Grinder Model 4732, Hobart MRG. Co., Troy, OH) and then blended with the fat and other spices in a mixer blender (Mixer Model I200DA70, Leland Southwest, Fort Worth, TX) for 2 minutes. The beef summer sausage recipe is shown in Table 2.1 below. Once the materials were properly blended they were then subjected to a final grind in the Hobart Grinder to 1/8 inch final grind size.

Premade batter batches of all beef summer sausage at low fat 8% and high fat 17% were provided for use in vacuum sealed packaging (~12.5 lbs. of batter per package). The two fat contents of summer sausage were chosen for testing to give a broad range from low to high fat as sausage products vary in fat content from one producer to another. Batter (both fat levels) were made fresh for each experimental run and were transferred to the BSL II laboratory where they were stored at 2-8°C until inoculation of with the prepared cocktail and stuffing of sausage casings could occur. All batter was used within ~24 hours of preparation for each experiment to ensure the reduced levels of natural bacteria and/or potential contaminants.

Table 2.1: Beef Summer Sausage Recipe

Ingredient	Amount added (lbs.)
Beef	11.600
Pork	8.400
Salt	0.600
Dextrose	0.150
Cane Sugar	0.400
Coarse Black Pepper	0.077
Prague Powder 6.25% Nitrite	0.042
Whole Mustard Seed	0.012
Garlic Powder	0.019
LACTACEL 115 Starter Culture (weight in grams)*	1.90

*LACTACEL 115 contains dextrose fermenting cultures *P. acidilactici*, *S. carnosus* and *P. pentosaceus* (Kerry Group, Beloit, WI, USA).

Preparation of the summer sausage sticks for use in testing occurred in the BSL II Laboratory. Batter was retrieved from storage at 2-8°C and placed into a cooled preparation area/room that was chilled to less than 4°C. Beef summer sausage sticks were prepared by first covering a stainless steel table with butcher paper. Two packages of batter at a given fat (either 8% or 17%) were then selected and weighed to confirm that they were at approximately 25 lbs. combined. Once weight was confirmed/adjusted (no batch exceeded 25 lbs.), the batter was removed aseptically as possible from the packaging and spread onto the butcher paper. Batter was spread evenly across the butcher paper to an approximate thickness of 1/2 inch for more uniform inoculation of organisms later on. Batter was spread across the butcher paper using gloved hands to press the meat flat and help ensure an even thickness.

Once batter was spread evenly then it was inoculated. Two types of summer sausage samples were made for each experimental run, inoculated test samples and un-inoculated control samples. Twenty five lbs. of batter of each fat type were used for each sample type (100 lbs. batter for each experimental run). Sterile deionized water was added to the un-inoculated sample batter so that the moisture adjustment would mimic the addition of inoculum to the inoculated batter. Inoculum or sterile deionized water, depending on sample batter type, was added at a rate of 20 ml per 25 lbs. of batter. Inoculum or deionized water was added in a drop by drop fashion while slowly moving back and forth across the batter to ensure that it was distributed as evenly as possible (Figure 2.1).

After inoculum had been added to the batter it was then hand blended for approximately 5 minutes to create a homogenous mixture of batter and inoculum. To hand blend, sterile gloves were worn to fold the sausage batter over itself followed by pressing flat and smashing the batter back together each time, kneading the batter like dough. This process was repeated multiple times by pulling batter from different edges toward the center to ensure a proper blending of the beef summer sausage batter and the inoculum. Once a proper blend had been achieved then summer sausage sticks were made.

The beef summer sausage batter at this point was transferred to the stuffing press to create summer sausage sample chubs for testing. A simple manual stuffing meat press (Meat Press, Friedr. DICK Corp, Farmingdale, NY) was used. The meat press uses force from cranking a lever to push the batter through a tube and stuffing horn into a non-edible, pre-tied, clear, and fibrous, 65mm sausage casing (Vista International Packaging, LLC, Kenosha, WI, USA - supplied by KSU Meat Lab) after which the casings were twisted to close off the open end. It is very important to note that the casings must be stuffed very tightly with sausage batter. This is to prevent fat from coming to the surface during the fermentation and thermal processing of the sausage and bleeding out. After filling casings with sausage batter to the desired length they were then closed by clipping with an aluminum clip using an air compressed clipper machine (Poly-clip System PCD8026, Poly-clip System LLC, Mundelein, IL) and labeled appropriately as to their fat level (8 or 17%) and sample type (inoculated, un-inoculated). Sausage sticks of each type and fat level were produced to lengths of ~6 inches for manufacturing experimental

samples and ~9 inches for HPP experimental samples to accommodate testing needs (HPP experimental samples will be discussed in later sections).

As the beef summer sausage was stuffed into casings, batter samples were pulled aside for each type and fat level for testing purposes. A total of approximately 40-50 sausage chubs were prepared for each test run consisting of approximately 10+ each of low fat un-inoculated, low fat inoculated, high fat un-inoculated, and high fat inoculated beef summer sausage sticks for use in manufacturing process and HPP testing.

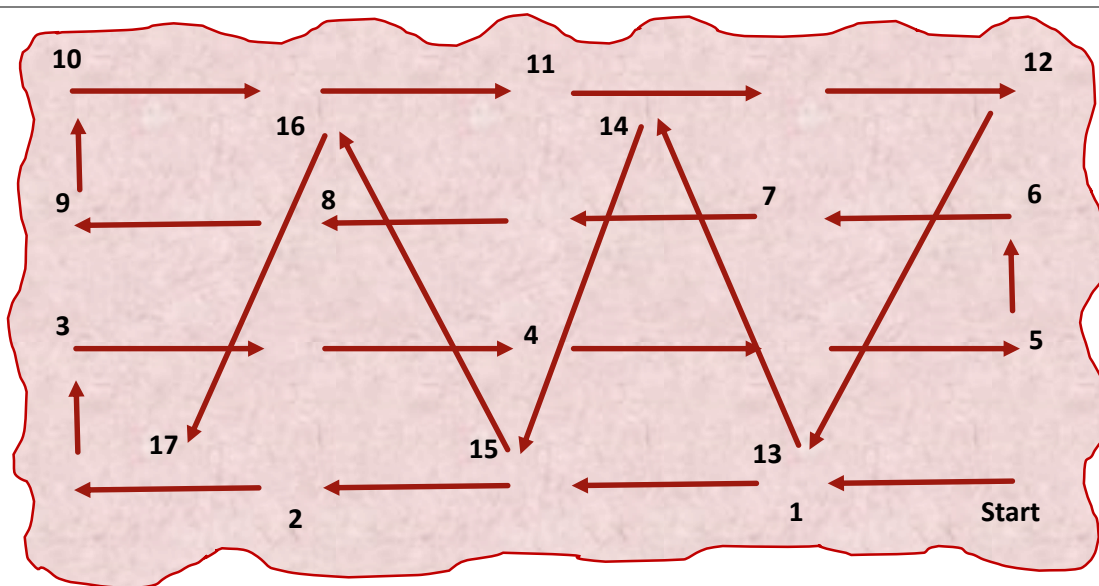


Figure 2.1: Beef Summer Sausage Inoculum Distribution Procedure. Figure displays the inoculation pattern for inoculating fermented beef summer sausage batter with *E. coli* cocktail.

Summer Sausage Processing Protocol

The production protocol used for the fermentation, heating, and chilling of the beef summer sausage was adapted from sausage manufacturing protocol design in Calicioglu et al., 1997 with several changes (Table 2.3). After preparation of production beef summer sausage chubs (8 and 17% fat) they were transported to the smokehouses (Maurer Oven D7752, Maurer-Atmos Middleby GmbH, Reichenau Germany) in closed containers for fermentation and heating procedures. Fermentation was initiated in the smokehouse at 29.4°C with a relative humidity of ~80%. Temperature was slowly ramped to 40.6°C during the first five stages (4-5 hours) in order to mimic a full smokehouse procedure in industry production of beef summer sausages instead of the mostly empty smokehouse that we are working with for testing purposes. After slowly ramping the temperature up, the summer sausage chubs were then held at 40.6°C until the desired pH level of approximately 5.0 was achieved.

During the fermentation step (stage 5) pH readings were taken (after about 9 to 10 hours of fermenting) using a hand held pH meter (Exstick PH100, Extech Instruments, Nashua, NH, USA). This was done to determine when the sausage chubs reached the desired pH level and stage 6 could be started. Samples were prepared for pH readings by removing a ~1 inch piece off the end of a control (un-inoculated) summer sausage chub and then extracting ~10.0 grams from that chub which was to be mixed with 100 ml deionized water in a beaker and blended using a food mixer (Essentials Hand Blender, Sabichi Homewares Limited, Perivale, Middlesex, UK) . The mixture of deionized water and sample were tested for pH by placing the hand held pH meter directly into the beaker.

This process was repeated several times until the desired pH of ~5.0 was achieved marking the end of the fermentation process.

Following fermentation an internal sausage temperature of 54.4°C was obtained by utilizing the following smokehouse stages (6 to 10); 1 h at 48.9°C wet bulb temp (WBT) 40.6°C, 1 h at 54.4°C WBT 45.6°C, 35 min at 60.0°C WBT 56.1°C and continuous at 65.6°C WBT 61.1°C until 54.4°C internal temperature was achieved. After internal temperatures of 54.4°C were achieved the smokehouse protocol was immediately stopped to prevent the product from going through a true thermal processing step. Instead of completing the thermal processing (heating) stage the sausage chubs were forced into the final cold shower stage instead, chill spraying for 15 minutes with cold water (~13°C), cooling the summer sausage chubs to below 48.9°C to prevent any further inactivation of the inoculated organisms and to meet the USDA-FSIS regulations for cooking sausage cooling product to below 48.9°C (Table 2.2). Once the cold shower was completed summer sausage sticks were removed from the smokehouse and placed into a cooler at 2-8°C for additional chilling and storage.

Samples were pulled for manufacturing process evaluation and for evaluating HPP as an alternative intervention to thermal processing at several different stages (at the same time). These stages included: post fermentation, 48.9°C internal temperature, 54.4°C internal temperature, and post chilling (post production). Samples were pulled from the smokehouse for post fermentation (stage 5) testing prior to manually starting stage 6 to

begin the heating treatment. An entire chub was removed from the smokehouse for each type of sample (representing 1 sample) including low fat inoculated, low fat un-inoculated, high fat inoculated, and high fat un-inoculated. This sampling pattern was used for pulling samples at all stages.

As the internal temperature of the beef summer sausage chubs reached 48.9°C (between stages 7 and 8) and 54.4°C (stage 9) the smokehouse schedule was temporarily halted so that samples could be safely removed from the smokehouse ovens. This process did not take more than 2 to 3 minutes each time after which the production schedule was promptly restarted and processing of the sausage resumed. Figure 2.2 on page 89 gives a visual representation for what type of testing samples were pulled for and at which manufacturing stages they were pulled at during the production process.

All samples that were pulled prior to the cold shower were immediately chilled in an ice water bath to rapidly reduce the core temperature of the sausage and stop the inactivation of the inoculated organisms. They were then transferred in closed containers to the laboratory for testing purposes. Beef summer sausage chubs that were pulled for high pressure process testing were transferred to a cooler at < 4.4°C until it was time for processing them for testing. All production samples pulled post cold shower were immediately placed into a cooler at < 4.4°C and held for 6 hours prior to testing the samples – HPP samples taken at this stage also were placed directly into the cooler at < 4.4°C.

Table 2.2: Beef Summer Sausage Smokehouse Protocol

Stage	Time (minutes)	Dry Bulb (°F)	Wet Bulb (°F)	Relative Humidity (%)
1	60	85	80	80
2	60	90	85	81
3	60	95	90	82
4	60	100	95	83
5	NA ^{1,3}	105	99	81
6	60	120	105	60
7	60 ³	130	114	60
8	35	140	133	82
9	NA ^{2,3}	150	142	81
10	15	Cold Shower	N/A	N/A

¹Stage 5 was stopped and manually forced into stage 6 after pH ~5.0 was achieved in product. ²Stage 9 was stopped and manually forced into stage 10 after internal temperature of product reached 54.4°C. ³Smokehouse schedule was interrupted for short periods of time to pull samples at Post Fermentation (pH ~5.0) Stage 5, internal temperature 48.9°C (in-between stages 7 and 8), and internal temperature 54.4°C Stage 9. Schedule was never down for more than 2 to 3 minutes to pull samples and then promptly restarted.

Protocol	All Beef	pH Levels	Heat to 54.4°C (internal Temperature) ¹	Dry Bulb Temp 60.0°C hold time	Dry Bulb Temp 65.6°C hold time	Hold time at 54.4°C (Internal Temperature)	Cold Shower	Chilling of Product	Vacuum Packaged Product	Storage Hold Time
Protocol Used	Yes	~ 5.0	Yes	0.6 hr	Undetermined ²	0 min	15 min	6 hr, < 4°C	No	Production Samples: None ⁴ HPP Samples: Minimum 7 days
Calicioglu	Yes	4.6, 5.0	Yes	1 hr	0.6 hr	0, 30, 60 min ³	15 min	6 hr, < 4°C	Yes	Production Samples: 7 days

Table 2.3: Beef Summer Sausage Production Protocol Comparison between Calicioglu et al., 1997 and Protocol Used for Testing. ¹Fermentation and heating procedures were: Fermentation; 1 hr @ 29.4°C, 1 hr @ 32.2°C, 1 hr @ 35.0°C, 1 hr @ 37.8°C, ~8 hr @ 40.6°C (sausage pushed to heating stage when desired pH attained) for both UNL and Calicioglu protocols. Heating; Oliver Protocol was 1 hr @ 48.9°C, 1 hr @ 54.4°C, .6 hr @ 60.0°C, and product was held @ 65.6°C until internal temperature of 54.4°C was achieved, Calicioglu Protocol was 1 hr @ 48.9°C, 1 hr @ 54.4°C, 1 hr @ 60.0°C, and 0.6 hr @ 65.6°C. ²Dry Bulb temperature at 65.6°C hold time is Undetermined because dry bulb temperature of 65.6°C was maintained until the product reached 54.4°C internal temperature at which point the cycle was stopped and forced into the cold shower stage instead of allowing the stage to run for a specified amount of time. ³Hold times at 54.4°C (internal temperature) were started after product had completed the 0.6 hr @ 65.6°C stage. Product was then pushed into a cold shower stage to reduce product temperature below 48.9°C. ⁴HPP (High Pressure Processing) samples were held for a minimum of 7 days to allow for any possible growth in order to mimic growth levels of Calicioglu et al., 1997 prior to subjecting the samples to HPP and further testing.

Production Sample Testing Procedure

Samples of the beef summer sausage were pulled for testing throughout the manufacturing process to verify the inactivation of *Escherichia coli* strains at the following stages; batter preparation, post fermentation (pH ~5.0), post fermentation internal temperature 48.9°C, post fermentation internal temperature 54.4°C, and post chill (post chill samples received a cold shower for 15 minutes and then were chilled at < 4.4°C for 6 hours prior to testing). A sample processing flow chart can be seen in Figure 2.2 pg. 89. At each testing stage one chub of each type of sample, low fat inoculated, low fat un-inoculated, high fat inoculated, and high fat un-inoculated, was pulled from the smokehouse and prepared for testing purposes. There are two exceptions to this procedure, for batter samples a representative amount of batter was collected for testing instead of a chub prior to the stuffing of the sausage casing and for post chill samples all remaining samples from the smokehouse were pulled and placed into the cooler at < 4.4°C. Testing of all manufacturing samples occurred following the same procedure which is described in Table 2.4.

The production samples testing procedure was as follows. Approximately 25.0 g of each sample type was extracted from the center of the chub and blended (Seward 400 Stomacher Blender, Seward Laboratory Systems Inc., Davie, FL, USA) in a sterile stomacher bag (Fisher Scientific, Fair lawn, NJ, USA). Samples were blended for 1 minute in 225 ml of either 0.1% sterile peptone water (BACTO™, Becton Dickinson and Company, Sparks, MD, USA) at 1 gram per liter for “un-inoculated samples” or 0.1% sterile peptone water containing 0.1 g per liter rifampicin for “inoculated samples”.

Rifampicin was utilized in the diluting solution for the inoculated samples to help control unwanted pathogens and background flora (strains utilized in cocktail are rifampicin resistant) as the beef being used for production was not sterilized or irradiated (natural/normal). It was necessary to break up the sausage sample inside the stomaching bag to facilitate proper mixing during the stomaching process. Samples were then serially diluted in either 0.1% sterile peptone water or 0.1% sterile PEPW + Rif as appropriate and plated on 3M Petrifilm™ (3M, St. Paul, MN, USA).

Inoculated samples were plated for *E. coli* and Coliforms (ECC) and Aerobic Plate Counts (APC) while the un-inoculated samples were plated for ECC, APC, and Enterobacteriaceae (EB). Additionally the inoculated samples were plated on Tryptic Soy Agar (TSA, Becton Dickinson and Company, Sparks, MD, USA) with a Violet Red Bile Agar (VRBA, Becton Dickinson and Company, Sparks, MD, USA) overlay to check for the survival of stressed or damaged cells (VRBA will be added 5-6 hours after initial plating to allow organisms to settle into the TSA). All plates were incubated for approximately 24 hours at ~35°C. Samples were plated in duplicate to verify accuracy of the results and techniques being used.

Table 2.4: Production Sample Testing Procedure Recap

-
1. Extract ~25.0 grams from center of beef summer sausage chub
 - a. Place extracted sausage piece in stomacher bag
 - b. Break up into smaller pieces
 2. Add 225 ml desired fluid
 - a. 0.1% PEPW for un-inoculated samples
 - b. 0.1% PEPW+RIF for inoculated samples
 3. Stomach for 1 minute
 4. Dilute sample (serial dilutions 10^1 - 10^6)
 5. Plate appropriate dilutions
 - a. 3M Pertrifilm™
 - b. TSA plates w/ VRBA overlay
 - c. Plate samples in duplicate
 6. Incubate for approximately 24 hours at ~35°C
 7. Record Results
-

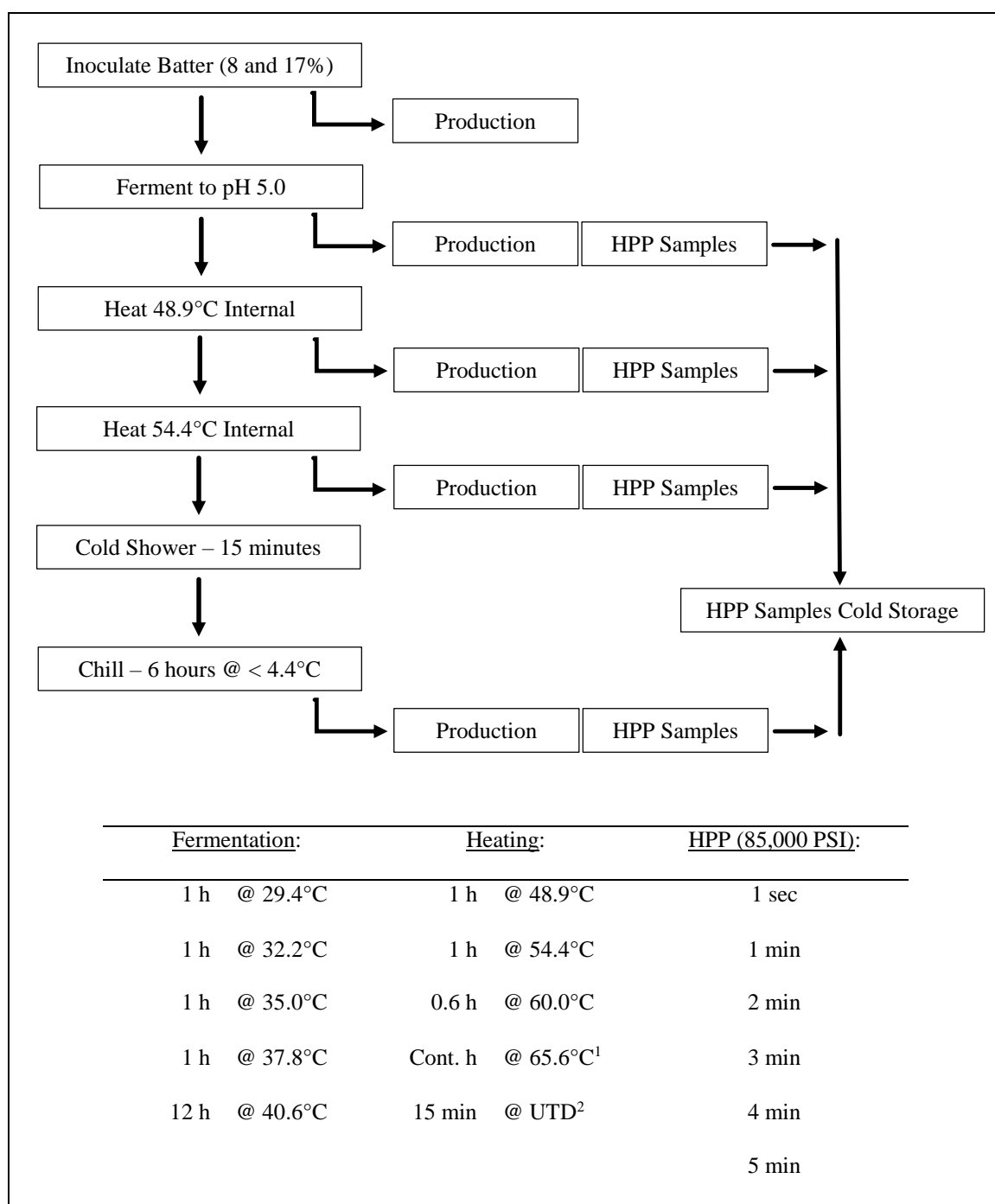


Figure 2.2: Beef Summer Sausage Sample Processing Flow Chart. Flow chart describes the manufacturing process for beef summer sausage and the points at which samples were taken. Additionally the fermentation, heating, and HPP treatments are also displayed.

¹Heating was continuous at 65.6°C until the internal temperature of the sausage reached 54.4°C. ²Product was sprayed with cold water to reduce the internal temperature below 48.9°C to meet USDA-FSIS standards.

High Pressure Processing Sample Preparation Procedure

In addition to evaluating the manufacturing stages of beef summer sausage production for the inactivation of foodborne pathogens, specifically *Escherichia coli*, HPP was also evaluated as an alternative intervention to the thermal processing (heat treatment) hurdle. In order to evaluate high pressure processing effectiveness, as mentioned previously, samples were pulled at the same stages (post fermentation, internal temperature 48.9°C, internal temperature 54.4°C, post chilling) and at the same time for HPP as they were for manufacturing samples (Figure 2.2 above). All high pressure processing samples were stored in a cooler at < 4.4°C until they were subjected to HPP and further testing. Samples of each type were pulled for high pressure processing including high fat inoculated, high fat un-inoculated, low fat inoculated, and low fat un-inoculated and placed into sealed containers to be transported to the University of Nebraska-Lincoln Applied Food Microbiology Laboratory. All products were packaged in plastic bags, tied closed, and placed on ice for transport in electric coolers to ensure the samples maintained < 4.4°C internal temperatures during transport. Upon arrival at UNL the samples were immediately placed into a cooler at < 4.4°C until they could be prepared for high pressure processing procedures.

All samples designated for HPP evaluation testing were held for a minimum of 7 days in the cooler at < 4.4°C before beginning the high pressure process preparation and testing procedures. This was to allow a worst case scenario situation where additional growth may occur prior to processing in a production facility and to allow any pathogenic bacterial cells injured during the manufacturing process to recover prior to being

subjected to another stressor. After a minimum of 7 days the samples, both inoculated and un-inoculated, and both fat levels (8 and 17%) for each stage being tested (post fermentation, internal temperature 48.9°C, internal temperature 54.4°C, and post chilling), were pulled and prepared to receive HPP treatments.

In order to prepare the samples to be treated by high pressure processing they were first cut into smaller pieces. Using a meat slicer (Chef's Choice Meat Slicer, Model 632, CHEFS, Colorado Springs, CO) several 1/8th inch pieces were removed from the beef summer sausage chub and then cut in half length wise creating two 1/8th inch thick half circles that were ~2.5 inches long. Each of these half pieces weighs approximately 10.0 grams so an entire half piece was used for testing.

Following the cutting the summer sausage samples were then triple vacuum packaged in a vacuum package sealer (Multivac A300/16 and C200, Multivac Inc., Kansas City, MO). Individual samples were first placed into the initial vacuum sealable pouch (3 mil STD Barrier, Prime Source / Clarity, Bunzl Processors Division of KOCH Supplies, London, England) and sealed with vacuum to less the 10 mbar (full vacuum). This pouch was then labeled with the replication number, sample type (inoculated, un-inoculated), the fat level (8 or 17%), and the dwell time for testing. Then the pouch was folded and placed into a second pouch that was then vacuum sealed to ~100 mbar. Finally the pouch was folded again and placed into a third vacuum pouch and vacuum sealed to ~100 mbar. At each time of sealing, the pouch was placed in the bottom of the previous vacuum pouch to reduce free space. This procedure was utilized to reduce the risk of the pouches leaking

during the high pressure process procedure. After vacuum sealing the beef summer sausage samples were placed back into the cooler at $< 4.4^{\circ}\text{C}$ until they could be transported to the appropriate location for high pressure processing treatments.

High Pressure Processing Treatments

Samples that had been properly cut, vacuumed, and sealed were then transported to the appropriate facility and subjected to high pressure processing treatments. Preliminary testing was performed on a HPP machine (HPP machine, FPG9400:922, Stansted Fluid Power, Harlow, Essex, UK) at the University of Nebraska-Lincoln (UNL) Food Processing Center. Based on results from the preliminary testing it was determined that commercial HPP would provide data more representative of a high pressure processing facility. Samples were transported on ice (or in electric coolers) to a commercial HPP facility in order to keep the temperature below 4.4°C . The commercial HPP facility utilized for testing is a 600,000 sq. ft. USDA/FDA approved facility that provides cold storage for manufacturers and performs high pressure processing on many products such as; pre-cooked whole meats, guacamole, salsas, dips and spreads, condiments, and pet food to name a few. It also is located approximately 6 miles from the laboratory at UNL reducing the risk of contamination and microbial growth during sample transport.

All types of samples (inoculated, un-inoculated) at both fat levels (8 and 17%), which were prepared previously in vacuum sealed pouches, were subjected to HPP treatments at 586 MPa (85,000 psi) for dwell times of 1 second, 1, 2, 3, 4 and 5 minutes in the high pressure processing machine (HPP machine, AV30, AVURE Technologies, Middleton,

OH). Additionally a control sample also was packaged that did not receive HPP treatment but was transported to the HPP facility so that the samples would be subjected to the same amount of stress as the samples receiving high pressure treatments. It is also important to note that the dwell time of 1 second is not necessarily 1 second due to the fact that the machine can take up to 3 seconds before switching off after reaching 85,000 psi. In light of this, the 1 second sample is considered representative of the come-up time of the machine to reach 85,000 psi and the data will be interpreted in that manner.

All samples being treated at the high pressure processing facility were kept at $< 4.4^{\circ}\text{C}$ while waiting to receive treatment (processing floor in the facility is kept below 4.4°C). The HPP machines utilize water that was run at water temperatures of $\sim 7^{\circ}\text{C}$ (not chilled) for the purpose of this experiment. Samples were only at temperatures $> 4.4^{\circ}\text{C}$ for the amount of time it took to run each treatment – see dwell times above. Once samples were finished being treated with HPP then they were placed back into the coolers and transported back to UNL where they were placed into a cooler at $< 4.4^{\circ}\text{C}$ until they could be evaluated/tested.

High Pressure Processing Sample Testing Procedure

After the beef summer sausage samples had been subjected to HPP treatments at the various dwell times then they were tested for the survival of pathogens. A very similar process was used to evaluate the HPP samples compared to the testing procedure for the production samples listed above in section “Production Sample Testing Procedure” and in Table 2.4 above. Since the two procedures are similar, and all of the details for testing

can be found in the mentioned section, then only the differences between the two procedures are given in the following paragraph as well as in Table 2.5 below.

HPP samples (entire sample ~10.0 g) were, aseptically as possible, removed from the vacuum sealed pouches and transferred to stomaching bags. Samples were transferred, stomached, serial diluted (1/10 dilutions in either 0.1% sterile peptone water or 0.1% sterile PEPW + Rif as appropriate), and plated in duplicate appropriately following the previously described procedure. However, instead of using 225 ml of stomaching solution, only 90 ml of the appropriate stomaching solution, depending on the sample type being either inoculated or un-inoculated, was utilized since the sample size was only 10 g instead of 25 grams. This sample size adjustment maintained the ratio of sample to dilution media used in the previous testing. Samples were plated as previously on 3M Petrifilm™ however, inoculated samples for this test will were not plated on TSA with VRBA overlay as it is not necessary. All plates were incubated for 24-48 hours at ~35°C. Samples were plated in duplicate to verify accuracy of the results and techniques being used. All plates were enumerated and resulting data was recorded appropriately.

Additionally the remaining fluid in the stomaching bag for each summer sausage HPP dwell time sample was utilized to perform enrichment testing. The remaining fluid and the sample were kept in the stomaching bag and the bag was incubated at ~35°C for 18-24 hours to attempt to grow any surviving bacteria that may be in solution but that are below the detectable limit. After incubation, the enrichment samples were streaked onto MacConkey Agar containing rifampicin added at 0.1 g per liter. The MacConkey plates

containing the streaks of each HPP sample were incubated at ~35°C for 18-24 hours and then checked to verify growth. Plates were evaluated on a Positive/Negative basis and then were compared to the results obtained from the 3M Petrifilm™ plates. All data was recorded appropriately.

Table 2.5: Beef Summer Sausage HPP Sample Testing Recap

1.	Aseptically remove entire (~10.0 g) sample from vacuum pouch
a.	Place sample into a stomacher bag
2.	Add 90 ml of desired sterile fluid
a.	0.1% PEPW for un-inoculated samples
b.	0.1% PEPW+RIF for inoculated samples
3.	Stomach for 1 minute
4.	Dilute sample (serial dilutions 10^1 - 10^6)
5.	Plate appropriate dilutions
a.	3M Petrifilm™
b.	Plate samples in duplicate
c.	Save stomaching bag + fluid for enrichment
6.	Incubate at ~35°C
a.	3M Petrifilm™ (42-48 h)
b.	Enrichment samples (18-24 h)
7.	Plate enrichment samples
a.	Incubate for 18-24 hours @ ~35°C
8.	Record Results
a.	Petrifilm results
b.	Enrichment results

Chemical Analysis of Beef Summer Sausage

Along with evaluating the inactivation of *Escherichia coli* during the described manufacturing stages and at various dwell times for high pressure processing at 586 mPa (85,000 psi), samples were taken for chemical analysis (pH, water activity and % acidity), spring form testing, and proximate analysis (Fat, Moisture, Protein, Salt, Ash).

The portions of samples used for spring form and proximate analysis testing were sent to the Department of Animal Sciences, Meat Science Laboratory at the University of Nebraska-Lincoln for processing. All samples sent for testing or used for chemical analysis verification were extracted from un-inoculated beef summer sausage chubs that were produced during the manufacturing process at Kansas State University and that had been stored in a cooler at $< 4.4^{\circ}\text{C}$.

Water activity was performed in the laboratory using a water activity (a_w) meter (AQUA Lab 3TE a_w Meter, AQUA Lab, Pullman, WA, USA) for all sausage sample readings. Samples were kept in sealed pouches in the refrigerator until just before readings to reduce the amount of evaporation. A small amount of meat (enough to cover bottom of water activity dish) was transferred to the water activity meter dish and sample was then placed into the meter to get result. The pH of the sausage was obtained using a pH meter (Accumet™ AB15, BASIC pH Meter, Fisher Scientific, Waltham, MA, USA) and a gel-filled polymer body single junction combination pH electrode (Accumet™ 3-620-108A, pH Combination Electrode, Fisher Scientific, Waltham, MA, USA) utilizing the following procedure. Values for pH were obtained by adding 10 grams of beef summer sausage into a stomaching bag with 100 ml of deionized water and blending/stomaching for 1 minute. A small portion of this solution (~5 ml) was then transferred to a sample cup and the readings for pH were taken.

RESULTS, DISCUSSION, AND CONCLUSION

Introduction

The following sections contain the bacterial results obtained during the manufacturing process of beef summer sausage as well as the results from the use of high pressure processing (HPP). First, we evaluated the adequacy of the manufacturing process itself in the destruction of *E. coli* species to determine if the manufacturing process was capable of achieving the United States Department of Agriculture Food Safety Inspection Service (USDA-FSIS) requirements of a 5.0 log reduction of *E. coli* and other foodborne pathogens (USDA 2001b). Then, we evaluated the effectiveness of HPP as an alternative to thermal processing for lethality within the manufacturing of fermented beef summer sausages (as a model sausage) to determine if it is a suitable alternative intervention to thermal processing and can be used in place of thermal processing to still meet the USDA-FSIS requirements mentioned above.

Fermented Beef Summer Sausage Manufacturing Process Results

Low (8%) and high fat (17%) fermented beef summer sausages were evaluated for the destruction of *E. coli* species at five different stages throughout manufacturing including: batter (BA), post fermentation ≤ 5.0 pH (PF), internal temperature 48.9°C (I-48.9°C), internal temperature 54.4°C (I-54.4°C), and post chilling (PC) (Figure 2.2).

Initial average aerobic plate counts (APC) of 7.3 (low fat) and 7.5 (high fat) log CFU/g were obtained in the batter samples (Table 2.6, Figure 2.3). Reductions of *E. coli* of 1.2,

1.3, 3.1 and 4.2 log CFU/g, and 1.3, 1.8, 3.5 and 4.5 log CFU/g were obtained subsequent to PF, I-48.9°C, I-54.4°C and PC, in low fat (8%) and high fat (17%) summer sausages, respectively (Table 2.7, Figure 2.4). The pH of fermented beef summer sausage samples were ≤ 5.0 at time of testing. *E. coli* coliform (ECC) testing also was performed. Initial average ECC counts for *E. coli* populations of 7.0 (low fat) and 7.2 (high fat) log CFU/g were obtained in the batter samples (Table 2.6, Figure 2.3). Reductions of *E. coli* of 1.0, 1.3, 3.0, and 3.8 log CFU/g, and 1.2, 1.8, 3.6, and 3.9 log CFU/g were obtained subsequent to PF, I-48.9°C, I-54.4°C and PC, in low fat (8%) and high fat (17%) summer sausages, respectively (Table 2.7, Figure 2.4).

Recovery of injured cells was performed using tryptic soy agar with violet red bile agar overlay (TSA VRBA Overlay). Fermented beef summer sausage samples were tested yielding initial *E. coli* populations of 8.0 and 8.2 log CFU/g in the batter for low (8%) and high fat (17%) samples respectively (Table 2.6, Figure 2.3). Reductions of *E. coli* of 1.6, 1.3, 3.5, and 3.7 log CFU/g, and 1.6, 2.2, 3.6, and 3.6 log CFU/g were obtained subsequent to PF, I-48.9°C, I-54.4°C and PC, in low fat (8%) and high fat (17%) summer sausages, respectively (Table 2.7, Figure 2.4). APC and TSA VRBA Overlay results for counts and/or log reductions were comparable (Figure 2.5, Figure 2.6). Additionally, APC and ECC results for counts and/or log reductions were also similar (Figure 2.7, Figure 2.8).

Utilizing Statistical Analysis System (SAS) software (SAS Institute Inc., Cary, NC, USA), it was determined using analysis of variance of the General Linear Model

procedure that fat content (low or high fat) of the fermented beef summer sausage did not affect ($p>0.05$) the *E. coli* reductions during beef summer sausage manufacture (Table 2.8). Fishers' Least Significant difference ($\alpha=0.05$) was used to separate means of each treatment.

Evaluation of High Pressure Processing Results

High pressure processing was performed, utilizing a commercial scale HPP machine, in place of thermal processing for the manufacturing process of fermented beef summer sausage at 586 MPa at dwell times of control (no HPP), 1 second, 1, 2, 3, 4, and 5 minutes for manufacturing stages PF, I-48.9°C, I-54.4°C and PC, in low fat (8%) and high fat (17%) summer sausages.

Initial average APC for PF samples of 6.1 (low fat) and 6.0 (high fat) log CFU/g were obtained in the control samples (Table 2.9, Figure 2.9, Figure 2.10, and Figure 2.14). Reductions of *E. coli* of 0.9, 2.0, 2.2, 2.3, 3.5 and 4.2 log CFU/g, and 1.1, 2.4, 3.0, 2.8, 4.2, and 4.9 log CFU/g were obtained subsequent to 1 sec, 1, 2, 3, 4, and 5 minutes of dwell time, in low fat (8%) and high fat (17%) summer sausages, respectively (Table 2.10, Figure 2.15, Figure 2.16, and Figure 2.20).

Initial average APC for I-48.9°C samples of 5.7 (low fat) and 5.5 (high fat) log CFU/g were obtained in the control samples (Table 2.9, Figure 2.9, Figure 2.11, and 2.16). Reductions of *E. coli* of 1.6, 2.5, 2.5, 3.9, 4.3, and 4.4 log CFU/g, and 1.5, 2.9, 3.7, 4.1, 5.0, and ≥ 5.5 log CFU/g were obtained subsequent to 1 sec, 1, 2, 3, 4, and 5 minutes of

dwelt time, in low fat (8%) and high fat (17%) summer sausages, respectively (Table 2.10, Figure 2.15, Figure 2.17, and Figure 2.20).

Initial average APC for I-54.4°C samples of 4.3 (low fat) and 4.3 (high fat) log CFU/g were obtained in the control samples (Table 2.9, Figure 2.9, Figure 2.12, and 2.16).

Reductions of *E. coli* of 3.5, 4.1, 4.1, and ≥ 4.3 log CFU/g, and 3.8 and ≥ 4.3 log CFU/g were obtained subsequent to 1 sec, 1, 2, and 3 minutes of dwelt time in low fat (8%), and 1 second and 1 minute of dwelt time in high fat (17%) summer sausages, respectively (Table 2.10, Figure 2.15, Figure 2.18, and Figure 2.20).

Initial average APC for PC samples of 3.4 (low fat) and 4.1 (high fat) log CFU/g were obtained in the control samples (Table 2.9, Figure 2.9, Figure 2.13, and Figure 2.14).

Reductions of *E. coli* of 2.6, 3.2 and ≥ 3.4 log CFU/g, and 3.8 and ≥ 4.1 log CFU/g were obtained subsequent to 1 sec, 1, and 2 minutes of dwelt time in low fat (8%), and 1 second and 1 minute of dwelt time in high fat (17%) summer sausages, respectively (Table 2.10, Figure 2.15, Figure 2.19, and Figure 2.20).

Initial average ECC counts for PF samples of 5.9 (low fat) and 5.9 (high fat) log CFU/g were obtained in the control samples (Table 2.11, Figure 2.9, and Figure 2.10).

Reductions of *E. coli* of 2.8 and ≥ 5.9 log CFU/g, and 3.6 and ≥ 5.9 log CFU/g were obtained subsequent to 1 second and 1 minute of dwelt time, in low fat (8%) and high fat (17%) summer sausages, respectively (Table 2.12, Figure 2.15, and Figure 2.16).

Initial average ECC counts for I-48.9°C samples of 5.3 (low fat) and 5.1 (high fat) log CFU/g were obtained in the control samples (Table 2.11, Figure 2.9, and Figure 2.11). Reductions of *E. coli* of 3.9 and ≥ 5.3 log CFU/g, and 4.3 and ≥ 5.1 log CFU/g were obtained subsequent to 1 second and 1 minute of dwell time, in low fat (8%) and high fat (17%) summer sausages, respectively (Table 2.12, Figure 2.15, and Figure 2.17).

Initial average ECC counts for I-54.4°C samples of 3.3 (low fat) and 3.4 (high fat) log CFU/g were obtained in the control samples (Table 2.11, Figure 2.9, and Figure 2.12). Reductions of *E. coli* of ≥ 3.3 and ≥ 3.4 log CFU/g were obtained subsequent to 1 second of dwell time, in low fat (8%) and high fat (17%) summer sausages, respectively (Table 2.12, Figure 2.15, and Figure 2.18).

Initial average ECC counts for PC samples of 2.5 (low fat) and 2.7 (high fat) log CFU/g were obtained in the control samples (Table 2.11, Figure 2.9, and Figure 2.13). Reductions of *E. coli* of ≥ 2.5 and ≥ 2.7 log CFU/g were obtained subsequent to 1 second of dwell time, in low fat (8%) and high fat (17%) summer sausages, respectively (Table 2.12, Figure 2.15, and Figure 2.19).

Enterobacteriaceae (EB) species populations were evaluated for fermented beef summer sausage for HPP at 586 MPa at dwell times of control (no HPP), 1 second, 1, 2, 3, 4, and 5 minutes for manufacturing stages PF, I-48.9°C, I-54.4°C and PC, in low fat (8%) and high fat (17%) summer sausages. EB plate counts were below the detectable limit for initial population in the all control samples except for high fat post fermentation No HPP

Control samples. These results showed an average of 0.47 log CFU/g EB count (for the high fat post fermentation control samples only). No EB counts were recovered from any other control samples or from samples that received HPP treatments (Figure 2.21).

Discussion

Escherichia coli is a very difficult organism to control, surviving throughout the manufacturing process, and causing major problems for food manufacturers. Many “hurdles” or interventions are in place throughout the production process of fermented beef summer sausages to help ensure the safety of these products from potentially deadly foodborne pathogens. These include temperature control, water activity, pH adjustment \leq 5.0, and the addition of competing cultures or salts, etc. (Incze 1998; Leroy, Verluyten, and De Vuyst 2006; USDA 1999a; Garriga et al., 2005; Comi et al., 2005). However, *E. coli* has the ability to survive these interventions which is potentially due to many factors.

E. coli has been known to survive in low pH (acidic) environments for periods of time due to its ability to regulate pH within its cells. One study showed that the internal pH of a specific *E. coli* strain (MC4100) and *Shigella flexneria* (3136) were maintained at 5.8 and 5.6, respectively, when the pH of the growth environment was only at 3.3 (Δ pH of 2.5 and 2.3, respectively). This was due to *rpoS* expression within the microorganisms when subjected to high acid concentrations for two hours at 25°C. Despite the acidic environment both the *E. coli* and *Shigella* strains maintained a survival rate of 100% (Small et al., 1994). This is a major problem for summer sausage manufacturers as pH adjustment by either acid addition or from fermentation via starter cultures is one of the

major interventions used in the sausage production industry for foodborne pathogen control. Another study showed when pH is adjusted via fermentation to pH levels of ≤ 5.0 using a probiotic lactic acid starter culture (*Lactobacillus rhamnosus* GG, *Lactobacillus rhamnosus* LC-705, *Lactobacillus rhamnosus* E-97800, and *Pediococcus pentosaceus* control (Condi Rasant)) that *E. coli* O157:H7 was only reduced by up to 3 log CFU/g (Erkkilä et al., 2000), potentially due to its ability to survive in acidic environments and the lower temperatures used during fermentation stages typically $\leq 43.3^{\circ}\text{C}$.

As mentioned previously, *E. coli* possess acid tolerance due to *rpoS* expression within their cells. Additionally they also have limited tolerance in higher NaCl^- (salt) environments via the *rpoS* gene allowing them to increase their halo-tolerance and not be destroyed by bursting. One study showed that in *E. coli* strains with a mutated *rpoS* gene, which was altered to be less effective, reduced the levels of acid tolerance and salt tolerance significantly resulting in additional reductions of 2.3 log CFU/g after 21 days of testing in dry fermented sausages (Cheville et al., 1996).

Temperature control is also important throughout the manufacturing process of fermented sausage products, beginning with the temperature of the raw materials, temperature of the processing environment, and the storage of the sausage post production. Literature shows that *E. coli* species in fermented sausages such as soudjouk style sausage, pepperoni, and salami have the ability to survive at cold temperatures during storage ranging from -20 to 4°C (Calicioglu et al., 2002; Faith et al., 1997; Faith et al., 1998) especially if other

interventions are not appropriately utilized (Calicioglu et al., 2002). One study showed that in soudjouk style sausage greater log reductions of *E. coli* were seen post fermentation (no thermal processing) in product that was stored at ambient temperatures of 25°C for 21 days than in sausage stored at 4 or 15°C for 21 days (Calicioglu et al., 2002). These data suggest that other interventions, in addition to low pH and temperature control, are necessary to control foodborne pathogens even if products are stored in freezer or refrigerated conditions.

There have been several studies performed looking at the viability of *E. coli* species in fermented sausages. In a study similar to the one described in this thesis, looking at the viability of *Escherichia coli* O157:H7 in beef summer sausages throughout the manufacturing process, it was shown that after fermenting the product to a pH of 5.0 and then heating the product to an internal temperature of 54.4°C that *E. coli* was reduced by 3.2 log CFU/g (Calicioglu et al., 1997). In comparison, under the same conditions, this study achieved log reductions of 3.1 and 3.5 (low fat) and 3.0 and 3.6 (high fat) log CFU/g for *E. coli*. These data suggest that the inactivation of *E. coli* species is consistent when subjected to the same processing parameters of pH adjustment and heating to an internal temperature of 54.4°C.

Another study also showed that total reductions of up to 4.4 log CFU/g of *E. coli* could be obtained post fermentation and drying (no thermal processing step) in soudjouk style fermented sausages that had been fermented to pH between 5.2 and 4.6 and then dried at 22°C to α_w of 0.92 to 0.86 (Hwang et al., 2009). A similar study performed by Porto-Fett

et al., 2008 also showed that the production process for fermented soudjouk style sausages with fermentation to pH between 5.3 and 4.8 and storage between 4 to 21°C, could obtain total reductions of *E. coli* up to 4.28 log CFU/g (Porto-Fett et al., 2008).

Based on the data from the studies above, the manufacturing processes for fermented dry and semi-dry sausages are not adequate to achieve the federal requirements for a 5.0 log reduction of foodborne pathogens in the United States on their own (USDA 2001b). In contrast, it was reported by Hinkens et al., 1996 that, in pepperoni, heating the sausage to an internal temperature of 53.3°C for 60 minutes was sufficient to obtain 5-6 log reduction of *Escherichia coli* O157:H7 without damaging the quality of the product (Hinkens et al., 1996; Calicioglu et al., 1997). Therefore, additional interventions such as thermal processing or high pressure processing would be necessary to achieve the requirement and ensure the safety of the fermented dry or semi-dry sausage regardless of sausage style or type.

High pressure processing has been evaluated in a few studies as an additional or alternative intervention for the control and reduction of *Escherichia coli* O157:H7 and the other STEC in beef and beef products such as fermented sausages. One study showed that *E. coli* O157:H7 and the other STEC can be controlled and react similarly under high pressure conditions when it is used as an additional intervention post production at isostatic pressures between 600 and 486 MPa for 1 to 12 minutes giving reductions of 4.7 to >5.8 log CFU/g (Porto-Fett et al., 2010). Even at lower pressures, which could be used to help preserve the quality of the beef products, it has been shown in ground beef at 400

MPa at 17°C that reductions of >2.0 log CFU/g of *E. coli* serogroups are achieved with the highest level of reductions in 90/10 ground beef at 4.31 log CFU/g (Jiang et al., 2015). Another study looking at lower pressures for the control of *Escherichia coli* serogroups showed that at pressures of 350 MPa for 30 minutes that *E. coli* O157:H7 and six STEC (O145:NM, O26:H11, O45:H2, O103:H2, O111:NM, O121:H19, O145) were reduced by 3.2 and 4.7 log CFU/g, respectively (Hsu et al., 2015).

Based on the results from literature and those obtained in this thesis, it can be stated that high pressure processing can be utilized as an alternative intervention to thermal processing in the production of fermented beef summer sausage with additional log reductions adequate to meet the USDA-FSIS federal safety guidelines of a 5.0 log reduction of *E. coli* in fermented meat products (USDA 2001b). When reviewing the data obtained from this study, looking at the most probable stages of the manufacturing process in which HPP might be applied starting with 54.4°C, HPP gave additional APC and *E. coli* log reductions of 3.5 and 3.8 log CFU/g at just 1 second of dwell time at 586 MPa in low fat (8%) and high fat (17%), respectively. These additional reductions when combined with the *E. coli* log reductions obtained from the manufacturing process yielded total *E. coli* log reductions of 6.6 and 7.3 log CFU/g for low fat (8%) and high fat (17%), respectively exceeding the 5.0 log CFU/g requirement.

Additionally, when reviewing the PC stage HPP gave additional APC and *E. coli* log reductions of 2.6 and 3.8 log CFU/g at just 1 second of dwell time at 586 MPa in low fat (8%) and high fat (17%), respectively. These additional reductions when combined with

the *E. coli* log reductions obtained from the manufacturing process yielded total *E. coli* log reductions of 6.8 and >7.4 log CFU/g for low fat (8%) and high fat (17%), respectively, also exceeding the 5.0 log CFU/g requirement. It is also important to note that total *E. coli* reductions were achieved within the beef summer sausage for product sampled at 54.4°C after 3 minutes in low fat and 1 minute in high fat, and for product tested PC after 2 minutes for low fat and 1 minute for high fat products.

Although the most probable stages for utilizing HPP as an alternative intervention are post internal temperature of 54.4°C and post chilling, HPP could potentially be utilized at earlier stages within the manufacturing process to achieve reduced pathogen numbers or potentially even achieve lethality requirements. At the PF stage, total log reductions of *E. coli* of 5.39 and 5.45 log CFU/g in low fat (8%) and high fat (17%) samples were achieved after 5 and 4 minutes of HPP at 586 MPa, respectively. During evaluation of product post internal temperature of 48.9°C total log reductions of *E. coli* of 5.16 and 5.49 log CFU/g in low fat (8%) and high fat (17%) samples were achieved after 3 and 2 minutes of HPP at 586 MPa respectively. Although the use of HPP at the PF and I-48.9°C stages would not necessarily occur, it is worth noting that it could be used if desired to help control foodborne pathogens like *Escherichia coli* at these important processing stages. However, the most logical stage in which HPP would be most effective would be post chilling as it would provide a final control intervention post production and handling of the sausage that is effective for controlling *E. coli* species and ensuring food safety.

Conclusions

In conclusion, it has been determined that the fermented beef summer sausage manufacturing process is not adequate, on its own, to meet the performance standards without additional interventions or lethality treatments such as thermal processing or HPP for the rifampicin resistant non-pathogenic *E. coli* species. The current beef summer sausage manufacturing process interventions such as pH, water activity, and temperature control, do cause inactivation of the rifampicin resistant non-pathogenic *E. coli* species throughout manufacturing of fermented beef summer sausages but they do not meet the 5.0 log CFU/g reduction regulation of *Escherichia coli* in fermented dry or semi-dry meat products put in place by the USDA-FSIS (USDA 2001a; USDA 2001b).

Application of a thermal processing step or alternately, a non-thermal processing intervention such as HPP, subsequent to processing, can meet the performance standard for rifampicin resistant non-pathogenic *E. coli*, and potentially for *E. coli* O157:H7, assuring the safety of fermented beef summer sausages. HPP has shown to be very effective in the inactivation of *Escherichia coli* species when utilized as an alternative intervention to thermal processing, especially when utilized at the post internal temperature of 54.4°C and post chilling stages of production. Therefore, HPP can be utilized as an alternative intervention to thermal processing subsequent to fermentation to achieve the required 5.0 log reduction of *E. coli* (surrogates) for the production of fermented beef summer sausages (of different fat levels) by manufacturers and aide in the safety of such products.

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Tables of Results for Manufacturing and HPP Processes

	APC				ECC				TSA VRBA Overlay			
	Low Fat		High Fat		Low Fat		High Fat		Low Fat		High Fat	
	Count	STDEV	Count	STDEV	Count	STDEV	Count	STDEV	Count	STDEV	Count	STDEV
Batter	7.32	0.47	7.52	0.20	6.97	0.49	7.17	0.26	8.02	0.16	8.15	0.33
PF	6.17	0.22	6.23	0.52	6.01	0.23	6.01	0.61	6.46	0.64	6.60	0.28
I48.9°C	6.02	0.10	5.77	0.37	5.64	0.21	5.39	0.51	6.74	0.96	6.00	0.53
I54.4°C	4.18	0.72	4.00	0.96	3.95	0.40	3.58	0.70	4.54	0.24	4.54	0.42
PC	3.13	0.72	2.93	2.30	3.17	0.41	3.29	0.94	4.33	0.15	4.61	0.95

Table 2.6: Average Counts CFU/g for Manufacturing of Fermented Beef Summer Sausages. Table contains the average aerobic plate counts (APC), *E. coli* coliform plate counts (ECC), and tryptic soy agar with a violet red bile agar overlay (TSA VRBA Overlay) counts from testing fermented beef summer sausage at five different stages of the manufacturing process. Processing stages tested were: batter, post fermentation (PF), internal temperature of 48.9°C (I48.9°C), internal temperature 54.4°C (I54.4°C), and post chilling (PC) of the sausage. Both low fat (8%) and high fat (17%) results are displayed. All counts are average counts displayed in log CFU/g with standard deviations (STDEV).

	APC				ECC				TSA VRBA Overlay			
	Low Fat		High Fat		Low Fat		High Fat		Low Fat		High Fat	
	Count	STDEV	Count	STDEV	Count	STDEV	Count	STDEV	Count	STDEV	Count	STDEV
PF	1.16	0.56	1.29	0.35	0.96	0.42	1.16	0.41	1.56	0.57	1.55	0.45
I48.9°C	1.31	0.40	1.76	0.26	1.33	0.32	1.78	0.34	1.29	0.93	2.15	0.45
I54.4°C	3.14	0.28	3.52	1.11	3.02	0.50	3.59	0.90	3.48	0.27	3.61	0.67
PC	4.19	0.25	4.51	2.49	3.80	0.55	3.88	1.07	3.69	0.31	3.55	1.12

Table 2.7: Average Log Reduction CFU/g for Manufacturing of Fermented Beef Summer Sausages. Table contains the average log reductions for aerobic plate counts (APC), *E. coli* coliform plate counts (ECC), and tryptic soy agar with a violet red bile agar overlay (TSA VRBA Overlay) counts obtained from testing fermented beef summer sausage at four different stages of the manufacturing process. Processing stages are: post fermentation (PF), internal temperature of 48.9°C (I48.9°C), internal temperature 54.4°C (I54.4°C), and post chilling (PC) of the sausage. Both low fat (8%) and high fat (17%) results are displayed. All counts are average log reduction counts displayed in log CFU/g with standard deviations (STDEV).

	Prod	HPP-0	HPP-1s	HPP-1m	HPP-2m	HPP-3m	HPP-4m	HPP-5m
High Fat	PF	6.2300 ^A	6.0333 ^A	4.9200 ^{ABCDE}	3.6733 ^{FGH}	2.9967 ^{FGHI}	3.2333 ^{FGHI}	1.8700 ^{JKLM}
Low Fat	PF	6.1667 ^A	6.0733 ^A	5.1900 ^{ABCD}	4.0567 ^{DEFGH}	3.8300 ^{DEFGH}	3.7533 ^{DEFGH}	2.6133 ^{HIJK}
High Fat	I48.9°C	5.7667 ^{AB}	5.5167 ^{ABC}	4.0433 ^{DEFGH}	2.6300 ^{GHIJK}	1.7900 ^{JKLM}	1.3900 ^{JKLMN}	0.5667 ^{LMN}
Low Fat	I48.9°C	6.0167 ^A	5.6767 ^{AB}	4.0733 ^{CDEFG}	3.1767 ^{FGHI}	3.1667 ^{FGHI}	1.8300 ^{JKLM}	1.3000 ^{JKLMN}
High Fat	I54.4°C	4.0000 ^{DEFGH}	4.3267 ^{BCDEF}	0.4933 ^{LMN}	-4.44E-16 ^N	0.0000 ^N	0.0000 ^N	0.0000 ^N
Low Fat	I54.4°C	4.1800 ^{CDEF}	4.3367 ^{BCDEF}	0.8467 ^{LMN}	0.2333 ^N	0.2333 ^N	-8.88E-16 ^N	-1.33E-15 ^N
High Fat	PC	2.9300 ^{FGHIJ}	4.1367 ^{CDEF}	0.3933 ^{MN}	-1.33E-15 ^N	0.0000 ^N	-4.44E-16 ^N	-4.44E-16 ^N
Low Fat	PC	3.1333 ^{FGHI}	3.3967 ^{FGH}	0.7667 ^{LMN}	0.2333 ^N	-2.22E-15 ^N	-1.78E-15 ^N	-1.78E-15 ^N

Table 2.8: Comparison of Low Fat and High Fat Fermented Beef Summer Sausage By General Linear Model. Table contains the least squares means (LSM) for aerobic plate counts (APC) obtained from testing fermented beef summer sausage at four different stages of the manufacturing process. Processing stages are: post fermentation (PF), internal temperature of 48.9°C (I48.9°C), internal temperature 54.4°C (I54.4°C), and post chilling (PC) of the sausage. The low fat (8%) and high fat (17%) results are both displayed. Superscripted letters denote the similarity between the different fat contents and the significance between different counts obtained in fat levels at different stages. Significance of the differences are calculated at the P>0.05 level.

HPP	Post Fermentation						148.9°C						154.4°C						Post Chill					
	Low Fat			High Fat			Low Fat			High Fat			Low Fat			High Fat			Low Fat			High Fat		
	Count	STDEV		Count	STDEV		Count	STDEV		Count	STDEV		Count	STDEV		Count	STDEV		Count	STDEV		Count	STDEV	
Cont	6.07	0.04		6.03	0.55		5.68	0.07		5.52	0.59		4.33	0.30		4.33	0.33		3.40	0.64		4.14	0.83	
1 se	5.19	0.62		4.92	0.16		4.07	1.14		4.04	0.33		0.85	0.78		0.85	0.49		0.77	0.68		0.39	0.68	
1 mi	4.06	1.83		3.68	1.50		3.18	1.03		2.63	0.92		0.23	0.40		0.23	0.00		0.23	0.40		0.00	0.00	
2 mi	3.83	1.69		3.00	2.60		3.17	0.86		1.79	1.55		0.23	0.40		0.00	0.00		0.00	0.00		0.00	0.00	
3 mi	3.75	0.80		3.23	0.82		1.83	1.58		1.39	1.32		0.00	0.00		0.00	0.00		0.00	0.00		0.00	0.00	
4 mi	2.61	0.88		1.87	1.64		1.39	1.27		0.57	0.98		0.00	0.00		0.00	0.00		0.00	0.00		0.00	0.00	
5 mi	1.84	1.62		1.14	1.98		1.30	1.15		0.00	0.00		0.00	0.00		0.00	0.00		0.00	0.00		0.00	0.00	

Table 2.9: Average APC Counts CFU/g from High Pressure Processing for Fermented Beef Summer Sausages. Table contains the average aerobic plate counts (APC) obtained from subjecting samples of fermented beef summer sausage from four different stages of the manufacturing process to high pressure processing (HPP). Manufacturing stages tested included: post fermentation, internal temperature of 48.9°C (148.9°C), internal temperature 54.4°C (154.4°C), and post chilling of the sausage. Both low fat (8%) and high fat (17%) results are displayed. Samples were subjected to HPP at 586 MPa for dwell times of Control (no HPP), 1 second (se), 1, 2, 3, 4, and 5 minutes (mi). All counts are average APC counts displayed in log CFU/g with standard deviations (STDEV).

Post Fermentation						I48.9°C						I54.4°C						Post Chill						
HPP	Low Fat		High Fat		Count	Low Fat		High Fat		Count	Low Fat		High Fat		Count	Low Fat		High Fat		Count	Low Fat		High Fat	
	Count	STDEV	Count	STDEV		Count	STDEV	Count	STDEV		Count	STDEV	Count	STDEV		Count	STDEV	Count	STDEV		Count	STDEV		
1 se	0.88	0.66	1.12	0.52	1.61	1.17	1.47	0.71	0.80	3.48	0.80	3.83	0.75	2.63	1.25	3.75	1.50							
1 mi	2.01	1.87	2.36	2.03	2.50	1.05	2.88	1.30	0.27	4.10	0.27	4.33	0.33	3.17	1.00	4.14	0.83							
2 mi	2.24	1.73	3.04	3.14	2.51	0.89	3.73	2.10	0.27	4.10	0.27	4.33	0.33	3.40	0.64	4.14	0.83							
3 mi	2.32	0.84	2.80	1.06	3.85	1.62	4.13	1.77	0.30	4.33	0.30	4.33	0.33	3.40	0.64	4.14	0.83							
4 mi	3.46	0.92	4.16	2.17	4.29	1.31	4.95	1.50	0.30	4.33	0.30	4.33	0.33	3.40	0.64	4.14	0.83							
5 mi	4.23	1.66	4.89	2.25	4.38	1.20	5.52	0.59	0.30	4.33	0.30	4.33	0.33	3.40	0.64	4.14	0.83							

Table 2.10: Average APC Log Reduction CFU/g from High Pressure Processing for Fermented Beef Summer Sausages. Table contains the average log reduction for aerobic plate counts (APC) obtained from subjecting samples of fermented beef summer sausage from four different stages of the manufacturing process to high pressure processing (HPP). Manufacturing stages tested included: post fermentation, internal temperature of 48.9°C (I48.9°C), internal temperature 54.4°C (I54.4°C), and post chilling of the sausage. Both low fat (8%) and high fat (17%) results are displayed. Samples were subjected to HPP at 586 MPa for dwell times of Control (no HPP), 1 second (se), 1, 2, 3, 4, and 5 minutes (mi). All counts are average log reduction APC counts displayed in log CFU/g with standard deviations (STDEV)

HPP	Post Fermentation						148.9°C						154.4°C						Post Chill					
	Low Fat			High Fat			Low Fat			High Fat			Low Fat			High Fat			Low Fat			High Fat		
	Count	STDEV	Count	STDEV	Count	STDEV	Count	STDEV	Count	STDEV	Count	STDEV	Count	STDEV	Count	STDEV	Count	STDEV	Count	STDEV	Count	STDEV	Count	STDEV
Cont	5.89	0.07	5.87	0.55	5.28	0.13	5.11	0.68	3.31	0.41	3.40	0.31	2.50	0.95	2.70	0.92								
1 se	3.10	2.70	2.29	2.14	1.38	1.33	0.81	0.88	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00								
1 mi	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00								
2 mi	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00								
3 mi	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00								
4 mi	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00								
5 mi	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00								

Table 2.11: Average ECC Counts CFU/g from High Pressure Processing for Fermented Beef Summer Sausages. Table contains the average *E. coli* coliform plate counts (ECC) obtained from subjecting samples of fermented beef summer sausage from four different stages of the manufacturing process to high pressure processing (HPP). Manufacturing stages tested included: post fermentation, internal temperature of 48.9°C (148.9°C), internal temperature 54.4°C (154.4°C), and post chilling of the sausage. Both low fat (8%) and high fat (17%) results are displayed. Samples were subjected to HPP at 586 MPa for dwell times of Control (no HPP), 1 second (se), 1, 2, 3, 4, and 5 minutes (mi). All counts are average ECC counts displayed in log CFU/g with standard deviations (STDEV).

	Post Fermentation						I48.9°C						I54.4°C						Post Chill					
	Low Fat			High Fat			Low Fat			High Fat			Low Fat			High Fat			Low Fat			High Fat		
	Count	STDEV	Count	STDEV	Count	STDEV	Count	STDEV	Count	STDEV	Count	STDEV	Count	STDEV	Count	STDEV	Count	STDEV	Count	STDEV	Count	STDEV	Count	STDEV
1 se	2.79	2.77	3.58	2.62	3.90	1.45	4.30	1.52	3.31	0.41	3.40	0.31	2.50	0.95	2.70	0.92								
1 mi	5.89	0.07	5.87	0.55	5.28	0.13	5.11	0.68	3.31	0.41	3.40	0.31	2.50	0.95	2.70	0.92								
2 mi	5.89	0.07	5.87	0.55	5.28	0.13	5.11	0.68	3.31	0.41	3.40	0.31	2.50	0.95	2.70	0.92								
3 mi	5.89	0.07	5.87	0.55	5.28	0.13	5.11	0.68	3.31	0.41	3.40	0.31	2.50	0.95	2.70	0.92								
4 mi	5.89	0.07	5.87	0.55	5.28	0.13	5.11	0.68	3.31	0.41	3.40	0.31	2.50	0.95	2.70	0.92								
5 mi	5.89	0.07	5.87	0.55	5.28	0.13	5.11	0.68	3.31	0.41	3.40	0.31	2.50	0.95	2.70	0.92								

Table 2.12: Average ECC Log Reduction CFU/g from High Pressure Processing for Fermented Beef Summer Sausages. Table contains the average log reduction for *E. coli* coliform plate counts (APC) obtained from subjecting samples of fermented beef summer sausage from four different stages of the manufacturing process to high pressure processing (HPP). Manufacturing stages tested included: post fermentation, internal temperature of 48.9°C (I48.9°C), internal temperature 54.4°C (I54.4°C), and post chilling of the sausage. Both low fat (8%) and high fat (17%) results are displayed. Samples were subjected to HPP at 586 MPa for dwell times of Control (no HPP), 1 second (se), 1, 2, 3, 4, and 5 minutes (mi). All counts are average log reduction ECC counts displayed in log CFU/g with standard deviations (STDEV).

Figures of Results for Manufacturing and HPP Processes

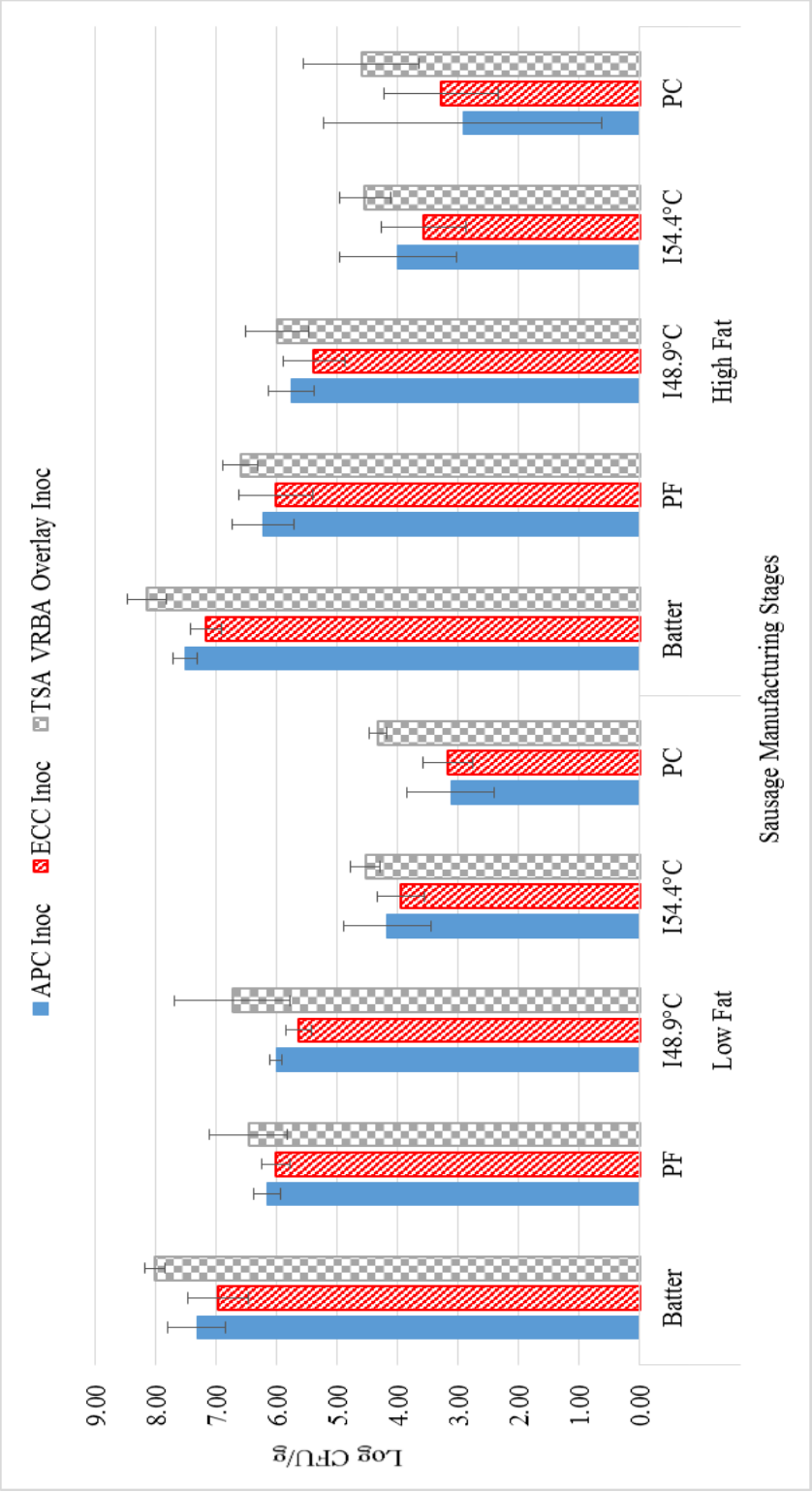


Figure 2.3: Average Counts Log CFU/g for Manufacturing of Fermented Beef Summer Sausages. Figure displays the average aerobic plate counts (APC), *E. coli* coliform plate counts (ECC), and tryptic soy agar with a violet red bile agar overlay (TSA VRBA Overlay) counts from testing fermented beef summer sausage inoculated with *Escherichia coli* surrogate species at five different stages of the manufacturing process. Processing stages tested were: batter, post fermentation (PF), internal temperature of 48.9°C (I48.9°C), internal temperature 54.4°C (I54.4°C), and post chilling (PC) of the sausage. Both low fat (8%) and high fat (17%) results are displayed. All counts are average counts displayed in log CFU/g.

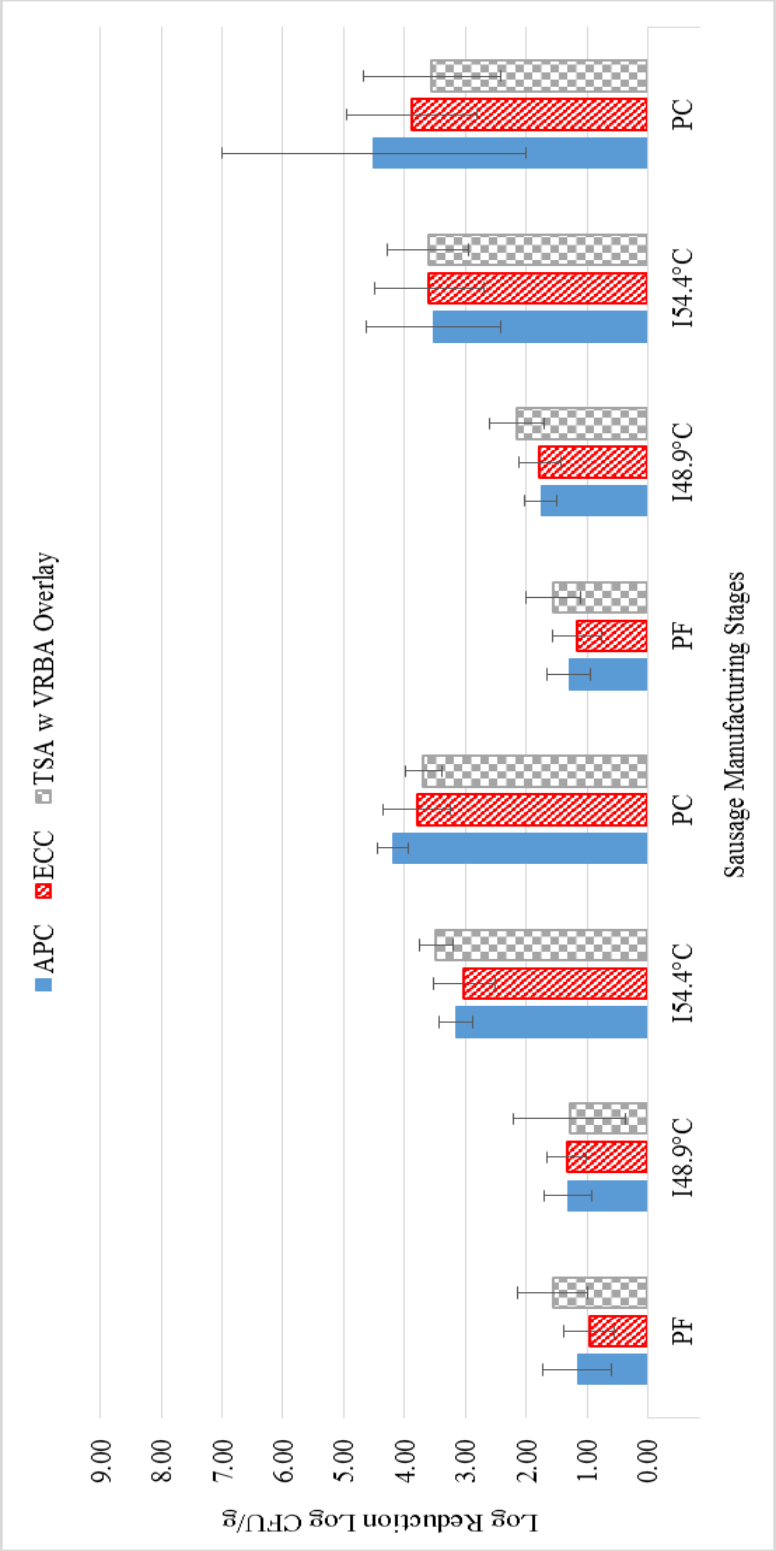


Figure 2.4: Average Log Reduction CFU/g for Manufacturing of Fermented Beef Summer Sausages. Figure displays the average log reductions for aerobic plate counts (APC), *E. coli* coliform plate counts (ECC), and tryptic soy agar with a violet red bile agar overlay (TSA VRBA Overlay) counts obtained from testing fermented beef summer sausage at four different stages of the manufacturing process. Processing stages are: post fermentation (PF), internal temperature of 48.9°C (I48.9°C), internal temperature 54.4°C (I54.4°C), and post chilling (PC) of the sausage. Both low fat (8%) and high fat (17%) results are displayed. All counts are average log reduction counts displayed in log CFU/g.

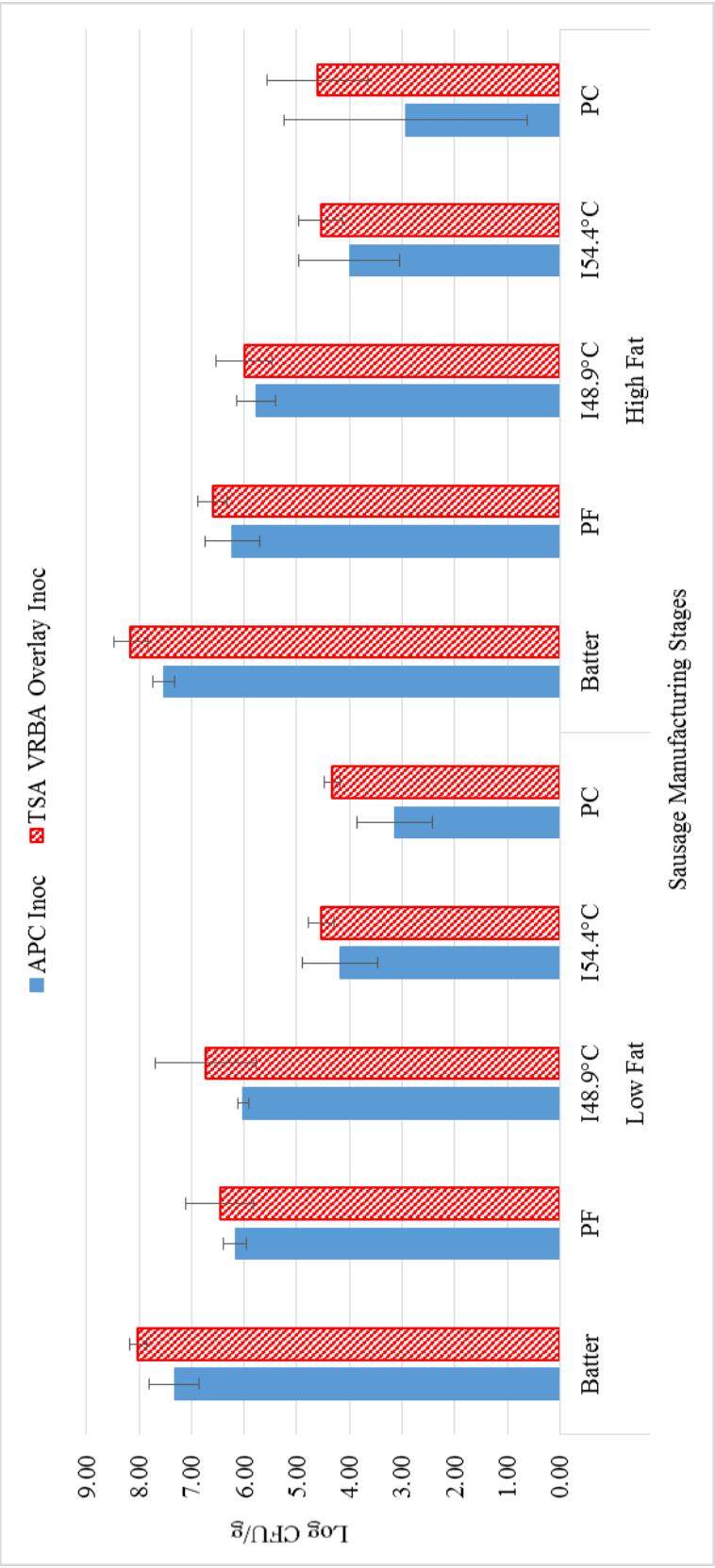


Figure 2.5: Comparison of APC and TSA VRBA Overlay Average Counts CFU/g for Manufacturing of Fermented Beef Summer Sausages. Figure displays the comparison between the average aerobic plate counts (APC) and the tryptic soy agar with a violet red bile agar overlay (TSA VRBA Overlay) counts from testing fermented beef summer sausage inoculated with *Escherichia coli* surrogate species at five different stages of the manufacturing process. Processing stages tested were: batter, post fermentation (PF), internal temperature of 48.9°C (I48.9°C), internal temperature 54.4°C (I54.4°C), and post chilling (PC) of the sausage. Both low fat (8%) and high fat (17%) results are displayed. All counts are average counts displayed in log CFU/g.

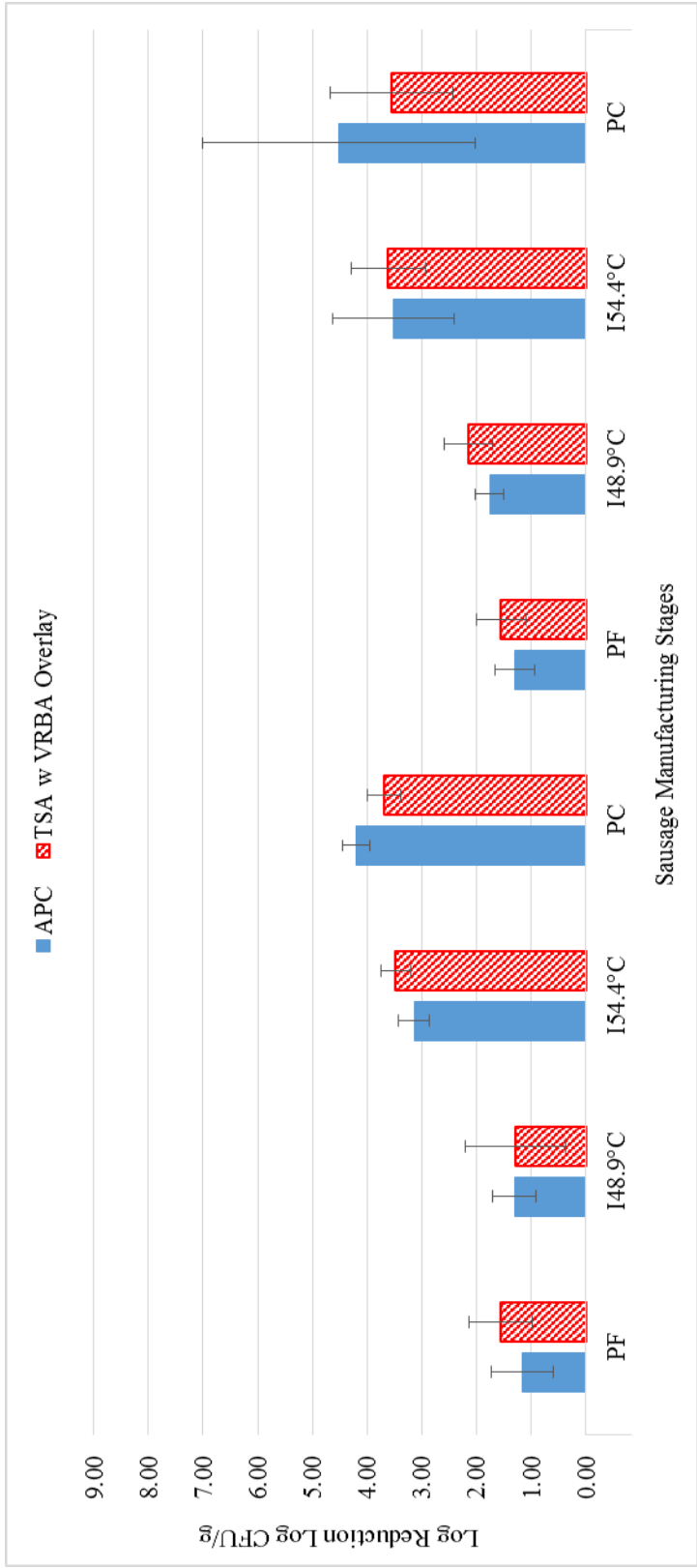


Figure 2.6: Comparison of APC and TSA VRBA Overlay Average Log Reduction CFU/g for Manufacturing of Fermented Beef Summer Sausages. Figure displays the comparison between the average log reductions for aerobic plate counts (APC) and the tryptic soy agar with a violet red bile agar overlay (TSA VRBA Overlay) from testing fermented beef summer sausage inoculated with *Escherichia coli* surrogate species at four different stages of the manufacturing process. Processing stages displayed are: post fermentation (PF), internal temperature of 48.9°C (I48.9°C), internal temperature 54.4°C (I54.4°C), and post chilling (PC) of the sausage. Both low fat (8%) and high fat (17%) results are displayed. All counts are average log reduction counts displayed in log CFU/g.

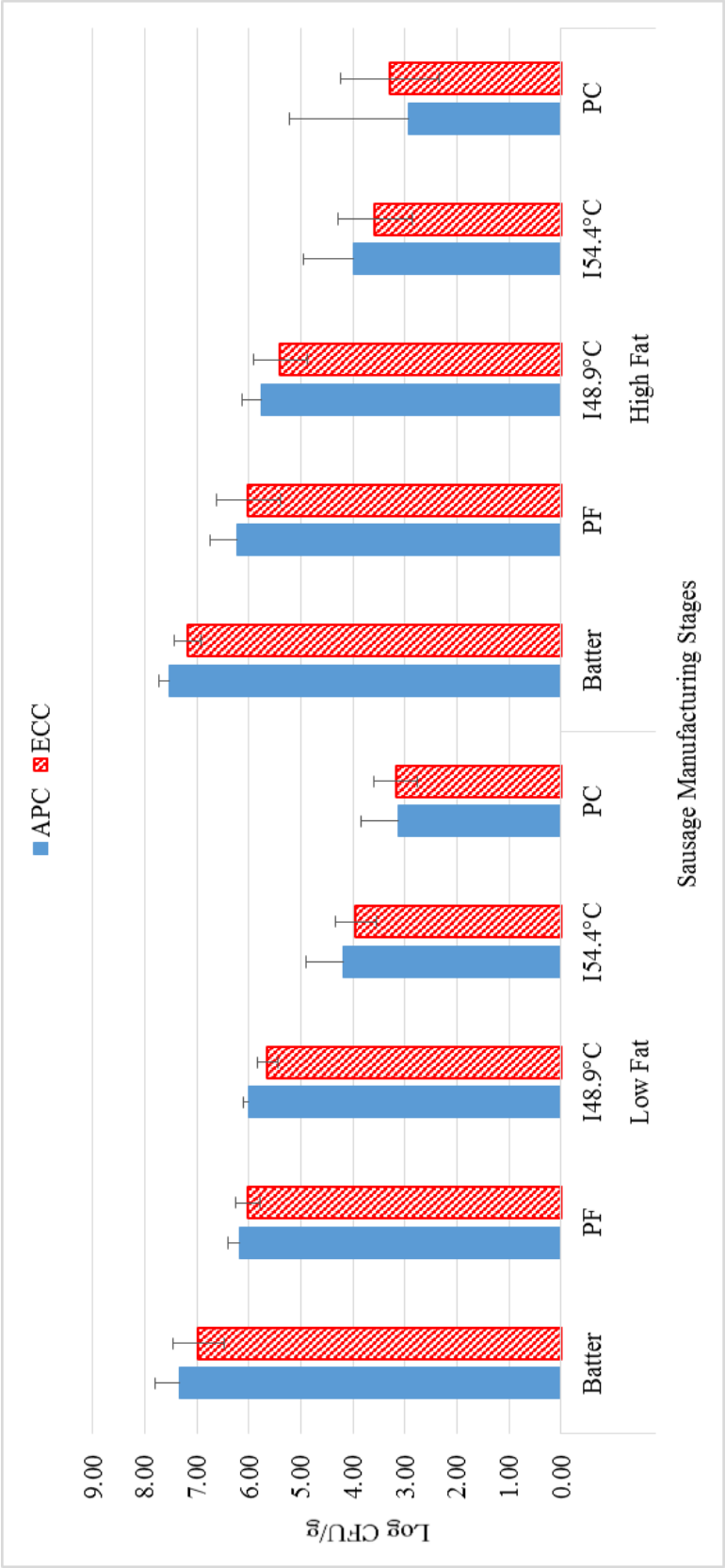


Figure 2.7: Comparison of APC and ECC Counts CFU/g for Manufacturing of Fermented Beef Summer Sausages. Figure displays the comparison between the average aerobic plate counts (APC) and the *E. coli* coliform (ECC) counts from testing fermented beef summer sausage inoculated with *Escherichia coli* surrogate species at five different stages of the manufacturing process. Processing stages tested were: batter, post fermentation (PF), internal temperature of 48.9°C (I48.9°C), internal temperature 54.4°C (I54.4°C), and post chilling (PC) of the sausage. Both low fat (8%) and high fat (17%) results are displayed. All counts are average counts displayed in log CFU/g.

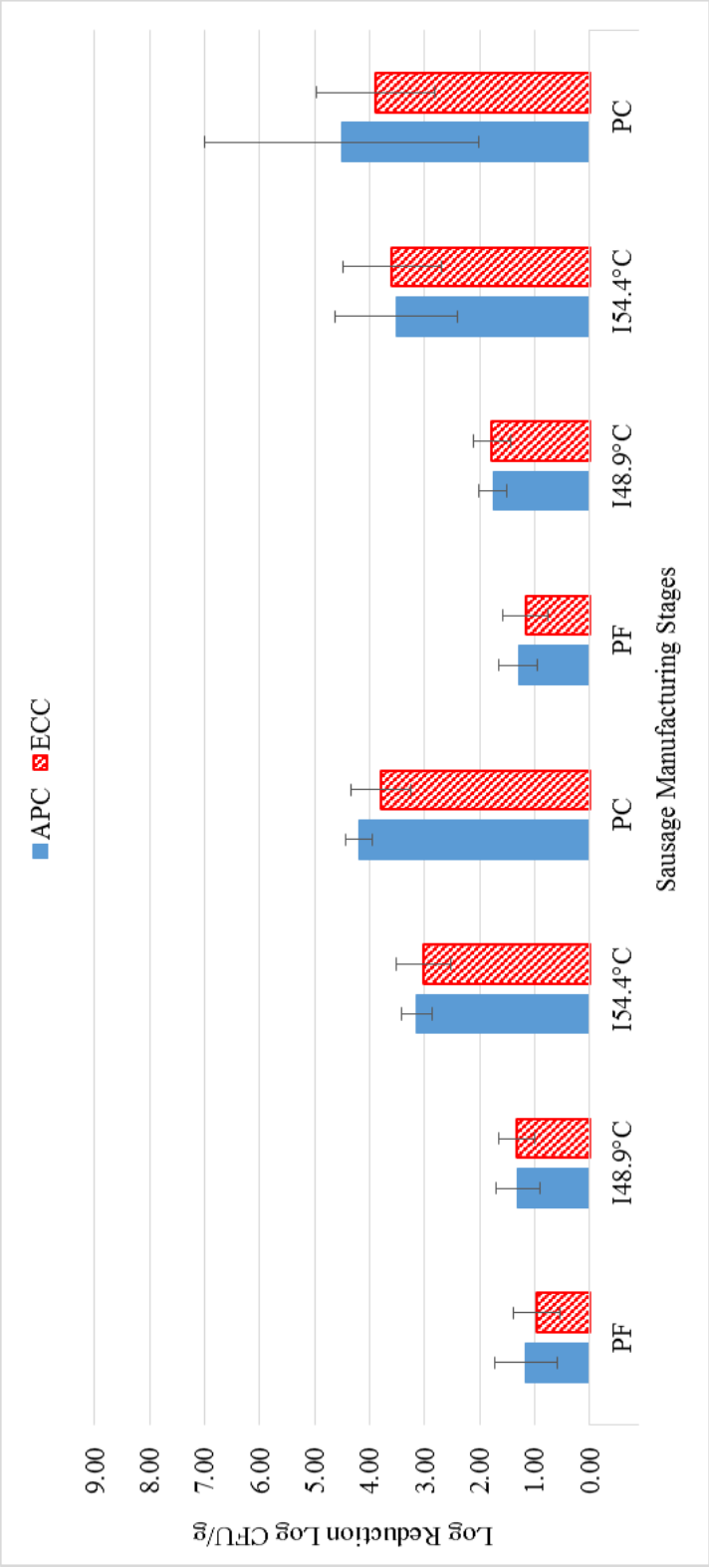


Figure 2.8: Comparison of APC and ECC Average Log Reduction CFU/g for Manufacturing of Fermented Beef Summer Sausages. Figure displays the comparison between the average log reductions for aerobic plate counts (APC) and the *E. coli* coliform (ECC) plate counts from testing fermented beef summer sausage inoculated with *Escherichia coli* surrogate species at four different stages of the manufacturing process. Processing stages displayed are: post fermentation (PF), internal temperature of 48.9°C (I48.9°C), internal temperature 54.4°C (I54.4°C), and post chilling (PC) of the sausage. Both low fat (8%) and high fat (17%) results are displayed. All counts are average log reduction counts displayed in log CFU/g.

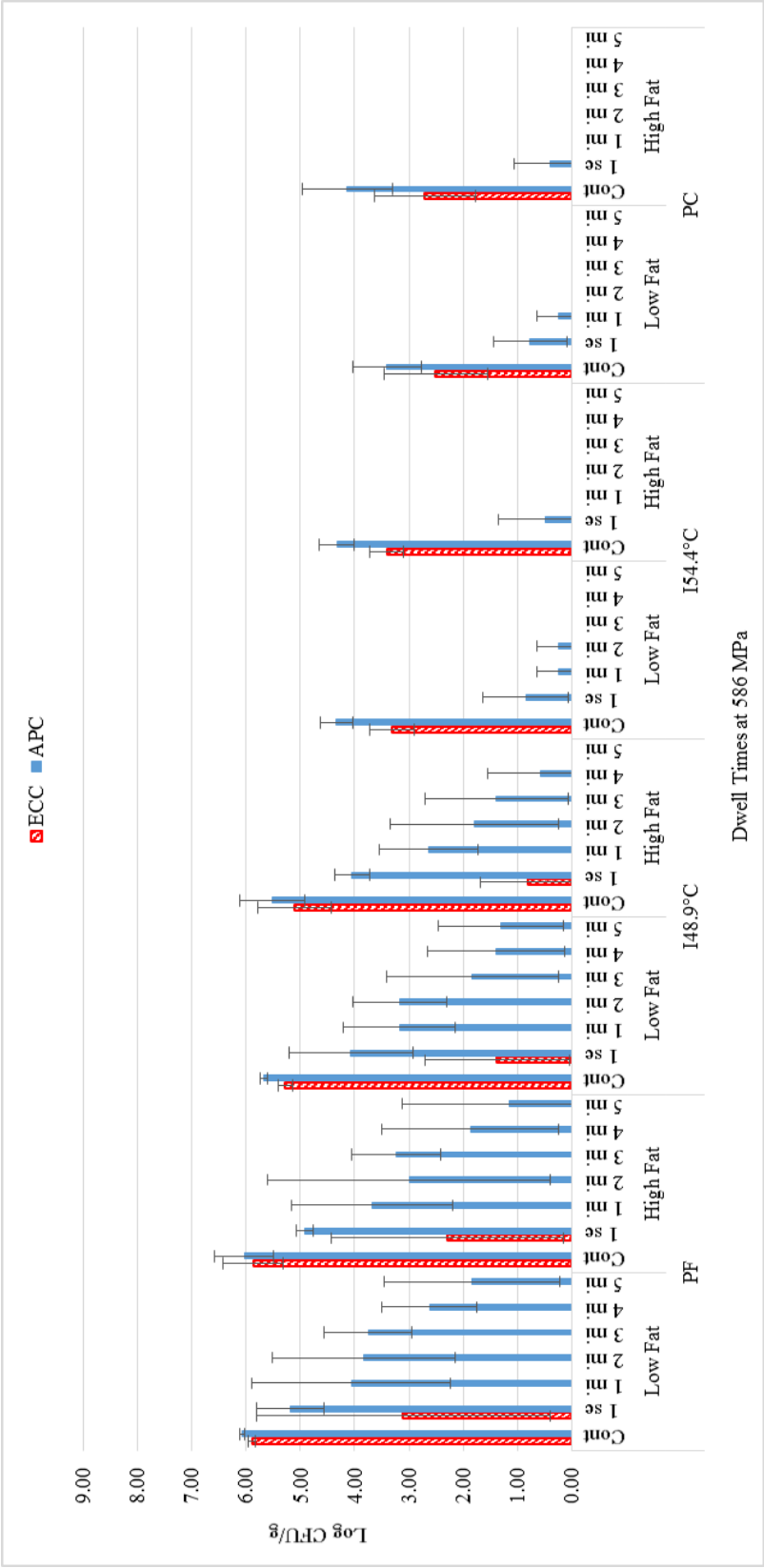


Figure 2.9: Average Counts Log CFU/g for High Pressure Processing of Fermented Beef Summer Sausages. Figure displays the average aerobic plate counts (APC) and the *E. coli* coliform (ECC) counts from high pressure processing (HPP) testing of fermented beef summer sausage inoculated with *Escherichia coli* surrogate species. Manufacturing stages tested include: post fermentation (PF), internal temperature of 48.9°C (148.9°C), internal temperature 54.4°C (154.4°C), and post chilling (PC) of the sausage. Both low fat (8%) and high fat (17%) results are displayed. Samples were subjected to HPP at 586 MPa for dwell times of Control (no HPP), 1 second (se), 1, 2, 3, 4, and 5 minutes (mi). All counts are average counts displayed in log CFU/g.

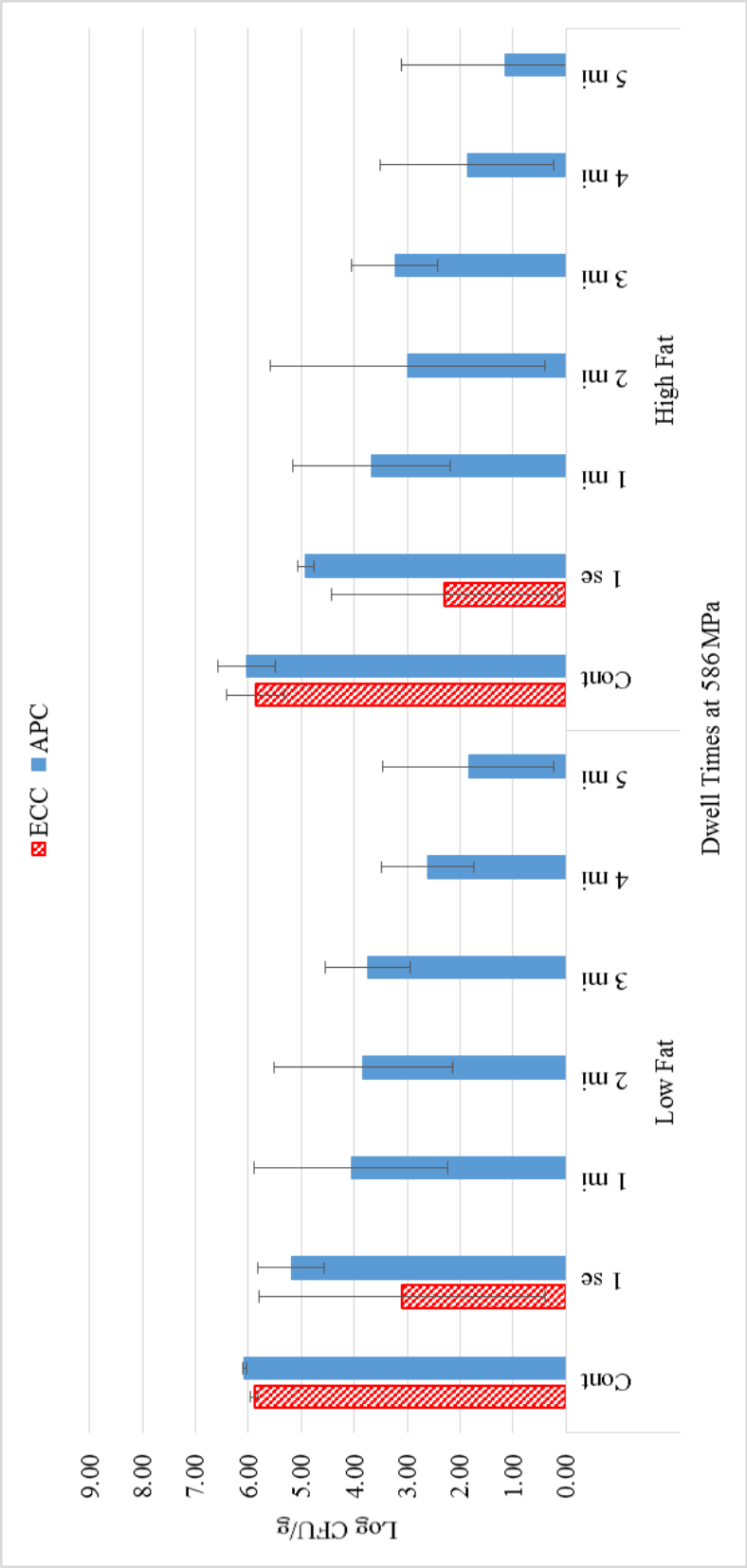


Figure 2.10: Post Fermentation Average Counts Log CFU/g from High Pressure Processing of Fermented Beef Summer Sausages. Figure displays the average aerobic plate counts (APC) and the *E. coli* coliform (ECC) counts from high pressure processing (HPP) testing of fermented beef summer sausage inoculated with *Escherichia coli* surrogate species from manufacturing stage post fermentation. Both low fat (8%) and high fat (17%) results are displayed. Samples were subjected to HPP at 586 MPa for dwell times of Control (no HPP), 1 second (se), 1, 2, 3, 4, and 5 minutes (mi). All counts are average counts displayed in log CFU/g.

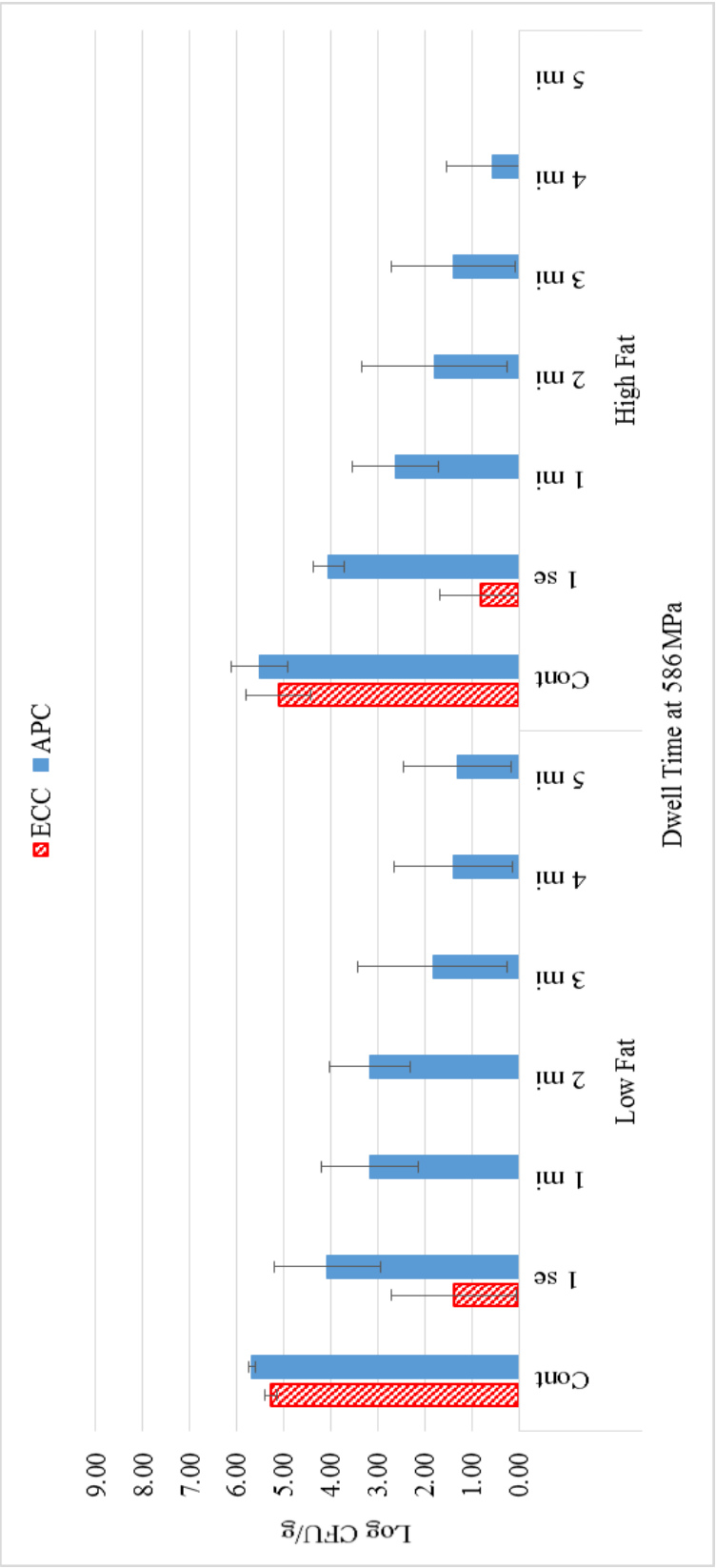


Figure 2.11: Internal Temperature 48.9°C Average Counts Log CFU/g from High Pressure Processing of Fermented Beef Summer Sausages. Figure displays the average aerobic plate counts (APC) and the *E. coli* coliform (ECC) counts from high pressure processing (HPP) testing of fermented beef summer sausage inoculated with *Escherichia coli* surrogate species from manufacturing stage internal temperature 48.9°C. Both low fat (8%) and high fat (17%) results are displayed. Samples were subjected to HPP at 586 MPa for dwell times of Control (no HPP), 1 second (se), 1, 2, 3, 4, and 5 minutes (mi). All counts are average counts displayed in log CFU/g.

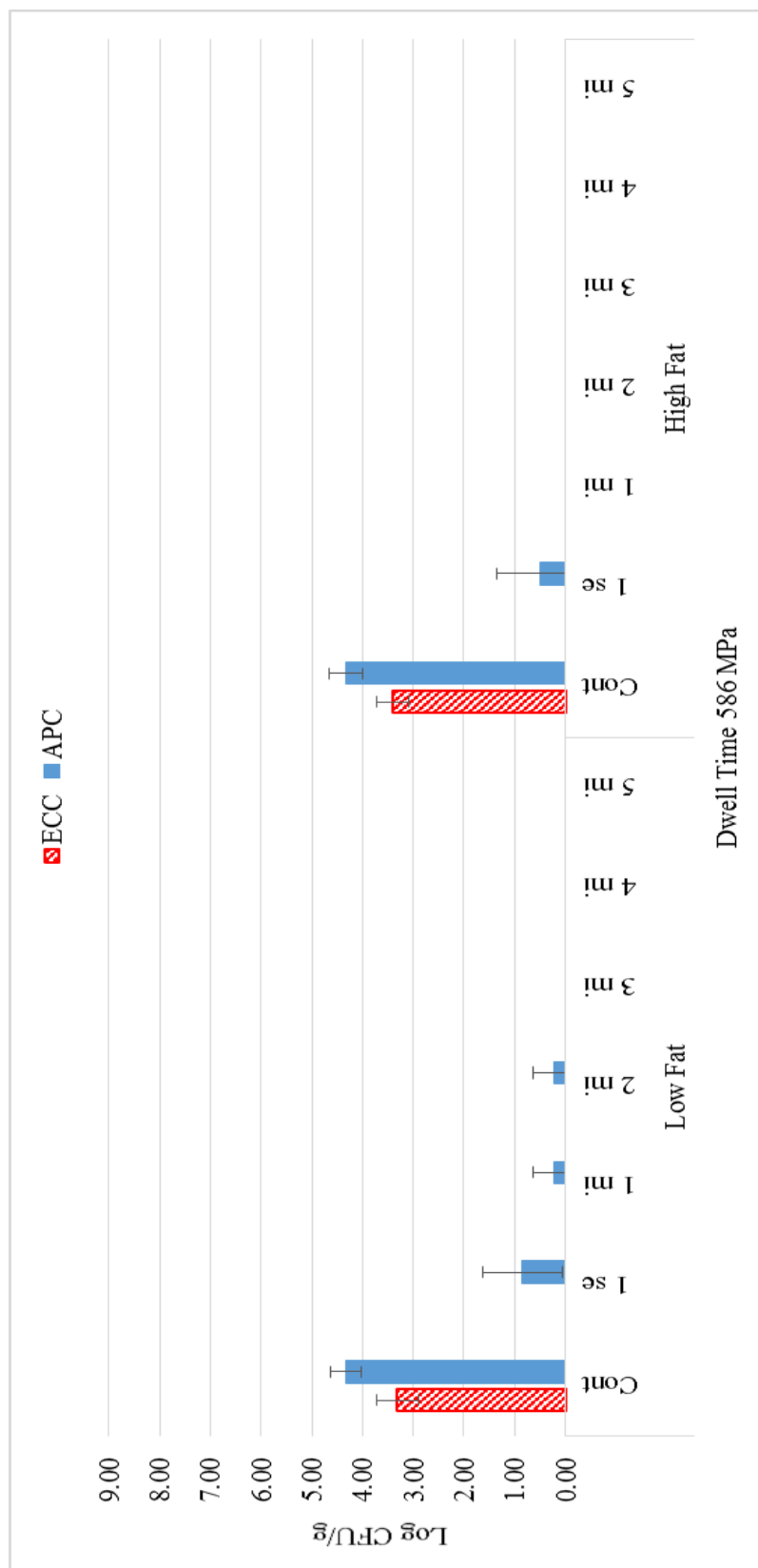


Figure 2.12: Internal Temperature 54.4°C Average Counts Log CFU/g from High Pressure Processing of Fermented Beef Summer Sausages. Figure displays the average aerobic plate counts (APC) and the *E. coli* coliform (ECC) counts from high pressure processing (HPP) testing of fermented beef summer sausage inoculated with *Escherichia coli* surrogate species from manufacturing stage internal temperature 54.4°C. Both low fat (8%) and high fat (17%) results are displayed. Samples were subjected to HPP at 586 MPa for dwell times of Control (no HPP), 1 second (se), 1, 2, 3, 4, and 5 minutes (mi). All counts are average counts displayed in log CFU/g.

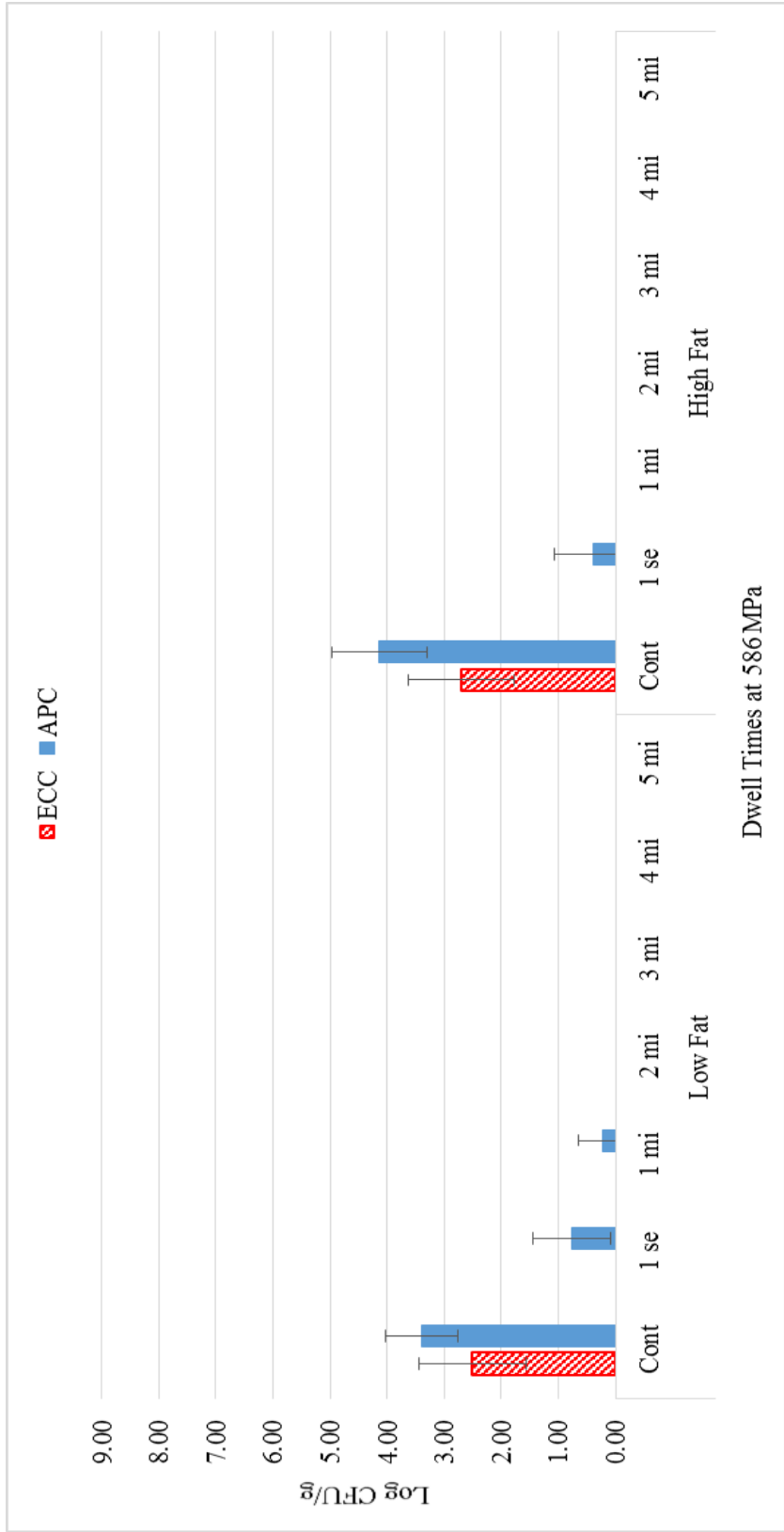


Figure 2.13: Post Chilling Average Counts Log CFU/g from High Pressure Processing of Fermented Beef Summer Sausages. Figure displays the average aerobic plate counts (APC) and the *E. coli* coliform (ECC) counts from high pressure processing (HPP) testing of fermented beef summer sausage inoculated with *Escherichia coli* surrogate species from manufacturing stage post chilling. Both low fat (8%) and high fat (17%) results are displayed. Samples were subjected to HPP at 586 MPa for dwell times of Control (no HPP), 1 second (se), 1, 2, 3, 4, and 5 minutes (mi). All counts are average counts displayed in log CFU/g.

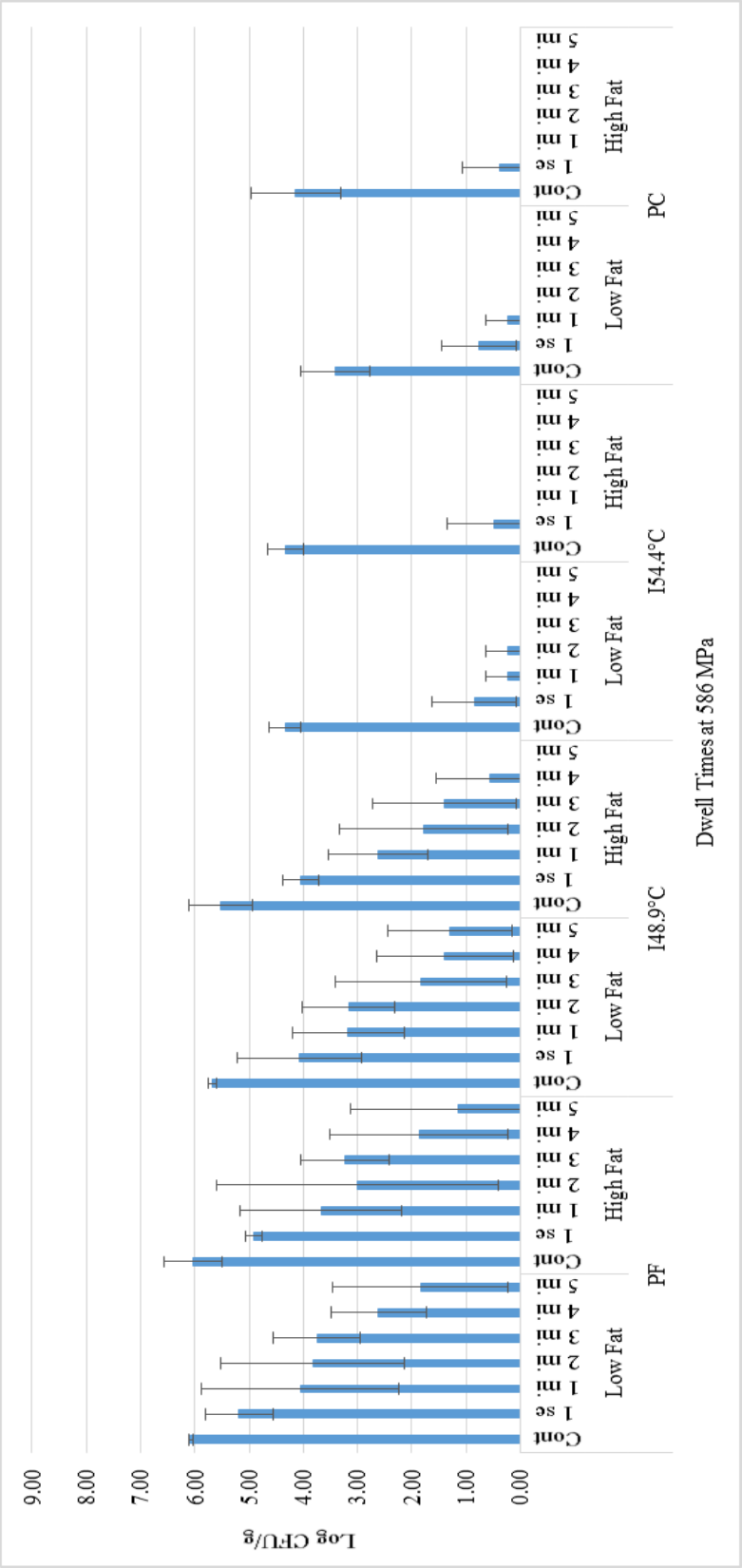


Figure 2.14: Average APC Counts Log CFU/g for High Pressure Processing of Fermented Beef Summer Sausages. Figure displays the average aerobic plate counts (APC) from high pressure processing (HPP) testing of fermented beef summer sausage inoculated with *Escherichia coli* surrogate species. Manufacturing stages tested include: post fermentation (PF), internal temperature of 48.9°C (I48.9°C), internal temperature 54.4°C (I54.4°C), and post chilling (PC) of the sausage. Both low fat (8%) and high fat (17%) results are displayed. Samples were subjected to HPP at 586 MPa for dwell times of Control (no HPP), 1 second (se), 1, 2, 3, 4, and 5 minutes (mi). All counts are average counts displayed in log CFU/g.

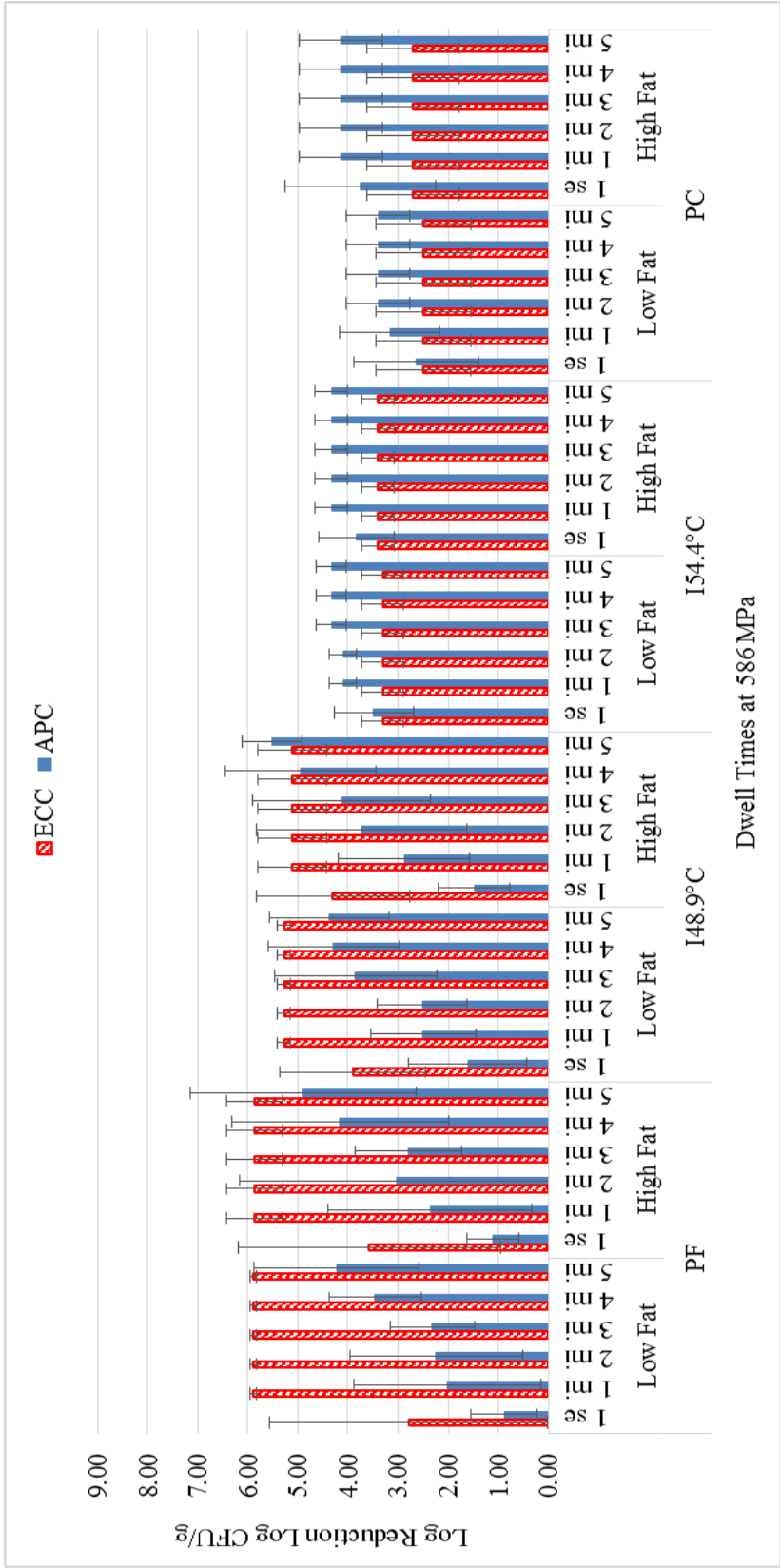


Figure 2.15: Average Log Reduction CFU/g for High Pressure Processing of Fermented Beef Summer Sausages. Figure displays the average log reduction for aerobic plate counts (APC) and the *E. coli* coliform (ECC) counts from high pressure processing (HPP) testing of fermented beef summer sausage inoculated with *Escherichia coli* surrogate species. Manufacturing stages tested include: post fermentation (PF), internal temperature of 48.9°C (148.9°C), internal temperature 54.4°C (154.4°C), and post chilling (PC) of the sausage. Both low fat (8%) and high fat (17%) results are displayed. Samples were subjected to HPP at 586 MPa for dwell times of 1 second (se), 1, 2, 3, 4, and 5 minutes (mi). All counts are average log reduction counts displayed in log CFU/g.

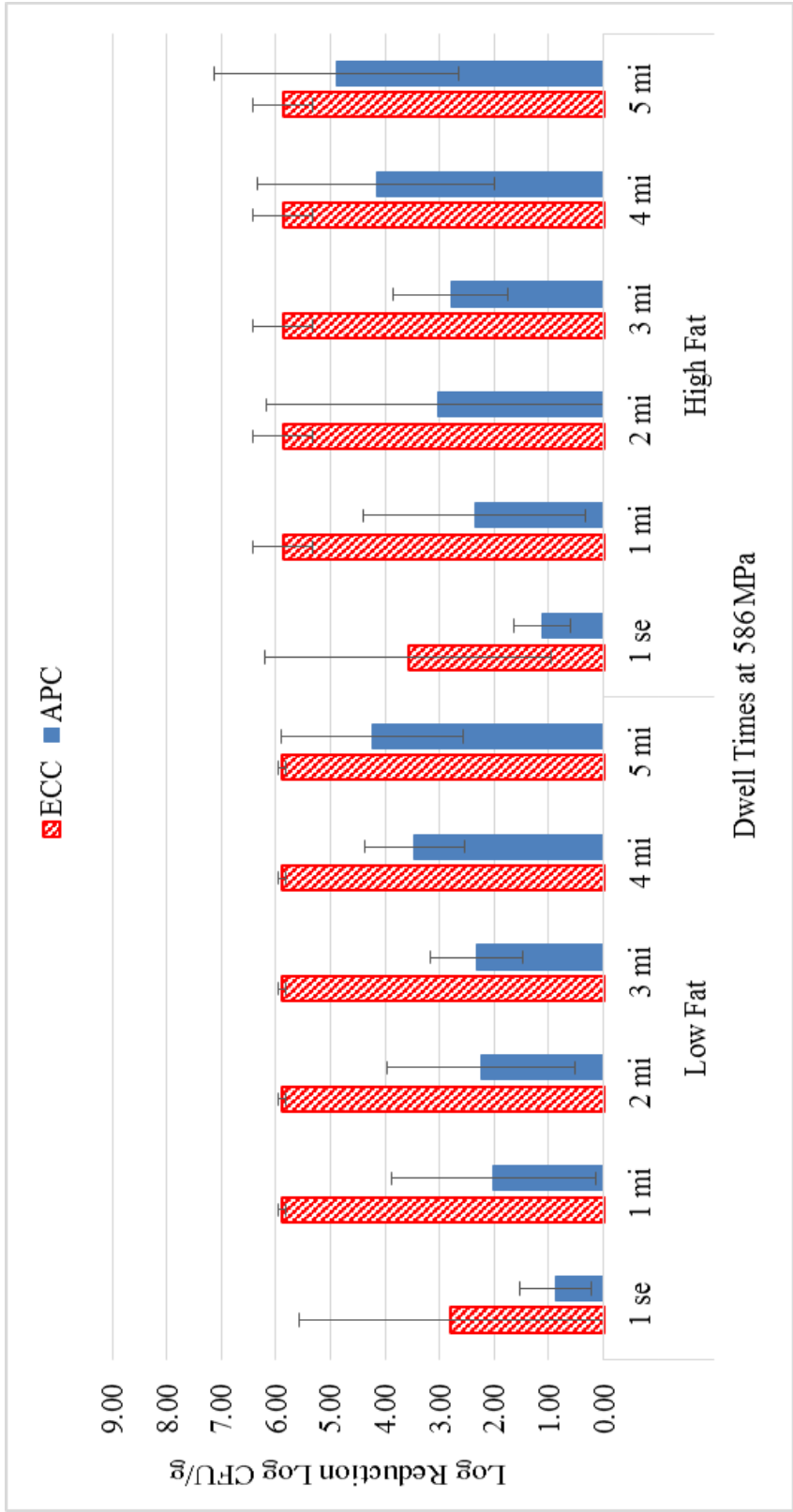


Figure 2.16: Post Fermentation Log Reductions CFU/g from High Pressure Processing of Fermented Beef Summer Sausages. Figure displays the average log reductions for aerobic plate counts (APC) and for *E. coli* coliform (ECC) counts from high pressure processing (HPP) testing of fermented beef summer sausage inoculated with *Escherichia coli* surrogate species from manufacturing stage post fermentation. Both low fat (8%) and high fat (17%) results are displayed. Samples were subjected to HPP at 586 MPa for dwell times of 1 second (se), 1, 2, 3, 4, and 5 minutes (mi). All counts are average log reduction counts displayed in log CFU/g.

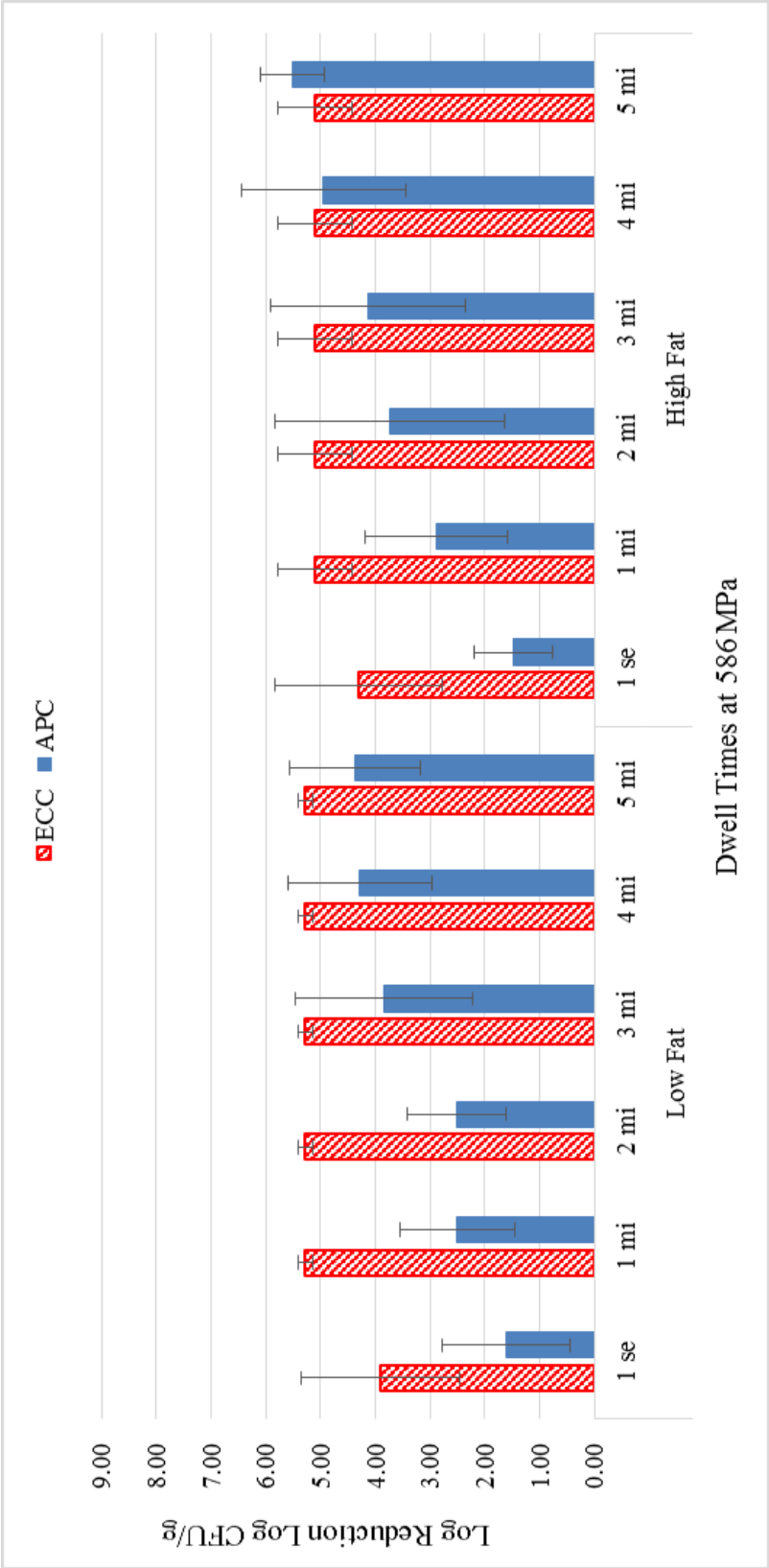


Figure 2.17: Internal Temperature 48.9°C Log Reductions CFU/g from High Pressure Processing of Fermented Beef Summer Sausages. Figure displays the average log reductions for aerobic plate counts (APC) and for *E. coli* coliform (ECC) counts from high pressure processing (HPP) testing of fermented beef summer sausage inoculated with *Escherichia coli* surrogate species from manufacturing stage internal temperature 48.9°C. Both low fat (8%) and high fat (17%) results are displayed. Samples were subjected to HPP at 586 MPa for dwell times of 1 second (se), 1, 2, 3, 4, and 5 minutes (mi). All counts are average log reduction counts displayed in log CFU/g.

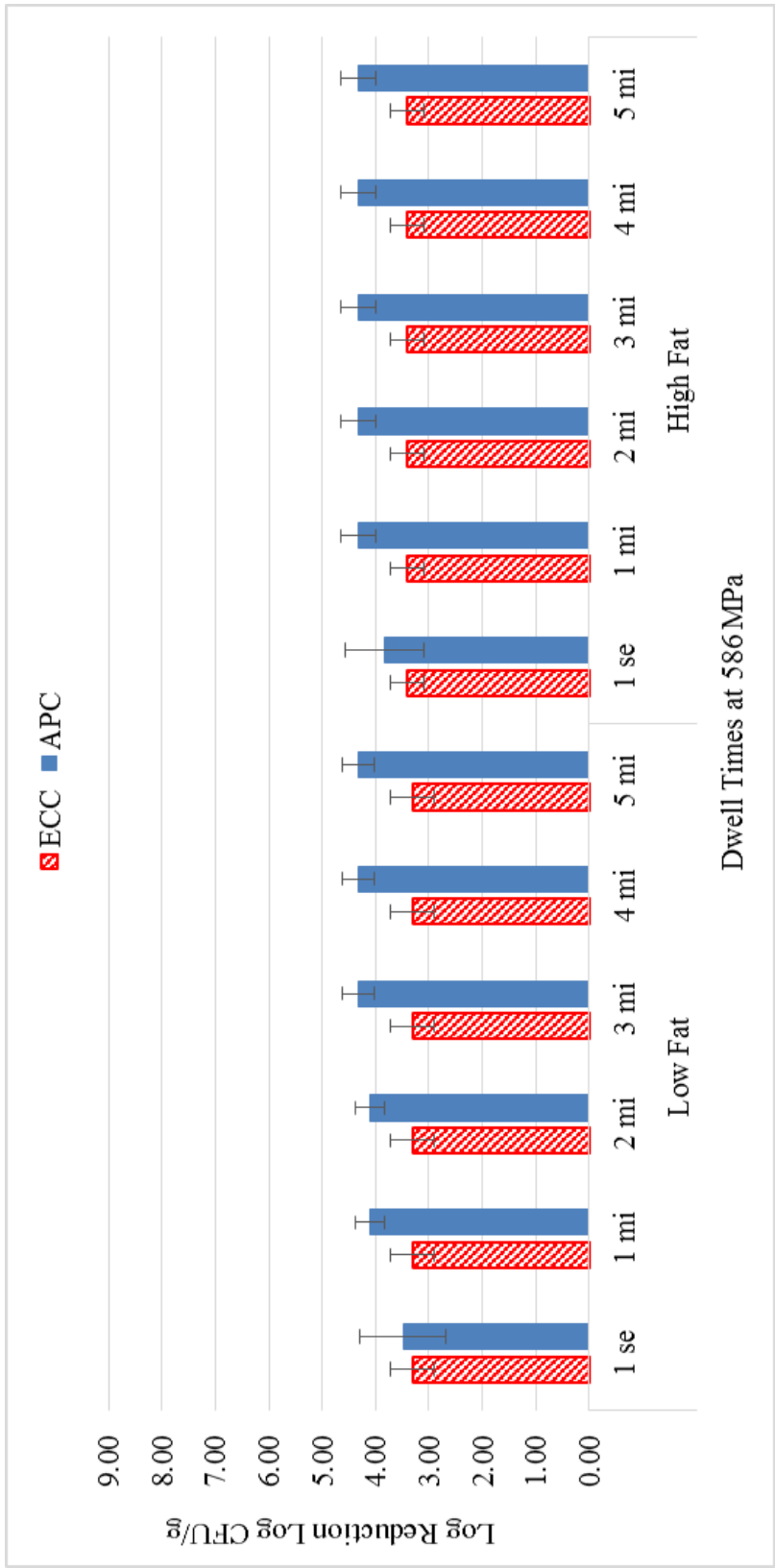


Figure 2.18: Internal Temperature 54.4°C Log Reductions CFU/g from High Pressure Processing of Fermented Beef Summer Sausages. Figure displays the average log reductions for aerobic plate counts (APC) and for *E. coli* coliform (ECC) counts from high pressure processing (HPP) testing of fermented beef summer sausage inoculated with *Escherichia coli* surrogate species from manufacturing stage internal temperature 54.4°C. Both low fat (8%) and high fat (17%) results are displayed. Samples were subjected to HPP at 586 MPa for dwell times of 1 second (se), 1, 2, 3, 4, and 5 minutes (mi). All counts are average log reduction counts displayed in log CFU/g.

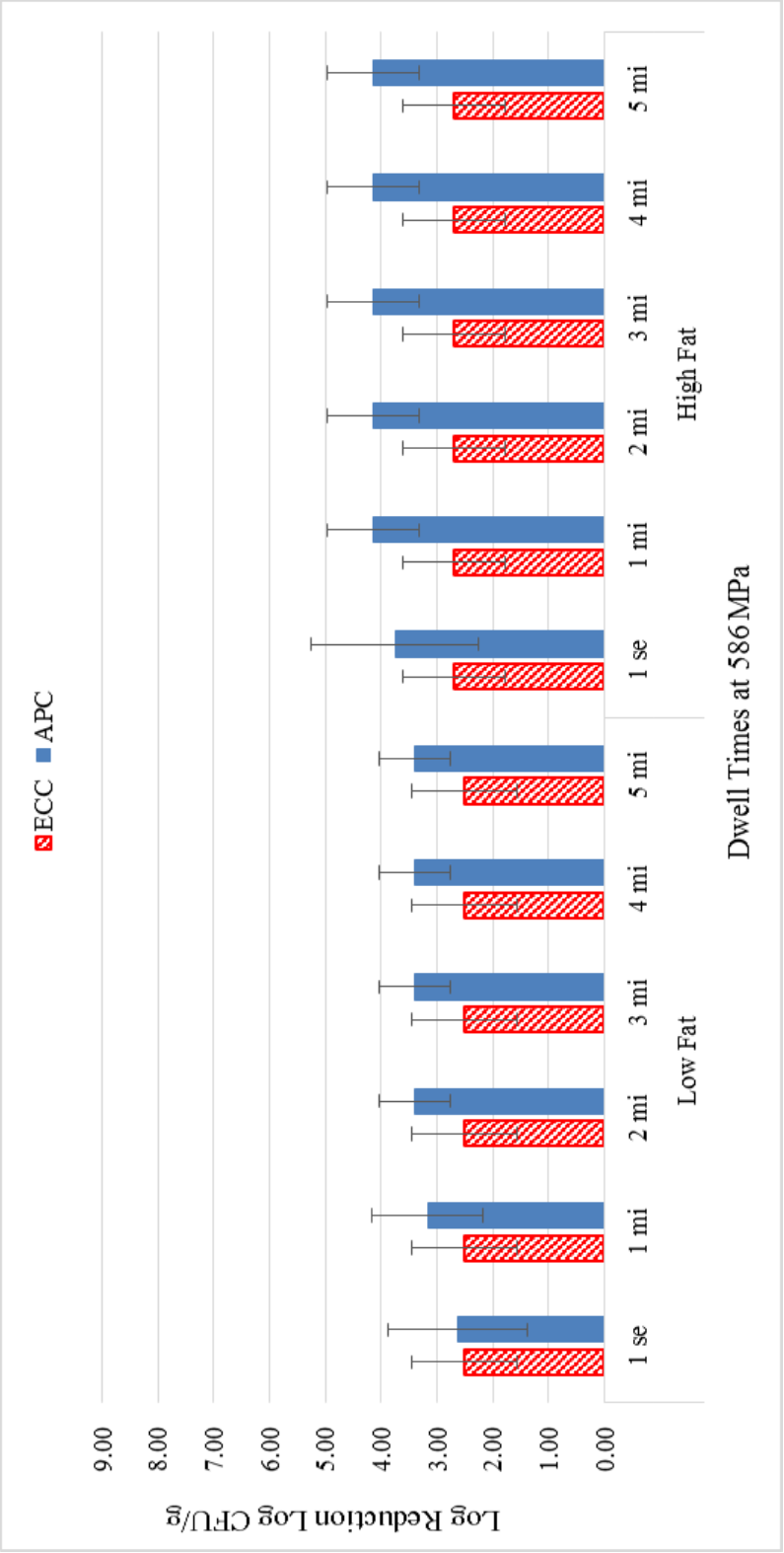


Figure 2.19: Post Chilling Log Reductions CFU/g from High Pressure Processing of Fermented Beef Summer Sausages. Figure displays the average log reductions for aerobic plate counts (APC) and for *E. coli* coliform (ECC) counts from high pressure processing (HPP) testing of fermented beef summer sausage inoculated with *Escherichia coli* surrogate species from manufacturing stage post chilling. Both low fat (8%) and high fat (17%) results are displayed. Samples were subjected to HPP at 586 MPa for dwell times of 1 second (se), 1, 2, 3, 4, and 5 minutes (mi). All counts are average log reduction counts displayed in log CFU/g.

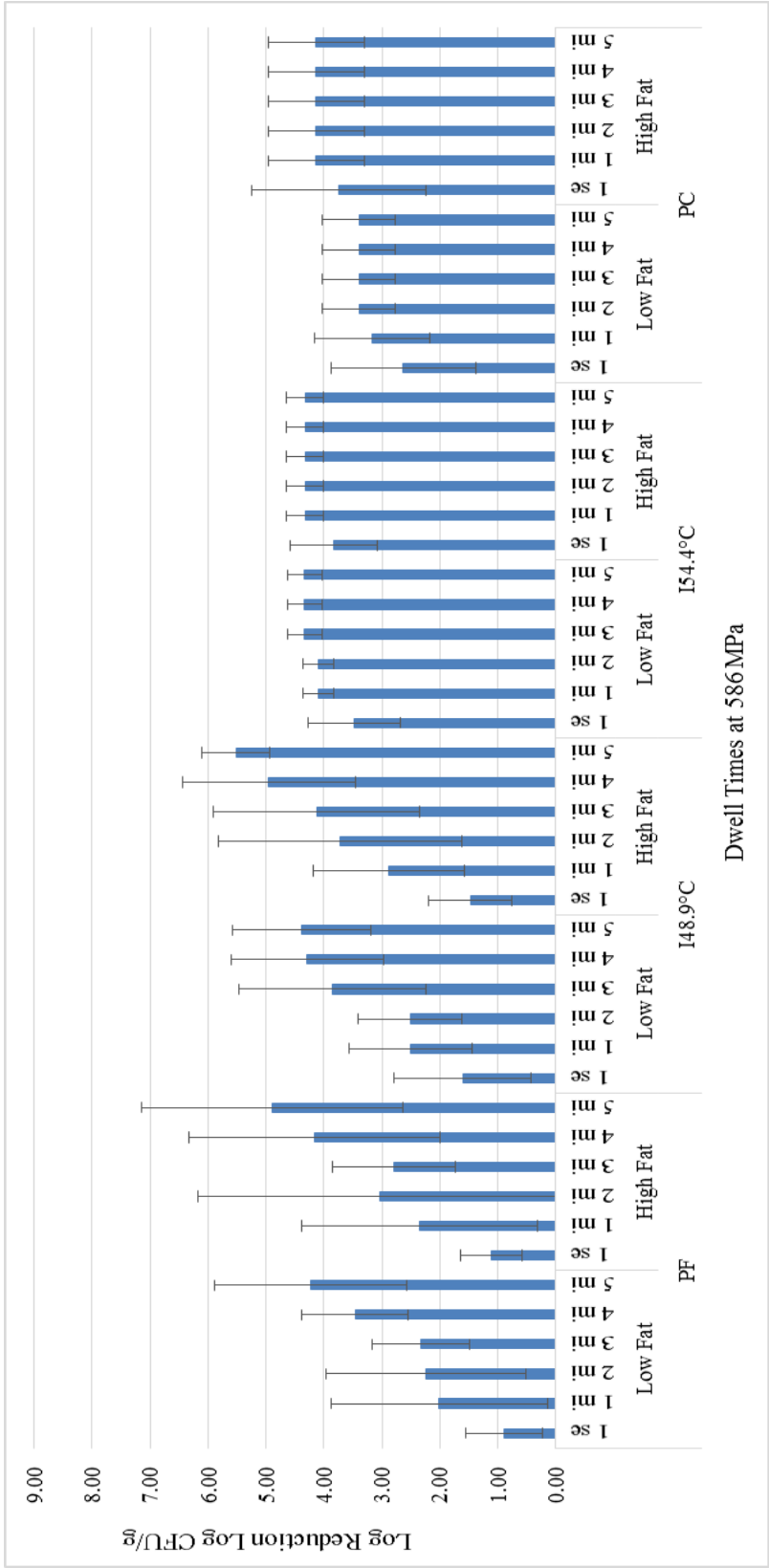


Figure 2.20: Average APC Log Reductions CFU/g for High Pressure Processing of Fermented Beef Summer Sausages. Figure displays the average log reductions for aerobic plate counts (APC) from high pressure processing (HPP) testing of fermented beef summer sausage inoculated with *Escherichia coli* surrogate species. Manufacturing stages tested include: post fermentation (PF), internal temperature of 48.9°C (I48.9°C), internal temperature 54.4°C (I54.4°C), and post chilling (PC) of the sausage. Both low fat (8%) and high fat (17%) results are displayed. Samples were subjected to HPP at 586 MPa for dwell times of Control (no HPP), 1 second (se), 1, 2, 3, 4, and 5 minutes (mi). All counts are average log reductions displayed in log CFU/g.

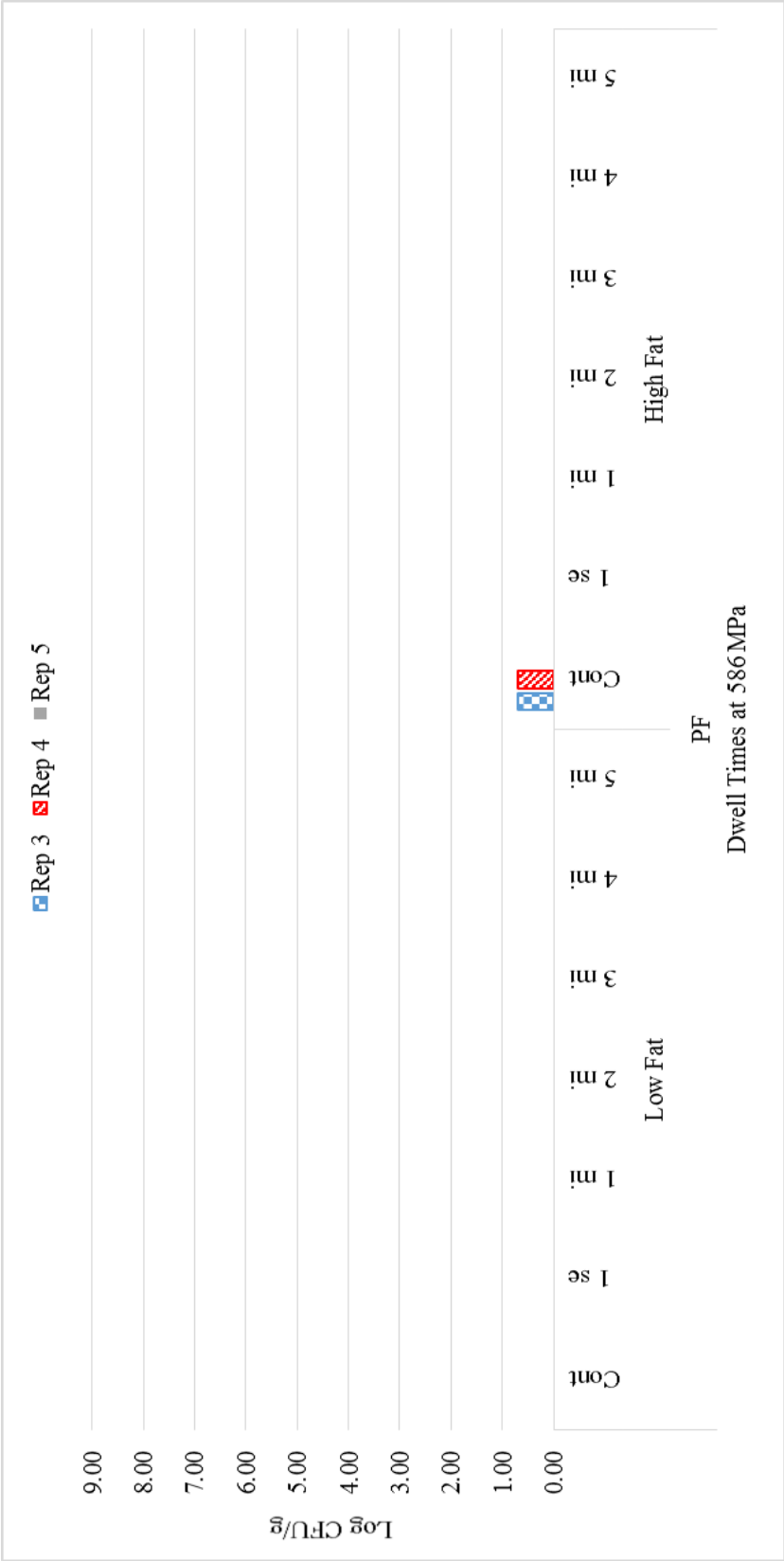


Figure 2.21: Enterobacteriaceae Counts CFU/g for High Pressure Processing of Fermented Beef Summer Sausages. Figure displays the Enterobacteriaceae (EB) counts from high pressure processing (HPP) testing of fermented beef summer sausage. Manufacturing stage displayed is post fermentation (PF) as no EB counts were seen in any other stage of production of the sausage. Both low fat (8%) and high fat (17%) results are displayed. Samples were subjected to HPP at 586 MPa for dwell times of Control (no HPP), 1 second (se), 1, 2, 3, 4, and 5 minutes (mi). All counts are average EB counts displayed in log CFU/g.

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CHAPTER 3

EVALUATION OF THE INACTIVATION OF *Escherichia coli* SPECIES IN BEEF SUMMER SAUSAGE VIA HIGH PRESSURE PROCESSING

INTRODUCTION

In the previous chapter *Escherichia coli* surrogates were evaluated throughout the manufacturing process of fermented beef summer sausage (as a model sausage) to determine the log reductions of *E. coli* at various production stages. This was done to determine if the manufacturing process for fermented beef summer sausage was adequate in achieving the lethality standard of a 5.0 log reduction of *E. coli* in fermented dry and semi-dry sausages directed by the United States Department of Agriculture and Food Safety Inspection Service (USDA 2001a; USDA 2001b). Additionally, beef summer sausage from that process was subjected to high pressure processing in place of the thermal processing (cook) stage to determine if HPP could be utilized within a sausage production protocol as an alternative intervention to thermal processing in meeting the USDA-FSIS lethality standard ensuring safe product for consumption. In this chapter we will continue to look at HPP but instead of looking at its use as an alternative intervention, we will instead evaluate the effectiveness of the high pressure process itself on the inactivation of *Escherichia coli* species (surrogates and pathogens) without the use of other interventions (stressors) from the manufacturing process.

Escherichia coli continues to cause problems for food manufacturers around the world invading food domains and causing outbreaks in a wide variety of food products including spinach (2006), pizza (2007), cookie dough (2009), cheese (2010), and even hazelnuts (2011) all being implicated in outbreaks of *E. coli* O157:H7 (CDC 2015). *E. coli*, as mentioned previously, has been implicated in many sausage outbreaks as well including outbreaks in products such as Genoa Salami in Canada (Williams et al., 2000),

uncooked fermented sausages in Australia (Ross and Shadbolt 2000; CDC 1995a), and fermented sausage products in Sweden from a butcher shop (Sartz et al., 2008). All of these outbreaks had confirmed illnesses from pathogenic *Escherichia coli*, and in many of those cases infected individuals developed hemolytic uremic syndrome and were hospitalized.

Outbreaks in beef as well as in fermented sausages are what led to regulations by the USDA-FSIS stipulating that fermented dry and semi-dry sausages must have reductions of a minimum of 5.0 logs of *E. coli* throughout the production process to ensure that the sausage is safe for consumption (USDA 2001a; USDA 2001b). Because of this regulation it is important to look at additional interventions that may help reduce the risk of *E. coli* infection, one such intervention is high pressure processing.

Since *E. coli* O157:H7, and the other Shiga Toxin Producing *Escherichia Coli* (STEC), O26:H11, O45:H2, O103:H2, O111:H⁻, O121:H19, and O145:NM (constituting the “Big Seven”), have been implicated in many foodborne illness outbreaks it becomes very important to understand how they react to alternative interventions such as high pressure processing. High pressure processing, as mentioned previously in this thesis, is the process of subjecting prepackaged (some products treated before packaging process) food products to isostatic pressures ranging from 300-600 megapascals (MPa) or higher (43,500 to 87,000 psi) (Hugas, Garriga, and Monfort 2002; Oey et al., 2008; Farkas and Hoover 2000) to achieve USDA-FSIS lethality standards.

Studies have been performed looking at the control of foodborne pathogens in sausage products including low acid sausage at 300 MPa with ripening (Marcos, Aymerich, and Garriga 2005) and slightly fermented Mediterranean style sausages at 400 MPa followed by ripening (Garriga et al., 2005) but, both of these studies focused primarily on *Listeria monocytogenes* and *Salmonella*, not *Escherichia coli* in their investigations. There is research that focused on the inactivation of *E. coli* in Genoa Salami at 483 MPa / 600 MPa showing additional reductions of *E. coli* from 4.7 to >5.8 logs post HPP (Porto-Fett et al., 2010). All of these studies however included additional hurdles and did not test the organisms in a non-stressed state.

Testing *Escherichia coli* via HPP, and without the added stress from manufacturing interventions such as; temperatures control 4.4°C during production, addition of salts and nitrates, pH to ≤ 5.3 (typically below 5.0), and water activity levels to ≤ 0.93 , (Barbuti and Parolari 2002; USDA 1999a; Incze 1998) is extremely valuable in helping to understand how these foodborne pathogens react to their environment, how they react to high pressure, if they react the same from strain to strain, and if HPP is capable of inactivation of *Escherichia coli* species (surrogates and pathogens) while they are in there normal state (non-stressed).

The overall objective of this study was to evaluate the effectiveness of high pressure processing on the inactivation of *Escherichia coli* species (surrogates and pathogens) on fermented beef summer sausages (as a model sausage) without subjecting the organisms to the additional stress of the manufacturing process which aides in making the pathogens

more susceptible to damage via high pressure. Already processed fermented beef summer sausage was inoculated with three different cocktails of *Escherichia coli* (surrogates, *E. coli* O157:H7, and STEC) and then subjected to various dwell times at 586 MPa (14 MPa below industry standard) to evaluate the industry standard for HPP of 600 MPa for the inactivation of this potentially deadly foodborne pathogen.

First, we evaluated whether or not HPP is effective at reducing *E. coli* (surrogate and pathogens) populations and at which dwell time HPP is most effective for reducing populations of *Escherichia coli* species (determine the minimum dwell time needed to see significant reductions). Additionally we evaluated the differences between the cocktails of *E. coli* species to determine if sensitivity to pressure is uniform across *Escherichia coli* species or if it is specific to each type of pathogenic strain. Finally, as our main objective, we looked at whether or not HPP “by itself” is capable of achieving the minimum 5.0 log reduction requirement for the inactivation of *E. coli* in fermented dry and semi-dry sausages regulated by the USDA-FSIS (USDA 2001a; USDA 2001b). This was performed to determine if HPP could be used to adequately ensure regulations could be met if contamination events occurred after sausage had already been subjected to stressors and other interventions during processing. All of these objectives will be discussed in the following sections.

MATERIALS AND METHODS

Bacterial Cultures and Inoculum Preparation

Experimentation conducted on the effectiveness of high pressure processing without additional interventions was done utilizing three separate cocktails of *Escherichia coli* (*E. coli*) including generic *E. coli*, *E. coli* O157:H7, and Shiga Toxin Producing *E. coli* (STEC). Set 1: *E. coli* O157:H7 – 7 Meyer TR-4, 8 Meyer 70 30-4, 9 Meyer 70 30-5, 10 Meyer 91 9-4 and 11 Meyer 91 9-5. Set 2: Rifampicin resistant non-pathogenic *E. coli* (surrogate) – BAA-1427 P1, BAA-1428 P3, BAA-1429 P8, BAA-1430 P14, and BAA-1431 P68 (same as previous study) from Dr. Gary Acuff at Texas A&M University Center for Food Safety. Set 3: STEC from Dr. John B. Luchansky's library (acquired via Dr. Rodney Moxley) – *E. coli* O145:NM 83-75, O26:H11 H30, O45:H2 CDC 963285, O103:H2 CDC 903128, O111:H⁻ JB195, O121:H19 CDC 973068 (Table 3.1).

All organism stocks (20% glycerol in tryptic soy broth) were reactivated from the deep freezer -80°C storage by aseptically transferring a loop full (<10 µl) of organism to tryptic soy agar plates. Plates of organism were streak plated to isolation to confirm that they were not contaminated and were pure cultures for testing. Plates were incubated for 18-24 hours at ~35°C. After incubation, plates were evaluated for purity and a single isolate was picked for each organism strain and transferred to 9 ml of Tryptic Soy Broth (TSB, Becton Dickinson and Company, Sparks, MD, USA) then incubated for 18-24 hours at ~35°C – individual organism strains grown in separate tubes.

Inoculum was then prepared from reactivated organisms by adding 5 ml of each grown culture from the TSB tubes to a centrifuge tube creating a 25 ml cocktail (keeping cocktails separate). The cultures were then centrifuged using a Beckman Centrifuge CS-15R (Beckman Coulter, Pasadena, CA, USA) at 5,150 x g (7,000 rpm) for 15 minutes at 4°C. After centrifuging, the supernatant was removed and pellets were suspended in 25 ml sterile 0.1% peptone water (BACTO™, Becton Dickinson and Company, Sparks, MD, USA). Inoculum was made fresh for each replication and used within ~1 hour of preparation. This process was used for each of the three sets of organism strains being evaluated in HPP testing procedures.

Inoculum for each cocktail was tested using the following procedure. A sample of beef summer sausage inoculated with each cocktail (1 sample per cocktail) was pulled aside during sample preparation and placed into a stomacher bag – see fermented beef summer sausage sample preparation section for details on sample preparation. Each stomacher bag was then filled with 90 ml of 0.1% peptone water (BACTO™, Becton Dickinson and Company, Sparks, MD, USA) and samples were blended (Nuetec Masticator 1378/471, Nuetec Group Inc., Farmingdale, NY, USA) for 1 minute. Samples were then serially diluted in 0.1% sterile peptone water and plated on 3M Petrifilm™ (3M, St. Paul, MN, USA). Samples were plated for *E. coli* and Coliforms (ECC) and Aerobic Plate Counts (APC). 3M Petrifilm™ plates were incubated for approximately 48 hours at ~35°C – see summer sausage sample testing section for more details.

Table 3.1: *Escherichia Coli* Strains used in Inoculating Beef Summer Sausage for HPP Evaluation

<i>Escherichia Coli</i> Cocktail Sets		
<i>E. coli</i> TAMU ¹ Surrogate (Rif Resistant) – Non Pathogenic	<i>E. coli</i> O157:H7	Shiga Toxin Producing <i>E. coli</i> (STEC) ²
BAA-1427 P1	7 Meyer TR-4	O145:NM 83-75
BAA-1428 P3	8 Meyer 70 30-4	O26:H11 H30
BAA-1429 P8	9 Meyer 70 30-5	O45:H2 CDC 963285
BAA-1430 P14	10 Meyer 91 9-4	O103:H2 CDC903128
BAA-1431 P68	11 Meyer 91 9-5	O111:H ⁻ JB195
		O121:H19 CDC973068

¹Texas A&M University (TAMU) acquired from Dr. Gary Acuff at Texas A&M University Center for Food Safety. This cocktail was also used to evaluate the manufacturing portion of the study. ²Cultures are originally from Dr. John B. Luchansky's organism library but were acquired from Dr. Rodney Moxley at the University of Nebraska-Lincoln School of Veterinary Medicine and Biomedical Sciences.

Fermented Beef Summer Sausage Sample Preparation

Samples were prepared for high pressure processing utilizing the beef summer sausage remaining from the manufacturing process evaluation procedure (mentioned above). Remaining control, un-inoculated, beef summer sausage chubs were removed from the cooler for both low and high fat (8 and 17%) and transported to the meat processing room. It is important to note that only un-inoculated control samples from post chill were used to create the samples for evaluating the three cocktails of *Escherichia coli*. The sausage chubs were then cut into ~3-4 mm thick sausage disks using a meat slicer (Deli Meat Slicer, B350M, BIRO Manufacturing, Marblehead, OH, USA). Beef summer sausage needed to be sliced to at least 3 mm thickness to accommodate the inoculation procedure. After slicing, the summer sausage was then transferred to the laboratory for

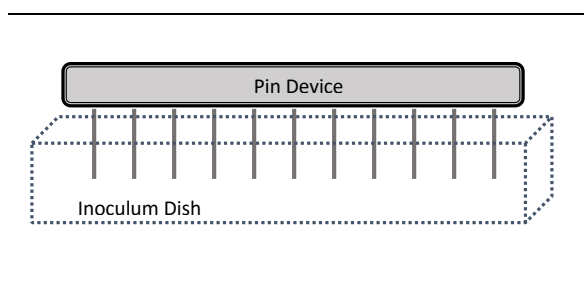
inoculating and packaging, making sure to keep the 8% low fat and the 17% high fat pieces separate from one another.

Next the beef summer sausage was inoculated using the following process. First the sausage was laid out in sterile petri dishes inside a biosafety cabinet with one sample per dish (a single ~3-4 mm thick round slice was considered 1 sample). The samples of summer sausage were then inoculated with the different cocktails of pathogens using a “Pin Inoculating System”. This system was utilized for inoculating the samples since it has been previously used with success in other laboratory procedures without cross contamination, and is very efficient for inoculating high volumes of individual samples with multiple cocktails. It is also useful in that a consistent amount of inoculum can be transferred to the sample surface, as well as the interior of sample, allowing us to evaluate the samples as if they were contaminated during production.

The Pin Inoculating System involves filling an autoclaved dish with the inoculum of choice for the inoculating procedure. Separate autoclaved dishes were used for each inoculum to reduce the possibility of cross contamination. Next the inoculating device, a block with multiple needles/pins pushed through it (Figure 3.1), was dipped into the dish containing the inoculum and then pressed into the flat exposed surface of the beef summer sausage sample (transferring ~16 μ L total to the sample) penetrating the surface and exposing the interior of the sample to the organism strains . The idea is to push the inoculum into the center of the sausage inoculating the interior of the sample as well as the surfaces touched by the pins to increase the inoculum coverage area of the sausage

samples. The pin inoculating device was cleaned in between each inoculum by soaking in 70% Ethanol to kill any remaining organisms left behind from the previous inoculum.

While still in the hood, the samples were aseptically transferred to vacuum sealable pouches (3 mil STD Barrier, Prime Source / Clarity, Buzl Processors Division of KOCH Supplies, London, England) and sealed using a vacuum sealer (Multivac A300/16 and C200, Multivac Inc., Kansas City, MO). The beef summer sausage samples were then vacuum sealed following the same procedure outlined in Chapter 2 of this thesis. After vacuum sealing, the beef summer sausage samples were placed back into the cooler at $< 4.4^{\circ}\text{C}$ until they were transported to the high pressure processing facility. Unlike the HPP samples from the manufacturing process these samples were held for a minimum of 14 days at $< 4.4^{\circ}\text{C}$ before HPP treatments were applied and testing continued. Fermented beef summer sausage samples were held for a minimum of 14 days in order to represent a worst case manufacturing scenario in industry. Typically, manufacturers do not have their own HPP machines and therefore would have to package the product and then ship it to a HPP facility where it would be held and then treated. The 14 days attempts to account for this time frame plus additional time for worst case situations.



*Figure 3.1: Pin Inoculating Set used for the inoculation of the beef summer sausage for the evaluation of different *E. coli* cocktails without additional hurdles (such as thermal processing) via HPP.*

High Pressure Processing Treatments without Hurdles

Samples that have been properly cut, inoculated with the appropriate cocktail of *Escherichia coli* and vacuum sealed (triple packaged) were transported on ice in marked biosafety containers to the University of Nebraska-Lincoln (UNL) HPP room in the Food Science and Technology Department (FDST). Samples were transported in this manner in order to keep the core temperature of the samples below 4.4°C during HPP processing. For high pressure processing purposes a dedicated BSL2 HPP machine (UNL-HPP, FPG9400:922, Stansted Fluid Power, Harlow, Essex, UK) was utilized for testing since pathogens are not allowed for testing in commercial facilities. There are no major differences in how the dedicated BSL2 machine and a commercial HPP machine function or in the effectiveness of the machines. There are some small differences however including the use of ethylene glycol instead of water for the processing fluid (which is discussed later in this section), and the size of the processing chamber being only 4” wide, but these differences do not affect the functionality of the machine or alter the process methodology.

Samples that were inoculated with different cocktails of organisms, at both fat levels (8 and 17%), which were prepared previously in vacuum sealed pouches, were subjected to HPP treatments at 586 MPa (85,000 psi) for dwell times of 1 second, 2, 4, and 6 minutes in the high pressure processing machine. Additionally control samples (for each fat and inoculum type) were also packaged that did not receive HPP treatment but were transported along with the other samples so they would be subjected to the same amount of stress as the samples receiving high pressure treatments. It is also important to note that the dwell time of 1 second is not necessarily 1 second due to the fact that the machine can take up to 3 seconds before switching off after reaching 85,000 psi (the HPP machine at University of Nebraska-Lincoln requires a minimum of 2 seconds at peak pressure before it may be shut off). In light of this the 1 second sample is considered the representative sample for the come-up of the machine to reach 85,000 psi (586 MPa) and samples were viewed in that manner.

All samples treated with high pressure processing were kept at $< 4.4^{\circ}\text{C}$ while waiting to receive treatment (HPP is not chilled therefore it is very important to keep the samples on ice packs until they can be processed). This procedure differs in the dwell times being evaluated from the commercial HPP samples evaluated during manufacturing testing in order to better cover the dwell times of the high pressure process and evaluate the range of the cocktails. These dwell time adjustments were based on the data acquired from the previous testing and changes were made accordingly to increase the value of this study. The UNL-HPP machine at UNL FDST utilizes ethylene glycol as its fluid medium for

high pressure treatments. The glycol was run at standard room temperatures of 25°C (not chilled) for the purpose of this experiment. Once samples were treated with HPP, they were placed back into the coolers and transported to the University of Nebraska-Lincoln Applied Microbiology Laboratory where they were placed into a cooler at < 4.4°C until they could be evaluated/tested.

Summer Sausage Sample Testing

Once the beef summer sausage samples were subjected to high pressure processing treatments, at various dwell times, they were then tested for the survival of the pathogens. The same process was used to evaluate the HPP samples from this experiment in comparison to the testing procedure for the HPP samples for manufacturing evaluation (Table 3.2). Each HPP sample (~3-4 mm thick) was aseptically removed from the vacuum sealed pouches and the entire sample was transferred to stomacher bags (Fisher Scientific, Fair lawn, NJ, USA). Ninety ml of 0.1% peptone water (BACTO™, Becton Dickinson and Company, Sparks, MD, USA) was added to the stomacher bag and samples were blended (Nuetec Masticator 1378/471, Nuetec Group Inc., Farmingdale, NY, USA) for 1 minute.

It is necessary to break up the sausage sample inside the stomaching bag to facilitate proper mixing during the stomaching process. In order to do this, a firm object (metal cell spreader) was pressed down on the beef summer sausage piece breaking it into smaller chunks. This was done through the stomacher bag while the sample was still inside the

stomacher bag to avoid unwanted contamination and the spread of pathogenic organisms. This process was continued until the sample was sufficiently broken apart to allow for proper stomaching of the sample.

The samples were serially diluted in 0.1% sterile peptone water and plated on 3M Petrifilm™ (3M, St. Paul, MN, USA). Samples were plated for *E. coli* and Coliforms (ECC) and Aerobic Plate Counts (APC). 3M Petrifilm™ plates were incubated for approximately 48 hours at ~35°C. Beef summer sausage inoculated samples were plated in duplicate on both ECC and APC 3M Petrifilm™ to verify accuracy of the results and techniques being used. All plates, for all cocktails of organisms, were enumerated and resulting data were recorded appropriately.

Table 3.2: Beef Summer Sausage Procedure for Evaluation of HPP Samples (Without Additional Hurdles)

1.	Aseptically remove entire (~3-4 mm thick) sample from vacuum pouch
a.	Place sample into a stomaching bag
2.	Add 90 ml of desired sterile fluid
a.	0.1% PEPW for all samples
3.	Stomach for 1 minute
4.	Dilute sample (serial dilutions 10^1 - 10^6)
a.	0.1% PEPW for all samples
5.	Plate appropriate dilutions
a.	3M Petrifilm™ APC, ECC
b.	Plate samples in duplicate
6.	Incubate at ~35°C
a.	3M Petrifilm™ (42-48 h)
7.	Enumerate Plates
8.	Record Results

RESULTS, DISCUSSION, AND CONCLUSIONS

Introduction

The following sections contain the bacterial results obtained from subjecting beef summer sausage to high pressure processing (HPP). Samples used for testing were preprocessed (manufactured) and then inoculated post manufacturing so that the organisms being evaluated would not be in a stressed state during HPP. Three separate cocktails of *Escherichia coli* (*E. coli*) including generic *E. coli* surrogates, *E. coli* O157:H7, and Shiga Toxin Producing *E. coli* (STEC) strains were chosen for testing including: Set 1, Non-pathogenic Rifampicin resistant *E. coli* (surrogates) – BAA-1427 P1, BAA-1428 P3, BAA-1429 P8, BAA-1430 P14, and BAA-1431 P68 from Dr. Gary Acuff's library at Texas A&M University Center for Food Safety; Set 2, *E. coli* O157:H7 – 7 Meyer TR-4, 8 Meyer 70 30-4, 9 Meyer 70 30-5, 10 Meyer 91 9-4, and 11 Meyer 91 9-5; Set 3, STEC from Dr. John B. Luchansky's library (acquired via Dr. Rodney Moxley) – *E. coli* O145: NM 83-75, O26:H11 H30, O45:H2 CDC 963285, O103:H2 CDC 903128, O111:H⁻ JB195, and O121:H19 CDC 973068. Samples were processed and then results were evaluated to determine the effectiveness of HPP on the inactivation of the different pathogenic strains of *E. coli*.

Two objectives were considered for these experiments. First, we evaluated the different strains of *E. coli*, whether they were non-pathogenic or pathogenic, to see if they were inactivated at the same rate via high pressure processing. This was to validate use of the non-pathogenic *E. coli* strains as a model (surrogate) to predict the inactivation of

pathogenic *Escherichia coli* during HPP. The second and main priority of this test process was to evaluate the effectiveness of HPP as a post processing cross contamination control procedure in the inactivation of *E. coli* and determine if HPP has the potential to be used in this manner to meet the USDA-FSIS requirements of a 5.0 log reduction of *E. coli* in fermented sausage products (USDA 2001b). This evaluation process will help to determine if HPP is adequate in meeting the regulations so that it might be used to ensure food safety after products have already been subjected to other processing measures.

High Pressure Processing Results for Non-Pathogenic *E. coli*, STEC, and *E. coli*

O157:H7 Cocktails

Fermented beef summer sausage inoculated with three different cocktails of *Escherichia coli*, nonpathogenic Rifampicin resistant *E. coli* (Sur), *E. coli* O157:H7 (O157), and shiga toxin producing *E. coli* (STEC), were evaluated via high pressure processing at 586 MPa with dwell times of 0 seconds (control), 1 second, 2, 4, and 6 minutes. Summer sausage was inoculated post manufacturing so that the *Escherichia coli* species were not stressed from other interventions within the manufacturing process.

Initial average populations of each inoculum on the sausage samples were: STEC – Aerobic Plate Counts (APC) 6.74 log CFU/g and *E. Coli* Coliform counts (ECC) 6.63 log CFU/g, *E. coli* O157:H7 – APC 6.78 log CFU/g and ECC 6.73 log CFU/g, and non-

pathogenic Rifampicin resistant *E. coli* (surrogate) – APC 6.06 log CFU/g and ECC 6.22 log CFU/g.

Initial average APC for STEC cocktail samples of 5.8 (low fat) and 5.9 (high fat) log CFU/g were obtained in the control samples (Table 3.3, Figure 3.2, and Figure 3.3). Reductions of *E. coli* of 1.2, 1.8, 3.2, and 4.0 log CFU/g, and 1.8, 2.4, 3.2, and 3.1 log CFU/g were obtained subsequent to 1 sec, 2, 4, and 6 minutes of dwell time at 586 MPa, in low fat (8%) and high fat (17%) summer sausages, respectively (Table 3.4, Figure 3.4, and Figure 3.7).

Initial average APC for nonpathogenic Rifampicin resistant *E. coli* (Surrogate (Sur)) cocktail samples of 5.8 (low fat) and 5.7 (high fat) log CFU/g were obtained in the control samples (Table 3.3, Figure 3.2, and Figure 3.3). Reductions of *E. coli* of 2.3, 1.9, 2.7, and 2.8 log CFU/g, and 1.9, 2.1, 2.7, and 2.1 log CFU/g were obtained subsequent to 1 sec, 2, 4, and 6 minutes of dwell time at 596 MPa, in low fat (8%) and high fat (17%) summer sausages, respectively (Table 3.4, Figure 3.4, and Figure 3.7).

Initial average APC populations for *E. coli* O157:H7 cocktail samples of 5.8 (low fat) and 5.9 (high fat) log CFU/g were obtained in the control samples (Table 3.3, Figure 3.2, and Figure 3.3). Reductions of *E. coli* of 1.1, 2.0, 2.4, and 2.1 log CFU/g, and 1.3, 2.2, 2.9, and 2.5 log CFU/g were obtained subsequent to 1 sec, 2, 4, and 6 minutes of dwell time at 586 MPa, in low fat (8%) and high fat (17%) summer sausages, respectively (Table 3.4, Figure 3.4, and Figure 3.7).

Initial average *E. Coli* Coliform counts (ECC) for STEC cocktail samples of 5.6 (low fat) and 5.7 (high fat) log CFU/g were obtained in the control samples (Table 3.5, Figure 3.2, and Figure 3.5). Reductions of *E. coli* of 3.5, 5.0, 5.3, and 5.3 log CFU/g, and 4.2, 4.9, 5.4, and 5.4 log CFU/g were obtained subsequent to 1 sec, 2, 4, and 6 minutes of dwell time at 586 MPa, in low fat (8%) and high fat (17%) summer sausages, respectively (Table 3.6, Figure 3.6, and Figure 3.7).

Initial average ECC for nonpathogenic Rifampicin resistant *E. coli* (Surrogate) cocktail samples of 5.5 (low fat) and 5.2 (high fat) log CFU/g were obtained in the control samples (Table 3.5, Figure 3.2, and Figure 3.5). Reductions of *E. coli* of 4.3 and ≥ 5.5 log CFU/g, and 4.6, 4.6, 4.8, and 4.8 log CFU/g were obtained subsequent to 1 second and 2 minutes in low fat (8%), and 1 second, 2, 4, and 6 minutes in high fat (17%) summer sausages at dwell time at 586 MPa, respectively (Table 3.6, Figure 3.6, and Figure 3.7).

Initial average ECC for *E. coli* O157:H7 cocktail samples of 5.7 (low fat) and 5.8 (high fat) log CFU/g were obtained in the control samples (Table 3.5, Figure 3.2, and Figure 3.5). Reductions of *E. coli* of 3.4, 5.2, 5.2, and 5.3 log CFU/g, and 2.9, 5.3, 5.1, and 5.2 log CFU/g were obtained subsequent to 1 sec, 2, 4, and 6 minutes of dwell time at 586 MPa, in low fat (8%) and high fat (17%) summer sausages, respectively (Table 3.6, Figure 3.6, and Figure 3.7).

Utilizing Statistical Analysis System (SAS) software (SAS Institute Inc., Cary, NC, USA), it was determined using analysis of variance of the General Linear Model

procedure that fat content (low or high fat) of the fermented beef summer sausage did not affect ($p>0.05$) the *E. coli* reductions during high pressure processing treatments for either aerobic plate count results (Table 3.7) or for *E. coli* coliform plate count results (Table 3.8). Fishers' Least Significant difference ($\alpha=0.05$) was used to separate means of each treatment.

Color change and other physical properties of the beef summer sausage were not monitored during testing. However, it is important to note that no notable color changes or visual product alterations were seen throughout testing the summer sausages at 586 MPa for dwell times evaluated.

Discussion

Escherichia coli has been a major problem for food manufacturers for 30+ years infiltrating food products with the ability to survive in multiple environments and causing foodborne disease in human beings (Rangel et al., 2005). Current manufacturing standards for fermented beef summer sausages involve the use of many "hurdles" or interventions throughout the production process to help ensure the safety of these products from potentially deadly foodborne pathogens including temperature control, water activity, pH adjustment ≤ 5.0 , addition of competing cultures or salts, and thermal processing, etc. (McQuestin, Shadbolt, and Ross 2009; Incze 1998; Leroy, Verluyten, and De Vuyst 2006; USDA 1999a; Garriga et al., 2005; Comi et al., 2005). Despite the use of these hurdles there are still outbreaks of *E. coli* O157:H7 and the other "Big Six" shiga toxin producing *E. coli* being reported. Fermented sausages are no exception with

outbreaks occurring worldwide in countries including the United States, Canada, Australia, and Sweden (Burros 1995; Williams et al., 2000; Ross and Shadbolt 2000; Sartz et al., 2008).

However, thermal processing, when used appropriately (Stringer, George, and Peck 2000) has been shown to be effective in the eradication of foodborne pathogens like *E. coli*. Literature has shown in beef and beef products like fermented sausages that *E. coli* can be controlled. A study performed looking at the inactivation of *E. coli* species in single and double cubed beef steak reported that cooking at 191.5°C on a griddle for at least 1.25 and 3.0 minutes for single cubed steak and double cubed steak, respectively would achieve greater than 5.0 logs of reduction in pathogen reaching levels up to ≥ 6.8 log CFU/g (Swartz et al., 2015). Another study showed that heating fermented sausages that had been pH adjusted to pH 5.0 and 4.6 to an internal temperature of 54°C that up to 4.58 and ≥ 7 log CFU/g reductions of *E. coli* could be obtained, respectively (Calicioglu et al., 1997). This difference in these obtainable reductions is most likely due to the expression of the *rpoS* gene within the *E. coli* strains cells increasing their acid tolerance levels and allowing their survival in the less acidic environment at pH 5.0 as mentioned in the previous chapter (Small et al., 1994; Riordan et al., 2000).

Although thermal processing can be effective in controlling *Escherichia coli* species, it has the potential to damage the physiochemical properties of the sausages making them undesirable to a consumer. Ferrini et al., 2014 showed that the cooked appearance (color), fat odor, flavor and texture all increased or were changed during thermal

processing as the length of processing increased (Ferrini et al., 2014). Therefore, increasing the time and/or temperature of the thermal processing step would not result in a desirable product and would not be recommended and other options should be explored to ensure food safety while maintaining the quality of the product.

In this thesis high pressure processing (HPP) was studied for the purpose of utilizing it as a post processing cross contamination control measure and as a potential alternative intervention to thermal processing. HPP has been evaluated for use in beef and beef products such as fermented sausages for quality and for the inactivation of *Escherichia coli* species and other foodborne pathogens such as *Salmonella enterica* and *Listeria monocytogenes* (Marcos, Aymerich, and Garriga 2005) giving a range in results for inactivation and for quality. It has been reported in literature that HPP can affect the physiochemical properties of food such as texture, structure, and color as well as reversibly modifying the physiochemical properties of water within the food products by decreasing the water volume by 4, 12, and 15% at 100, 400, and 600 MPa, respectively (Cheftel and Culioli 1997).

In a study looking at the use of HPP in the low acid fermented sausages, Chorizo and Fuet, it was reported that treating the sausage at 300 MPa for 10 minutes at 17°C caused a noticeable change in the color of the sausage causing it to look pale pink in Fuet and a duller overall appearance in Chorizo (Marcos, Aymerich, and Garriga 2005). Lowder, Waite-Cusic, and Mireles DeWitt 2014 reported that in beef that had been subjected to HPP at 551 MPa for 4 minutes at 3°C that color change with a loss of redness was seen,

amount of loss (purge) during cooking increased, and that the tenderness of the cook beef decreased compared to beef that was not treated by HPP (Lowder, Waite-Cusic, and Mireles DeWitt 2014). In this study, there were no notable color changes in the fermented beef summer sausage post HPP.

This attribute of high pressure processing was also seen in a study performed by Mor-Mur and Yuste 2003 in which cooked sausages were subjected to HPP at 500 MPa for 5 and 15 minutes at 65°C and then sensory was performed on the sausages in comparison with sausage manufactured using thermal processing at 80-85°C for 40 minutes. They found that although the texture of the pressure treated sausage was less firm, that the color was unchanged, and that the sensory panel did not detect a flavor difference between the two sausages, actually showing a preference for the high pressure processed sausage over the thermally processed sausage, and concluding that HPP could be used as an alternative to thermal processing for cooked sausages (Mor-Mur and Yuste 2003).

One study looking at the inactivation of *Escherichia coli* species in ground beef found at moderate high pressure that *E. coli* O157:H7 and the other “Big Six” STEC (O26, O45, O103, O111, O121, and O145) were reduced by ≥ 5 log CFU/g at 450 MPa for 15 minutes at refrigerated temperatures and also reported that in general the “Big Six” STEC were more sensitive to HPP than *E. coli* O157:H7 concluding that HPP standards for *E. coli* O157:H7 could be used for the STEC (Hsu et al., 2015). In another similar study it was reported that *E. coli* O157:H7 and non-O157:H7 STEC (O26, O45, O103, O111, O121, and O145) when treated for HPP at 400 MPa for 4 (60 second) cycles at 17°C, that

E. coli species populations were reduced by up to 3.88 and 4.31 log CFU/g in high fat (80/20) and low fat (90/10) beef patties, respectively (Jiang et al., 2015).

In a study more similar to the one performed in this thesis, Omer et al. (2010) reported that Norwegian dry fermented sausages inoculated with *Escherichia coli* O103:H25 with samples treated by HPP at 600 MPa for 10 minutes and for three cycles of 200 seconds per cycle, gave reductions of 2.9 and 3.3 log CFU/g respectively (Omer et al., 2010). In comparison this study treated fermented beef summer sausage at 586 MPa for 6 minutes at ~7°C yielded log reductions for nonpathogenic *E. coli* surrogate, shiga toxin producing *E. coli*, and *E. coli* O157:H7 cocktails of ≥ 5.5 and 4.8, 5.3 and 5.4, and 5.3 and 5.2 log CFU/g in low fat (8%) and high fat (17%) beef summer sausage, respectively. Although Omer et al. (2010) reported log reductions were lower, it is important to note that they were using a specific strain of *E. coli*, *E. coli* O103:H25, for their testing which was not included in our cocktail and could potentially be more resistant than the other strains. Additionally, only ECC results are discussed above for this portion of the study as ECC results are more relevant since Rifampicin was not used for testing cocktails and background flora may be present that is potentially pressure resistant altering the final counts.

In another similar study it was reported that additional reductions (post production) of *Listeria monocytogenes*, *E. coli* O157:H7, and *Salmonella spp.* in Genoa salami HPP treated at 600 and 483 MPa for 1 to 12 minutes were achieved of 1.6 to >5.0, 4.7 to >5.8,

and 1.9 to 2.4 log CFU/g, respectively (Porto-Fett et al., 2010) with the *E. coli* O157:H7 reductions being more in line with the log reductions we received from the study in the previous chapter of this thesis.

Statistical analysis of the data (see Tables 3.7 and 3.8) in this study shows that at the shortest dwell time of 1 second the nonpathogenic Rifampicin resistant *E. coli* (surrogate) cocktail was significantly different in log reduction of *Escherichia coli* at $P > 0.05$ when compared to either the STEC cocktail or the *E. coli* O157:H7 cocktail for high fat product. The pathogenic cocktails, STEC and *E. coli* O157:H7, were not significantly different from each other at this same dwell time. Additionally, the low fat Rifampicin resistant *E. coli* cocktail was significantly different at $P > 0.05$ from the STEC and *E. coli* O157:H7 cocktails at 1 second dwell time as well, but was not significantly different from the high fat Rifampicin resistant *E. coli* or high fat STEC cocktails.

To compare the log reductions, the nonpathogenic Rifampicin resistant *E. coli* (surrogate) cocktail gave 4.3 (low fat) and 4.6 (high fat) log CFU/g reductions at 1 second of dwell time at 586 MPa. In comparison the STEC and *E. coli* O157:H7 cocktails gave 3.5 and 3.4 (low fat) and 4.2 and 2.9 (high fat) log CFU/g reductions in beef summer sausage. It is important to note that the *E. coli* O157:H7 cocktail high fat count at 1 second was significantly different ($p > 0.05$) from the STEC cocktail high fat count at 1 second but was not significantly different from the STEC or *E. coli* O157:H7 low fat counts.

This however does not hold true at longer dwell times of 2, 4, and 6 minutes where all cocktails are considered to be the same statistically ($p>0.05$) and with log reductions of *E. coli* species being very similar. For instance looking at the ECC data for log reduction of *E. coli* species (all cocktails) at 6 minutes of dwell time at 586 MPa, we can see that the log reductions are 5.3, 5.5, and 5.3 log CFU/g for low fat (8%), and 5.4, 4.8, and 5.2 log CFU/g for high fat (17%) beef summer sausage in STEC, Rifampicin resistant *E. coli*, and *E. coli* O157:H7 cocktails, respectively. These reductions are significantly similar at the $P>0.05$ level.

Therefore it can be determined that, based on the statistical analysis and total log reductions, that nonpathogenic Rifampicin resistant *E. coli* (surrogate) cocktail should not be utilized as a surrogate for pathogenic *Escherichia coli* strains at short dwell times for high pressure processing at 586 MPa. The Surrogate cocktail results are not conservative enough to accurately depict the true reductions that are being seen with the pathogenic strains of shiga toxin producing *E. coli* species without further comparison and research. However, the nonpathogenic Rifampicin resistant strains of *Escherichia coli* have the potential to be utilized as suitable surrogates for testing pathogenic strains at longer dwell times exceeding 2 minutes of HPP at 586 MPa. If more research is completed it is plausible to conclude that the Rifampicin resistant strains of *Escherichia coli* might be suitable for use as a surrogate cocktail in evaluating HPP.

Comparison of Stressed and Non-Stressed HPP Results

The data from this study shows that high pressure processing is more effective on stressed cells than on unstressed cells. For instance, in the first study in this thesis inoculated fermented beef summer sausage chubs were subjected to the entire manufacturing process and the interventions that were within that process including pH adjustment, temperature control, etc. The sausage chubs were subjected to high pressure processing in place of the thermal processing stage of production. This process resulted in log reductions of *Escherichia coli* species (nonpathogenic Rifampicin resistant surrogate) post chilling of ≥ 2.5 and ≥ 2.7 log CFU/g in low fat (8%) and high fat (17%) beef summer sausage respectively, at just 1 second of dwell time of HPP at 586 MPa which constituted total reduction of the pathogen. In comparison, this study, taking preprocessed fermented beef summer sausage and inoculating it post processing to bypass the added stressors/interventions of the manufacturing process, resulted in log reductions of *Escherichia coli* species from a nonpathogenic Rifampicin resistant surrogate cocktail of 4.3 and ≥ 5.6 (low fat) and 4.6, 4.6, 4.8, and 4.8 (high fat) log CFU/g at 1 second and 2 minutes (low fat 8%) and 1 second, 2, 4, and 6 minutes (high fat 17%) in beef summer sausage for HPP at 586 MPa.

As data shows that the non-stressed organism of nonpathogenic Rifampicin resistant surrogate cocktail survived longer than their stressed counterparts. In fact, in high fat product for the non-stressed organism they were not completely inactivated with approximately 0.3 log CFU/g surviving at 6 minutes of dwell time at 586 MPa whereas

the stressed organisms in high fat product were eradicated after just 1 second of high pressure processing at 586 MPa as mentioned previously.

Conclusions

In conclusion, the data suggests that the three separate cocktails of *Escherichia coli* species including nonpathogenic Rifampicin resistant *E. coli*, shiga toxin producing *E. coli*, and *E. coli* O157:H7 cocktails, are similar in the inactivation of pathogens seen when subjected to high pressure processing at 586 MPa of dwell times 2, 4, and 6 minutes in fermented beef summer sausages. It also suggests that while STEC and *E. coli* O157:H7 cocktails are statistically the same ($p>0.05$) that the nonpathogenic surrogate cocktail is not similar to the pathogenic *Escherichia coli* species cocktails at lower dwell times below 2 minutes. However, based on the results from the higher dwell times, it is plausible that with more data the Rifampicin resistant strains of *Escherichia coli* would become significantly similar at lower dwell times making them a suitable surrogate for the pathogenic strains.

Based on this data it would not be recommended to utilize the surrogate cocktail in place of pathogenic strains of shiga toxin producing *Escherichia coli* until further testing can be done to verify if the nonpathogenic strains react differently at lower dwell times or if they are in fact the same as they currently would not accurately predict the survival of those pathogens. However, the nonpathogenic Rifampicin resistant strains of *Escherichia coli* could be utilized as suitable surrogates for testing pathogenic strains in fermented beef

summer sausage at longer dwell times exceeding 2 minutes of HPP at 586 MPa as mentioned above.

High pressure processing has the potential to be utilized as a post processing contamination control procedure for the eradication of foodborne pathogens such as *E. coli*. However, as mentioned previously, HPP is not nearly as effective at inactivating *E. coli* species in fermented beef summer sausages when the organisms have not been stressed via some other intervention such as pH, temperature control, water activity, etc. That being said, when looking at ECC data from the high pressure treatments, it can be seen that a 5.0 log reduction of pathogen is potentially obtainable at 586 MPa between 4-6 minutes of dwell time without other stressors. Therefore, if longer dwell times are utilized, HPP could potentially be used as a standalone lethality treatment to meet the USDA-FSIS guidelines of a 5.0 log reduction of *Escherichia coli* in fermented meat products (USDA 2001a; USDA 2001b). Because of this HPP can be used as an additional intervention within the manufacturing process of fermented beef summer sausage by manufacturers to help control contamination events and further ensure the safety of fermented beef summer sausages.

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Tables of Results for High Pressure Processing Procedure

Dwell Time	STEC				Surrogate				<i>E. coli</i> O157:H7			
	Low Fat		High Fat		Low Fat		High Fat		Low Fat		High Fat	
	Count	STDEV	Count	STDEV	Count	STDEV	Count	STDEV	Count	STDEV	Count	STDEV
Control	5.75	0.06	5.87	0.14	5.76	0.10	5.67	0.25	5.75	0.17	5.93	0.08
1 second	4.52	0.52	4.02	0.57	3.49	0.65	3.80	0.17	4.70	0.07	4.64	0.12
2 minute	3.98	0.56	3.49	0.42	3.82	0.39	3.58	0.24	3.76	0.07	3.72	0.20
4 minute	2.51	0.60	2.68	0.35	3.04	0.64	3.00	0.78	3.40	0.43	3.03	1.06
6 minute	1.73	0.67	2.79	0.12	2.94	0.87	3.59	0.33	3.63	0.39	3.39	0.76

Table 3.3: Average Aerobic Plate Counts Log CFU/g for HPP of Fermented Beef Summer Sausages. Table contains the average aerobic plate counts (APC), from testing fermented beef summer sausage at five different dwell times of high pressure processing (HPP) at 586 MPa at standard temperatures inoculated with three separate cocktails of *E. coli* species including; shiga toxin producing *E. coli* (STEC), non-pathogenic Rifampicin resistant *E. coli* Surrogate (Surrogate), and *E. coli* O157:H7 cocktails. Dwell times evaluated were: Control (no HPP), 1 second, 2, 4, and 6 minutes for the fermented beef summer sausage. Both low fat (8%) and high fat (17%) results are displayed. All results are average APC counts displayed in log CFU/g with standard deviations (STDEV).

STEC				Surrogate				<i>E. coli</i> O157:H7			
		Low Fat		High Fat		Low Fat		High Fat		Low Fat	
Dwell Time	Count	STDEV	Count	STDEV	Count	STDEV	Count	STDEV	Count	STDEV	Count
		Count	STDEV	Count	STDEV	Count	STDEV	Count	STDEV	Count	STDEV
1 second	1.23	0.57	1.84	0.67	2.27	0.58	1.87	0.24	1.05	0.11	1.29
2 minute	1.76	0.62	2.38	0.36	1.94	0.33	2.09	0.31	1.99	0.11	2.22
4 minute	3.24	0.56	3.19	0.43	2.72	0.72	2.67	0.67	2.35	0.60	2.90
6 minute	4.01	0.68	3.07	0.03	2.82	0.79	2.08	0.55	2.12	0.40	2.54

Table 3.4: Average Aerobic Plate Count Log Reductions Results Log CFU/g for HPP of Fermented Beef Summer Sausages. Table contains the average log reductions for aerobic plate counts (APC), from testing fermented beef summer sausage at five different dwell times of high pressure processing (HPP) at 586 MPa at standard temperatures inoculated with three separate cocktails of *E. coli* species including; shiga toxin producing *E. coli* (STEC), non-pathogenic Rifampicin resistant *E. coli* Surrogate (Surrogate), and *E. coli* O157:H7 cocktails. Dwell times evaluated were: 1 second, 2, 4, and 6 minutes for the fermented beef summer sausage. Both low fat (8%) and high fat (17%) results are displayed. All log reduction results are average APC counts displayed in log CFU/g with standard deviations (STDEV).

Dwell Time	STEC				Surrogate				<i>E. coli</i> O157:H7			
	Low Fat		High Fat		Low Fat		High Fat		Low Fat		High Fat	
	Count	STDEV	Count	STDEV	Count	STDEV	Count	STDEV	Count	STDEV	Count	STDEV
Control	5.56	0.02	5.74	0.17	5.46	0.54	5.15	0.23	5.66	0.19	5.77	0.23
1 second	2.08	0.61	1.56	0.36	1.15	0.88	0.56	0.37	2.30	0.77	2.84	0.92
2 minute	0.61	0.46	0.80	0.72	0.00	0.00	0.59	0.36	0.51	0.53	0.49	0.47
4 minute	0.29	0.51	0.36	0.48	0.00	0.00	0.37	0.63	0.47	0.46	0.63	0.40
6 minute	0.32	0.55	0.35	0.46	0.00	0.00	0.33	0.31	0.37	0.63	0.60	0.52

Table 3.5: Average *E. coli* Coliform Counts Log CFU/g for HPP of Fermented Beef Summer Sausages. Table contains the average *E. coli* coliform counts (ECC), from testing fermented beef summer sausage at five different dwell times of high pressure processing (HPP) at 586 MPa at standard temperatures inoculated with three separate cocktails of *E. coli* species including; shiga toxin producing *E. coli* (STEC), non-pathogenic Rifampicin resistant *E. coli* Surrogate (Surrogate), and *E. coli* O157:H7 cocktails. Dwell times evaluated were: Control (no HPP), 1 second, 2, 4, and 6 minutes for the fermented beef summer sausage. Both low fat (8%) and high fat (17%) results are displayed. All results are average ECC counts displayed in log CFU/g with standard deviations (STDEV).

Dwell Time	STEC				Surrogate				<i>E. coli</i> O157:H7			
	Low Fat		High Fat		Low Fat		High Fat		Low Fat		High Fat	
	Count	STDEV	Count	STDEV	Count	STDEV	Count	STDEV	Count	STDEV	Count	STDEV
1 second	3.48	0.63	4.19	0.28	4.31	0.71	4.59	0.42	3.36	0.67	2.93	0.72
2 minute	4.95	0.46	4.94	0.56	5.46	0.54	4.56	0.47	5.15	0.35	5.28	0.57
4 minute	5.27	0.52	5.38	0.40	5.46	0.54	4.78	0.48	5.19	0.28	5.14	0.19
6 minute	5.24	0.57	5.39	0.38	5.46	0.54	4.82	0.19	5.29	0.46	5.17	0.30

Table 3.6: Average *E. coli* Coliform Log Reduction Results Log CFU/g for HPP of Fermented Beef Summer Sausages. Table contains the average log reductions for *E. coli* coliform counts (ECC), from testing fermented beef summer sausage at five different dwell times of high pressure processing (HPP) at 586 MPa at standard temperatures inoculated with three separate cocktails of *E. coli* species including; shiga toxin producing *E. coli* (STEC), non-pathogenic Rifampicin resistant *E. coli* Surrogate (Surrogate), and *E. coli* O157:H7 cocktails. Dwell times evaluated were: 1 second, 2, 4, and 6 minutes for the fermented beef summer sausage. Both low fat (8%) and high fat (17%) results are displayed. All log reduction results are average ECC counts displayed in log CFU/g with standard deviations (STDEV).

		HPP-0	HPP-1s	HPP-2m	HPP-4m	HPP-6m
High Fat	Sur	5.670000 ^A	3.803333 ^{CDE}	3.576666 ^{7DEFG}	2.996666 ^{7FGHIJ}	3.593333 ^{3DEFG}
Low Fat	Sur	5.763333 ^A	3.493333 ^{3DEFGH}	3.820000 ^{CD}	3.040000 ^{0EFGHIJ}	2.943333 ^{3GHIJ}
High Fat	STEC	5.866666 ^{7A}	4.020000 ^{0BCD}	3.490000 ^{0DEFGH}	2.676666 ^{7IJ}	2.793333 ^{3HIJ}
Low Fat	STEC	5.746666 ^{7A}	4.520000 ^{0BC}	3.983333 ^{3BCD}	2.510000 ^{0JK}	1.733333 ^{33K}
High Fat	O157H7	5.930000 ^{0A}	4.640000 ^{0B}	3.713333 ^{3DEFG}	3.030000 ^{0EFGHIJ}	3.386666 ^{7DEFGHI}
Low Fat	O157H7	5.753333 ^{3A}	4.703333 ^{3B}	3.760000 ^{0CDEF}	3.403333 ^{3DEFGHI}	3.626666 ^{7DEFG}

Table 3.7: Comparison of Low Fat and High Fat Aerobic Plate Counts for Fermented Beef Summer Sausage by General Linear Model. Table contains the least squares means (LSM) for aerobic plate counts (APC) obtained from treating fermented beef summer sausage with high pressure processing (HPP) at five different dwell times at 586 MPa at standard temperatures. Dwell times used were: control-no HPP (HPP-0), 1 second (HPP-1s), 2 (HPP-2m), 4 (HPP-4m), and 6 (HPP-6m) minutes. *E. coli* species evaluated included: shiga toxin producing *E. coli* (STEC), non-pathogenic Rifampicin resistant *E. coli* Surrogate (Sur), and *E. coli* O157:H7 (O157H7) cocktails. The low fat (8%) and high fat (17%) results are both displayed. Superscripted letters denote the similarity between the different fat contents and the significance between different counts obtained in fat levels at different stages. Significance of the differences are calculated at the $P>0.05$ level.

		HPP-0	HPP-1s	HPP-2m	HPP-4m	HPP-6m
High Fat	Sur	5.14666667 ^A	0.56000000 ^{EF}	0.59000000 ^{EF}	0.36666667 ^{EF}	0.33333333 ^F
Low Fat	Sur	5.46000000 ^A	1.15333333 ^{DE}	0.00000000 ^F	0.00000000 ^F	-0.00000000 ^F
High Fat	STEC	5.74333333 ^A	1.55666667 ^{CD}	0.80000000 ^{DEF}	0.36000000 ^{EF}	0.35333333 ^{EF}
Low Fat	STEC	5.56333333 ^A	2.07333333 ^{BC}	0.60666667 ^{EF}	0.29333333 ^F	0.31666667 ^F
High Fat	O157H7	5.77000000 ^A	2.84000000 ^B	0.49000000 ^{EF}	0.63000000 ^{EF}	0.60333333 ^{EF}
Low Fat	O157H7	5.66000000 ^A	2.30000000 ^{BC}	0.51333333 ^{EF}	0.47000000 ^{EF}	0.36666667 ^{EF}

Table 3.8: Comparison of Low Fat and High Fat *E. coli* Coliform Counts for Fermented Beef Summer Sausage by General Linear Model. Table contains the least squares means (LSM) for *E. coli* coliform counts (ECC) obtained from treating fermented beef summer sausage with high pressure processing (HPP) at five different dwell times at 586 MPa at standard temperatures. Dwell times used were: control-no HPP (HPP-0), 1 second (HPP-1s), 2 (HPP-2m), 4 (HPP-4m), and 6 (HPP-6m) minutes. *E. coli* species evaluated included: shiga toxin producing *E. coli* (STEC), non-pathogenic Rifampicin resistant *E. coli* Surrogate (Sur), and *E. coli* O157:H7 (O157H7) cocktails. The low fat (8%) and high fat (17%) results are both displayed. Superscripted letters denote the similarity between the different fat contents and the significance between different counts obtained in fat levels at different stages. Significance of the differences are calculated at the P>0.05 level.

Figures of Results for High Pressure Processing Procedure

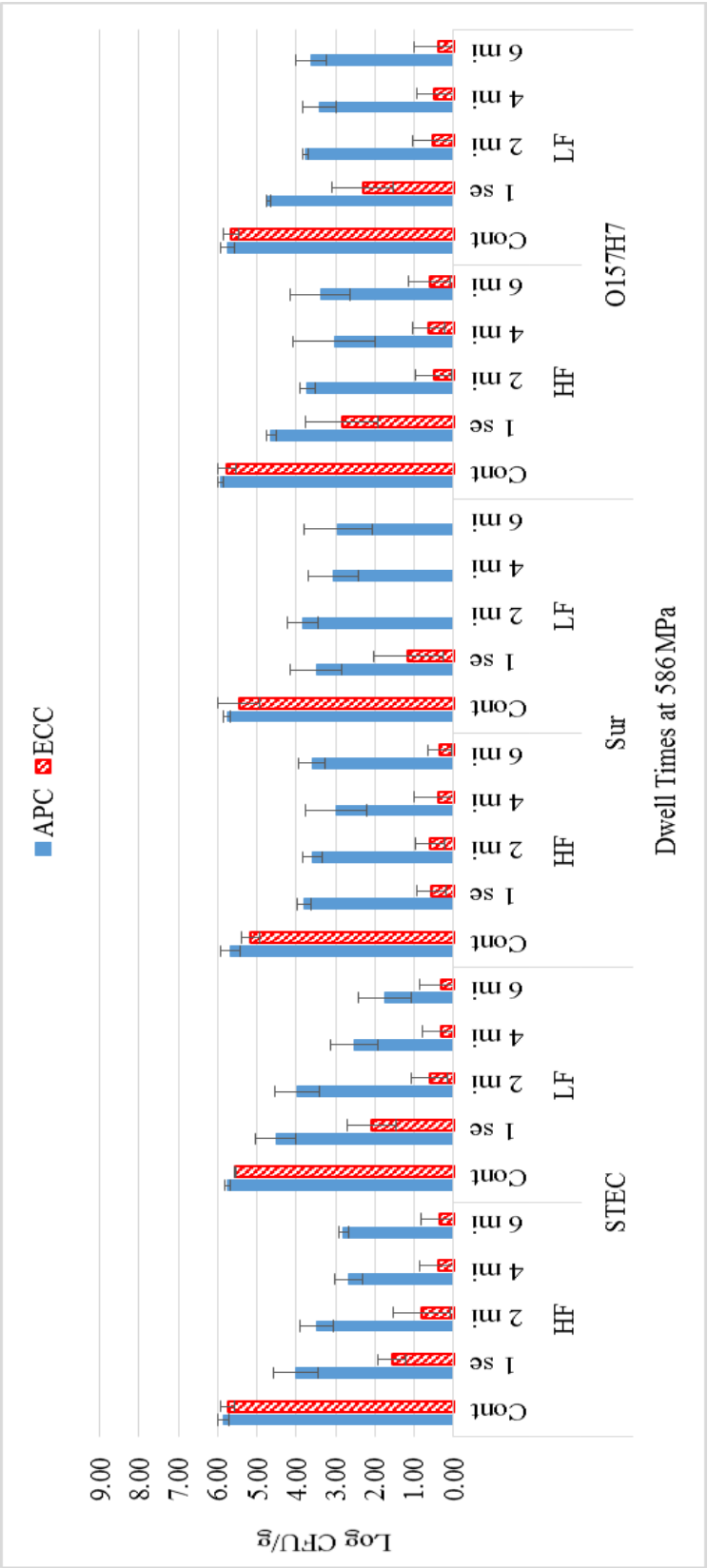


Figure 3.2: Average Counts Log CFU/g for High Pressure Processing of Fermented Beef Summer Sausages. Figure displays the average aerobic plate counts (APC) and average *E. coli* coliform counts (ECC), from testing fermented beef summer sausage at five different dwell times of high pressure processing (HPP) at 586 MPa at standard temperatures inoculated with three separate cocktails of *E. coli* species including; shiga toxin producing *E. coli* (STEC), non-pathogenic Rifampicin resistant *E. coli* Surrogate (Sur), and *E. coli* O157:H7 (O157H7) cocktails. Dwell times evaluated were: control-no HPP (Cont), 1 second (se), 2, 4, and 6 minutes (mi) for the fermented beef summer sausage. Both low fat (LF, 8%) and high fat (HF, 17%) results are displayed. All results are average bacterial counts displayed in log CFU/g.

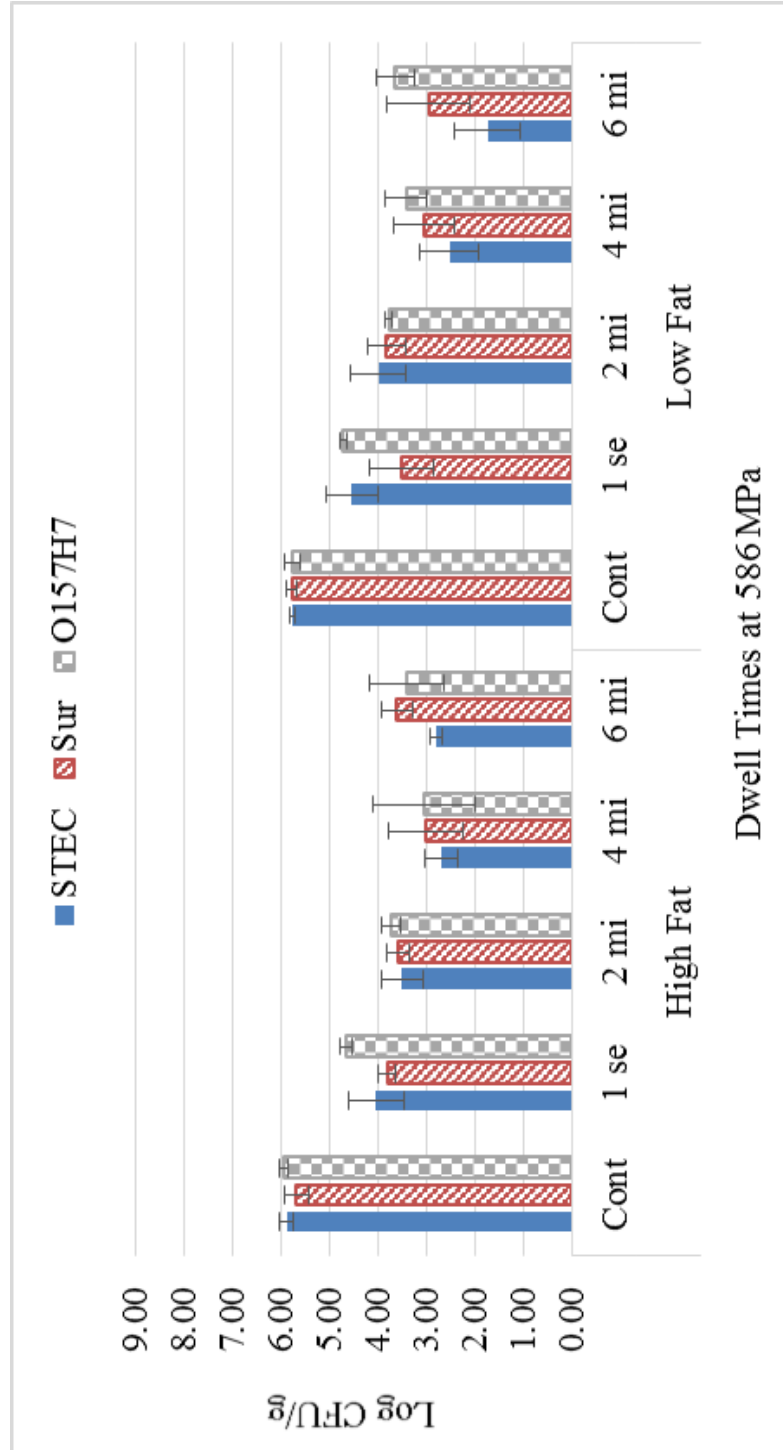


Figure 3.3: Average Aerobic Plate Counts Log CFU/g for High Pressure Processing of Fermented Beef Summer Sausages. Figure displays the average aerobic plate counts (APC) from testing fermented beef summer sausage at five different dwell times of high pressure processing (HPP) at 586 MPa at standard temperatures inoculated with three separate cocktails of *E. coli* species including; shiga toxin producing *E. coli* (STEC), non-pathogenic Rifampicin resistant *E. coli* Surrogate (Sur), and *E. coli* O157:H7 (O157H7) cocktails. Dwell times evaluated were: control-no HPP (Cont), 1 second (se), 2, 4, and 6 minutes (mi) for the fermented beef summer sausage. Both low fat (LF, 8%) and high fat (HF, 17%) results are displayed. All results are average bacterial counts displayed in log CFU/g.

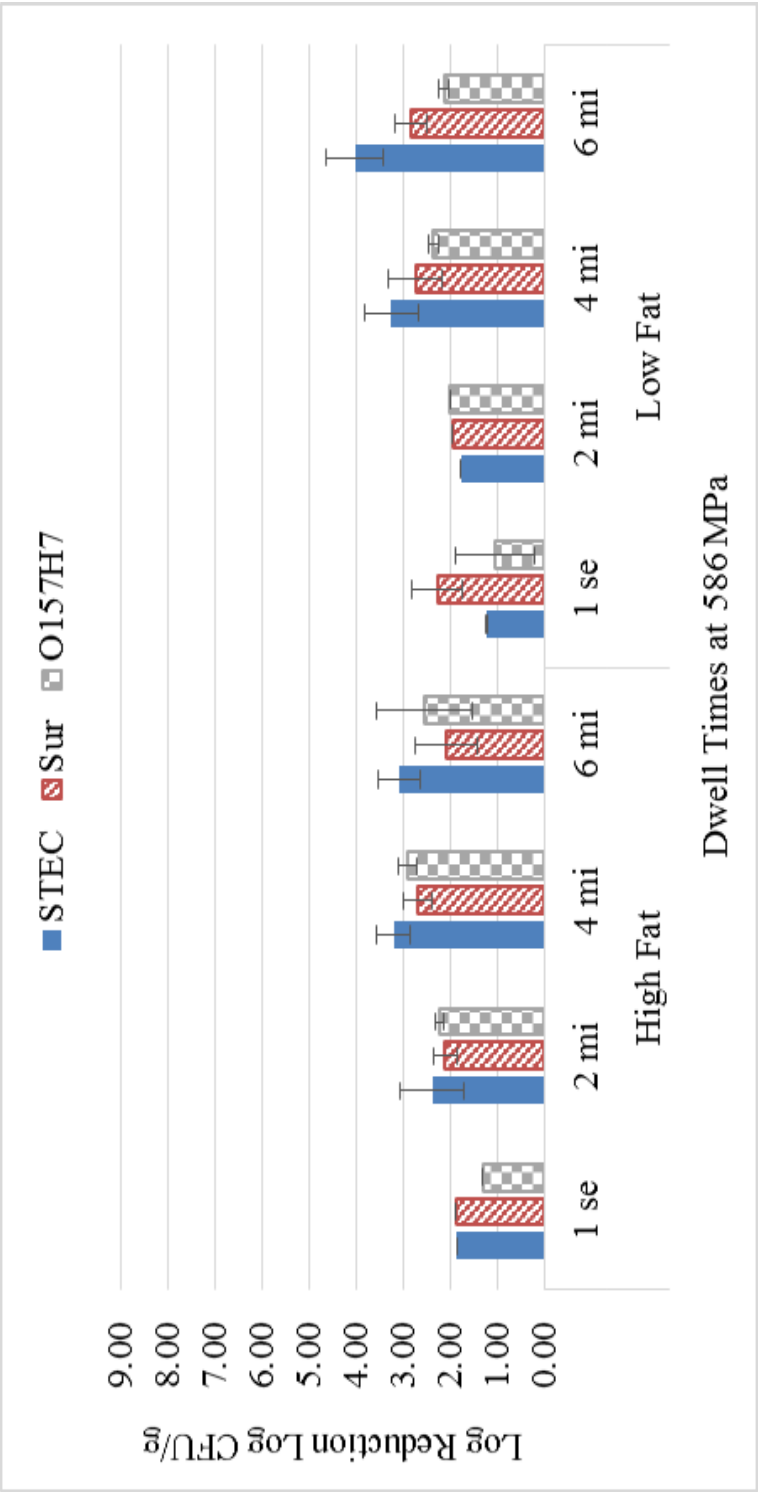


Figure 3.4: Average Log Reduction APC Log CFU/g for High Pressure Processing of Fermented Beef Summer Sausages. Figure displays the average log reduction for aerobic plate counts (APC) from testing fermented beef summer sausage at 4 different dwell times of high pressure processing (HPP) at 586 MPa at standard temperatures inoculated with three separate cocktails of *E. coli* species including; shiga toxin producing *E. coli* (STEC), non-pathogenic Rifampicin resistant *E. coli* Surrogate (Sur), and *E. coli* O157:H7 (O157H7) cocktails. Dwell times evaluated were: 1 second (se), 2, 4, and 6 minutes (mi) for the fermented beef summer sausage. Both low fat (LF, 8%) and high fat (HF, 17%) results are displayed. All results are average log reduction bacterial counts displayed in log CFU/g.

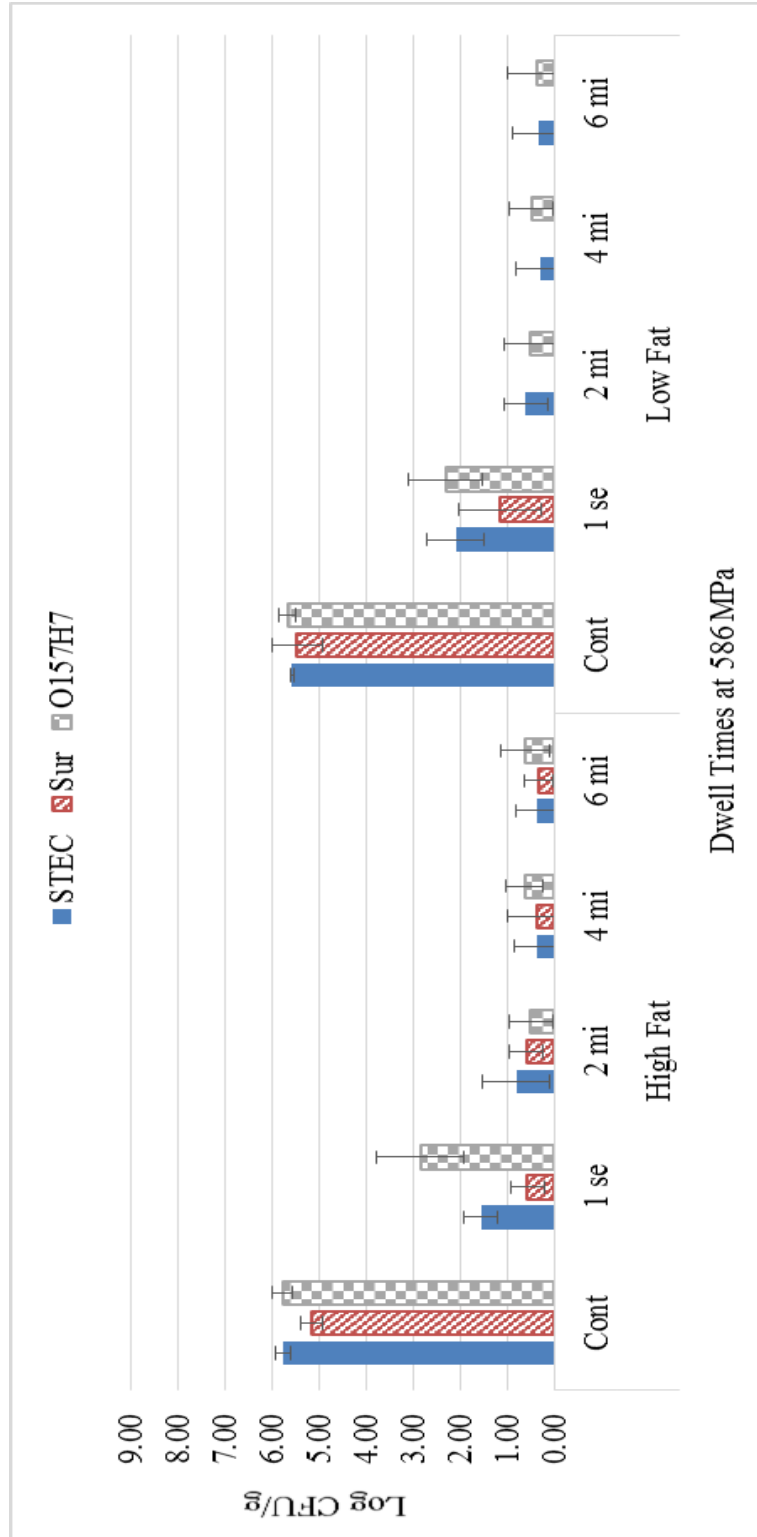


Figure 3.5: Average *E. coli* Coliform Counts Log CFU/g for High Pressure Processing of Fermented Beef Summer Sausages. Figure displays the average *E. coli* coliform counts (ECC) from testing fermented beef summer sausage at five different dwell times of high pressure processing (HPP) at 586 MPa at standard temperatures inoculated with three separate cocktails of *E. coli* species including; shiga toxin producing *E. coli* (STEC), non-pathogenic Rifampicin resistant *E. coli* Surrogate (Sur), and *E. coli* O157:H7 (O157H7) cocktails. Dwell times evaluated were: control-no HPP (Cont), 1 second (se), 2, 4, and 6 minutes (mi) for the fermented beef summer sausage. Both low fat (LF, 8%) and high fat (HF, 17%) results are displayed. All results are average bacterial counts displayed in log CFU/g.

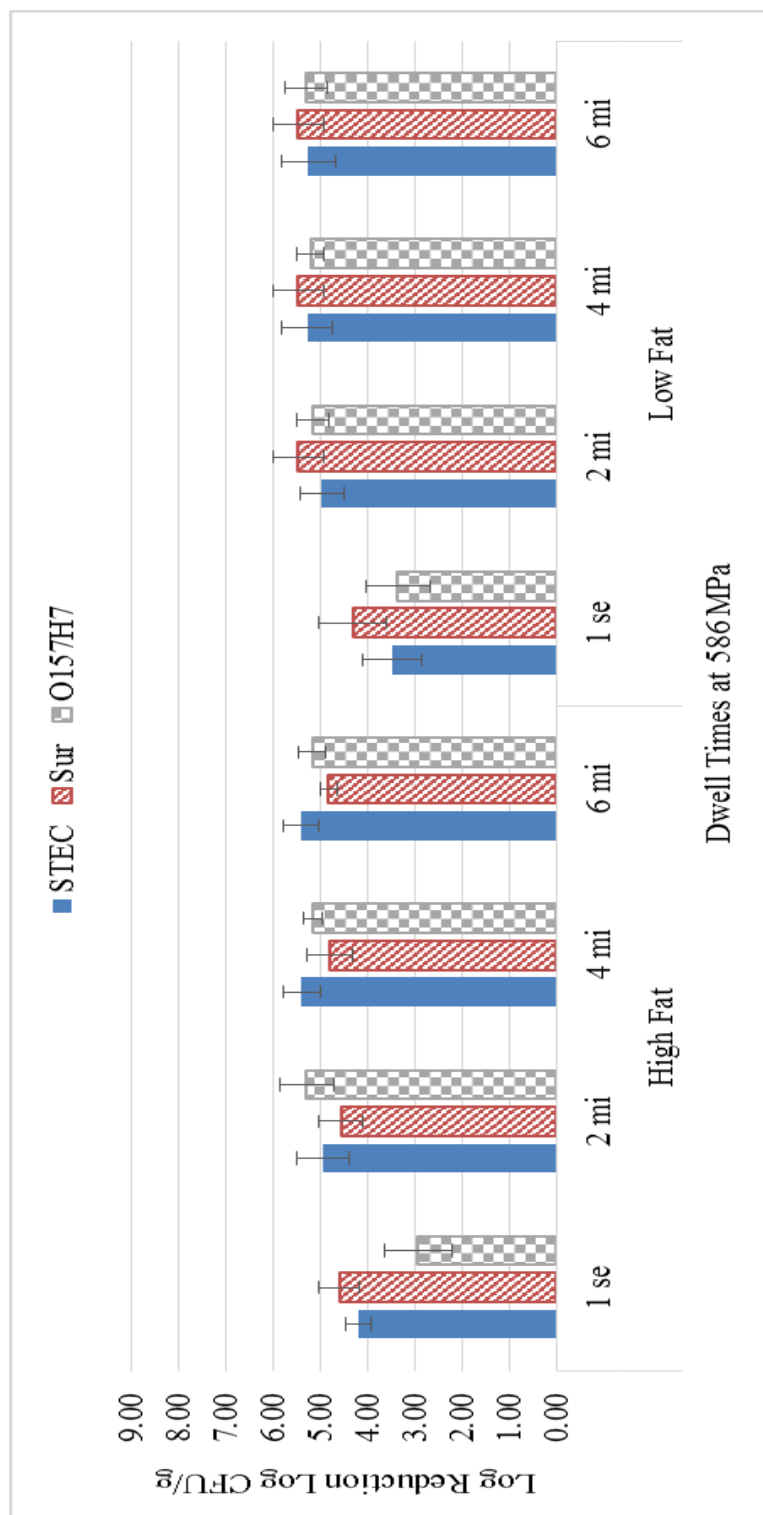


Figure 3.6: Average Log Reduction ECC Log CFU/g for High Pressure Processing of Fermented Beef Summer Sausages. Figure displays the average log reduction for *E. coli* coliform counts (ECC) from testing fermented beef summer sausage at 4 different dwell times of high pressure processing (HPP) at 586 MPa at standard temperatures inoculated with three separate cocktails of *E. coli* species including; shiga toxin producing *E. coli* (STEC), non-pathogenic Rifampicin resistant *E. coli* Surrogate (Sur), and *E. coli* O157:H7 (O157H7) cocktails. Dwell times evaluated were: 1 second (se), 2, 4, and 6 minutes (mi) for the fermented beef summer sausage. Both low fat (LF, 8%) and high fat (HF, 17%) results are displayed. All results are average log reduction bacterial counts displayed in log CFU/g.

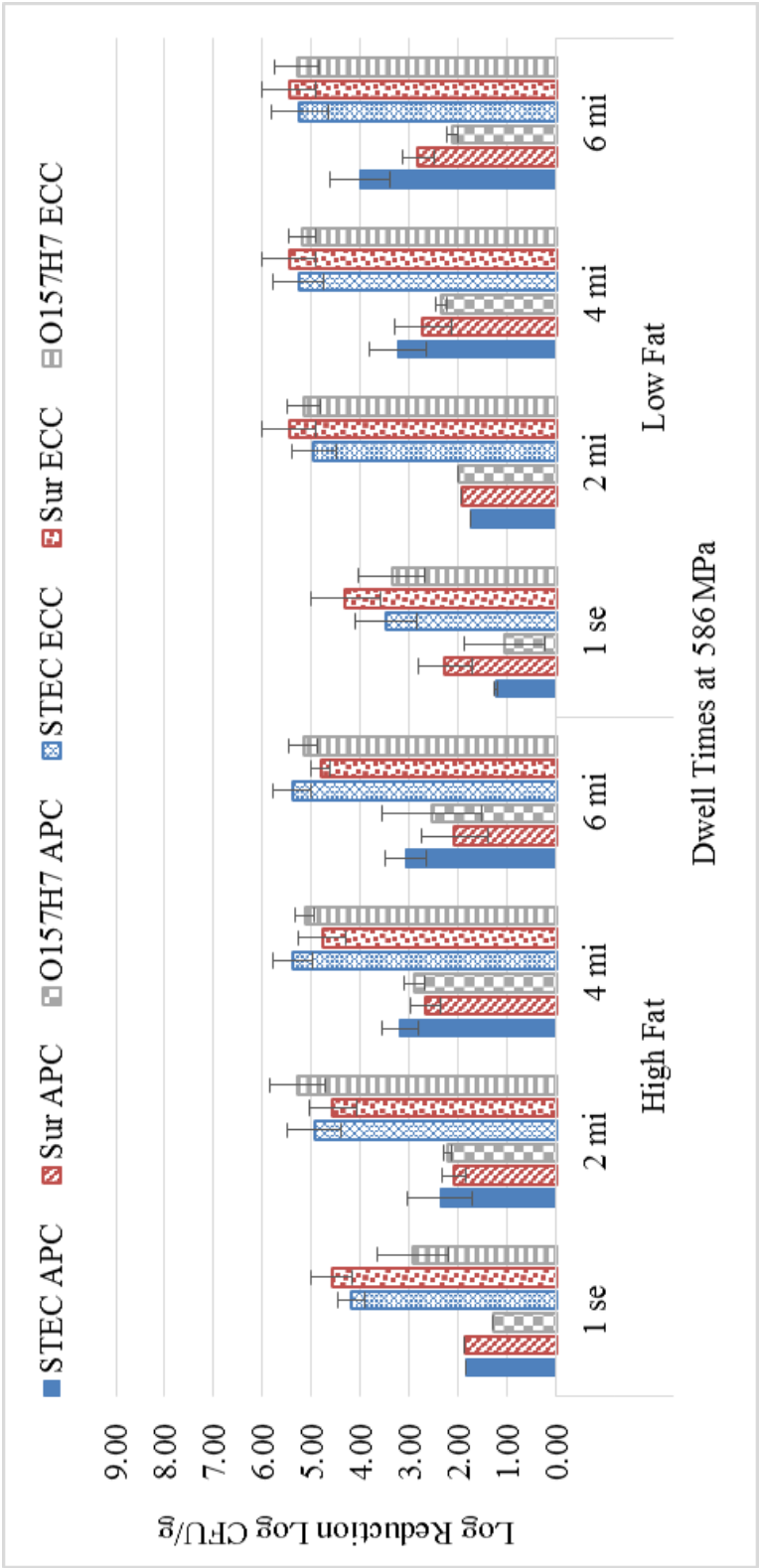


Figure 3.7: Average Log Reduction Log CFU/g for High Pressure Processing of Fermented Beef Summer Sausages. Figure displays the average log reduction for aerobic plate counts (APC) and *E. coli* coliform counts (ECC) from testing fermented beef summer sausage at 4 different dwell times of high pressure processing (HPP) at 586 MPa at standard temperatures inoculated with three separate cocktails of *E. coli* species including; shiga toxin producing *E. coli* (STEC), non-pathogenic Rifampicin resistant *E. coli* Surrogate (Sur), and *E. coli* O157:H7 (O157H7) cocktails. Dwell times evaluated were: 1 second (se), 2, 4, and 6 minutes (mi) for the fermented beef summer sausage. Both low fat (LF, 8%) and high fat (HF, 17%) results are displayed. All results are average log reduction bacterial counts displayed in log CFU/g.

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CHAPTER 4

SUMMARY, THE FUTURE, AND CLOSING REMARKS

SUMMARY OF RESULTS

Throughout this thesis we have discussed and studied *Escherichia coli* species and evaluated their destruction within fermented beef summer sausage production and via high pressure processing. The following paragraphs are a summary of the conclusions and findings stated within the results sections for each research chapter. We will first recap the initial study performed looking at inactivation of *E. coli* species in fermented beef summer sausage production, followed by high pressure processing as an alternative intervention, and then will conclude with a recap looking at high pressure processing as a “post processing contamination control measure” procedure for the control of *E. coli* species. All information shared in this section can be found in greater detail throughout the other chapters.

Escherichia coli can be controlled during the manufacturing of fermented beef summer sausages when proper processing techniques are utilized. The first study performed focused on the inactivation of *E. coli* species throughout the normal production process of fermented beef summer sausage utilizing the “hurdles” that are currently in place such as processing temperature control, competing cultures, and pH adjustment through fermentation, to control foodborne pathogen growth. This study was performed to determine if the manufacturing process for fermented beef summer sausage is adequate on its own to control *E. coli* species or if additional and/or alternative interventions are necessary to achieve compliance regulations of a 5.0 log reduction of *E. coli*. We found that, although the manufacturing process did cause a significant reduction in *E. coli* species (4.2 log CFU/g in low fat 8% post chilling), that is was not adequate on its own to

achieve the compliance guidelines and therefore additional interventions such as thermal processing or high pressure processing would be necessary in order for manufacturers of fermented beef summer sausages to meet the guidelines and sell their products to consumers within the United States.

With our initial study showing that the manufacturing process was not adequate to control *E. coli* species, it became even more important to evaluate the use of additional and/or alternative interventions. We chose to evaluate the use of high pressure processing in place of the thermal processing stage of production of fermented beef summer sausages to determine if HPP could be used as a suitable alternative intervention to thermal processing which could potentially lead to improved quality of the fermented sausage products. It was found that HPP could achieve the 5.0 log reduction regulation thus capable of meeting the requirement in post manufacturing sausage samples with just 1 second of dwell time at 586 MPa. Data showed additional reductions of *E. coli* of 2.6 log CFU/g with total reductions of 6.8 log CFU/g in low fat (8%) fermented beef summer sausages exceeding the compliance regulation when used in place of thermal processing.

After determining that additional or alternative interventions were necessary to control *Escherichia coli* species during the manufacturing of fermented beef summer sausages, and that high pressure processing could be used in place of thermal processing to meet the regulatory guidelines of a 5.0 log reduction of *E. coli*, we then set out to determine if high pressure processing could be used to control post manufacturing contamination events or potentially as a standalone procedure. In order for HPP to be considered as a

standalone procedure it would have to be able to meet the regulatory guidelines without the additional hurdles that are utilized within the manufacturing process to help control foodborne pathogens such as *E. coli*. We found that HPP can be used as a post processing contamination control measure and that it has the potential to be used as a standalone procedure to eradicate *E. coli* species from fermented beef summer sausage. However, it was determined that HPP works much better as an additional hurdle than as a standalone intervention. When reviewing the *E. coli* coliform counts (ECC) our data showed that > 4.6 log CFU/g reductions of *E. coli* could be obtained from dwell times ranging between 2-6 minutes at 586 MPa with many dwell times giving > 5.0 log CFU/g reductions depending on the cocktail of *E. coli* species being evaluated.

This data shows that *E. coli* is susceptible to high pressure processing without the additional stressors of the manufacturing process, but it takes much longer dwell times to eradicate the pathogens then when the additional manufacturing stress has occurred which could negate the quality aspect of utilizing HPP over thermal processing. Our testing procedure did not evaluate the quality of the fermented sausage or a dwell time that was long enough to properly determine at which point HPP could be used as a standalone intervention to adequately destroy pathogen and preserve quality of the sausage products.

FUTURE RESEARCH

Escherichia coli is still a major concern for the food industry and continues to be a problem in many different food mediums, including fermented sausages, causing foodborne illness outbreaks around the world. It continues to make headlines in the news and also continues to be on the forefront of regulation being pushed by the United States Department of Agriculture and Food and Drug Administration. Because of this it is important to continue to do research such as the research that was performed within this thesis to help us better understand how *Escherichia coli* species are evolving and to better evaluate how to control them to make food products safe for all consumers.

Based on the research that was performed in this thesis there are several more experiments that should be considered to further the understanding of *E. coli* species in fermented beef summer sausage products. During our initial study we only evaluated *E. coli* surrogates throughout the manufacturing process of fermented beef summer sausage. It would be valuable information to know if all other shiga toxin producing *E. coli* behave the same as the surrogates and are inactivated at the same rate given the same stressors (hurdles) of the manufacturing process, or if some of them are more resistant and require greater control parameters.

Other experiments that should be done are directly related to high pressure processing. Several experiments need to be done to further verify our findings. The studies performed on the surrogates from the manufacturing process utilizing HPP as an alternative intervention should be performed on the STEC. Additionally, HPP should be evaluated

utilizing *E. coli* species on packaged sausages that are packaged similar to store bought pre-sliced sausages to mimic what a consumer would actually purchase. One study of interest, but may not be necessary, would be to evaluate HPP and inactivation of foodborne pathogens on whole chubs of sausage, instead of slices to see if HPP would be a valuable alternative intervention for whole stick summer sausages sold at market or to determine if whole chubs could be processed pre-slicing during manufacturing as an additional control point.

Finally, experiments need to be done to assess the quality of the sausage products post high pressure processing to determine if there are any negative quality attributes seen. Although we did not see any negative quality attributes during our testing, official testing should be conducted to measure all of the organoleptic and physiochemical properties of the sausage pre and post high pressure processing to truly determine if it improves and/or maintains the quality of fermented sausage products.

CLOSING REMARKS

In closing, it has been my privilege to study *Escherichia coli* and learn how to better control this deadly foodborne pathogen during the production of fermented beef summer sausages. Additionally, it has been exciting to learn about *E. coli* species and to get the opportunity to apply alternative solutions to help better control *E. coli* which could eventually lead to safer higher quality food products for consumers.

Doing research that not only provides pathways for safer products but also improves the quality of the foods we eat is essential for the growth and progress of the food industry. Continued research and advancements in alternate technologies, such as HPP, not to replace but to improve our current production system for food products like fermented beef summer sausages is not only necessary, but is vital for the future of the food industry and the health of the human population. If we continue to do research to better understand foodborne pathogens and continue to look at not just our current production processes, but also alternative solutions and interventions, we will ultimately find those solutions we need to make our food products safer for everyone around the globe. If we do not continue to do these things then foodborne disease will continue to be a major problem for all mankind, and a leading cause of death and disease each year.

I am very grateful that I am apart of helping to find the solutions to fixing our foodborne pathogen problem and look forward to continuing with research to help make food safer so that consumers can be worry free when enjoying their food!