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Determining the Impact on Varying Methods for Acid Adaptation on Thermal Resistance of Shiga Toxigenic *Escherichia coli* (STEC), *Listeria monocytogenes*, and *Salmonella enterica* in Orange Juice

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To the Graduate Council:

I am submitting herewith a thesis written by Valerie Eunice Nettles entitled "Determining the Impact on Varying Methods for Acid Adaptation on Thermal Resistance of Shiga Toxigenic *Escherichia coli* (STEC), *Listeria monocytogenes*, and *Salmonella enterica* in Orange Juice." I have examined the final electronic copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Food Science and Technology.

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Determining the Impact on Varying Methods for Acid Adaptation on Thermal Resistance of Shiga Toxigenic *Escherichia coli* (STEC), *Listeria monocytogenes*, and *Salmonella enterica* in Orange Juice

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ABSTRACT

Foodborne pathogens such as *Listeria monocytogenes, Salmonella enterica, and* Shiga-toxigenic *Escherichia coli* (STEC) are consistently an issue for food safety. Controlling and preventing the spread of these pathogens is paramount for food processors. In January of 2001, the Food and Drug Administration (FDA) implemented mandatory hazard analysis critical control points (HACCP) for juice manufactures to prevent the number of foodborne illnesses related to juice outbreaks that were increasingly frequent. Juice HACCP does not require traditional pasteurization of juice with heat but does require juice processors achieve a 5-log pathogen reduction of any pertinent foodborne pathogens.

It is important to validate any process to assure consistent lethality when processing. Microorganisms, including foodborne pathogens, when given enough time and nutrients can adapt to a wide variety of environments (*e.g.* low pH, desiccation) in food production. These adaptations may confer a competitive advantage when confronted with environmental stressors. To that end, it is important to understand what role, if any, acid adaptation may play in thermal inactivation rates of foodborne pathogens.

The thermal inactivation rates of *Salmonella* and STEC cocktails were determined for three different temperatures (60°C, 65°C and 70°C) with prior acid adaptation through two different mechanisms (hydrochloric acid and glucose supplementation) as well as a control. In a similar fashion, inactivation rates of *L. monocytogenes* were determined for these treatments at 60°C and 70°C. The thermal death time curves were compiled from the observations of six independent replicates and evaluated for fit using linear and Weibull regression models, with Weibull models resulting in a better fit (R^2>0.925). Overall, STEC cocktail was the most thermally resistant, and acid adaptation did not impact thermal resistance.

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CHAPTER I

LITERATURE REVIEW

Burden of Foodborne Illness

Foodborne illnesses are an ever-pertinent topic in food safety. For over the past half century 30% of all outbreaks were caused by pathogens that are often transmitted though food *(58)*. It is a worldwide public health concern affecting every country; rich or poor, small or large, agricultural or industrial. In the United States viruses, specifically norovirus, cause the most foodborne illnesses per year, but bacterial pathogens cause a disproportionate amount of hospitalizations and deaths *(58)*. Bacterial pathogens may only cause 39% of all foodborne illnesses per year, but they are the leading cause of hospitalizations and deaths related to foodborne illness *(58)*.

One common bacterial pathogen is *Escherichia coli* (*E. coli*). In the United States O26, O45, O103, O111, O121, O145 and O157 are the STEC serotypes that cause the most illness amongst STEC serotypes *(25, 41, 50)*. STEC is estimated to cause about 175,000 cases annually in the United States *(58)*. STEC can cause illness with a very small number of cells *(11)*. Shiga-toxigenic *E. coli* or STEC is characterized by the ability to produce Shiga toxins that can cause gastrointestinal disease and hemolytic uremic syndrome (HUS) *(11, 50)*. The severity of STEC can be anywhere from mild to life-threating due to its ability to lead to HUS *(11, 25, 61)*. HUS destroys red blood cells which results in internal bleeding and often bloody diarrhea *(27)*. The loss of blood and fluids

lead to kidney failure and sometimes death *(11)*. The harmful long term effects of HUS can last the rest of one's lifetime *(50)*. Ina meta-analysis of 82 studies reporting complications and chronic sequalae after *E. coli* O157:H7 infection, HUS development was reported in 17.2 to 4.2% of cases *(36)*. The development of HUS after *E. coli* O157:H7 infection is a major cause for concern due to 12% of HUS cases resulting in death or end-stage renal disease *(34, 36)*. Thrombotic thrombocytopenic purpura (TTP) is a blood disorder that causes very small blood clots, these blood clots can block oxygen rich blood from reaching vital organs in the body *(54)*. Blood clots and reduced red blood cell counts lead to hemolytic anemia *(54)*. TTP development after *E. coli* O157:H7 infection were reported between 7% and 2% of cases *(36)*.TTP-HUS has an extremely high mortality rate since they both limit the amount of red blood cells to vital organs *(8)*.

Salmonella enterica, the pathogen that causes salmonellosis is, another foodborne pathogen that frequently draws public attention. Historically, *Salmonella* was most prevalently associated with illnesses where eggs and poultry were the vehicle for contamination. Since that time, there have been several instances of contamination and outbreaks associated with an array of foods *(5, 10, 11, 51, 60)*. Today *Salmonella* outbreaks are seen more frequently because of its ability to survive in so many different types of foods with adverse intrinsic properties such as low water activity, acidic pH, nutrients, oxidation reduction potential, and inhibitory substances *(33, 59)*. *Salmonella* causes about

1,000,000 cases of foodborne illness annually which is about 11% of all foodborne illnesses, the second most cases annually. *Salmonella* is the number one cause of hospitalizations and deaths due to foodborne illness *(58)*.

Listeria monocytogenes is an intercellular pathogen which allows it to grow within cells and spread cell to cell resulting in septicemia *(35)*. Another concerning trait of *L. monocytogenes* that it is a psychrophilic organism, giving it the ability to grow at refrigeration temperature, which explains why it has been associated with deli meats and dairy products *(35)*. *L. monocytogenes* causes about 1,600 cases annually *(58)*. Although the number of cases are far fewer than STEC and *Salmonella, L. monocytogenes* is associated with a 15.9% mortality rate making it the causative agent in 19% of all deaths associated with foodborne pathogens *(58)*. Due to the high case fatality rate and the ability to survive and grow in certain food and food processing environments, in the United States *L. monocytogenes* is a zero-tolerance pathogen in ready-to-eat foods.

Food Vehicles Associated with STEC, *Listeria monocytogenes*, and *Salmonella enterica* Outbreaks

STEC, *Salmonella*, and *L. monocytogenes* all have the ability to cause significant foodborne illness outbreaks. In 2015 alone, the CDC reported that 34 foodborne outbreaks were caused by STEC, 158 due to *Salmonella*, and two

because *of L. monocytogenes (5)*. During this period, 56% of all multistate outbreaks were caused by *Salmonella* and 33% were caused by STEC *(5)*.

As previously discussed, *Salmonella* is well known for its ability to cause outbreaks in fruits, vegetables, eggs and meat *(11)*. From 1999 to 2008 *Salmonella* caused foodborne outbreaks in beef, beverages, baked goods, dairy, eggs, deli meats, complex foods, pork, poultry, produce and seafood *(7)*. The majority of the outbreaks from this time period were found in poultry *(7)*. Although there are many commonly associated *Salmonella* outbreaks, there have recently been outbreaks in foods that are not typically associated with *Salmonella.* The presence of *Salmonella* in low water activity (a_w) foods, such as peanut butter, powdered milk, chocolate, and bakery products demonstrates that it has the ability to adapt to environments that were initially considered less than optimal for survive *(6, 16, 51, 59)*. Since *Salmonella* does not grow in a_w controlled foods, its presence is likely the result of cross-contamination with a source *(51)*. There is a growing concern that when *Salmonella* adapts to a low a_w environment, it will allow for cross-protection to other processes that have previously been used for inactivation *(10, 51)*.

When most Americans recall past STEC outbreaks, beef is typically the food vehicle that comes to mind. This is an accurate association since beef did cause the majority of STEC outbreaks from 1999 to 2008 *(7)*. Transmission of

STEC to foods is most commonly related to fecal contamination *(11)*. Contamination of meat products with STEC often occurs during the slaughtering process *(11)*. More recently, STEC has also been associated with low a_w foods. From December 21, 2015 to September 5, 2016 there was an outbreak of STEC that contaminated flour and sickened 56 people *(21)*. STEC serogroup O121 and O26 were the causative agents in this outbreak demonstrating that like *Salmonella,* STEC can cause illnesses arising from vegetative cells that persist in environments that do not support growth *(10)*.

L. monocytogenes' psychrophilic nature makes it unlike other foodborne pathogens where refrigeration temperatures typically prevent growth *(11)*. *L. monocytogenes* is often related to produce, lunch meat, milk, cheese, and ice cream outbreaks *(11)*. From 1999 to 2008 the majority of outbreaks caused by *L. monocytogenes* were in dairy products *(7)*. During that same period, there was a decline in outbreaks associated with ready-to-eat meat products *(15, 17)*. The same trend was not seen in dairy products, unfortunately there was an increase in outbreaks of foods that were previously considered to be "low-risk" or "moderate-risk" such as foods stored in the freezer and fresh produce *(15)*. In 2010 there was a foodborne outbreak of *L. monocytogenes* contamination on celery causing illness in 10 individuals *(15)*. The very next year there was a large multi-state outbreak of *L. monocytogenes* affecting 147 individuals through contaminated cantaloupes *(15)*. In 2014 mung bean sprouts, stone fruit, and

caramel apples were products related to *L. monocytogenes* outbreaks *(15)*. From 2010 to 2015 *L. monocytogenes* was present in the processing plant of a large ice cream producer *(15, 52)*. This outbreak was noteworthy because *L. monocytogenes* was associated with illnesses that stretched back to 2010 and the recall of all of the brands products did not occur until 2015 *(52)*. Much like *Salmonella* and STEC, *L. monocytogenes* is thought to be more persistent in the food processing environment leading to outbreaks in foods that were thought to be “low-risk” or “moderate-risk” *(15)*.

Outbreaks in Juices

In the 1990’s juices became a growing concern in food safety due to an increasing frequency of outbreaks (Table 1). The CDC reported 21 juice-associated outbreaks from 1995 to 2005 causing 1,366 illnesses *(67)*. During those ten years, 35 states were affected by at least one juice outbreak *(67)*. *Salmonella* caused five outbreaks, STEC caused five, *Cryptosporidium parvum* was the cause of two, and both *E. coli* O111 and *C. parvum* were found to be the causative agent in one outbreak. For eight outbreaks, the cause of illness was not determined *(67)*. All but 15 states were affected by the large number of outbreaks during this 10 year period in which 48 and 38% were linked to apple juice and orange juice *(67)*.

In 1995, there was an orange juice outbreak that was caused by *Salmonella* Hartford and Gaminara in Florida that caused 62 illnesses *(20, 67)*. In 1999 there were four orange juice related outbreaks with more than 350 Illnesses associated with *Salmonella* Muenchen contamination *(67)*. The very next year there were two additional outbreaks in orange juice, one due to *Salmonella* Enteritidis *(67)*. This trend caught the attention of regulators, resulting in the Food and Drug Administration (FDA) to develop a regulation mandating Hazard Analysis Critical Control Points (HACCP) in 2001 for all juice manufacturers *(3)*.

Juice HACCP

HACCP was developed by Pillsbury Company and The National Aeronautics and Space Administration (NASA) as a method for food processing *(31)*. The goal was to analyze each step in the production process to ensure that foods were processed in a safe manor and potential hazards present during processing were monitored *(31)*. Pillsbury and NASA designed this process to achieve a “zero defect” product with the intent to guarantee product safety *(31)*. HACCP contains seven principles that are used as a guide to start and maintain a processing environment with limited hazards *(31)*.

The first principle is conduct a hazard analysis, this means examining every step in processing and determining if there is a potential for a biological, physical, or chemical hazard to make its way into the product (Figure 1) *(31)*. The second principle is to identify the critical control points (CCPs), these are the points that will prevent the potential hazards from entering the product. The third principle is to establish critical limits for each identified CCP, this principle sets a requirement specific to its product. The fourth principle is monitoring of the CCPs and limits, this ensure that every product that leaves the facility has meet requirements. The firth principle is to establish corrective actions Corrective actions are in place as instructions for what to do if the CCP or CCP monitoring fails. The sixth principle is to keep records, as evidence that the HACCP plan is being implemented as described when operating. The last principle is to establish verification procedures. Verification procedures prove that the HACCP plan is working correctly.

The seven principles of HACCP are specific, at the same time it is also adaptable to various food products. As different categories of food products were increasingly associated with foodborne illness, product-specific HACCP regulations were mandated to decrease the burden of foodborne illness associated with these foods. The FDA first utilized HACCP principles in 1973 with the low-acid canned foods to control the presence of *Clostridium botulinum* in the canning industry *(31)*. In 1998, the FDA implemented the juice labeling

regulation mandating that juice processors that did not process juice to achieve a pathogen reduction provide a warning label on their products *(67)*. This regulation was intended to educate consumers that the juice or juice ingredients had not been processed to reduce potential pathogens. When juice related foodborne illness continued to be a concern, the FDA developed Juice HACCP to attempt to reduce the number of juice associated outbreaks *(67)*. The final ruling was published in January 2001, and unless exempt, processors of all sizes, domestic and international, had been compliant with this regulation by January 2004 *(3, 47)*.

The most significant requirement of the new regulation was a mandate for all processors to achieve a five-log reduction of pertinent pathogens in their products *(62, 67)*. Pertinent pathogens are the pathogens that cause the most risk to public health. In this case *Salmonella* and *E. coli* had caused the most outbreaks in juice *(42, 45, 49, 67)*. Since the survival of *L. monocytogenes* in juice is possible it is also considered a pertinent pathogen *(42, 45, 57)*. The regulation is not specific regarding what inactivation techniques can be used to achieve the 5-log pathogen reduction, leaving processors open to choose between thermal and non-thermal processes as long as they are scientifically valid.

While thermal pasteurization is straightforward and cost effective for many juice processors, there are other methods that have proven to be successful in juice processing. Some processors chose to use UV light treatment since bacterial pathogens are extremely susceptible to UV treatment and a 5-log reduction of bacterial pathogens can be achieved *(64)*. Usaga et al., 2015 has demonstrated a 5-log reduction in various strains of *E. coli* in apple juice *(64)*. Their research not only looked at unadapted *E. coli* but also the effects of UV inactivation on acid adapted and acid shocked *E. coli (64)*. They were able to demonstrate over a 5-log reduction in all treatments with constant UV treatment *(64)*. UV processing of juice for microbial pathogens can be an effective method of processing although, molds and yeasts are not as susceptible to UV processing *(64)*. Molds and yeast do not cause as large of a concern for food safety, but it would affect the shelf life of the juice. One limitation of UV treatment is that the UV light does not pass through liquids therefore requiring tubular flow to ensure that the entire volume of liquid has been exposed to the UV light treatment *(28)*.

High-pressure pasteurization uses a combination of pressure and temperature to achieve the combination that will provide a pathogen reduction *(48)*. One of the major benefits to high-pressure pasteurization is that it can preserve some of the desired sensory attributes such as flavor and color of juice. The combination of pressure and temperature can be adjusted to fit the product

and targeted pathogens. Pressure and temperature combination can also be manipulated to achieve the least amount of sensory harm to the product *(48)*. Citrus juice producers are also allowed to treat the outside of the citrus fruit in addition to the juice to obtain a cumulative 5-log reduction, but citrus fruits are only fruits allowed to use a surface treatment for these purposes *(62)*

Juice HACCP was an important regulation for food safety. In the years immediately following the implementation of juice HACCP there was a decline in juice associated outbreaks *(67)*. In six juice associated outbreaks from 2002 to 2005, only one of the processors were using methods that met juice HACCP requirements *(67)*. It has been estimated that there are between 462 and 508 less juice associated foodborne illnesses per year *(47)*. Not only has juice HACCP reduced the number of annual illnesses but it has also reduced some of the financial burden associated with foodborne illnesses by $59 million to $82 million per year *(47)*. Overall there was a significant reduction in the number of juice related foodborne outbreaks after juice HACCP was implemented.

The seven principles of HACCP are only effective if the methods used at CCPs are valid methods for elimination of the hazard *(31)*. In juice processing, having a validated process for 5-log pathogen reduction is important to ensure that these criteria for minimum pathogen reductions are actually being achieved. Juice processors often use scientific literature to determine what method to use.

Validation studies are conducted to provide evidence that the process as implemented is effective at achieving a 5-log pathogen reduction mandate of Juice HACCP *(43)*.

Microbial Adaption

In 2000, *Salmonella* caused a multistate outbreak in orange juice *(67)*. Juice HACCP had been introduced although processors were not yet required to adhere to the regulation. In this outbreak, the juice processor was adhering to what they believed was a scientifically-valid series of pasteurization steps and unfortunately it was not effective. After the event, there was a great deal of analysis determining the root cause of the contamination event. It is possible that their juice HACCP plan was not followed correctly, but there was not any evidence of a breakdown during process. An additional hypothesis was that the pathogens present with in the juice processing plant adapted to the harsh environment that was designed to inactivate them and survived in large enough numbers to cause illness.

Since 2004, all juice processors have been compliant with juice HACCP unless otherwise exempt. There have been seven domestic and five imported juice related outbreaks since 2004 *(22, 32)*. Manufactures that export juice to the U.S. are required to follow juice HACCP just like domestic processors. Three of

the domestic outbreaks were apple juice related and caused by *E. coli* O157:H7 *(22)*. All three were unpasteurized, falling under the juice HACCP exemption. Only one of the three were sold retail *(22)*. In 2005 a large juice outbreak of *Salmonella* Typhimurium and *Salmonella* Saintpaul caused 157 illnesses across 24 states *(32)*.

Literature supports the theory that pathogens have the ability to adapt to stressful environments. The idea of cross-protection has been demonstrated by various studies and shows that when microorganisms are exposed to a stressful stimulus within the environment, they have the ability to adapt if not inactivated. This adaptation will often result in increased resistance to other stressors as well, thus why it is referred to as cross-protection *(10, 30, 38, 39, 44, 51, 66)*. There is some literature that suggests that cells that have been grown in an optimal environment, non-stressed and grown in rich media, are not an accurate representation of cells that would be found in a food processing environment *(38)*. In 1993 Leyer and Johnson evaluated acid adaptation and cross-protection to stressors commonly implemented during food processing in *Salmonella* Typhimurium *(57)*. They demonstrated that acid adaptation can affect a cell's resistance to a variety of stressors, including thermal treatment *(38)*.

Some studies suggest that cells begin to produce proteins, referred to as shock proteins, to repair damages giving it the ability to be more tolerant to

secondary stressors *(66)*. Another theory is that cells increase the ratio of saturated fatty acids to unsaturated fatty acids and this decreases membrane fluidity *(2, 66)*. Velliou et al., 2011 was able to demonstrate that the level of thermal resistance was corelated to the type of acid used for acidification *(66)*. While the observation that acid adaptation can result in cross-protection, many of the mechanisms for adaptation have yet to be elucidated.

Doyle et al., 2001 studied the heat resistance of *L. monocytogenes* in beef, pork, eggs, seafood, vegetables, milk and dairy products *(23)*. They stated that thermal resistance of *L. monocytogenes* could be induced by multiple factors such as strain variation, stressful growth condition, heat shock or acid shock *(23)*. *L. monocytogenes* is a target organism for thermal processed foods that are sold ready to eat. It is important to understand what impact prior adaptation to stressors can have on subsequent thermal resistance.

Juice HACCP does not require juice processors to use methods that are approved by the FDA *(32)*. Instead companies are required to validate the process that they choose to use *(32)*. Since thermal processing is proven to be an effective way to achieve a specific pathogen reduction, it is a preferred method of processing for many juice processors. The juice processing environment exposes pathogens to high acid and temperatures making the opportunity for acid induced heat tolerance likely.

Differing Approaches for Acid Adaption of Foodborne Pathogens

There are two common methods currently being utilized for acid adaption of foodborne pathogens. One method is to use an acid such as citric acid, acetic acid, lactic acid or hydrochloric acid (HCl) to allow the cells to adjust and adapt to the low pH *(26, 55)*. The other method utilizes additional glucose supplemented into the growth media. In this method the cell uses self-induced acid adaption. In a glucose rich environment, organic acids such as acetic, lactic and formic acid are produced during fermentation. The presence of organic acids gradually lowers the pH of the medium leading to acid stress and, as a reaction, (self-induced) acid adaptation *(55, 66)*.

Acid adaption through an immediate drop in pH provides acid shock initially, with the surviving cells adapting to this stressor over prolonged exposure. Acid adaption through a slowly changing pH during metabolic processes, allows the cells to gradually adapt to the acidic environment. Many studies use 1% glucose, weight by volume (w/v), enriched media to encourage self-induced acid adaption of *E. coli*, *Salmonella*, and *L. monocytogenes (55, 63)*.

Mazzotta, in 2001 used HCl to observe the effect of thermal inactivation on *E. coli* O157:H7, *Salmonella* and *L. monocytogenes* in apple, orange and

white grape juice *(45)*. This research found that HCl acid adapted *E. coli* O157:H7 had the highest thermal resistance of all three organisms and recommended a processing time of 3 seconds at 71.1°C *(66)*. This study is recommended by the FDA for juice processors with a pH of less than 4.0 *(4)*. The state of New York is a large producer of the nation's apple juice and apple cider. The state of New York recommends that its juice processers pasteurize their juice at 71.1°C for 6 seconds *(4, 42)*.

Velliou et al., 2011 used *E. coli* K12 to analyze the affect glucose adaption had on thermal resistance *(66)*. They found that *E. coli* K12 demonstrated increased thermal resistance in a glucose rich environment *(66)*. Ryu and Beuchat, 1998 demonstrated the same type of thermal cross-protection of *E. coli* but in the presence of apple cider and orange juice after it had been acidified in glucose enriched media, allowing the cells to slowly adapt to the low acid environment *(55)*. Topalcengiz and Danyluk, 2017 used glucose to acid adapt three separate strains of STEC, *Salmonella* and *L. monocytogenes* in orange juice *(63)*. They found that most strains that were acid adapted had higher D-values than those that were not acid adapted but since they tested strains individually they did identify a few exceptions *(63)*.

Since each acid adaptation method has demonstrated the ability to provide increased thermal resistance to pathogens, both methods have been

utilized in peer-reviewed research *(26, 38, 45, 55, 63, 66)*. Unfortunately, there has not been a comparison of the two methods to determine if one method or the other provides thermal resistance that is greater than the other.

The association of *E. coli* and *Salmonella* in acidic foods, such as fruit juices, indicates that they can adapt and survive in acidic environments. Organisms that are acid adapted are more likely to be resistant to heat than organisms that are acid shocked *(55)*. Ryu and Beuchat, 1998 were able to demonstrate that acid adapted cells had higher D-values than acid shocked cells *(55)*. They found that acid shocked cells had D-values that were similar to cells that were not acid adapted *(55)*. Microorganisms are exposed to multiple stressors in the processing environment, understanding these stressors and how they further affect they adapt is important to preventing further adaption and potentially further resistance in processing environments. Orange juice processing environments are typically an acidic environment allowing organisms that are present the opportunity to adapt. It is important to understand how pathogens will respond to processing steps, such as thermal pasteurization, under the worst case scenarios if we are to develop robust processing systems.

CHAPTER II

THERMAL INACTIVATION OF ACID ADAPTED SHIGA TOXIGENIC *ESCHERICHIA COLI* (STEC), *LISTERIA MONOCYTOGENES*, AND *SALMONELLA ENTERICA* IN ORANGE JUICE

Abstract

When microorganisms are exposed to a stressful environment they adapt to survive, in some instances adaption to one stressor may provide protection against other environmental stressors, called cross protection. Previous studies have shown connections/correlations between acid adapted microorganisms and increased thermal resistance. Food processing environments, such as juice processors, may allow for such adaptations in which a subpopulation would be resistant to subsequent thermal processing, rendering the pasteurization process less effective. Additionally, there is a lack of consensus regarding what method of acid adaptation should be used in order to evaluate thermal inactivation of pertinent foodborne pathogens. This study evaluated the thermal resistance of *Listeria monocytogenes*, *Salmonella,* and STEC with and without prior acid adaption. Two common methodologies for acid adaptation of microorganisms were evaluated via exposure to hydrochloric acid or growth in media with additional glucose. The thermal death time curves were compiled from the observations of six replicates and evaluated for fit using linear and Weibull regression models, with Weibull models resulting in a better fit (R^2>0.925). Overall, STEC was the most thermally resistant organism, and differences in inactivation rates based on acid adaption were not consistent across treatment temperatures for any pathogen.

Introduction

Foodborne illnesses often go unreported since the distress that they cause is non-life threatening *(58)*. Although symptoms can be mild and can pass within 24 to 48 hours, the overall burden that foodborne illness play on society is significant *(33, 58)*. It is estimated that foodborne pathogens cause 9.4 million cases of illness in the United States alone every year *(58)*. *Escherichia coli, Salmonella enterica* and *Listeria monocytogenes* are bacterial pathogens that contribute significantly to these foodborne illnesses, outbreaks, and quality adjusted life years.

Salmonella has a history of causing foodborne illness in poultry products while foodborne outbreaks due to *E. coli* contamination were historically linked to beef and are typically the result of fecal contamination *(11)*. *L. monocytogenes* is a psychrophile allowing it to grow at refrigeration temperature and cause outbreaks in in refrigerated foods such as dairy products and deli meats *(7)*. As epidemiological methods for determining the source of foodborne outbreaks have improved, an increasing array of foods, some that have been previously thought to have an environment that does not support pathogen survival, have been the source of outbreaks *(1)*.

Fruit juice was not a product of concern until the 1990s. In a ten year time frame, there were 21 outbreaks that affected 35 states and caused over 1,000 illnesses *(67)*. The rise of foodborne outbreaks in juice received the attention of the Food and Drug Administration (FDA) and eventually led to a regulation which mandated minimum processing guidelines for the safe production of juice *(67)*. Reducing the number of foodborne illnesses and outbreaks will not only prevent people from getting sick but can also provide a financial benefit and save millions by eliminating the cost of treating foodborne illnesses and managing outbreaks *(47, 58)*.

Hazard Analysis Critical Control Points, otherwise known as HACCP, was designed to identify points in production that may allow hazardous material into the product and provides steps to prevent products from being exposed to biological, chemical or physical hazards *(31)*. Juice HACCP was implemented in 2001 in an attempt to reduce the number of foodborne outbreaks related to juice *(46)*. The regulation requires juice processors to implement a kill step in their process to achieve a 5-log reduction of pertinent foodborne pathogens *(3)*.

In order to meet juice HACCP processing requirements, a processor must implement a validated method that will consistently achieve the desired inactivation. There has been increasing evidence that that pathogens are adapting and surviving in environments that were thought to be undesirable *(10)*.

Researchers have been able to determine that the adaption of organisms to stressors can result in increased resistance to other stressor referred to as cross protection *(55)*. This cross protection against multiple stressors can make it more difficult to inactivate and control pathogens in a food processing environment. From a juice processing perspective, it is important to understand if pathogens have increased thermal resistance once acid adapted. Some studies have demonstrated prior acid adaptation increases thermal resistance, and it is important to understand what steps need to be taken to kill the most thermally resistant organism likely to be present in juice *(24, 38, 41, 44, 45, 55, 63, 64, 66)*.

An additional factor driving uncertainty when developing a validated process, is the fact that a common methodology for acid adapting organisms has yet to be agreed upon. There are two methods of acid adaption that are the most common, the first is acid adaption through enrichment of media by adding a fermentable sugar, such as glucose, and the second is acid adaption with a strong acid, such as hydrochloric acid (HCl) supplementation of growth media. Acid adaptation in a high sugar environment allows the organism to ferment the sugar and self-induced acid adaption via organic acid production during metabolic processes *(55, 66)*. Acid adaption with a strong acid provides an immediate shock and allows surviving cells to adapt and grow within the acidic environment *(55)*. To assure sufficient inactivation amongst pertinent foodborne

pathogens, it is important to know what impact the mode of acid adaption plays, if any, on thermal resistance.

The traditional approach to analyze the rate of thermal inactivation in microorganisms has been first order kinetics *(37)*. This method analyses the amount of time at which it takes to kill 1 log of microorganisms expressed as decimal reduction time or D-value *(37)*. Once a D-values for multiple temperatures have been established, the relationship between the D-values and temperature can be evaluated to obtain a z-value *(37)*. Since first order kinetics is a linear model, z-value represents the temperature change to change the D-value by 10-fold *(37)*. While this method has been used by the food industry to validate thermal processing, it is not always the best fit. Thermal inactivation is often a nonlinear survival curve and a linear model results in diminished R^2 values and skewed observations from the predicted values *(65)*.

The Weibull model can account for the nonlinear data that is observed during thermal inactivation of microorganisms *(65)*. Using two parameters, α and β, to better explain how the organisms react to heat. For example when β is less than one the model is concave up and shows that the remaining cells have the ability to adapt, however when β is greater than 1 the model is concave down and shows that the remaining cell populations are increasingly inactivated over time *(37)*. Since the Weibull model is nonlinear, a z-value cannot be calculated,

although specific D-values can be obtained from the model. The Weibull model has proven to provide a better fit when comparing R^2 values of thermal inactivation data *(12-14, 37, 53)*.

Materials and Methods

Orange Juice and Physicochemical analysis

Frozen concentrated orange juice was rehydrated with sterile water to create a large single representative lot of orange juice used for all treatments within a replicate.

The pH of the representative lot was recorded with Accumet Excell XL 15 pH meter (Fisher Scientific, Waltham, MA).

The sugar content of the representative lot was recorded with a portable refractometer (NuLine, China).

Inoculum preparation

Strains used in this experiment are listed in Table 2.1. Each STEC and *Salmonella* strain was plated and individually grown on Difco™ Tryptic Soy Agar (TSA; Becton, Dickerson and Company, Sparks, MD) and incubated at 37 ± 2°C for 24 ± 2 h. A single colony from the TSA plates was transferred to Bacto™ Tryptic Soy Broth (TSB; Becton, Dickinson and Company, Sparks, MD) and incubated at 37 ± 2°C for 24 ± 2 h with three subsequent transfers prior to use.

Each strain of *Listeria monocytogenes* was plated and individually grown on Bacto™ Brain Heart Infusion agar (BHI; Becton, Dickerson and Company, Sparks, MD) and incubated at 32 ± 2°C for 24 ± 2 h. A single colony was

transferred into a BHI broth (Becton, Dickerson and Company, Sparks, MD) and incubated at 32 ± 2°C for 24 ± 2 h with three subsequent transfers prior to use.

Acid adaption in hydrochloric acid enriched media

STEC and *Salmonella* strains were transferred to TSB supplemented with 1N hydrochloric acid (TSBH; La-Mar-Ka, Baton Rouge, LA) to achieve a pH of 5.0 ± 0.1 and incubated at 37±2°C for 18±2 h, with two subsequent transfers. *L. monocytogenes* strains were transferred to BHI supplemented with 1N hydrochloric acid (BHIH) to achieve a pH of 5.0±0.1 and incubated at 32±2°C for 18±2 h, with two subsequent transfers.

Acid adaption in glucose enriched media

STEC and *Salmonella* strains were individually transferred to TSB supplemented with 1% (w/v) glucose (TSBG; Mallinckrodt®, Paris, KY) and incubated at 37 ± 2°C for 18 ± 2 h, with two subsequent transfers. *L. monocytogenes* strains were transferred to BHI supplemented with 1% (w/v) glucose, (BHIG) and incubated at 32 ± 2°C for 18±2 h, with two subsequent transfers.

No acid adaption (Control)

STEC*, Salmonella*, *and L. monocytogenes* strains were cultivated as previously described and incubated at 37±2°C for 18±2 h, with two subsequent transfers prior to use.

Preparation of inoculum cocktail

An equal volume from each strain/serovar (1 ml) was combined to create a five-strain cocktail for each pathogen. The inoculum cocktail was centrifuged with an Eppendorf 5804R centrifuge at 5,000 *x g* for 10 min at 4 °C. The TSB/BHI broth was removed from the centrifuge tube and cells were resuspended in 5 mL of 0.1% peptone. The cells were centrifuged a second time at 5,000 *x g* for 10 min at 4°C. The 0.1% peptone was removed and cells were resuspended in 5 mL 0.1% peptone once more. The cells were centrifuged a final time at 5,000 *x g* for 10 min at 4 °C. The 0.1% peptone was removed, and the cells were resuspended in 5 mL orange juice and would subsequently be used to inoculate the orange juice

Thermal inactivation

Ninety-nine mL of orange juice was heated in a sterile flask containing a water-resistant traceable thermometer (Traceable® Products, Webster, TX). The flask of orange juice was placed into a Precision circulating water bath (MFG, city, state) and stirred using a VWR™ 230 submersible magnetic stirrer (VWR™,

Radnor, PA). The juice was heated to the target temperature; once that temperature was achieved, 1 mL of orange juice inoculum cocktail was added to the 99 mL of orange juice. At six time points which varied based on temperature (Table 2.2), 5 mL of orange juice was removed and immediately placed into a sterile Falcon tube (Corning Scientific, Tamaulipas, Mexico) then immersed in an ice bath for at least one minute until the temperature was brought below 25°C.

Microbial analysis

Each 5 mL sample was used to determine the population of survivors. All organisms were diluted using 0.1% peptone water. All samples were spiral plated using an Eddy Jet 2 spiral plater (IUL, Barcelona, Spain). STEC and *Salmonella* strains were plated on TSA in duplicate and incubated at 37 ± 2°C for 24 ± 2 h. *L. monocytogenes* strains were plated on BHI+ 0.1% sodium pyruvate (VWR, Solon, OH) in duplicate and incubated at 32 ± 2°C for 24 ± 2 h. After incubation, plates were enumerated to determine microbial populations using a IUL Sphere Flash automatic plate counter (IUL, Barcelona, Spain). Surviving colonies were counted as colony forming units (CFU) per milliliter of orange juice.

Design of experiments

The experiment was independently replicated six times for each treatment and temperature combination as shown in Table 2.2.

Calculation of thermal inactivation

Counts from each replicate were averaged and logarithmically transformed and set to an inverse scale using the following equation:

$$log\frac{N(t)}{N_0} = -b \times t^{\beta}$$

b is defined as follows:

$$b = 1/2.303 \times (1/\alpha)^{\beta}$$

Alpha and β are scale and shape parameters, respectively. Alpha was calculated using the following equation:

$$\alpha = 1/(b \times 2.303)^{\frac{1}{\beta}}$$

The Weibull model can provide D-values for specific log reductions. Time for a 5-log reduction (5-log D-values) in seconds were calculated using the following equation:

$$t_{5D} = (5/b)^{\frac{1}{\beta}}$$

Statistical analysis

Statistical analysis was preformed using SPSS version 24 statistical package (IBM Corporation, Armonk, NY). Parameter estimates were obtained, and analysis of variance was preformed to calculate the R^2 value.

Results and Discussion

Physicochemical analysis

The pH of the representative juice lot was recorded at 3.96. The soluble sugar solids of the representative juice lot were recorded at 11.9 °Brix. Research analyzing the heat resistance of *E. coli* in apple juice states that Degrees Brix ranging from 11.8 to 16.5 had no significant effect on the heat resistance of *E. coli*. They also found that the pH range of 3.6 to 4.0 was not a significant factor with respect to thermal inactivation *(24)*.

Weibull model shape parameters and model fit

A Weibull model was created for each organism, temperature, and treatment variables. The specific *b*, α and β parameters for each model are listed in Table 4, 5 and 6 for *Salmonella,* STEC and *L. monocytogenes*, respectively. The shape and scale parameters provide an accurate model for each organism, temperature, and treatment combination. As can ben see in Figures 2-9, generally a concave upward curve was observed. As the temperature increased to 70°C, more rapid inactivation was observed at earlier time points, with slower rates of inactivation observed after 40 s.

When evaluated against linear models, in all cases the Weibull model resulted in a better fit resulting in higher R^2 values (Table 4 – 6). The largest

difference between models was seen with STEC at 60°C where R^2 values ranged from 0.99 to 0.97 for the Weibull model compared to 0.91 to 0.44 for the linear regression model (Table 5). It is important to make sure that the model is an accurate representation of the data that it is referencing. Every R^2 value obtained from each Weibull model was 0.925 or higher indicating that each model will well fit and appropriately represented the data that was collected. This gives great assurance for estimated values obtained within the range of data observed.

Many studies that evaluate thermal inactivation in juices use first order kinetics to analyze data *(26, 45)*. The main concern with this is that they may not have an accurate model to calculate D-values from *(37, 65)*. Survival curves observed in thermal inactivation of microorganisms are concave upward and showing that initially organisms die off quickly yet over time the ones that survive will not die off at the same rate *(65)*. Assuming that the rate of thermal inactivation is constant is a dangerous assumption. In the beginning a linear analysis will overestimate the rate of thermal inactivation. While not reflective of the true population, this would result in a more conservative approach to estimating thermal inactivation. However, as time goes on it will underestimate the number of survivors. The graphed results from this study provide a visual representation to show that microorganisms did not die off at a steady rate making the Weibull model a more appropriate model for data analysis of thermal

inactivation of microorganisms. For STEC and *Listeria*, greater discrepancies were observed at 60°C when using the linear model (Table 5 and 6), indicating the potential for survivors when D- and z-values calculated from data collected at lower temperatures were extrapolated to higher temperatures.

Thermal inactivation

The time that it takes for each organism, treatment, and temperature combination to reach a 1- and 5-log reduction is listed in table 2.6 and 2.7, respectively. This study found non-acid adapted STEC to be the most thermally resistant organism of those studied. Indicating that at 70°C it would take 155 seconds (2.6 minutes) to achieve a 5-log reduction. All strains evaluated in this study were associated with outbreaks (Table 2) as is common practice when conducting challenge studies. Three of the five STEC strains selected were associated with juice outbreaks, demonstrating a previous-association with virulence in a low pH environment.

Acid-adapted *Salmonella* was more heat resistant than non-acid adapted *Salmonella* at all processing temperatures when comparing D-values (Table 7). Glucose and HCl modes of adaptation were both found to result in longer processing times to achieve a 1-log reduction. Similar trends were also found in the 5-D calculations (Table 8) While with *L. monocytogenes,* HCl adapted cells

were consistently more heat resistant than when acid adapted through glucose supplementation or compared to the control at 60 and 70°C (Table 7 and 8).

It is commonly found that acid adaptation at lower processing temperatures can affect thermal resistance *(45, 55, 56, 63)* Topalcengiz and Danyluk (2017) found that differences amongst acid adapted (glucose) STEC at temperatures ranging from 56-60°C compared to unadapted cells, but these trends did not hold true with *Salmonella* and *L. monocytogenes (63)*. They determined that there is strain to strain variability when it comes to thermal resistance due to acid adaptation in STEC strains *(63)*.

Sharma et al. (2005) found similar trends with acid adapted (glucose) *E. coli* O157:H7, *Salmonella* and *L. monocytogenes* in cantaloupe juice and watermelon juice *(40)*. They found that acid adapted cells of *Salmonella* and *E. coli* O157:H7 had increased thermal resistance but *L. monocytogenes* did not show increased resistance from acid adaption *(40)*. This may also be the result of strain variability within an organism.

More recently Haberbeck et al. (2017) tested 48 strains of *E. coli* and found acid adaptation at a pH of 5.5 will increase thermal resistance at 58°C *(29)*. They did observe a large amount of variability in D-values of each strain at 58°C *(29)*. Their observed D-values ranging from 1 to 69 min, agrees with the findings

of Topalcengiz and Danyluk (2017) and strain variability *(29, 63)*. These findings suggest that inclusion of *E. coli* serotypes beyond O157:H7 is imperative as we seek to further validate processes in future work. The drastically extended processing times reported in this work for STEC may be a result of resistance tied to a subset of strains used.

Mazzotta (2001) analyzed the effects of acid adapted *L. monocytogenes, Salmonella* and *E. coli* O157:H7 in orange juice compared to non-adapted cells *(45)*. A D-value of 0.35 min for HCl acid adapted *Salmonella* in orange juice at 60°C and 0.21min for stationary phase cells was determined, as well as a D-value of 1.7 and 1.1 min for acid adapted and unadapted *E.coli* O157:H7 under the same conditions *(45)*. This study also saw similar trends in HCl adapted cells having higher D-values for *L. monocytogenes* with stationary phase cells having a D-value of 0.43 min at 60°C and 0.67 min for acid adapted. The time to achieve a 1-log reduction for *Salmonella* in this study were lower (0.03 – 0.12 min) but showed similar trends.

While this study can confirm that STEC is the most thermally resistant, when comparing STEC, *Salmonella* and *L. monocytogenes*, this study did not find that acid adapted STEC was more thermally resistant than non-acid adapted (Table 7 and 8). Amongst all foodborne pathogens, *Salmonella* was the least resistant to heat (Table 7 and 8). At 60°C, acid adapted cells did result in higher

D-values for *Salmonella* and *L. monocytogenes*, with HCl showing longer processing time than glucose enriched cells. At higher processing temperatures, there was no clear trend in thermal inactivation tied to the method of acid adaptation. For *Salmonella* and *L. monocytogenes,* at 65 and 70°C, one of the acid adaptation treatments resulted in the longest recommended processing time to achieve a 1-log (Table 7) or 5-log (Table 8) reduction.

The FDA highlights two different validation studies that provides suggestions and guidance for juice processors on juice HACCP *(4)*. Mazzotta (2001) provides a recommendation to ensure a 5-log reduction of *E. coli* O157:H7, *Salmonella* and *L. monocytogenes* for juice with pH less than 4.0 *(45)*. This study looked at acid adapted (with HCl) and non-acid adapted *E. coli* O157:H7, *Salmonella* and *L. monocytogenes* in apple juice, orange juice and white grape juice and recommends thermal processing at 71.1°C for 3 seconds *(45)*. Juice processors that are looking to process their juice at a lower temperature to protect flavor or color characteristics may look at the second study. The second study looks at *E. coli* O157:H7, *Salmonella* and *L. monocytogenes* in apple cider *(43)*. In this study Mak et al. (2001) were able to demonstrate a 5-log reduction of *E. coli* O157:H7, *Salmonella* and *L. monocytogenes* in apple cider at 68.1°C for 14 seconds *(43)*.

Mazzotta (2001) was able to achieve a 5-log pathogen reduction at 71.1°C for 3 seconds *(45)*. Based on the results from this research, 3 seconds would not be sufficient to inactivate the strains utilized. The other processing recommendation at 68.1°C for 14 seconds is also insufficient based on the results in this study *(43)*. Further evaluation is warranted to determine if the extrapolated values from the Weibull models accurately predict data when extrapolated. If so, further evaluation for STEC inactivation would be warranted to determine which strain(s) amongst the five utilized are driving these observations and if they are representative of organisms which should be targeted for Juice HACCP.

Conclusion

While this study did not find that acid adaption played a role in the thermal resistance of STEC, this study did show increased thermal resistance in *Salmonella* and *L. monocytogenes*. This data is also in agreement that Shiga toxigenic *E. coli* are the most resistant organisms when compared to *Salmonella* and *L. monocytogenes*. The 5-log reduction times observed in this study suggest that 71.1°C for 3 s would not be sufficient to achieve a 5-log reduction of STEC strains evaluated. Finally, first order kinetics did not provide the best fit model when compared to Weibull models. Use of linear models may result in surviving populations as greater inactivation rates are desired for product safety.

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APPENDIX

Table 1. Early reports of juice-associated outbreaks *(9, 18, 19, 53)*

Organism	*Strain/Serovar*	*Juice*	*Year*	*Number of Illness caused*
Salmonella	Hartford	Orange	1995	62
	Muenchen	Orange	1999	298
STEC	O157:H7	Apple Cider	1991	23
	O157:H7	Apple	1996	70

Table 2. Strains/serovars used to evaluate the thermal resistance in orange juice, the outbreak they were initially associated with and source they were obtained from.

Cocktail Organism	***Strain/Serovar***	***Outbreak***	***Source***
L. monocytogenes	LM 101	Cantaloupe	The University of Tennessee culture collection
	Scott A	Milk	The University of Tennessee culture collection
	V7	Milk	The University of Tennessee culture collection
	301	Goat Cheese	The University of Tennessee culture collection
	101	Salami	The University of Tennessee culture collection
STEC	O157:H7 C7927	Cider	The University of Tennessee culture collection
	O111 MDD338	Apple Cider	The University of Florida, Danyluk Lab
	O157:H7 MDD339	Apple Juice	The University of Florida, Danyluk Lab
	O157:H7 CDC 658	Cantaloupe	The University of Tennessee culture collection
	O157:H7 ATCC 43895	Hamburger	The University of Tennessee culture collection
Salmonella	Gaminara	Orange Juice	The University of Tennessee culture collection
	Typhimurium ATCC 14028	Orange Juice	The University of Tennessee culture collection
	Enteritidis ATCC BAA-1045	Almond	American Type Culture Collection
	Montevideo	Tomato	The University of Tennessee culture collection
	Muenchen MDD30	Orange Juice	The University of Tennessee culture collection

Table 3. Temperature and time combinations evaluated to determine the inactivation of *Salmonella enterica, Listeria monocytogenes,* and Shiga toxigenic *Escherichia coli*.

60°C	**65°C**[1]	**70 °C**
0 seconds	0 seconds	0 seconds
5 seconds	5 seconds	5 seconds
30 seconds	20 seconds	20 seconds
60 seconds	40 seconds	40 seconds
90 seconds	60 seconds	60 seconds
120 seconds	80 seconds	80 seconds
	100 seconds	

[1]Only *Salmonella* and Shiga toxigenic *E. coli* were evaluated at 65°C

Table 4. Coefficients of Weibull model survival curve of *Salmonella* in orange juice during thermal inactivation with and without prior acid adaptation.

		Weibull Model				Linear Regression
Temperature (°C)	**Treatment**	**β** ± std error	**b** ± std error	**α**	**R^2**	**R^2**
60	Control	0.358±0.028	0.840±0.101	0.158	0.994	0.976
	1 N HCl	0.484±0.037	0.376±0.061	1.341	0.994	0.976
	1 % Glucose	0.434±0.023	0.554±0.056	0.569	0.997	0.944
65	Control	0.470±0.027	0.487±0.055	0.785	0.995	0.943
	1 N HCl	0.514±0.039	0.374±0.061	1.334	0.992	0.947
	1 % Glucose	0.592±0.031	0.288±0.037	1.997	0.996	0.919
70	Control	0.054±0.008	4.798±0.138	0.000 (6.99E-20)	0.999	0.986
	1 N HCl	0.078±0.012	4.087±0.170	0.000 (3.05E-13)	0.997	0.980
	1 % Glucose	0.102±0.013	3.510±0.172	0.000 (1.2E-9)	0.997	0.980

Table 5. Coefficients of Weibull model survival curve of STEC in orange juice during thermal inactivation with and without prior acid adaptation

		Weibull Model				Linear Regression
Temperature (°C)	**Treatment**	**β** ± std error	**b** ± std error	**α**	**R^2**	**R^2**
60	Control	0.267±0.024	0.183±0.019	25.738	0.993	0.445
	1 N HCl	0.568±0.091	0.103±0.042	12.598	0.972	0.691
	1 % Glucose	0.478±0.041	0.251±0.045	3.248	0.992	0.910
65	Control	1.188±0.086	0.013±0.005	19.795	0.992	0.824
	1 N HCl	0.429±0.037	0.6346±0.098	0.413	0.990	0.940
	1 % Glucose	0.501±0.034	0.408±0.059	1.130	0.993	0.839
70	Control	0.486±0.044	0.430±0.075	1.020	0.991	0.944
	1 N HCl	0.198±0.011	2.358±0.094	0.00019	0.998	0.945
	1 % Glucose	0.435±0.039	0.777±0.120	0.262	0.992	0.928

Table 6. Coefficients of Weibull model survival curve of *L. monocytogenes* in orange juice during thermal inactivation with and without prior acid adaptation.

		Weibull Model				Linear Regression
Temperature (°C)	**Treatment**	**β** ± std error	**b** ± std error	**α**	R^2	R^2
60	Control	0.963±0.058	0.031±0.008	15.284	0.996	0.868
	1 N HCl	1.434±0.359	0.001±0.001	76.979	0.925	0.716
	1 % Glucose	0.879±0.109	0.037±0.018	16.478	0.983	0.872
70	Control	0.358±0.030	1.266±0.190	0.050	0.989	0.988
	1 N HCl	0.402±0.056	0.934±0.207	0,149	0.981	0.957
	1 % Glucose	0.152±0.023	3.261±0.277	0.000 (1.691E-6)	0.992	0.947

Table 7. Time in seconds to achieve a 1-log reduction based upon Weibull regression models with and without cells which had been previously acid adapted.

Organism	Treatment	Temperature (°C)		
		60	**65**	**70**
Salmonella	None	1.63	4.62	3.13 E-13
	HCl	7.27	6.77	1.38 E-8
	Glucose	3.89	8.17	4.37 E-6
STEC	None	588.7	39.95	5.67
	HCl	54.78	2.89	1.3 E-2
	Glucose	18.04	5.98	1.79
L. monocytogenes	None	36.34	nd[1]	0.52
	HCl	137.8	nd	1.18
	Glucose	42.55	nd	4.13 E-4

[1]Not determined.

Table 8. Time in seconds (minutes) to achieve a 5-log reduction based upon Weibull regression models with and without cells which had been previously acid adapted.

Organism	Treatment	Temperature (°C)		
		60	65	70
Salmonella	None	146.5 (2.44)	141.4 (2.36)	2.13 (0.04)
	HCl	189.3 (3.15)	155.5 (2.59)	13.7 (0.23)
	Glucose	159.0 (2.65)	123.6 (2.06)	32.4 (0.54)
STEC	None	na[1]	154.9 (2.58)	155.5 (2.59)
	HCl	933.3 (15.5)	122.9 (2.05)	44.9 (0.75)
	Glucose	523.3 (8.72)	148.6 (2.48)	72.4 (1.21)
L. monocytogenes	None	193.3 (3.22)	nd[2]	46.3 (0.77)
	HCl	423.3 (7.06)	nd	65.0 (1.09)
	Glucose	265.3 (4.42)	nd	16.7 (0.28)

[1]Value not determined due to extended extrapolation.

[2]Not determined

Figure 1. Principles of HACCP *(31)*

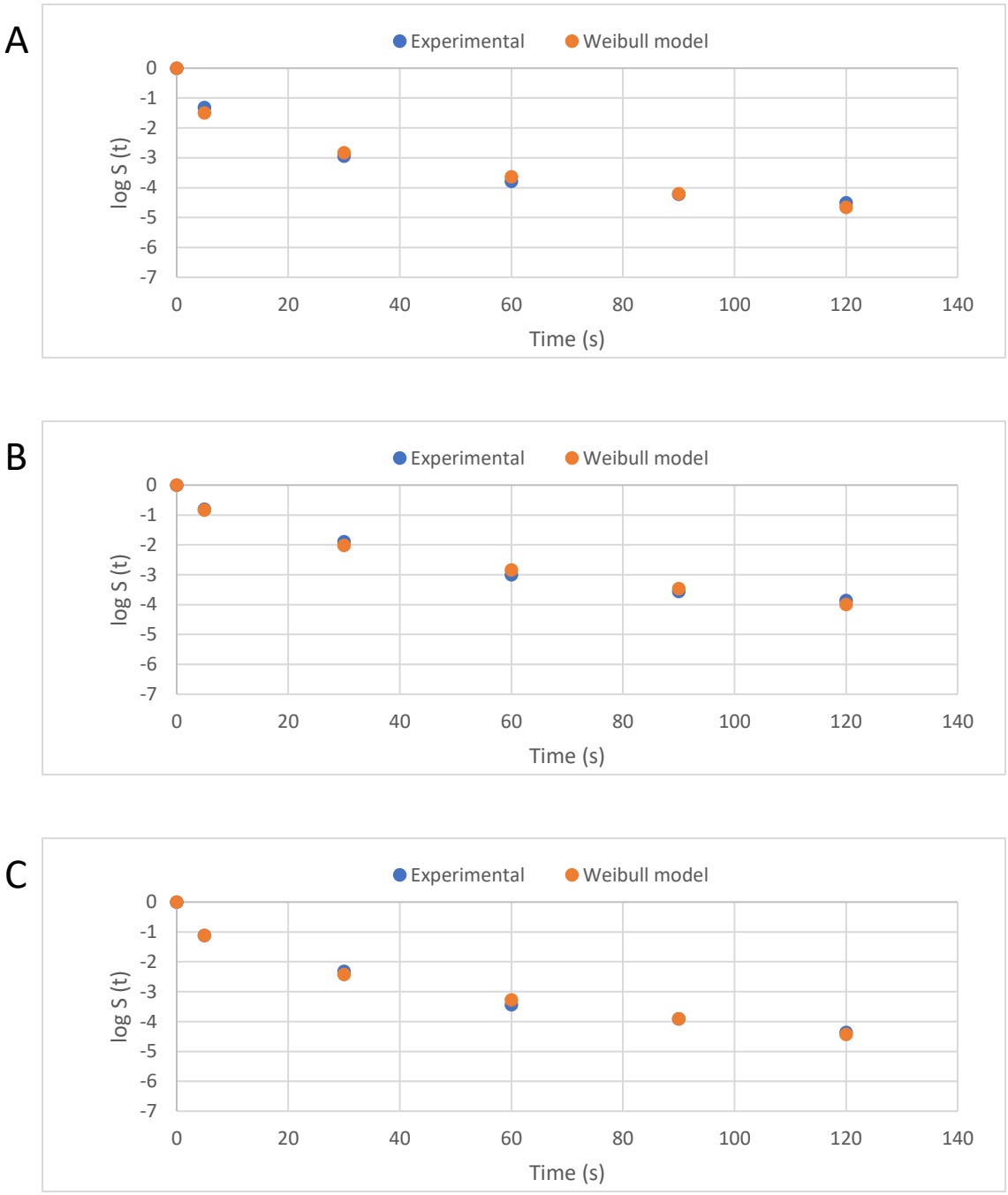

Figure 2. Thermal inactivation curves for *Salmonella enterica* at 60°C with a) no acid adaption, b) acid adaptation with 1N HCl, and c) acid adaptation through fermentation with 1% (w/v) glucose.

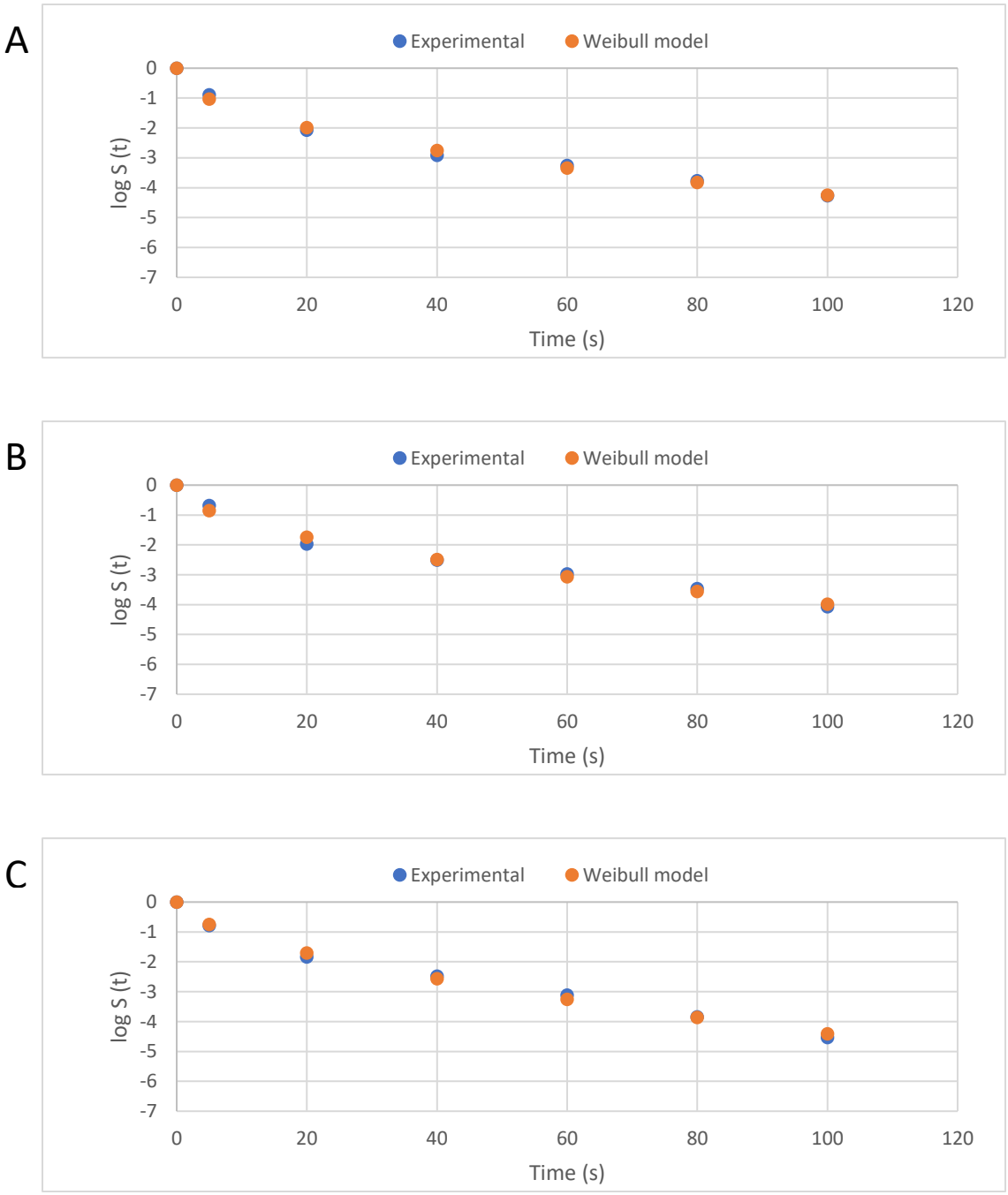

Figure 3. Thermal inactivation curves for *Salmonella enterica* at 65°C with a) no acid adaption, b) acid adaptation with 1N HCl, and c) acid adaptation through fermentation with 1% (w/v) glucose.

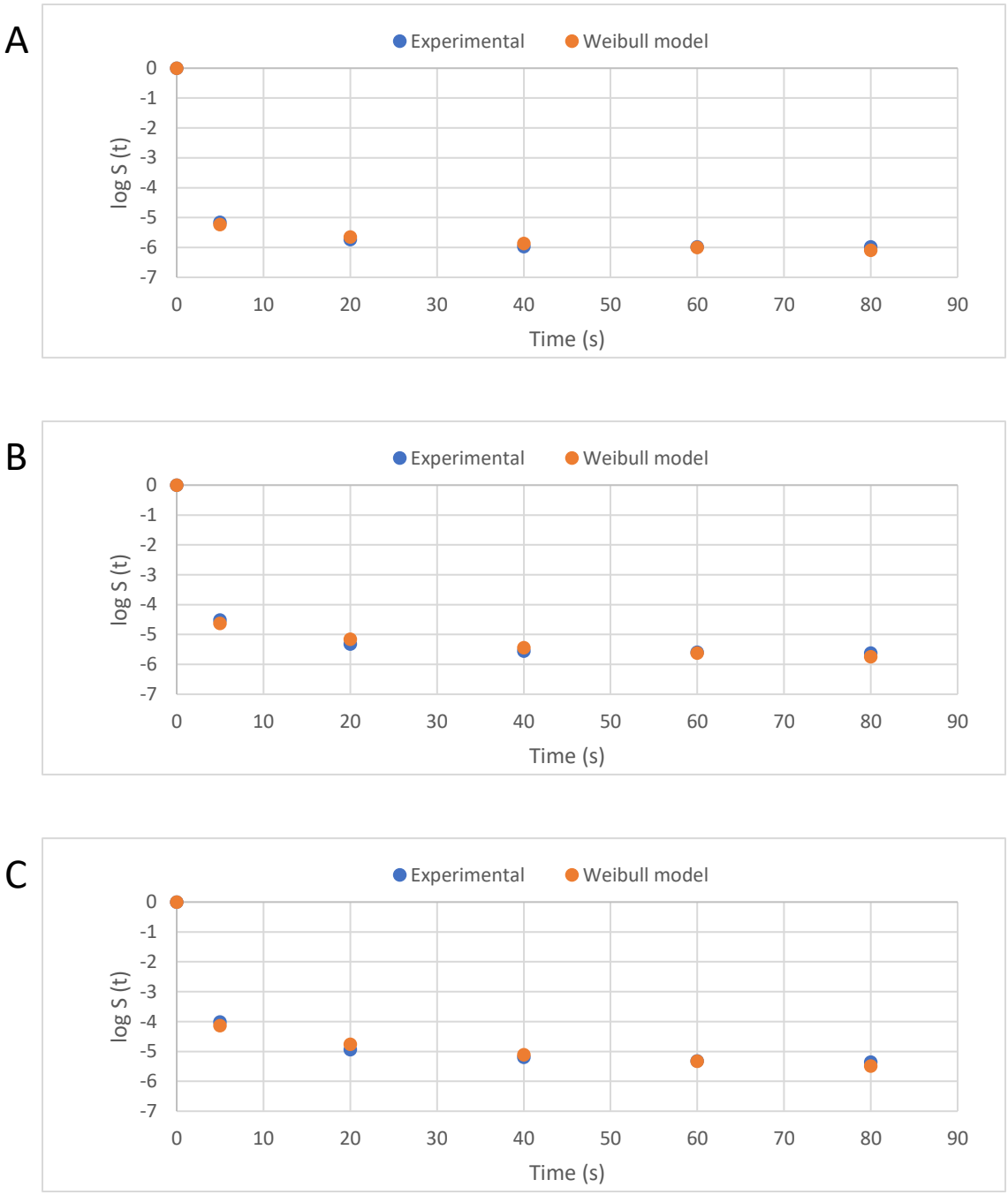

Figure 4. Thermal inactivation curves for *Salmonella enterica* at 70°C with a) no acid adaption, b) acid adaptation with 1N HCl, and c) acid adaptation through fermentation with 1% (w/v) glucose.

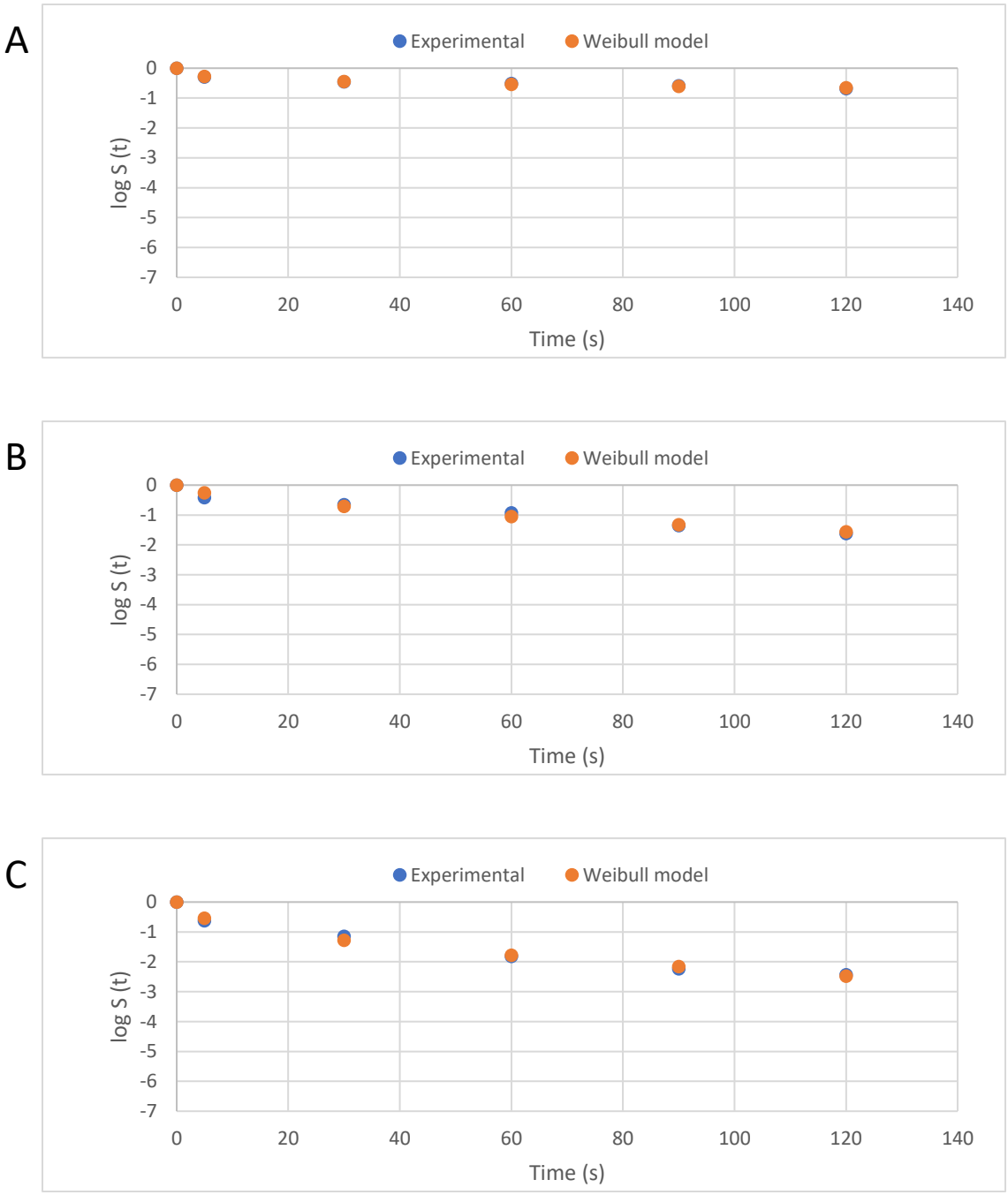

Figure 5. Thermal inactivation curves for Shiga toxigenic *Escherichia coli* at 60°C with a) no acid adaption, b) acid adaptation with 1N HCl, and c) acid adaptation through fermentation with 1% (w/v) glucose.

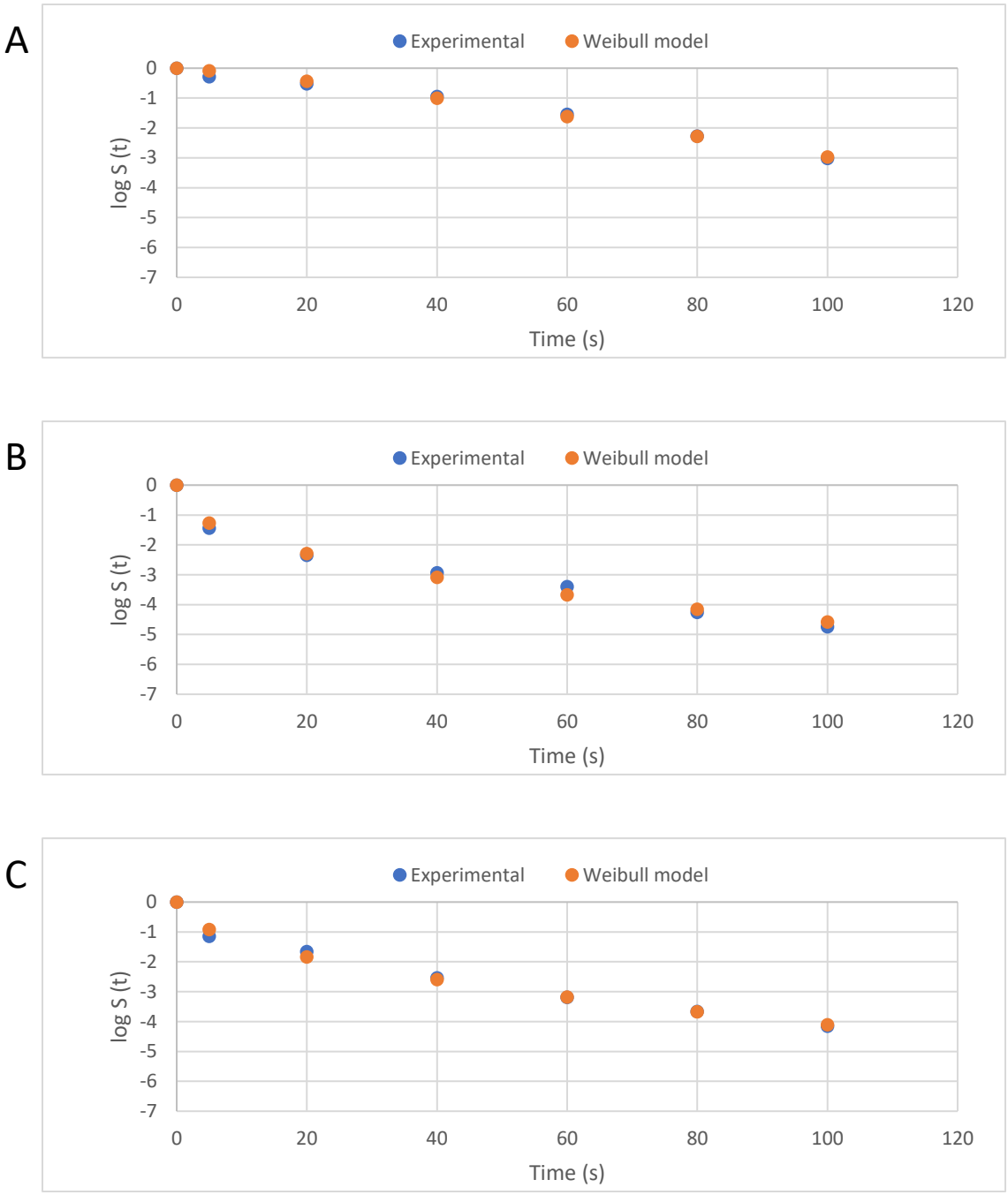

Figure 6. Thermal inactivation curves for Shiga toxigenic *Escherichia coli* at 65°C with a) no acid adaption, b) acid adaptation with 1N HCl, and c) acid adaptation through fermentation with 1% (w/v) glucose.

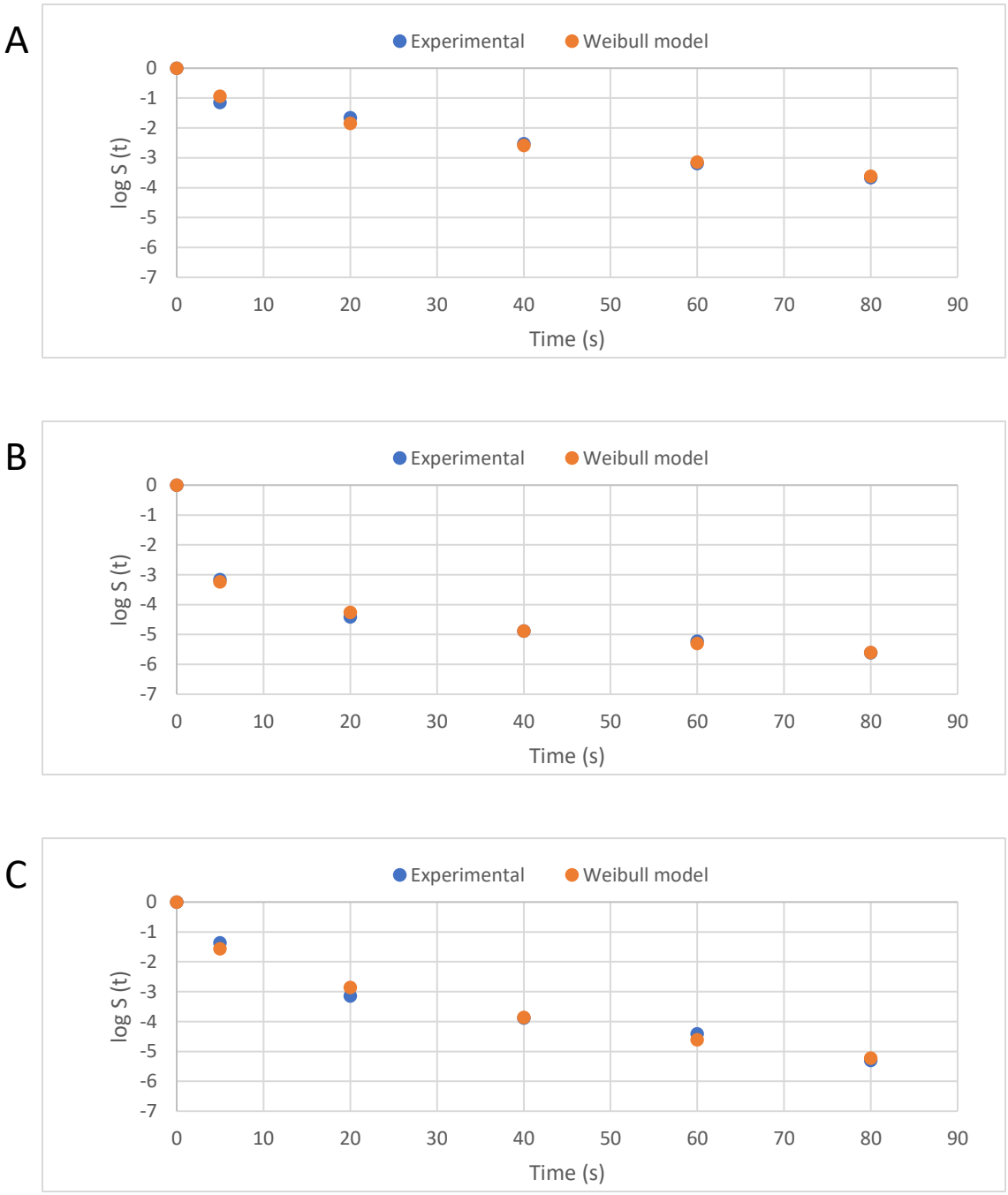

Figure 7. Thermal inactivation curves for Shiga toxigenic *Escherichia coli* at 70°C with a) no acid adaption, b) acid adaptation with 1N HCl, and c) acid adaptation through fermentation with 1% (w/v) glucose.

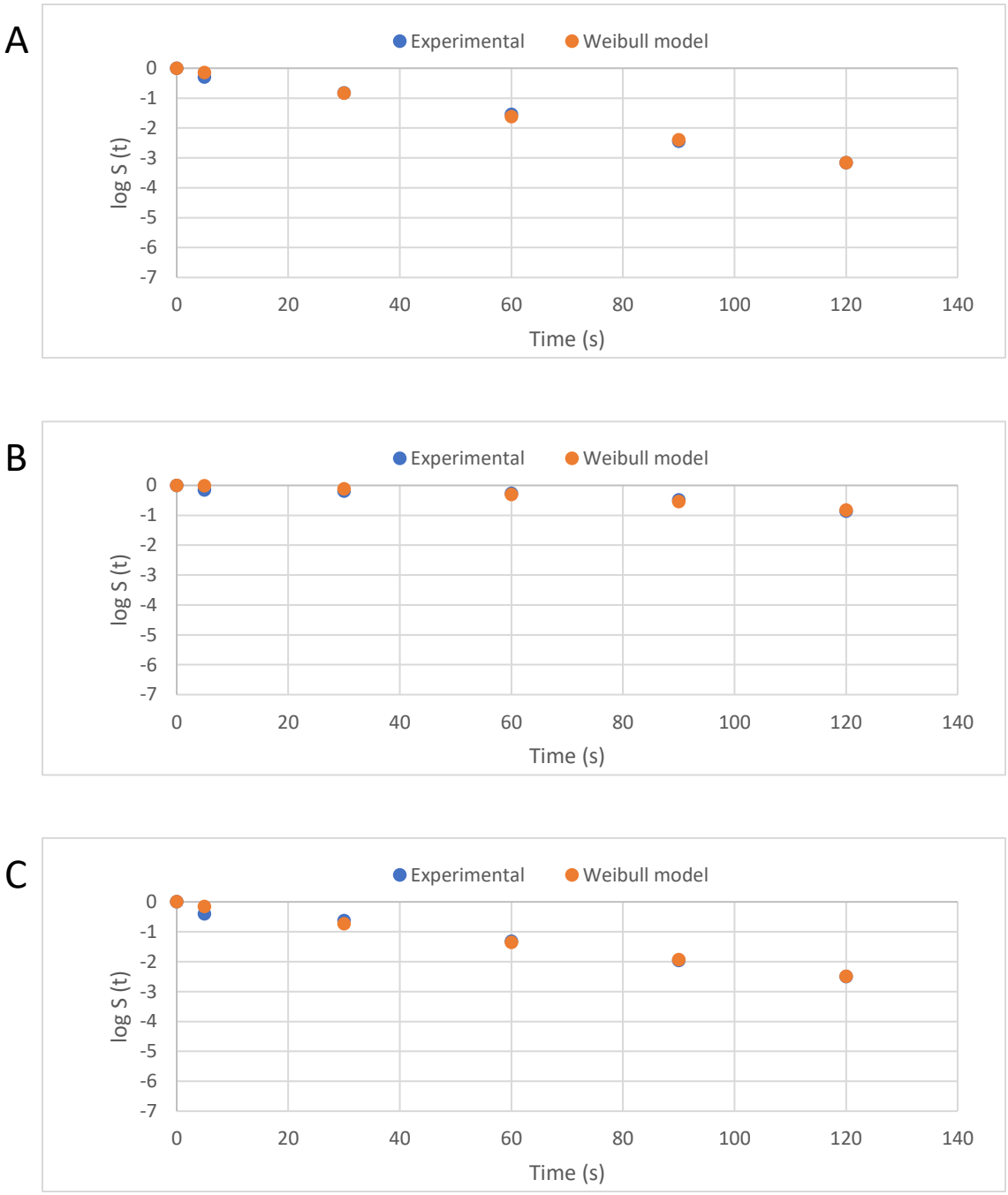

Figure 8. Thermal inactivation curves for *Listeria monocytogenes* at 60°C with a) no acid adaption, b) acid adaptation with 1N HCl, and c) acid adaptation through fermentation with 1% (w/v) glucose.

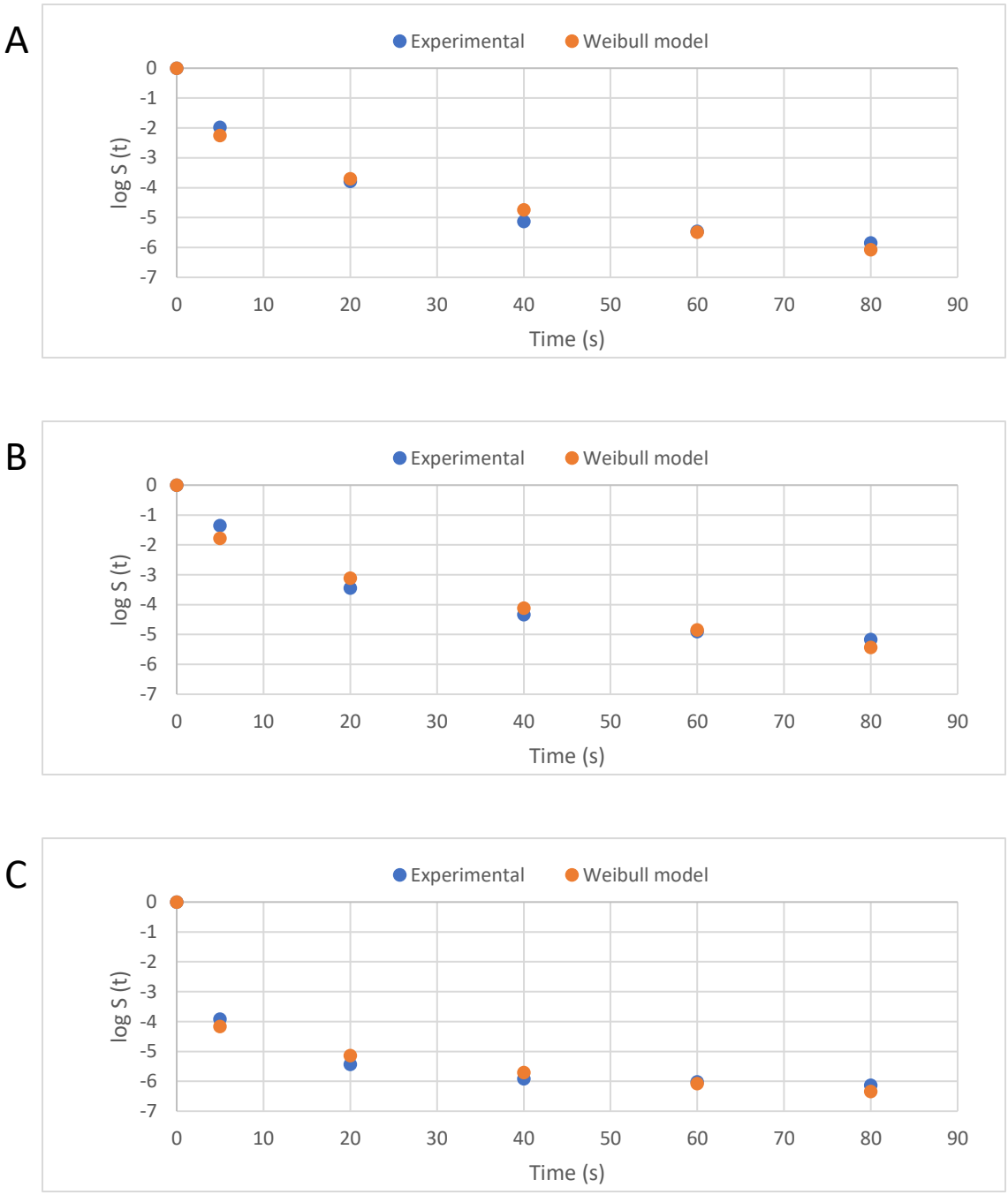

Figure 9. Thermal inactivation curves for *Listeria monocytogenes* at 70°C with a) no acid adaption, b) acid adaptation with 1N HCl, and c) acid adaptation through fermentation with 1% (w/v) glucose.

VITA

Valerie Nettles was born in Long Beach, California on August 6th, 1992 to parents Reginald and DeLorri Nettles. Valerie's family moved to Portland, Oregon where she grew up and graduated from Beaverton High School in 2010. Her family then moved back to Long Beach where she attended Long Beach City College. In 2014, she moved to Knoxville, Tennessee to attend the University of Tennessee where she received her Bachelor of Science degree in Food Science and Technology in 2016. After graduating, Valerie started the graduate program in Food Science at the University of Tennessee where she studied food microbiology.