Impacts of low-water activity food type on inactivation kinetics and models of foodborne pathogens treated with low-temperature, vacuum-assisted steam processing

Jennifer Claire Acuff

Dissertation submitted to the faculty of the Virginia Polytechnic Institute and State University in partial fulfillment of the requirements for the degree of

Doctor of Philosophy
In
Food Science and Technology

Monica A. Ponder, Committee Chair
Daniel Gallagher
Haibo Huang
Robert C. Williams

March 16, 2020
Blacksburg, VA

Keywords: low-water activity food safety, macadamia nuts, raisins, apricots, vacuum-steam, surrogate, inactivation kinetics, models, Shiga toxin-producing *Escherichia coli*, *Salmonella*, *Listeria monocytogenes*, *Pediococcus*

Copyright 2020

Impacts of low-water activity food type on inactivation kinetics and models of foodborne pathogens treated with low-temperature, vacuum-assisted steam processing

Jennifer C. Acuff

Abstract - SCIENTIFIC

Low water activity foods (LWAF), specifically nuts and dried fruits, have been generally considered safe because they do not support the growth of foodborne pathogens. However, many pathogens have been noted to survive in LWAF for considerable periods of time, and a number of recent outbreaks and recalls have implicated various types of nuts and dried fruits. The Food Safety Modernization Act requires food processors to develop preventive control plans that make ready-to-eat LWAF safer for consumers. The presented research was designed to investigate several aspects of LWAF safety by evaluating a steam process as a strategy to remove pathogen contamination from LWAF, modeling the inactivation of such treatments, and studying the thermal resistances of two *E. coli* strains in low-water activity solutions. Low-temperature, vacuum-assisted steam (vacuum-steam) was evaluated as a potential intervention and preventive control to remove pathogens from the surface of LWAF without using high-heat treatments that could damage product quality. The presented work examined the efficacy of vacuum-steam (<85°C) as a means to decontaminate the surface of whole macadamia nuts, dried apricot halves, and raisins from *Salmonella* spp., *Listeria monocytogenes*, and Shiga toxin-producing *Escherichia coli* (STEC) contamination. The low-temperature steam treatments successfully reduced all pathogens by >4 log CFU/g from the surfaces of the foods. Additionally, *Pediococcus acidilactici*, proved to be a surrogate organism for these pathogens and could be

used to challenge and validate similar treatments within processing plants. The data were fit to models, which showed that food type significantly impacted the fit, with the Weibull model best describing bacterial inactivation kinetics on raisins and macadamia nuts, and the Gompertz model best describing reductions on the apricot halves. The models were challenged for validation of their abilities to predict times required for 3-log reductions using internal and external datasets, determining the usefulness to industry members who wish to design similar thermal treatments for LWAF. Comparing predicted values from internally constructed models to observed values generated from external data, models were shown to be limited in scope and application and could only be applied to pathogen inactivation on different LWAF or thermal processes in certain circumstances. First-order and Weibull model predictions of bacterial reductions on dried apricots had varied success in predicting times for 3-log reductions on other thermally treated LWAF. However, the models of bacterial reductions on thermally treated macadamia nuts frequently overestimated the times required for 3-log bacterial reductions for other LWAF. In an effort to understand the effect that reduced water activity has specifically on STEC, two strains were investigated for induced thermal resistance due to osmotic stress. Thermal resistance of STEC strains (O121:H19 and O157:H7) were evaluated on the basis of strain variation, culture preparation, and water activity (*D*- and *z*-values). At the lowest treatment temperature (56°C), O121 displayed greater heat resistance than O157, and the broth-grown samples exhibited greater heat resistance than the lawn-grown cells, but significant differences were not observed at higher temperatures. Samples in reduced-water activity solutions displayed reduced thermal resistance at 56°C, but the *z*-values were 29-43% higher than those of high-water activity samples. While water activity has been shown to impact thermal resistance of pathogens, comparisons of STEC thermal resistance according to the *D*- and *z*-values revealed

that other factors also play roles in pathogen thermal resistance on LWAF. Results from the collection of experiments conclude that efficacy of thermal treatments is impacted by the physiological state of the cells, stress experienced in the food matrix, and characteristics of the food, including water activity and composition.

Impacts of low-water activity food type on inactivation kinetics and models of foodborne pathogens treated with low-temperature, vacuum-assisted steam processing

Jennifer C. Acuff

Abstract - PUBLIC

Consumers expect foods they purchase to be safe to consume by themselves and family members, particularly those that are ready-to-eat with no additional cooking requirements. Many of these foods are low-water activity foods (LWAF), like nuts and dried fruits, with very little water content that could be used by bacteria. These foods may be preferred snack foods due to their affordability, long shelf lives, and health benefits over other types of snack foods. Until recently, LWAF were generally considered safe because they do not support the growth of foodborne pathogens due to the lack of moisture or water within the food. However, a number of recent outbreaks related to various types of nuts and dried fruits have proven that many pathogens can survive in dried foods, even if not actively growing, for considerable amounts of time. Designed to address these types of food safety issues, the Food Safety Modernization Act recognizes risks associated with foods and responded with regulations requiring food processors to take steps to make ready-to-eat LWAF, like nuts and dried fruits, safer for consumers. A popular strategy is to treat foods with heat to destroy pathogens, however the quality attributes of some nuts and dried fruits could be damaged by high-heat treatments like roasting. An alternative process uses a vacuum to form steam at lower temperatures, allowing for efficient heat transfer through water droplets to the surface of the foods, thus causing less damage to the foods without introducing too much moisture. This research evaluated how this process could be

used by food processors to remove harmful bacteria from the surfaces of whole macadamia nuts, dried apricot halves, and raisins. Results indicated that the low-temperature steam treatments successfully reduced *Salmonella*, *Listeria monocytogenes*, and Shiga toxin-producing *Escherichia coli* (STEC) by >4 log CFU/g (>99.99%) from the surfaces of the foods. Additionally, a nonpathogenic lactic acid bacterium, *Pediococcus acidilactici*, exhibited similar or greater heat tolerance, which would allow food processors to use it as a substitute, or surrogate, for in-plant studies without introducing harmful bacteria into the food processing environment. Mathematical models were used to describe the trends of bacterial death due to the steam treatments, and the results indicated that the type of food significantly impacted the reduction of bacteria. The models were tested using additional data collected within our own laboratory, as well as others. Results indicated that some of the models could be used as predictors of bacterial death for similar LWAF but can only be applied with caution and consideration for the type of food and process. Additionally, two different *E. coli* strains associated with outbreaks (O121:H19 and O157:H7) were investigated to understand impacts of strain variation, growth method, and water activity on thermal resistance. Some differences in heat resistance were observed between the strains and between the growth methods. Additionally, the reduced water activity seemed to decrease the bacteria's ability to withstand some heat treatments. Overall, thermal resistance studies indicated that several factors, in addition to water activity, impact pathogens' development of resistance to heat treatments. The experiments' results show that there are complex relationships between bacteria and the food they inhabit. Food processors must consider these relationships in order to design the best thermal processes to make LWAF safe for consumers.

Acknowledgements

All work presented in this dissertation are results of collaborative efforts of dozens of individuals. I desire to acknowledge these persons for their assistance and encouragement, which were vital to my success.

Dr. Jian Wu has graciously served as a mentor for me over the last three years. Since I arrived at Virginia Tech, Dr. Wu made himself available to help with the needs of my project. I could not have designed and built the steam system in the BSL-2 processing plant without his assistance, and I am grateful for all the time he generously gave to help with each replication of the steaming projects. I am also grateful for the friendship we developed over the long hours of steaming and teaching food microbiology labs, where we bonded over our love of classical music, among many other things.

I cannot possibly thank Kim Waterman enough for her contributions to each and every one of the experiments presented in this dissertation. Kim helped with every aspect of the experiments, from beginning to end. I appreciated her willingness to arrive early on experiment mornings, flexibility to do whatever was needed to complete the projects, and wonderful attitude that kept a smile on everyone's faces. She provided both mental support and encouragement and was there for every success and pitfall. Without Kim, her technical expertise and knowledge of food safety research, I'm certain I would not have been able to complete the experiments on time or with success!

Claire Marik was connected with me and this project during her year in Blacksburg, and I am so grateful for the timing! Claire was helpful in so many ways, from the experiment preparation through execution. I was so lucky to have a partner who was so willing and capable of working in the BSL-2 lab. It took very little time for me to learn how competent, trustworthy,

and reliable Claire is. I enjoyed all of our conversations in the lab while harvesting lawns, and I particularly enjoyed solving the legendary puzzle together, along with Kim, during long treatments in the processing plant!

I wish to thank Brett Driver, Brian Wiersema, and Roman (Rusty) Rustia for the roles they played in building the steam system. Brett and Brian were particularly helpful in explaining how the steam system had been previously used years before I began my project, and they also provided ideas and suggestions that led to the reconstruction. Rusty was vital in building the system as it exists currently and was so helpful in troubleshooting to optimize the design.

We thank Dr. Hengjian Wang for his contributions to the work. Dr. Wang graciously performed all proximate analyses at minimal cost. We appreciate his expertise and willingness to accommodate our needs.

I am thankful for the role Dr. Robert Williams served as committee member. Dr. Williams consistently offered thought-provoking questions and was always willing to serve as a sounding board for ideas. I also appreciated his support and encouragement to do my best, while also reminding me to maintain a healthy perspective by acknowledging the reality of research, time constraints, and the truly important things in life.

I am honored that Dr. Haibo Huang agreed to serve as a committee member. His expertise was extremely valuable as we designed the steam system and parameters for treating the foods. Dr. Huang was happy and willing to meet with me on many occasions to answer my many questions and provide an engineer's perspective for the problems we came across.

I would like to enthusiastically thank Dr. Daniel Gallagher for his help with the work presented in this dissertation. Without his expertise, I would have been left to process massive amounts of data in Excel, and I surely would have lost my mind. Dr. Gallagher not only helped

develop code that could process all of my data and model the inactivation curves, but he met regularly with me to patiently answer my many questions. I learned exponentially more about data analysis and modeling by working with him and I am so grateful for his willingness to play an active role in this project. Dr. Gallagher sought to both challenge and encourage me over the last several years in my academic endeavors, and I am grateful for his mentorship.

For the last three years, I have felt an overwhelming sense of gratitude toward Dr. Monica Ponder. After only brief email exchanges and a few phone calls, Dr. Ponder agreed to advise me at Virginia Tech. Taking on a PhD student is a huge investment of both time and money, and while I will never know what prompted Dr. Ponder to take a chance on me, I am enormously grateful. Dr. Ponder taught me so much over the last three years about microbial ecology, food industries, research design, teaching, and so much more. She provided me every opportunity possible to grow academically and professionally and allowed me to own my successes and learn from my failures. Dr. Ponder has given me tools throughout her mentorship that I know will serve me throughout my career, and I only hope to be able to pass them along one day to my own students. I value Dr. Ponder's tremendous mentorship and friendship. I hope that I have made her proud and represented her well over the last three years, and hope that I continue to do so throughout my future career.

When I began my time at Virginia Tech, I found myself surrounded by dozens of other graduate students who became the best Foodie Friends I could ask for. I am so grateful for the friendships I found in my cohort. We learned alongside each other, encouraged one another, and commiserated together. Specifically, I want to thank Zachary and Rebekah Taylor for being kindred spirits, Erika Estrada Martinez for being the best office mate, Cameron Bardsley and Lester Schonberger for being wonderful collaborators, Kat Racine for being a fellow transplant

Texan and source of encouragement, and Dr. Lily Yang for managing to be a confidant, cheerleader, mentor, and close friend all in one.

I also wish to thank other friends who have been with me along this journey for many years. Dr. Minto Michael has been both a mentor and friend for the last five years, and I cannot express how much I have learned from him. Even while I was a master's student, he offered opportunities for me to learn as much I could, and I am still grateful for the leadership he displayed while we worked together at Kansas State University. Sarah L. Jones has been one of my closest friends and colleagues since 2015, and I know our friendship and collaboration will last beyond our years of graduate school. I am grateful to know her and to have experienced her brilliance, sense of humor, and kindness. Finally, I wish to thank Stephen and Bronwyn Case who have been nothing short of the best friends we could possibly ask for. Our friendship has withstood the tests of thousands of miles and several decades, and we are so blessed to have had their encouragement and kindness throughout this journey.

While my husband's entire family has been both understanding and loving throughout the last five years, I want to specifically thank Don, Gale, and Ryan Fleer. They have been immensely supportive as we have moved around the country for graduate school. They have accommodated our busy schedules, shortened holidays, and highly stressed lives, and I cannot express how much I appreciate their prayers and encouragement.

For all of my 28 years of life, my constant source of love and encouragement has been my family. My mom, dad, and sister have all believed in my abilities to achieve my goals and have been with me for each step of the journey. I am so grateful for my mom, Awilda Acuff, and her patience and willingness to work through math problems me, even throughout my graduate school years! She is the best and kindest mathematician I know. For as long as I can remember,

my mom always encouraged me to do my best and glorify God in all my actions. I'm grateful for her example of those actions and am blessed to have had her friendship and support throughout my academic career and life. I can never repay her for the nights she stayed up late with me to study and complete school projects, or for the number of times she brought said projects to my school when I forgot them at home. I only hope I can be half as wonderful of a mother one day.

My dad, Gary Acuff, undoubtedly inspired and sparked my interest in food microbiology, so I must thank him for his guidance as I began navigating a career in food safety. My early training as a microbiologist took place in his lab and with his research associate, Lisa Lucia, and I learned more during these years than I can even list. I made more mistakes in his lab than I'd like to admit, but it was there that I learned my aseptic technique, how to plan research projects efficiently, that cleanliness is next to godliness, and how to make 50-year-old glassware shine like new. More importantly, though, I want to thank him for always encouraging me to chase my passions and goals, whatever they may be. While I am certain he would have supported my interest in any career I pursued, I love that we have such unique opportunities to attend conferences and work together from time-to-time. I have learned so much from him as a scientist, but I have learned even more from him about hard work and sacrifice, love for one's family, and how to be a life-long fan of losing football teams.

I cannot express how grateful I am to my sister, Laura Tolentino, for our lifelong friendship. Her encouragement and impeccable ability to send the right meme at just the right time helped me through some of my toughest days of graduate school. I also owe a great deal to her for being my first brutal editor. She taught me to write and copy edit like a journalist, and to take constructive criticism as opportunities to learn. Laura has also served as my personal graphic designer for a surprising number of microbiology-related projects, and I admire her

creative spirit. She, her husband, Paolo, and their children have given me more smiles than I deserve, and I'm so thankful for their love and support.

Finally, I want to thank my husband, Justin Fleer. Justin and Walter, our fur-child, are my biggest sources of joy and what I look forward to at the end of each day. Justin has been a part of my academic journey since we were freshmen at Abilene Christian University, and I can honestly say I would not have made it to or through graduate school without his unending support. He has moved across the country with me, stayed up late to help me study, fixed my computers when they died, sorted through data when my eyes crossed, helped me understand and write lines of code, waited outside the lab on weekends so I wouldn't be alone, helped decorate cookies and make goody bags for my friends and lab mates, made me breakfast every morning, and much, much more. For every step of this journey, he has given me encouragement to reach my full potential and believe in myself. Justin has been my shoulder to cry on when days didn't go as I had hoped, and he's been my loudest cheerleader for even the smallest of successes. He is my biggest blessing – one that I don't deserve but will hold onto with every bit of life that I have. I can't thank God enough for putting him in my life, and I can't thank Justin enough for loving and supporting me so well.

Attributions

Many professionals at Virginia Tech contributed greatly to the work presented in this dissertation, including faculty, staff, and students.

Dr. Monica A. Ponder, Associate Professor in the Department of Food Science and Technology, is the primary advisor and committee chair for this research. She provided extensive direction and guidance for every aspect of the projects. Her mentorship, expertise, and knowledge about the food industry and low-water activity food safety was integral. Dr. Ponder is a coauthor on all manuscripts.

Dr. Daniel Gallagher, Professor in the Department of Civil and Environmental Engineering, is a committee member for this research. Dr. Gallagher helped analyze all results related to Chapters 3-4. He coded frameworks in order to process and analyze data in efficient manners and prompted many of the ideas for data analyses in these chapters. Dr. Gallagher is coauthor on manuscripts associated with Chapters 3 and 4.

Dr. Haibo Huang, Assistant Professor in the Department of Food Science and Technology, is a committee member for this research in the Department of Food Science and Technology. Dr. Huang helped develop the low-temperature, vacuum-assisted steam system used to collect data, and he provided food engineering expertise and guidance as projects were designed. Dr. Huang is coauthor on manuscripts associated with Chapters 3 and 4.

Dr. Robert Williams, Professor and Extension Food Microbiologist in the Department of Food Science and Technology, is a committee member for this research. He provided valuable guidance, mentorship, and direction as the research was developed and executed. Dr. Williams is a coauthor on manuscripts associated with Chapters 3 and 4.

Ms. Kim Waterman, Research Associate in the Department of Food Science and Technology was integral in the execution of these research projects. Her expertise in laboratory techniques was highly valued and appreciated and she is coauthor on all manuscripts.

Dr. Jian Wu, Research Associate and Teaching Lab Manager in the Department of Food Science and Technology, provided expertise in both food microbiology and engineering, which contributed to the success of this research. Dr. Wu is coauthor on manuscripts associated with Chapters 3 and 4.

Ms. Claire Marik, Graduate Research Assistant in the Department of Food Science and Technology, was instrumental in the data collection for vacuum-assisted steam projects. Ms. Marik provided technical support for the research described in Chapters 3 and 4, and her time and efforts warranted her co-authorship on the associated manuscripts.

Jahnavi Ramakrishnan, undergraduate student at Virginia Tech, provided technical support for a portion of the research presented in Chapter 5. She is a coauthor on the associated manuscript.

Table of Contents

Abstract - SCIENTIFIC ... ii

Abstract - PUBLIC ... v

Acknowledgements ... vii

Attributions ... xiii

Table of Contents ... xv

List of Figures ... xviii

List of Tables ... xxi

Chapter 1 Introduction & Significance ... 1

Objectives ... 1

Justification ... 1

Use of Low-Temperature, Vacuum-Assisted Steam (Chapter 3) ... 3

Bacterial Reductions from Vacuum-Steam ... 3

***Pediococcus acidilactici* as a Surrogate Microorganism for Pathogens during Vacuum-Steam Processes ... 5**

Modeling Bacterial Inactivation Trends from Vacuum-Assisted Steam ... 6

Exploration of Model Flexibility and Limitations (Chapter 4) ... 7

Impacts of Reduced Water Activity on Thermal Resistance of Shiga Toxin-Producing *E. coli* (Chapter 5) ... 8

Summary ... 10

References ... 12

Chapter 2 Literature Review ... 16

1. Background and Significance ... 16

2. Survival of microorganisms on LWAF ... 21

3. Organisms ... 28

4. Relevant Research Methods ... 38

References ... 48

Chapter 3 Thermal Inactivation of *Salmonella*, Shiga Toxin-Producing *Escherichia coli*, *Listeria monocytogenes*, and a Surrogate (*Pediococcus acidilactici*) on Raisins, Apricot Halves, and Macadamia Nuts Using Vacuum-Steam Pasteurization ... 60

Abstract: ... 61

1. Introduction ... 63

2. Materials and Methods ... 67

3. Results **73**
4. Discussion **78**
5.0 Conclusions **85**
Figures **87**
Tables **91**
Supplemental Materials **93**
References **98**
Chapter 4 Challenge and validation of kinetic models of bacterial thermal inactivation on whole macadamia nuts and dried apricot halves using low-temperature, vacuum-assisted steam 104
Abstract: **105**
1. Introduction **107**
2. Materials & Methods **109**
3. Results and Discussion **115**
4. Conclusions **124**
Figures **126**
Tables **137**
Supplemental Materials **141**
References **145**
Chapter 5 Inactivation Kinetics of Shiga Toxin-Producing *Escherichia coli* O121:H19 and O157:H7 as Impacted by Culture Preparation Method and Water Activity 148
Abstract: **149**
Highlights **150**
1. Introduction **151**
2. Materials and Methods **153**
3. Results **155**
4. Discussion **156**
Figures **162**
Tables **163**
References **164**
Chapter 6 Conclusions & Future Directions 168
Chapter 7 Appendix 175
Combined average log bacterial reductions of low-temperature, vacuum-assisted steam on raisins, apricot halves, apricot pieces, whole macadamia nuts, and macadamia nut pieces. **175**

Combined temperature profile parameters averaged across each treatment combination. 185

Bacterial reductions (log CFU/g ± standard deviation) on apricot pieces resulting from 72°C and 82°C vacuum-steam treatment of various times. 188

Bacterial reductions (log CFU/g ± standard deviation) on macadamia nut pieces resulting from 72°C and 82°C vacuum-steam treatment of various times. 189

Proximate analyses of raisins before and after low-temperature, vacuum-assisted treatments. 190

Proximate analyses of dried apricot halves before and after low-temperature, vacuum-assisted treatments. 191

Proximate analyses of dried apricot pieces before and after low-temperature, vacuum-assisted treatments. 192

Proximate analyses of whole macadamia nuts before and after low-temperature, vacuum-assisted treatments. 193

Proximate analyses of macadamia nut pieces before and after low-temperature, vacuum-assisted treatments. 194

Code written in R for data compilation, model construction, analyses, and temperature profile compilation. 195

***D*- and *z*-values of each experiment. 210**

List of Figures

Figure 3.1. Bacterial reductions (log CFU/g ± standard deviation) on apricot halves resulting from 72°C (a) and 82°C (b) vacuum-steam treatment of various times. Asterisk (*) next to treatment times indicate significant differences of bacterial reductions from the reductions of a 0-min treatment, while lowercase letters above bars indicate significant differences between organisms within a treatment from pairwise comparisons (p<0.05). Lack of letters indicate no significant difference between organisms within the treatment. 87

Figure 3.2. Bacterial reductions (log CFU/g ± standard deviation) on macadamia nuts resulting from 72°C (a) and 82°C (b) vacuum-steam treatment of various times. Asterisk (*) next to treatment times indicate significant differences of bacterial reductions from the reductions of a 0-min treatment, while lowercase letters above bars indicate significant differences between organisms within a treatment from pairwise comparisons (p<0.05). Lack of letters indicate no significant difference between organisms within the treatment. 87

Figure 3.3. Bacterial reductions (log CFU/g ± standard deviation) on raisins resulting from 62°C (a) and 72°C (b) vacuum-steam treatment of various times. Asterisk (*) next to treatment times indicate significant differences of bacterial reductions from the reductions of a 0-min treatment, while lowercase letters above bars indicate significant differences between organisms within a treatment from pairwise comparisons (p<0.05). Lack of letters indicate no significant difference between organisms within the treatment... 88

Figure 3.4. Figure 4. One-to-one ratio plots of pathogens vs. Pediococcus reductions (log CFU/g) for apricot halves (a, b), macadamia nuts (c, d), and raisins (e, f) with confidence and prediction intervals. (○E. coli, +L. monocytogenes, ◆Salmonella) 89

Figure 3.5. Inactivation kinetics of L. monocytogenes, Salmonella, and Pediococcus on apricots (●), macadamia nuts (☐), and raisins (╳), respectively, when treated with 72°C vacuum-steam treatment. Lines shown are Gompertz and Weibull models that were best fit to the datasets...... 90

Figure 3.6. Schematic of equipment design for vacuum-assisted steam delivery. 95

Figure 3.7. Temperature profile example of 62°C vacuum-steam cycle on raisins..................... 96

Figure 3.8. Temperature profile example of 72°C vacuum-steam cycle on raisins..................... 96

Figure 3.9. Temperature profile example of 82°C vacuum-steam cycle on apricot halves.......... 97

Figure 4.1. Comparisons of predicted vs. observed times for 3-log reductions made by first-order models of Salmonella, Pediococcus, L. monocytogenes, and E. coli on whole macadamia nuts (predicted) vs. macadamia nut pieces (observed) and apricot halves (predicted) vs. apricot pieces (observed) vacuum-steamed at 72 and 82°C. * indicates relative error values within the acceptable range (-0.30-0.15). 126

Figure 4.2. Comparisons of predicted vs. observed times for 3-log reductions made by Weibull models of Salmonella, Pediococcus, L. monocytogenes, and E. coli on whole macadamia nuts

(predicted) vs. macadamia nut pieces (observed) and apricot halves (predicted) vs. apricot pieces (observed) vacuum-steamed at 72 and 82°C. * indicates relative error values within the acceptable range (-0.30-0.15). 127

Figure 4.3. Comparisons of predicted vs. observed times for 3-log reductions made by Gompertz models of Salmonella, Pediococcus, L. monocytogenes, and E. coli on whole macadamia nuts (predicted) vs. macadamia nut pieces (observed) and apricot halves (predicted) vs. apricot pieces (observed) vacuum-steamed at 72 and 82°C. * indicates relative error values within the acceptable range (-0.30-0.15). 128

Figure 4.4. Heat map of relative error between predicted and observed times required for 3-log reductions of microorganisms based on first-order model parameters of externally acquired data of thermally treated LWAF at 72°C. Area within the bold lines reflects internal generated data compared using the same vacuum-steam process. The acceptable relative error ranged from -0.30 (fail-safe; blue) to 0.15 (fail-dangerous; red). Externally acquired data is blocked and on the outside of the graph. 129

Figure 4.5. Heat map of relative error between predicted and observed times required for 3-log reductions of microorganisms based on first-order model parameters of externally acquired data of thermally treated LWAF at 82°C. Area within the bold lines reflects internal generated data compared using the same vacuum-steam process. The acceptable relative error ranged from -0.30 (fail-safe; blue) to 0.15 (fail-dangerous; red). Externally acquired data is blocked and on the outside of the graph. 130

Figure 4.6. Heat map of relative error between predicted and observed times required for 3-log reductions of microorganisms based on Weibull model parameters of externally acquired data of thermally treated LWAF at 72°C. Area within the bold lines reflects internal generated data compared using the same vacuum-steam process. The acceptable relative error ranged from -0.30 (fail-safe) to 0.15 (fail-dangerous). Externally acquired data is blocked and on the outside of the graph. 132

Figure 4.7. Heat map of relative error between predicted and observed times required for 3-log reductions of microorganisms based on Weibull model parameters of externally acquired data of thermally treated LWAF at 82°C. Area within the bold lines reflects internal generated data compared using the same vacuum-steam process. The acceptable relative error ranged from -0.30 (fail-safe) to 0.15 (fail-dangerous). Externally acquired data is blocked and on the outside of the graph. 133

Figure 4.8. Heat map of relative error between predicted and observed times required for 3-log reductions of microorganisms based on internally constructed Gompertz model parameters of thermally treated LWAF at 72°C. The acceptable relative error ranged from -0.30 (fail-safe) to 0.15 (fail-dangerous). 135

Figure 4.9. Heat map of relative error between predicted and observed times required for 3-log reductions of microorganisms based on internally constructed Gompertz model parameters of thermally treated LWAF at 82°C. The acceptable relative error ranged from -0.30 (fail-safe) to 0.15 (fail-dangerous). 136

Figure 5.1. Averaged log D-values of STEC O121 lawn (▲), broth and high-water activity (a_w 0.99 ●), intermediate-water activity (a_w 0.75 ●), and low-water activity (a_w 0.50 ○). 162

Figure 5.2. Averaged log D-values of STEC O157 lawn (▲), broth and high-water activity (a_w 0.99 ●), intermediate -water activity (a_w 0.75 ●), and low-water activity (a_w 0.50 ○). 162

Figure 7.1. Bacterial reductions (log CFU/g ± standard deviation) on apricot pieces resulting from 72°C (a) and 82°C (b) vacuum-steam treatment of various times. Asterisk (*) next to treatment times indicate significant differences of bacterial reductions from the reductions of a 0-min treatment, while lowercase letters above bars indicate significant differences between organisms within a treatment from pairwise comparisons ($p<0.05$). Lack of letters indicate no significant difference between organisms within the treatment. 188

Figure 7.2. Bacterial reductions (log CFU/g ± standard deviation) on macadamia nut pieces resulting from 72°C (a) and 82°C (b) vacuum-steam treatment of various times. Asterisk (*) next to treatment times indicate significant differences of bacterial reductions from the reductions of a 0-min treatment, while lowercase letters above bars indicate significant differences between organisms within a treatment from pairwise comparisons ($p<0.05$). Lack of letters indicate no significant difference between organisms within the treatment. 189

List of Tables

Table 3.1. First-order model inactivation rate coefficients (k [log CFU/g min^{-1}]), $D_{62°C}$, $D_{72°C}$, or $D_{82°C}$ values (min), and statistical results (R^2, RMSE [log CFU/g], AIC) for each food, organism, and treatment combination. 91

Table 3.2. Weibull (*k* (log CFU/g min^{-1}) and β) and Gompertz (A, μ_M [log CFU/g min^{-1}], λ [min]) model parameter coefficients and statistical results (RMSE [log CFU/g], AIC) for each food, organism, and treatment. Best values are bolded. Notable deviances from the observed trends related to food type are marked with (*). 92

Table 3.3. Temperature profile parameters averaged across each treatment combination. 93

Table 3.4. Average bacterial reductions of Shiga toxin-producing *E. coli*, *L. monocytogenes*, *Salmonella*, and *Pediococcus acidilactici* on apricot halves, macadamia nuts, and raisins treated for various lengths at 62, 72, and 82°C. 94

Table 4.1. External datasets acquired for independent validation and comparison against internally constructed models. 137

Table 4.2. First-order (k [log CFU/g min^{-1}]), Weibull (k (log CFU/g min^{-1}) and β) and Gompertz (A, μ_M [log CFU/g min^{-1}], λ [min]) model parameter coefficients and statistical results (RMSE [log CFU/g], AIC) for organisms (STEC, *Salmonella*, *L. monocytogenes*, and *Pediococcus acidilactici*) on dried apricot halves, whole macadamia nuts, dried apricot pieces, and macadamia nut pieces treated with 72°C and 82°C vacuum-steam. 138

Table 4.3. Predictions of 3-log STEC reductions based on D- and z-values acquired from thermally treated reduced-water activity solutions compared to reductions of an STEC cocktail on dried apricot halves and whole macadamia nuts. 140

Table 4.4. Comparisons of times (min) for 3-log reductions of E. coli, Salmonella, L. monocytogenes, and Pediococcus acidilactici on thermally treated low-water activity foods based on first-order models. Foods were compared based on similar water activity and comparisons were evaluated based on relative error with acceptable fail-safe, fail-dangerous range of -0.30-0.15 142

Table 4.5. Comparisons of times (min) for 3-log reductions of E. coli, Salmonella, L. monocytogenes, and Pediococcus acidilactici on thermally treated low-water activity foods based on Weibull models. Foods were compared based on similar water activity and comparisons were evaluated based on relative error with acceptable fail-safe, fail-dangerous range of -0.30-0.15. 143

Table 4.6. Comparisons of times (min) for 3-log reductions of E. coli, Salmonella, L. monocytogenes, and Pediococcus acidilactici on thermally treated low-water activity foods based on Gompertz models. Foods were compared based on similar water activity and comparisons were evaluated based on relative error with acceptable fail-safe, fail-dangerous range of -0.30-0.15 144

Table 5.1. Calculated D-values (min ± SD) and z-values (°C ± SD) for each experiment using lawn and broth culture preparation methods and reduced-a_w solutions of E. coli O121:H19 and O157:H7 163

Table 7.1. Average bacterial reductions (Log CFU/g) of E. coli, Listeria monocytogenes, Pediococcus, and Salmonella on raisins ("Raisins"), apricot halves ("Apricot"), apricot pieces ("ApriPie"), whole macadamia nuts ("MacaNut"), and macadamia nut pieces ("MacaPie"). ... 175

Table 7.2. Temperature profile parameters averaged across each treatment combination for dried apricot halves, whole macadamia nuts, raisins, dried apricot pieces, and macadamia nut pieces. 185

Table 7.3. Decimal reduction times (min) for E. coli O121 broth cultures at 56, 59, and 62°C. 210

Table 7.4. Decimal reduction times (min) for E. coli O121 lawn cultures at 56, 59, and 62°C. 210

Table 7.5. Decimal reduction times (min) for E. coli O156 broth cultures at 56, 59, and 62°C. 210

Table 7.6. Decimal reduction times (min) for E. coli O157 lawn cultures at 56, 59, and 62°C. 211

Table 7.7. Decimal reduction times (min) for E. coli O121 in low-water activity solution (a_w 0.50) at 56, 59, and 62°C. 211

Table 7.8. Decimal reduction times (min) for E. coli O121 in intermediate-water activity solution (a_w 0.75) at 56, 59, and 62°C. 211

Table 7.9. Decimal reduction times (min) for E. coli O157 in low-water activity solution (a_w 0.50) at 56, 59, and 62°C. 212

Table 7.10. Decimal reduction times (min) for E. coli O157 in intermediate-water activity solution (a_w 0.75) at 56, 59, and 62°C. 212

Table 7.11. Calculated z-values (°C) based on the log D-values for each culture. 212

Chapter 1 Introduction & Significance

Objectives

The objectives of the presented research were to 1) evaluate the efficacy of lab-scale, low-temperature, vacuum-assisted steam as an intervention to reduce *Salmonella* spp., Shiga toxin-producing *E. coli* (STEC), and *L. monocytogenes* from macadamia nuts, apricot halves, and raisins; 2) investigate *P. acidilactici* as a potential surrogate for these pathogens and the vacuum-steam process on the chosen LWAF; 3) build models to describe pathogen inactivation during the steam process and explore the respective limitations; and 4) investigate impacts of the water activity of a food matrix on thermal resistance of pathogens.

Justification

Foodborne illnesses originate from all sectors of the food system. More than 50% of outbreaks can be assigned to known food sources, and of these, nearly all of them are attributed to aquatic animals, land animals, or plant groups (Richardson et al., 2017). Within the plant category, a number of fruits and vegetables are frequently dried and considered to be low water activity foods (LWAF). Because LWAF do not support the growth of foodborne pathogens, consumption of LWAF (a_w <0.86) has not previously been considered to be associated with increased risk of foodborne illness. While LWAF do not present the same amount of risk as some other foods, a number of outbreaks involving LWAF have encouraged further investigation into their safety (CDC, 2012; CDC, 2018a; CDC, 2018b; CDC, 2019a; CDC, 2019b; CFIA, 2011; FDA, 2016b; FDA, 2017a; Finn et al., 2013; Painter et al., 2013). Between 2007-2012 globally, there were 41 outbreaks associated with LWAF that caused 7,315 illnesses from pathogens including Shiga toxin-producing *Escherichia coli* (STEC) and *Salmonella* spp.

(Farakos and Frank, 2014). Growth of microorganisms is limited in LWAF by the small amount of free water available for biological use, but *Salmonella*, as well as other pathogens, can persist for long periods of time if contamination occurs (Forghani et al., 2018; Keller et al., 2015; Suehr et al., 2019).

Dried fruits and nuts, in particular, are broadly distributed for inclusion into other products, as well as considered ready-to-eat (RTE) by consumers. Macadamia nuts, raisins, and dried apricots, for example, are common ingredients in trail mixes, bakery products, or RTE without alteration. The impact of an outbreak implicating these types of foods would be far-reaching, involving recalls of a broad array of products produced by multiple companies that could lead to consumer illnesses. From 2015-2018, macadamia nuts were recalled for presence of *Listeria monocytogenes*, *Salmonella* spp., and STEC (FDA, 2015a; FDA, 2015b; FDA, 2015c; FDA, 2016b; FDA, 2017b; FDA, 2018b). Dried fruits have also been recalled in the past for pathogen contamination (Callejón et al., 2015; FDA, 2018a; FDA, 2019b; FSN, 2018; FSN, 2019). Although nuts and dried fruits will not promote the growth of human pathogens, some are considered raw, agricultural products and all are prone to pre- and post-harvest environmental contamination.

The risk of foodborne illnesses associated with LWAF that are also considered RTE cannot be overlooked. However, determining the safety of some dried fruits and nuts can be challenging, since many are imported from different regions with unknown or variable processing histories (Bourdoux et al., 2016). Nevertheless, the Food Safety Modernization Act (FSMA) requires all processors to identify possible risks, coupled with the implementation of relevant interventions through the Preventive Control Rule (FDA, 2016a). Preventive controls should reduce risks by preventing contamination events or reducing the impact of a

contamination event through some type of process or intervention. To improve LWAF safety, various processing techniques have been investigated, particularly to address RTE products. In this project, one such strategy will be explored by evaluating the efficacy of vacuum-steam.

Use of Low-Temperature, Vacuum-Assisted Steam (Chapter 3)

Bacterial Reductions from Vacuum-Steam

In an effort to comply with the FSMA regulations, post-harvest pasteurization strategies, such as high-heat treatments, propylene oxide, or radiation have been explored for treating LWAF (Ban and Kang, 2016; Brar et al., 2015; Danyluk et al., 2005; Farakos et al., 2017; Karagöz et al., 2014; Saunders et al., 2018). However, these processes may come at large expense, be inefficient for LWAF, or potentially damage the quality or desirable characteristics of the food. The use of low-temperature, vacuum-assisted steam should be explored as a process control for Preventive Control Plans, as it has been documented to be successful in removing pathogens from LWAF such as nuts, grains, and spices, as the steam can access and transfer heat to the small crevices and nonuniform surfaces (Chang et al., 2010; Newkirk et al., 2018; Shah et al., 2017). Using a vacuum to create negative pressure, steam is formed at temperatures below 100°C. Lower treatment temperatures of vacuum-steam can maintain the quality attributes of the food (Duncan et al., 2017). For foods like macadamia nuts, raisins, and dried apricot halves, a low-temperature treatment to remove pathogens without harming the foods' characteristics would be valuable to food processors.

Macadamia nuts, raisins, and dried apricot halves were mist-inoculated with a cocktail of eight pathogens and a non-pathogenic microorganism. A mist inoculation was deemed most appropriate for the food matrix, as the high inoculum level was ideal for demonstrating large bacterial reductions and the foods required little time to dry to initial water activity levels. The

method has also been shown to have high retention on similar foods following drying back to original water activity levels (Beuchat and Mann, 2014).

Inoculum used to contaminate foods included *L. monocytogenes* 1/2a (FSL R2-499, sliced turkey isolate), 1/2b (FSL R2-502, chocolate milk isolate), and 4b (ScottA, milk outbreak isolate). There are no known outbreaks of *L. monocytogenes* associated with a LWAF, so strains were chosen on the basis of known resistances or disease implications rather than as isolates from LWAF. Each strain was selected for having a known history of causing human illnesses from a contaminated RTE food. *Salmonella* serovars chosen for the presented experiments (Montevideo [1449, peppercorn-associated outbreak], Newport [allspice isolate], and Tennessee [K4643, peanut butter outbreak]) were isolated from LWAF for potential tolerance or resistance to desiccation. Other experiments using these serovars also noted desiccation resistance during storage on peppercorns, cashews, macadamia nuts (Bowman et al., 2015; Saunders et al., 2018). STEC strains were chosen based on demonstrated abilities to cause human illnesses, as well as represent diverse characteristics based on associated outbreaks. *E. coli* O121:H19 (FNW19M81, wheat flour outbreak isolate) was chosen to represent a strain with potential desiccation and thermal resistance, since it was isolated from a LWAF and likely endured long-term storage and possible thermal treatments. *E. coli* O157:H7 F4546 (alfalfa sprout outbreak isolate) was also used, as it has generally only been investigated in the context of produce.

Relevant Hypotheses:

H_{01}: Characteristics of the food products *will not* affect the efficacy of the vacuum-steam treatments, and the *same* treatment (time and temperature) will be adequate to significantly reduce each type of pathogen (STEC, *Salmonella*, and

L. monocytogenes) from each food type (macadamia nuts, dried apricot halves, and raisins).

H_{A1}: Characteristics of the food products *will* affect the efficacy of the vacuum-steam treatments, and *different* treatments (time and temperature) will be required to significantly reduce pathogens (STEC, *Salmonella*, and *L. monocytogenes*) from each food type (macadamia nuts, dried apricot halves, and raisins).

H_{02}: There *will not* be significant differences between the bacterial log reductions of the pathogens resulting from the vacuum-steam.

H_{A2}: There *will* be significant differences between the bacterial log reductions of the pathogens resulting from the vacuum-steam.

***Pediococcus acidilactici* as a Surrogate Microorganism for Pathogens during Vacuum-Steam Processes**

Any new process or intervention should be validated in-plant to be certain the preventive control is effective in reducing or eliminating pathogens from foods. To prevent contamination of food products with the introduction of pathogens, non-pathogenic bacteria, referred to as surrogates, should be used. Many types of surrogates are used for in-plant validations, but they must be chosen to appropriately represent the pathogen targeted by the steam intervention and be comparable or more resistant to the treatment (Hu and Gurtler, 2017). When selecting a surrogate, the processing method, type of applied stress (thermal, osmotic, etc.), and characteristics of the food must be considered. *Enterococcus faecium* NRRL B-2354 has been used on several different LWAF as a surrogate for thermally treated foodborne pathogens, but some studies have shown it to be either less resistant or excessively resistant than the target

pathogens, leading to under- or over-processing of the food (Verma et al., 2018). *Pediococcus acidilactici* ATCC 8042 has more recently been investigated as a possible surrogate to be used on LWAF for various types interventions and may be more suitable for low-temperature thermal treatments (Ceylan and Bautista, 2015; Saunders, 2017; Saunders et al., 2018).

Relevant Hypotheses:

H_{03}: *Pediococcus acidilactici will not* be an appropriate surrogate for the pathogens on LWAF treated with vacuum-assisted steam by having *less* survival compared to the pathogens.

H_{A3}: *Pediococcus acidilactici will* be an appropriate surrogate for the pathogens on LWAF treated with vacuum-assisted steam by having *greater* survival compared to the pathogens.

Modeling Bacterial Inactivation Trends from Vacuum-Assisted Steam

As a supplement to in-plant validations, mathematical models that can describe pathogen inactivation based on process parameters, such as time and temperature, can be useful for food processors. Models can be used to optimize a process, as well as predict the response of a microbial population to a treatment (Marks, 2019). First-order models describe linear bacterial reductions or inactivation and are often used to determine *D*-values (time required for a 1-log CFU/g reduction of a microorganism at a constant temperature). These experiments are usually highly controlled and specifically applied to single strains or uniform food matrices. First-order models generally assume the population of microorganisms is homogenous and consistent in its response to the heat (Blackburn et al., 1997).

The physiological state of the cells, composition of the food matrix, and other processing parameters can greatly impact the linearity of inactivation curves (Bevilacqua et al., 2015). If the

true inactivation trends are non-linear, first-order inactivation curves could be dangerously applied to overestimate pathogen reduction. Using non-linear models allow for visualization of heterogeneous microbial populations. Non-linear inactivation can be described by models such as the Weibull or Gompertz, which depict shouldering, tailing, or curvature that are indicative of variable levels of susceptibility and resistance of the microorganisms. These types of models may be more appropriate in describing the unique and complex relationships between the food matrix, bacteria, and treatment effects.

Relevant Hypotheses:

H_{04}: A single model *will* adequately describe the inactivation of all pathogens on each of the different LWAF treated with vacuum-steam, indicating *little* variation.

H_{A4}: A single model *will not* adequately describe the inactivation of all pathogens on each of the different LWAF treated with vacuum-steam, and different models will need to be fit to appropriate describe all trends, indicating *substantial* variation.

Exploration of Model Flexibility and Limitations (Chapter 4)

Detailed models that incorporate multiple process parameters can be accurate predictors of pathogen reduction, but if the model is not robust enough or has not been validated for additional use, it should not be applied to different microorganisms, food matrices, or processes. Most models do not account for non-isothermal conditions of a food matrix or food processing environment, which limits the accuracy and applicability of a model to an actual food processing scenario (Marks, 2019). Parameters used to validate or evaluate the accuracy and bias of models can help indicate if models are influenced by the nature of the microorganism being inactivated, the properties of the food, or the treatment conditions themselves (Farakos et al., 2013). While

the primary models may be appropriate for describing the inactivation of pathogens under specific circumstances, they should only be applied to other microorganisms or conditions if appropriately validated.

Challenging the model with external data or in-plant validations helps determine the limitations of the model. The use of independently gathered data is key to validating models, but the availability of relevant datasets is limited. The presented research used both internally and externally acquired datasets to determine if the constructed models could accurately predict bacterial reductions from other similar thermally treated LWAF by comparing predicted and observed inactivation.

Relevant Hypotheses:

H_{05}: Primary models constructed from whole foods treated with vacuum-steam *will not* accurately predict bacterial reduction on other foods from similar thermal treatments.

H_{A5}: Primary models constructed from whole foods treated with vacuum-steam *will* accurately predict bacterial reduction on other foods from similar thermal treatments.

Impacts of Reduced Water Activity on Thermal Resistance of Shiga Toxin-Producing *E. coli* (Chapter 5)

Thermal resistance of pathogens is greatly impacted by a number of environmental characteristics (relative humidity, temperature, pressure, composition of matrix, etc.). Recognized as one of the most influential factors on the thermal resistance of pathogens, water activity must be considered carefully when determining appropriate treatment parameters to reduce pathogens on foods (Syamaladevi et al., 2016). Furthermore, characteristics of the pathogen, such as genetics or cell history can also affect thermal resistance. Many studies that

examine thermal resistance relative to reduced water activity offer conclusions impacted to unknown extents by the aforementioned variables.

Inoculation procedures have been investigated for the purposes of understanding how best to conduct applied experiments with LWAF, specifically in terms of physiological cell state, as the propagation method may stress cells in ways that lead to resistance against osmotic or thermal stress (Bowman et al., 2015). Additionally, noted examples of increased heat resistance of pathogens isolated from LWAF is of great interest and value to LWAF processors (Lang et al., 2017; Laroche et al., 2005). Because the food microenvironment is extremely complex, it is difficult to isolate the impact of water activity alone on pathogen thermal resistance. Experiments have examined the inoculation method as it pertains to survival or thermal resistance on LWAF but have not exclusively studied the impact of culture preparation method on thermal resistance.

Two STEC strains were examined to identify differences in thermal resistance, specifically as effected by culture preparation and reduced water activity. Experiments were conducted to determine *D*- and *z*-values from thermal treatment of STEC O121:H19 and O157:H7 when the strains were grown in broth or as a lawn on agar plates. Strains were also thermally treated in reduced water activity solutions to examine how low and intermediate water activities impact thermal resistance.

H_{06}: There will be *no differences* between the *D*- and *z*-values of the two STEC strains at the same temperature.

H_{A6}: There will be observed *differences* between the *D*- and *z*-values of the two STEC strains at the same temperature.

H_{07}: Culture preparation method of the pathogens *will not* impact the *D*- and *z*-values of the two STEC strains.

H_{A7}: Culture preparation method of the pathogens *will* impact the *D*- and *z*-values of the two STEC strains.

H_{08}: Suspensions of cells in low- and intermediate-water activity solutions *will not* result in significant differences of the *D*- and *z*-values of the two STEC strains relative to a high-water activity solution.

H_{A8}: Suspensions of cells in low- and intermediate-water activity solutions *will* result in significant differences of the thermal resistance of the two STEC strains relative to a high-water activity solution.

Summary

The complexities of a LWAF matrix, as well as the physiological state and response of foodborne pathogens create many challenges for food processors. Choosing an intervention is complicated by issues such as the thermal resistance of pathogens and the possibility of quality degradation. Many experiments seek to determine how foodborne pathogens are impacted by the LWAF in terms of thermal resistance and the effectiveness of interventions that can reduce pathogen contamination while maintaining desirable characteristics of the food.

In summary, the presented research will contribute to overall knowledge about pathogen survival on LWAF, as well as provide resources that can help LWAF processors design and optimize a novel intervention: low-temperature, vacuum-assisted steam. Vacuum-steam can provide pathogen reductions on foods that could be damaged by high-heat treatments, which could prevent the degradation of product quality and thus the loss of profit. Furthermore, the use of this intervention could prevent recalls and outbreaks of LWAF, the cost of which could be

devastating. The models generated in this work accurately described the inactivation trends of STEC, *L. monocytogenes*, and *Salmonella* on raisins, whole macadamia nuts, and dried apricot halves, but these models, as well as others should not be applied to other foods or processes without extensive validation. While in-plant validations using a surrogate organism, such as *Pediococcus acidilactici*, is the surest way of determining the effectiveness of an intervention, robust models could provide guidance by estimating pathogen reductions.

References

1. Ban, G.-H. and Kang, D.-H. (2016). Effectiveness of superheated steam for inactivation of *Escherichia coli* O157:H7, *Salmonella* Typhimurium, *Salmonella* Enteritidis phage type 30, and *Listeria monocytogenes* on almonds and pistachios. *International Journal of Food Microbiology, 220*(C), 19–25.
2. Beuchat, L. R. and Mann, D. A. (2014). Survival of *Salmonella* on dried fruits and in aqueous dried fruit homogenates as affected by temperature. *Journal of Food Protection*, *77*, 1102–1109. http://doi.org/10.4315/0362-028X.1FP-13-549. (Date accessed: 14 June 2018).
3. Bevilacqua, A., Speranza, B., Sinigaglia, M., Corbo, M. 2015. A focus on the death kinetics in predictive microbiology: benefits and limits of the most important models and some tools dealing with their application in foods. *Foods*, *4*(4), 565–580.
4. Blackburn, C. de W., Curtis, L. M., Humpheson, L., Billon, C., and McClure, P. J. (1997). Development of thermal inactivation models for *Salmonella enteritidis* and *Escherichia coli* O157:H7 with temperature, pH and NaCl as controlling factors. *International Journal of Food Microbiology*, *38*, 31–44.
5. Bourdoux, S., Li, D., Rajkovic, A., Devlieghere, F., and Uyttendaele, M. (2016). Performance of drying technologies to ensure microbial safety of dried fruits and vegetables. *Comprehensive Reviews in Food Science and Food Safety*, *15*(6), 1056–1066. http://doi.org/10.1111/1541-4337.12224
6. Bowman, L. S., Waterman, K. M., Williams, R. C., and Ponder, M. A. (2015). Inoculation Preparation Affects Survival of *Salmonella enterica* on whole black peppercorns and cumin seeds stored at low water activity. *Journal of Food Protection*, *78*(7), 1259–1265. http://doi.org/10.4315/0362-028X.JFP-14-483
7. Brar, P.K., Proano, L.G., Friedrich, L.M., Harris, L.J., Danyluk, M.D. (2015). Survival of *Salmonella*, *Escherichia coli* O157:H7, and *Listeria monocytogenes* on raw peanut and pecan kernels stored at −24, 4, and 22°C. *Journal of Food Protection, 78*(2), 323–332. http://doi.org/10.4315/0362-028X.JFP-14-327
8. Callejón, R.M., Rodríguez-Naranjo, M.I., Ubeda, C., Hornedo-Ortega, R., Garcia-Parrilla, M.C., Troncoso, A.M. (2015). Reported foodborne outbreaks due to fresh produce in the United States and European Union: trends and causes. *Foodborne Pathogens and Disease, 12*(1), 32–38.
9. Canadian Food Inspection Agency (CFIA). (2011). Health hazard alert: certain walnuts and walnut products may contain *E. coli* O157:H7 bacteria. Available from: http://www.inspection.gc.ca/about-the-cfia/newsroom/food-recall-warnings/complete-listing/2011-04-03/eng/1359548340192/1359548340223.
10. Centers for Disease control and Prevention (CDC). (2012). Foodborne outbreak online database. Available from: http://www.cdc.gov/foodborneoutbreaks/.
11. Centers for Disease Control and Prevention (CDC). (2018a). Burden of foodborne illness: findings. Available from: https://www.cdc.gov/foodborneburden/2011-foodborne-estimates.html
12. Centers for Disease Control and Prevention, National Outbreak Reporting System (CDC). (2018b). NORS dashboard. Available from: https://wwwn.cdc.gov/norsdashboard/
13. Centers for Disease Control and Prevention (CDC). (2019a). Reports for selected *E. coli* outbreak investigations. Available from: https://www.cdc.gov/ecoli/outbreaks.html.

14. Centers for Disease Control and Prevention (CDC). (2019b). Reports for selected *Salmonella* outbreak investigations. Available from: https://www.cdc.gov/salmonella/outbreaks.html.
15. Ceylan, E. and Bautista, D. (2015). Evaluating *Pediococcus acidilactici* and *Enterococcus faecium* NRRL B-2354 as a thermal surrogate microorganism for *Salmonella* for in-plant validations studies of low-moisture pet food products. *Journal of Food Protection, 72*(5), 934-939.
16. Chang, S.S., Han, A.R., Reyes-De-Corcuera, J.I., Powers, J.R., and Kang, D.H. (2010). Evaluation of steam pasteurization in controlling *Salmonella* serotype Enteritidis on raw almond surfaces. *Letters in Applied Microbiology, 50*(4), 393–398.
17. Danyluk, M.D., Uesugi, A.R., and Harris, L.J. (2005). Survival of *Salmonella* Enteritidis PT 30 on inoculated almonds after commercial fumigation with propylene oxide. *Journal of Food Protection, 68*, 1613–1622.
18. Duncan, S.E., Moberg, K., Amin, K.N., Wright, M., Newkirk, J.J., Ponder, M.A., Acuff, G.R., and Dickson, J.S. (2017). Processes to preserve spice and herb quality and sensory integrity during pathogen inactivation. *Journal of Food Science, 82*(5), 1208–1215.
19. Farakos, S. M. S., Frank, J. F., and Schaffner, D. W. (2013). Modeling the influence of temperature, water actvity and water mobility on the persistence of *Salmonella* in low-moisture foods. *International Journal of Food Microbiology*, *166*(2), 280–293. http://doi.org/10.1016/j.ijfoodmicro.2013.07.007
20. Farakos, S.M.S., and Frank, J.F. (2014). Challenges in the control of foodborne pathogens in low-water activity foods and spices. In Gurtler, J. B., Doyle, M. P., and Kornacki, J. L. (Eds.), *The microbiological safety of low water activity foods and spices,* pp. 15-34. New York: Springer New York.
21. Farakos, S.M.S., Pouillot, R., and Keller, S.E. (2017). *Salmonella* survival kinetics on pecans, hazelnuts, and pine nuts at various water activities and temperatures. *Journal of Food Protection, 80*(5), 879–885.
22. Finn, S., Condell, O., McClure, P., Amézquita, A., and Fanning, S. (2013). Mechanisms of survival, responses, and sources of *Salmonella* in low-moisture environments. *Frontiers in Microbiology, 4*(331)*,* 1–15.
23. Food Safety News (FSN). (2018). Dates from Iran linked to Hepatitis A outbreak for second time in 2 years. Available from: https://www.foodsafetynews.com/2019/05/dates-from-iran-linked-to-hepatitis-a-outbreak-in-sweden/
24. Food Safety News (FSN). (2019). Snack mix recall expanded as patient count increases in *Salmonella* outbreak. Available from: https://www.foodsafetynews.com/2019/03/snack-mix-recall-expanded-as-patient-count-increases-in-salmonella-outbreak/.
25. Forghani, F., den Bakker, M., Futral, A.N., and Diez-Gonzalez, F. (2018). Long-term survival and thermal death kinetics of enterohemorrhagic *Escherichia coli* serogroups O26, O103, O111, and O157 in wheat flour. *Applied Environmental Microbiology, 84*, e00283–18.
26. Hu, M. and Gurtler, J.B. (2017). Selection of surrogate bacteria for use in food safety challenge studies: a review. *Journal of Food Protection, 80*(9), 1506–1536. http://doi.org/10.4315/0362-028X.JFP-16-536.

27. Karagöz, I., Moreira, R. G., and Castell-Perez, M. E. (2014). Radiation D10 values for *Salmonella* Typhimurium LT2 and an *Escherichia coli* cocktail in pecan nuts (Kanza cultivar) exposed to different atmospheres. *Food Control, 39*(C), 146–153.
28. Keller, S.E., Stam, C.N., Gradl, D.R., Chen, Z., Larkin, E.L., Pickens, S.R., and Chirtel, S.J. (2015). Survival of *Salmonella* on chamomile, peppermint, and green tea during storage and subsequent survival or growth following tea brewing. *Journal of Food Protection, 78*(4), 661–667.
29. Lang, E., Chemlal, L., Molin, P., Guyot, S., Alvarez-Martin, P., Perrier-Cornet, J.-M., Dantigny, P., and Gervais, P. (2017). Modeling the heat inactivation of foodborne pathogens in milk powder: High relevance of the substrate water activity. *Frin*, *99*(Part 1), 577–585. http://doi.org/10.1016/j.foodres.2017.06.028
30. Laroche, C., Fine, F., and Gervais, P. (2005). Water activity affects heat resistance of microorganisms in food powders. *International Journal of Food Microbiology*, *97*(3), 307–315. http://doi.org/10.1016/j.ijfoodmicro.2004.04.023
31. Marks, B. P. (2008). Status of microbial modeling in food process models. *Comprehensive Reviews in Food Science and Food Safety*, *7*, 137–143.
32. Newkirk, J.J., Wu, J., Acuff, J.C., Caver, C.B., Mallikarjunan, K., Wiersema, B.D., Williams, R.C., and Ponder, M.A. (2018). Inactivation of *Salmonella enterica* and surrogate *Enterococcus faecium* on whole black peppercorns and cumin seeds using vacuum steam pasteurization. *Frontiers in Sustainable Food Systems*, *2*, 252–12.
33. Painter, J.A., Hoekstra, R.M., Ayers, T., Tauxe, R.V., Braden, C.R., Angulo, F.J., and Griffin, P.M. (2013). Attribution of foodborne illnesses, hospitalizations, and deaths to food commodities by using outbreak data, United States, 1998–2008. *Emerging Infectious Diseases, 19,* 407–415.
34. Richardson, L. C., Bazaco, M. C., Parker, C. C., Dewey-Mattia, D., Golden, N., Jones, K., Klontz, K., Travis, C., Kufel, J. Z., and Cole, D. (2017). An Updated scheme for categorizing foods implicated in foodborne disease outbreaks: a tri-agency collaboration. *Foodborne Pathogens and Disease*, *14*(12), 701–710. http://doi.org/10.1089/fpd.2017.2324
35. Saunders, T. (2017). Inactivation of *Salmonella* and surrogate bacteria on cashews and macadamia nuts exposed to saturated steam and propylene oxide treatments. (MS Thesis). Virginia Tech. Blacksburg, VA.
36. Saunders, T., Wu, J., Williams, R.C., Huang, H., and Ponder, M.A. (2018). Inactivation of *Salmonella* and surrogate bacteria on cashews and macadamia nuts exposed to commercial propylene oxide processing conditions. J. Food Prot. 81(3), 417–423.
37. Shah, M.K., Asa, G., Sherwood, J., Graber, K., and Bergholz, T.M. (2017). Efficacy of vacuum steam pasteurization for inactivation of *Salmonella* PT 30, *Escherichia coli* O157:H7 and *Enterococcus faecium* on low moisture foods. *International Journal of Food Microbiology, 244*(C), 111–118.
38. Suehr, Q.J., Anderson, N.M., Keller, S.E. (2019). Desiccation and thermal resistance of *Escherichia coli* O121 in wheat flour. *Journal of Food Protection, 82*(8), 1308–1313.
39. Syamaladevi, R. M., Tang, J., Villa-Rojas, R., Sablani, S., Carter, B., and Campbell, G. (2016). Influence of Water activity on thermal resistance of microorganisms in low-moisture foods: a review. *Comprehensive Reviews in Food Science and Food Safety*, *15*(2), 353–370. http://doi.org/10.1111/1541-4337.12190
40. U.S. Food and Drug Administration (FDA). (2015a). Natural Grocers® issues recall on

10oz. Caribbean Nut & Fruit Mix due to possible health risk. Available from: https://wayback.archive-it.org/7993/20170723005254/https://www.fda.gov/Safety/Recalls/ArchiveRecalls/2015/ucm453516.htm.
41. U.S. Food and Drug Administration (FDA). (2015b). Rocky Mountain Foods, Inc. voluntarily recalls Free Range Snack Co. Brand 16 oz. Island Fruit and Nut Trail Mix and bulk macadamia nuts because of possible health risk. Available from: https://wayback.archive-it.org/7993/20170406094935/https://www.fda.gov/Safety/Recalls/ArchiveRecalls/2015/ucm453116.htm.
42. U.S. Food and Drug Administration (FDA). (2015c). Texas Star Nut and Food Co. Inc. Natural Macadamia Nuts are being voluntarily recalled due to possible *Salmonella* contamination. Available from: https://wayback.archive-it.org/7993/20170723005958/https://www.fda.gov/Safety/Recalls/ArchiveRecalls/2015/ucm439283.htm.
43. U.S. Food and Drug Administration (FDA). (2016a). Draft guidance for industry: hazard analysis and risk-based preventive controls for human food; Chapter 4 Preventive Controls. Available from: https://www.fda.gov/media/99572/download.
44. U.S. Food and Drug Administration (FDA). (2016b). Mahina Mele Farms, LLC expands voluntary recall of macadamia nut products due to possible health risk. Available from: https://wayback.archive-it.org/7993/20170112133521/http://www.fda.gov/Safety/Recalls/ucm484851.htm.
45. U.S. Food and Drug Administration (FDA). (2017a). FDA investigated multistate outbreak of *E. coli* O157:H7 infections linked to soynut butter. Available from: https://www.fda.gov/Food/RecallsOutbreaksEmergencies/Outbreaks/ucm544964.htm.
46. U.S. Food and Drug Administration (FDA). (2017b). Kroger recalls simple truth dry roasted macadamia nuts because of possible health risk. Available from: https://www.fda.gov/safety/recalls-market-withdrawals-safety-alerts/kroger-recalls-simple-truth-dry-roasted-macadamia-nuts-because-possible-health-risk.
47. U.S. Food and Drug Administration (FDA). (2018a). FDA investigated *Salmonella* Typhimurium outbreak linked to dried coconut. Available from: https://www.fda.gov/food/outbreaks-foodborne-illness/fda-investigated-salmonella-typhimurium-outbreak-linked-dried-coconut.
48. U.S. Food and Drug Administration (FDA). (2018b). Mauna Loa Macadamia Nut Corporation voluntarily recalls. Available from: https://www.fda.gov/safety/recalls-market-withdrawals-safety-alerts/mauna-loa-macadamia-nut-corporation-voluntarily-recalls.
49. U.S. Food and Drug Administration (FDA). (2019). Archive for recalls, market withdrawals & safety alerts. Available from: www.fda.gov/safety/recalls-market-withdrawals-safety-alerts/archive-recalls-market-withdrawals-safety-alerts.
50. Verma, T., Wei, X., Lau, S.K., Bianchini, A., Eskridge, K.M., and Subbiah, J. (2018). Evaluation of *Enterococcus faecium* NRRL B-2354 as a surrogate for *Salmonella* during extrusion of low-moisture food. *Journal of Food Science, 83*(4), 1063–1072.

Chapter 2 Literature Review

1. Background and Significance

There are over 48 million estimated cases of foodborne illness in the United States per year, with approximately 3,000 cases resulting in death (CDC, 2019a). Of those foodborne illnesses, *Salmonella* spp. is responsible for more than 1 million cases and 27% of foodborne illness-related deaths. Additionally, it is estimated that *Salmonella*-associated outbreaks and illnesses result in approximately $3.7 billion annually, taking into account hospitalizations and treatment, work absences, and death (USDA ERS, 2015). While *Listeria monocytogenes* causes far fewer illnesses than *Salmonella* spp., it is still responsible for nearly 20% of deaths related to foodborne illnesses (CDC, 2016; Scallan et al., 2011). High percentages of hospitalizations due to listeriosis also creates a high economic burden of more than $2.6 billion (USDA ERS, 2015). Similarly, while numbers of reported and laboratory-confirmed cases of Shiga toxin-producing *E. coli* (STEC) O157:H7 are much lower than those of *Salmonella* (5,283 STEC cases vs. 41,930 *Salmonella* cases), STEC infections account for more than 59% of hospitalizations for foodborne illnesses, compared to 27% for *Salmonella* infections (Scallan et al., 2011). Combined, the annual economic burden in the United States of these three pathogen genera is greater than $6.3 billion, which includes healthcare costs, lost wages, etc. (USDA ERS, 2015). Furthermore, infections of these three particular pathogen groups can lead to complications like meningitis, kidney failure, or sepsis, or even cause or chronic conditions such as arthritis, kidney disease, or recurring inflammation.

1.1. Low-water activity food safety

The numbers of reported foodborne illnesses in the US and worldwide are staggering, and it should be noted that foodborne illnesses are drastically underreported (Mead et al., 1999). The

complexities of surveillance and underreporting create challenges for scientists and regulatory agencies when assessing risk or resolving outbreaks. However, recent advances in outbreak detection, including advanced subtyping and whole genome sequencing, have provided stronger evidence for epidemiologists to connect outbreak pathogens to particular food vehicles. In some cases, the foods associated with these outbreaks were not previously considered high-risk foods and assumed to be safe, such as in the cases of caramel apples and other ready-to-eat products (Buchanan et al., 2017). Similarly, low-water activity foods (LWAF) are not generally identified to be associated with high risks of foodborne illnesses, since the lack of available water for use of microorganisms will restrict the growth of pathogens, as long as the water activity is maintained below 0.86. However, illnesses and outbreaks that result from many different food commodities other than those identified as high-risk heightens awareness of potential contamination hazards associated with *Salmonella, L. monocytogenes*, and STEC contamination on LWAF.

Salmonella contamination caused a staggering 94% of LWAF recalls and 53% of the 7,315 worldwide LWAF outbreaks between 2007-2012. These statistics reasonably influence processors' production design to target *Salmonella* as a threat to LWAF production (Scott et al., 2009). However, STEC and *L. monocytogenes* have also been responsible for recalls of LWAF in the past, some of which were connected to illnesses and outbreaks. *E. coli* O157:H7 was the source of several recalls and outbreaks in LWAF, and resulted in many illnesses, and hospitalizations from the contamination of raw cookie dough (CDC, 2019b). Raw flour was assumed, though not confirmed, to be the most likely contaminant. Additionally, recalls and outbreaks have been associated with flour, raw shelled walnuts, in-shell hazelnuts, organic cacao

nibs, and hazelnut and mixed nut products (CDC, 2012; CFIA, 2011; FDA, 2017; Painter et al., 2013).

Moreover, *Listeria* presence has been detected on LWAF in the past and resulted in recalls of walnuts, peanut butter, pine nuts, snack mixes, popcorn flavorings, RTE sandwiches, and other commodities (Farakos and Frank, 2014; FDA, 2019b). In 2015, as well as more recently in 2019, stone fruits (peaches, plums, pluots, and nectarines) were recalled for possible *L. monocytogenes*-contamination due to in-house testing results (FDA, 2019a). In this case, as in several other recalls and outbreaks like ice cream or caramel apples, *L. monocytogenes* contamination was unexpected by consumers and experts, as these foods are generally not identified as high-risk (Buchanan et al., 2017; Glass, 2015; Jackson et al., 2015). The notion that LWAF pose no risks for *Listeria*-associated outbreaks and recalls is obsolete, and risk assessments will surely be updated in the future to investigate the risks of LWAF as vehicles for foodborne pathogens like *L. monocytogenes*. The incidence of *Listeria* and STEC contamination may be much lower than that of *Salmonella*, but the dire consequences of listeriosis or STEC-associated infections make the pathogens key focuses for the presented research.

1.2. Risks associated with LWAF

LWAF are often considered RTE foods themselves or are incorporated as ingredients in other products that may be RTE, as in the case with trail mixes. RTE foods do not require further processing, giving consumers the notion that the food is without risk. However, some LWAF are raw and minimally processed, despite the fact that they may originate from agricultural environments, where they are susceptible to environmental contamination. In addition to *Salmonella* and *L. monocytogenes* contamination, LWAF may be at risk for post-harvest contamination by various pathogens during processing, packaging or transport. In 2007 alone,

there were 45 STEC illnesses associated with attributed to consumption of contaminated rice, seeds, nuts, and almonds among other commodities (EFSA, 2009). In 2011, the deadliest outbreak of pathogenic *E. coli* and deadliest outbreak in Europe occurred when contaminated fenugreek sprout seeds caused more than 4,000 illnesses and over 50 deaths (EFSA, 2011). Although the fenugreek sprout seeds were a raw agriculture product, it is notable that the pathogen, which was not previously classified as a major contaminant of LWAF, survived long-term in the low-water activity environment.

Several studies have investigated prevalence of pathogens on LWAF, particularly nuts, to evaluate both contamination level and likelihood. Prevalence studies of *Salmonella* spp. on nuts showed estimates of 0.04-6.90% ranging from <0.003 to 0.092 MPN/g, the largest of both prevalence and contamination levels belonging to macadamia nuts (Zhang. G. et al., 2017). A similar study specifically examining almonds found prevalence to be 0.87% ± 0.2% with *Salmonella*-contamination levels at 1.2-1.9 MPN/g (Danyluk et al., 2007). Prevalence studies investigating *E. coli* contamination on nuts, particularly O157:H7, have not found conclusive evidence of contamination (Davidson et al., 2015; Eglezos, Huang, and Stuttard, 2008). However, recalls, as well as outbreaks, would suggest that contamination occurs even if at low, irregular levels that challenge the capabilities of testing methods. Because there have been no cases of listeriosis associated with LWAF, risk assessments have been focused on other RTE foods that have been sources of documented *L. monocytogenes*-related outbreaks. However, their ability to survive in dried foods with low water activities should not be overlooked (Beuchat et al., 2013; Brar et al., 2015). Risks assessments focused on the bacterium have noted fresh produce as potential vehicles of contamination (Hitchins and Whiting, 2001). Furthermore, the recalls of stone fruits due to possible *L. monocytogenes* contamination prompt considerations of

risks that the pathogen could also be present on dried stone fruits (FDA, 2019a; Jackson et al., 2015). The high sugar content and low water activity of dried stone fruits would certainly prevent active growth of *L. monocytogenes* and other pathogens, but if contamination were to occur, survival may be possible if not likely.

1.3. Efforts to increase LWAF safety

The recently enacted Food Safety Modernization Act (FSMA) now mandates preventive controls to mitigate risks of pathogen contamination through implementation of good agricultural and manufacturing practices, as well as control procedures that result in a significant reduction of frequently occurring pathogens. In response, processors of LWAF have turned to thermal and gas fumigation as a strategy to reduce bacterial contamination, such as roasting or the use of sulfur dioxide or propylene oxide (PPO). These strategies may not always be the best suited means of decontamination for certain LWAF if they change the quality of the product. Additionally, *Salmonella*, as well as *E. coli* and *Cronobacter sakazakii*, have been known to be more thermally resistant to dry heat treatments when on a LWAF, adding another layer of complication (Grasso et al., 2014; Juneja and Eblen, 2000; Lang et al., 2017; Laroche and Gervais, 2003; Laroche, Fine, and Gervais, 2005). The ability of some pathogens to survive in the low water activity environments and delicacy of some food products highlight the need for processors and researchers to develop protocols that can eliminate the threat of contamination, while preserving the integrity of the product.

Steam treatments may be an appealing method to processors because a dry heat treatment is generally less efficient than moist heat. Dry heat treatments may require longer treatment times or higher temperatures, which can be very damaging to certain product qualities (Denyer, Hodges, and Gorman, 2011; Fudge et al., 2016). A vacuum-assisted, low-temperature steam

treatment can be an effective treatment to achieve an acceptable reduction. Vacuum-assisted steam can be used from 60-100ºC when steam is injected into a chamber that is placed under a vacuum. The negative pressure allows steam to form at lower temperatures, thus preserving certain qualities of foods that could be degraded at higher temperatures. The steam can penetrate small cavities, completely filling the space in the chamber, and efficiently transferring heat to decontaminate the surfaces of foods or objects within.

A number of studies have investigated vacuum-assisted steam as a treatment for LWAF, particularly spices, grains, and nuts. Shah et al. showed successful reductions (> 3 log CFU/g) of *Salmonella* and *E. coli* O157:H7 from 75°C steam treatments on whole flaxseed, quinoa, and sunflower kernels after 1 min (2017). Also investigating vacuum-steam pasteurization, Newkirk et al. showed 5-log reduction of *Salmonella* from 85°C steam treatments on whole black peppercorns and cumin seeds after 1 min (2018). *Salmonella* were also successfully reduced (5 log CFU/g) from almond surfaces using a 95°C steam treatments for 35 sec (Chang et al., 2010). Low-temperature, vacuum-assisted steam under 85°C may be successful in reducing pathogen contamination, but quality aspects of the foods may be damaged, which would require further sensory studies in the future (Chang et al., 2010; Duncan et al., 2017). Further discussion of vacuum-steam treatments can be found in section 4.1. of this review.

2. Survival of microorganisms on LWAF

2.1. Interactions of foodborne pathogens with the food[1]

[1] Acuff, J. C. and Ponder, M. A. (2019). "Interactions of foodborne pathogens with the food matrix." In Food Safety Engineering. Edited by Demirci A, Feng H. Springer, Boca Raton. In Press.

Presence of unbound or free water is a key indicator of microbial growth in foods. Water activity (a_w), or the ratio of free, unbound water compared to pure water can be an important indicator of microbial safety. Water activity can be reduced whether through the introduction of osmolytes such as sugars or salts, or by removal of water through drying or desiccation. Reduced water activity results in localized increases to osmotic pressure that causes diffusion of water across the cytoplasmic membrane or cytoplasm shrinkage when the microenvironment exposes cells to a lower gas phase (Burgess et al., 2016). A higher a_w will provide microorganisms with enough water to carry out normal metabolic functions, resulting in growth, and may be at risk for association with foodborne outbreaks because they can stimulate and sustain the growth of pathogens. Foods with lower water activities, however, may not support active growth of organisms, but can still support survival of some organisms. Many Gram-negative foodborne pathogens, like *E. coli*, *Salmonella*, and *Vibrio* spp. require at least 0.95 a_w for growth (Maserati et al., 2017). Other organisms, such as *Staphylococcus aureus* and *Listeria monocytogenes* can grow at lower a_w of 0.86 and 0.92, respectively (Nolan, Chamblin, and Troller, 1992; Sperber, 1983).

Reduced water activity has historically been used to prevent proliferation of pathogens, but growth is not necessary for persistence of foodborne pathogens. Human illnesses are increasingly associated with low water activity foods (LWAF) because many organisms, particularly *Salmonella* spp., can survive for long periods of time in LWAF. Often, outbreaks associated with LWAF indicate a low infectious dose of the contaminant, and it is believed that these organisms maintain their virulence even under stress (Andino and Hanning, 2015). This can make LWAF vehicles for disease, despite the stress placed on bacterial cells, and provide

incentive to understand how desiccation stress impact microorganisms when water activity in food is manipulated.

Reduction of water activity can be achieved through desiccation or the addition of salt or sugar, which function to increase localized osmolyte concentrations and decrease water available for biological use. The removal of water limits the cells' abilities to carry out normal metabolic functions for growth. During desiccation or drying processes, energy is transferred from the environment to the food, in the form of heat, and in turn, moisture from the food is evaporated into the environment (Raponi et al., 2017). Enzymatic reactions are limited at low a_w, and the rate of reactions decreases while energy is funneled into pathways that promote survival or persistence, including those that increase nutrient sources and trans fatty acids proportions in the cell membrane (Burgess et al., 2016; Cronan, 2002; Deng et al., 2017). Cells must adapt to desiccation or else damage to the cell membrane, DNA, and metabolic functions occurs (Venkitanarayanan, et al. 2017). Despite the extreme circumstances, however, many pathogens can survive at $a_w<0.87$, though growth may not occur.

Pathogens have varying limits for growth at certain a_w, but the method of lowering the a_w, whether by desiccation or addition of solutes, may impact the organisms' reactions and adaptations. *L. monocytogenes* is capable of growth and survival through a variety of strategies in approximately 0.93 a_w achieved through an 11% NaCl solution (1.9 M), such as the expression of genes under the control of σ_B that impact the structure of the cell wall (Ribeiro et al., 2014). Although these displays of resistance to salt stress were conducted *in vitro*, the stress responses are likely maintained and applied even within the food matrix. While some organisms have novel mechanisms of resistance against salt, most organisms are susceptible to high concentrations. Salt is commonly used to lower the a_w of Gouda cheese, and combined with lactic acid

production, inhibit growth of *L. monocytogenes* (Wemmenhove et al., 2016). On the surface of vacuum packaged hams with 2.35% NaCl (0.99 a_w), *L. monocytogenes* has been shown to grow in filaments (Liu et al., 2014). *Listeria* on the surface of ham responds to reduced osmotic pressure by down regulating genes involved in cell division. Other foodborne bacteria including strains of *E. coli* and *Salmonella enterica* have been shown to form filaments under osmotic stress typically at the lower threshold associated with microbial growth. Filament formation in hyperosmotic and high-a_w environments can confer protection if the cells are later faced with certain desiccation stresses (Stackhouse et al., 2012). Upon rehydration, the increased biomass could result in a massive surge of cell numbers, heightening the risk of foodborne illness.

As a reaction to low water activities, cells may use the solutes within the food as part of their defense and resistance. When certain disaccharides are present (trehalose and sucrose), they can confer some protection during desiccation by taking the place of the lost water, as well as the formation of glass structures within the cytoplasmic membrane (García, 2011). The soluble sugars become more concentrated from the removal of water to the point that they essentially crystallize, causing vitrification, or the formation of glasses within the cell. Glasses form to prevent the destruction of cell layers and maintain the membrane's structure, as well as physically block chemical reactions from occurring that could lead to cell degradation, thus promoting a state of dormancy (Burgess et al., 2016; Koster, 1991). Cells may enter states of dormancy where they are more difficult to cultivate and recover on media due to injury, but can still be resuscitated, as was seen in high-protein, dried milk powder contaminated with *Cronobacter sakazakii* and *Salmonella* Typhimurium (Lang et al., 2017).

Comparisons of low- and intermediate-water activity foods provide insight into pathogens' abilities to survive in desiccated states. Following inoculations of peanut butter

(intermediate-water activity) and nonfat dry milk powder (low-water activity), greater survival was observed on peanut butter (Li et al., 2014). The rate of a_w reduction also plays an important role in both altering the food environment and damaging pathogens during desiccation. Slower drying rates are associated with increased lethality compared to faster drying rates (Raponi et al., 2017). *Salmonella* survival on inoculated ginger root was increased when drying was performed at a high rate (a_w reduction from 0.99 to 0.1 in 4 hours) compared to a slower reduction in a_w (0.99 to 0.4) over a longer period of time (Gradl et al., 2015). During storage of the dried ginger at ambient temperature (25°C), it was noted that *Salmonella* survived longer (365 days) when the relative humidity and a_w were kept low (33% and 0.35 a_w, respectively), as opposed to 25 days when the relative humidity and a_w were higher (97% and 0.85 a_w). Similar trends of higher levels of survivability were noted in loose-leaf teas that were stored at low relative humidity (<30%) than high relative humidity (>90%). *Salmonella* has been shown to survive as high as 7-8 log CFU/g on peppermint, chamomile, and green tea leaves for over 6 months at low relative humidity (Keller et al., 2015). It may be inferred that certain cellular processes are arrested due to the low-water activity responses, leading to heightened use of survival strategies. Clearly, temperature, relative humidity, and rates of desiccation widely vary between food-drying processes and understanding how each parameter affects a pathogen's survival and resistance to damage is vital for the food industry.

Desiccation of foods is often used for a processing control or preservation, but there are many observed accounts of increased thermal resistance of *Salmonella* serovars in low water activity foods, such as dried milk powder, flour, cocoa, etc. (Burgess et al., 2016). These particular findings lead to concerns about low water activity foods that are minimally processed by low heat treatments, since desiccation and osmotic stress are often used to control foodborne

pathogens (Capozzi et al., 2009). Low heat treatments may not be sufficient in eliminating populations that have acquired resistance from overcoming desiccation.

2.2. Cross-Protection

Cross protection is a phenomenon that has been observed on occasions when a microorganism's exposure and resistance to a particular stress provides additional protection or resistance to another stress. Protein moonlighting may occur during these circumstances, in which a protein functions in multiple ways to respond to various stresses (Venkitanarayanan et al., 2017). Resistance to low water activity and desiccation has also been associated with resistance to thermal treatments, but there are many varying parameters that may influence these conclusions.

Thermal resistance of pathogens in LWAF has also been observed, prompting questions regarding cross-protection from the impacts of water activity on thermal resistance. *Salmonella* in particular has been seen to have notable thermoresistance in LWAF. For example, only 3.2-log reductions of certain *Salmonella* serovars (Agona, Enteritidis, and Typhimurium) were achieved after a 90°C treatment of peanut butter for 50 min (Shachar and Yaron, 2006). Additionally, *Salmonella* survived in dried milk powder for 10 h at 76°C (McDonough and Hargrove, 1968). Archer et al. demonstrated that reducing the water activity of wheat flour increased the *D*-value of *Salmonella* Weltevreden substantially (1998). While several examples of increased thermal resistance have been revealed, there is still some debate as to how the cells develop heat resistance. The cell history and adaptation, microenvironment (food or other substance), and cultivation or culture preparation method all likely play roles in the development of heat resistance through cross-protection.

It has been noted in many instances that *L. monocytogenes* may gain resistance to certain conditions, such as antimicrobials or pH adjustments, when exposed to sublethal stressors (Poimenidou et al., 2016). Sublethal stressors could include forced osmotic or pH changes to the environment or changes in temperature (Skandamis et al., 2008). One study noted that cold shock reduced thermal resistance of *L. monocytogenes* on tomatoes, lettuce, and tryptic soy broth (TSB), but increased tolerance to osmotic or acid stress in some cases (Poimenidou et al., 2016). *Salmonella* and *E. coli* O157:H7 have also displayed the ability to develop resistance to various sublethal stressors (Burgess et al., 2016).

The presented research examines the different effects LWAF and intermediate-water activity food (IWAF) may have on thermal resistance, since the conditions may stress microorganisms to varying degrees. The variation in water activity, moisture content, or other composition characteristics could cause certain pathogens to develop more thermal resistance than others. The water activity of macadamia nuts and raisins are relatively comparable at approximately a_w 0.5-0.6, respectively. Alternatively, the water activity of other dried fruits, including apricot is slightly higher at a_w 0.65-0.70. This higher water activity of dried fruit with a moisture content around 30% may impact survival of microorganisms, specifically foodborne pathogens, and subsequent reactions to thermal treatments.

Raisins, apricot halves, and macadamia nuts were chosen for presented experiments because they are marketed as RTE, but they may be subject to contamination. Raisins and apricots, which are often sundried, may be untreated entirely following the drying process. Sun drying has not been proven to be an adequate intervention for pathogen reduction, and the prolonged exposure to the environment may actually increase the likelihood of contamination incidents (Bourdoux et al., 2016). Macadamia nuts are the second most popularly consumed tree

nut in the United States after almonds and are high in both fat and expense compared to other nuts (Brežná, Piknová, and Kutcha, 2009; Lee et al., 2011). Macadamia nuts are dried while still within the kernel, which holds approximately 30% moisture, for about 3 weeks until the moisture content lowers to 1.5% and the kernel can be easily removed from the nut ("Processing Macadamia Nuts," 2020). If the drying process is slow enough, it could allow pathogens that contaminated the kernel to acclimate and adapt as the moisture content and water activity slowly decreases.

For experiments, foods with additives should be avoided, such as citric acid, additional sugars, or sulfur dioxide. Citric acid, an organic acid, can be used as an antimicrobial. Additional sugar coatings could interfere with inoculation of pathogens on the product. Sulfur dioxide is used as an antioxidant and for antimicrobial purposes, particularly in dried fruits that are prone to discoloration from browning reactions (Ough and Were, 2005). Sulfur dioxide has been seen to be inhibitory to Gram-negative organisms, such as *E. coli* and *Pseudomonas*, even more so than Gram-positive organisms, which has been demonstrated by the use of sulfites in meat products. Bound sulfur dioxide is less antimicrobial, but free, unbound residues may continue to have antimicrobial effects on organisms, thus interfering with the observation of the effects of steam on the inoculated pathogens. Sulfur dioxide levels have been seen to be especially bacteriostatic to many lactic acid bacteria, so it could be possible that the surrogate, *Pediococcus acidilactici*, would be especially inhibited by these residues or be reduced to a viable but nonculturable state.

3. Organisms

3.1. *Salmonella*

Salmonella spp. are a diverse genus of Gram-negative rods that are responsible for causing salmonellosis. *Salmonella* infections can lead to intense gastroenteritis, which may include symptoms of nausea, vomiting, abdominal cramps, and fever. The infection typically persists 4 to 7 days and is generally self-limiting, though individuals can require treatment or hospitalization for dehydration (CDC, 2018b). *Salmonella* infections are usually acquired through the consumption of contaminated foods or through contact with pets and wildlife that may carry the bacterium (Maurer, 2017). The pathogen can be easily spread from food handlers through poor hygiene.

Both the food microenvironment and the human GI tract place *Salmonella* cells under great amounts of stress, eliciting stress responses that repair and protect cellular components and DNA (Horn and Bhunia, 2018). When *Salmonella* is ingested, it colonizes the intestine and invades intestinal epithelial cells, allowing access to macrophages using the Type III Secretion system or through phagocytosis. The pathogen survives in the *Salmonella*-containing vacuole, where there is protection from antibacterial attacks from the human immune response. *Salmonella* may also further replicate intracellularly within the vacuole if nutrients are available. To survive intracellularly, *Salmonella* Pathogenicity Islands encode for factors that enable processes, ion transporters, nutrient uptake, and extracellular surface structures like flagella and fimbriae that may aid in adhesion and other survival strategies. This action furthers the propagation and spread of the pathogen throughout the body, prompting an immune response (Ibarra and Steele-Mortimer, 2009).

Because of the association of *Salmonella* with wildlife or other animals, the pathogen is often associated with raw meat and poultry. However, recent analyses of outbreak data indicate that fresh produce pose a greater threat for *Salmonella* contamination than previously assumed.

Meat, poultry, and egg products combined were associated with approximately 50% of salmonellosis cases, while fruits, vegetables, and other forms of produce were responsible for approximately 47% of *Salmonella*-related illnesses in 2016 (IFSAC, 2018). *Salmonella* is surely not confined to a single category of food but should rather be considered an environmental contaminant with access to raw foods prior to harvest, as well as to processed foods through worker transmission. *Salmonella* also possesses abilities to survive desiccation and dehydration, which results in persistence on foods that may not be considered "raw" by a consumer and would not receive an additional kill-step once purchased. This may be the case for many LWAF, such as dried fruits and nuts.

The majority of outbreaks associated with LWAF result from *Salmonella* contamination. Widespread contamination and circumstantially low infectious doses make *Salmonella* a formidable opponent to LWAF safety. Because of the high association of *Salmonella* with outbreaks of LWAF, their persistence in LWAF and mechanisms to resist desiccation stress, osmotic stress, and thermal stress have been extensively studied (Burgess et al., 2016). Salmonellae are known to gather compatible solutes that prevent the destruction of cellular membrane components when the water activity surrounding the cells is lowered (Rychlik and Barrow, 2005). A number of proteins are involved in the uptake of osmoprotectants, but *Salmonella* can also enter dormant state in which the cell is physiologically inactive or minimally active in genome transcription (Deng et al., 2012; Rychlik and Barrow, 2005). In these cases, the cells may be viable but nonculturable (VBNC), which presents additional challenges in terms of estimating pathogen reduction due to thermal interventions and prompts additional consideration of recovery methods used in laboratory experiments (Gruzdev, Pinto, and Saldinger, 2012; Podolak et al., 2010).

The infectious dose for *Salmonella* varies depending on the food and at-risk populations. The typical infectious dose for a healthy, fit human is assumed to be between 3-5 log CFU/g, as evidenced by volunteer studies (Kothary and Babu, 2001). However, LWAF outbreaks have been associated with cases where a low infectious dose is believed to cause the outbreak (Finn et al., 2013). Small concentrations (as few as 10-100 organisms) of contamination have been detected on chocolate products that were linked to outbreaks (Greenwood and Hooper, 1983; Kapperud et al., 1990; Werber et al., 2005). These low numbers of cells detected likely result from post-processing contamination and pose significant threats to consumers. Rare instances of low infectious doses could illustrate how composition of the food matrix could impact the persistence and survival of pathogens. Specifically, a high-fat food, like chocolate, could provide greater protection for the cells.

3.2. Listeria monocytogenes

L. monocytogenes, the causative agent of listeriosis, is a ubiquitous organism in the environment that creates many challenges for the food industry. Food processors must conform to the “zero tolerance” policies in the United States for RTE foods. *L. monocytogenes* naturally exists in the environment and must overcome a number of harsh conditions, such as starvation, oxidative stress, and temperature stress to survive. These conditions force the organism to employ genes that trigger repair enzymes meant to help the cells maintain homeostasis while under sublethal stresses. Little research has been conducted to investigate mechanisms of desiccation tolerance of *L. monocytogenes*, but their ability to survive in a desiccated environment has been observed (Hansen and Vogel, 2011). Furthermore, *L. monocytogenes* is a known biofilm-producer, which has also been shown to enhance desiccation tolerance. Additionally, *L. monocytogenes* strains have been compared on the basis of different stress

responses, including desiccation, and learned that the serotypes with increased desiccation tolerance did not contain a fully expressed *inlA* (Internalin A; Hingston et al., 2017).

Additionally, the organism can develop cross-adaptation or cross-protection, in which exposure to one stress may provide strength against another stress in the future, or there may also be overlap of expression of stress response genes (Hansen and Vogel, 2011). Minimally processed foods pose special threats of creating cross-adaptation events in pathogens (Falerio, 2017). These threats are especially relevant when considering a low-temperature thermal treatment on a low-water activity food. It was observed that decreasing the water activity from a_w 0.69 to a_w 0.57 of black peppercorns increased thermal resistance of *L. monocytogenes* to a mild steam treatment, indicating stress responses were triggered (Zhou et al., 2019).

A "zero tolerance policy" in the USA on RTE products was enacted due to the heavy consequences of listeriosis for at-risk populations, which prompts product recalls when *L. monocytogenes* is detected on foods or in food contact zones (Archer, 2018). Immunocompetent individuals generally experience non-gastrointestinal symptoms that present in a flu-like manner, or mild gastroenteritis, which likely contributes to underreporting illnesses. However, young children, elderly individuals, and other immunocompromised populations can develop meningitis or septicemia. Furthermore, pregnant women are at risk of miscarriages due to listeriosis, as the organism can pass the blood-brain and placental barriers with little response from the immune systems due to the lowered cellular immunity. These results of listeriosis lead to high hospitalization rates (80%) and represent nearly 30% of foodborne-associated fatalities in the US (Silk et al., 2012; Mead et al., 1999).

L. monocytogenes is an invasive organism with abilities to cross the blood-brain barrier and placental barrier. During an infection, the organism travels from cell-to-cell, so a cell

mediated response from the host is necessary for defense against the infection. A typical humoral immune response is ineffective against *L. monocytogenes*, as the cell-to-cell infection allows the bacterium to avoid antibodies and continue invading and replicating intracellularly (Vázquez-Boland et al., 2001; Ward et al., 2004). *L. monocytogenes* begins an infection by making contact with and invading the mammalian cell using *inlA*, which is a surface protein that promotes invasion through a phagosome (Ragon et al., 2008; Farber and Peterkin, 1991). Listeriolysin O (*hly*), along with the help of *plcB*, a membrane-hydrolyzing enzyme, lyses the host phagosome, which releases *L. monocytogenes* into the cytoplasm of the host cell where it may replicate. The *mpl-actA-plcB* operon is directly responsible for producing the proteins that aid in successful cell-to-cell invasion (Vázquez-Boland, et al., 2001). The bacterium uses the ActA protein (*actA*) to polymerize the host cell's actin, which propels it into the adjacent cell so that it may further the infection. Furthermore, the functionality of these genes have been observed to impact virulence.

Listeria spp. are serotyped and categorized based on O (somatic) and H (flagellar) antigens, but there is great diversity amongst these identified serotypes. Furthermore, the majority of illnesses are caused specifically by four serotypes: 1/2a, 1/2b, 1/2c, and 4b, which are members of lineages I and II (Ragon et al., 2008; Farber and Peterkin, 1991; McLauchlin, 1990). Within these serotypes, however, there are variations in the level of virulence. For example, many serotypes isolated from food are found to contain premature stop codon mutations in *inlA*, which impacts the organisms' virulence. This has specifically been seen in *L. monocytogenes* 1/2a, which is not linked to as many clinical cases or outbreaks in the United States as is *L. monocytogenes* 4b (Buchanan et al., 2017; van Stelten et al., 2010). Although these serotypes have been commonly identified as the source of illnesses and outbreaks, there have not been any

identified serotypes associated specifically with LWAF outbreaks, so prevalence of particular disease-causing strains on dried or low-moisture foods have not been established.

L. monocytogenes has been the source of illness in a variety of RTE foods, such as ice cream and caramel apples (Glass et al., 2015). In these instances, it was speculated that contamination occurred during processing, and no additional steps were taken to ensure the safety of the product (Buchanan et al., 2017). Because *L. monocytogenes* is considered to be ubiquitous in the environment, it has been associated with outbreaks of fresh produce such as pre-cut celery, cantaloupe, and mung bean sprouts that resulted in staggering numbers of hospitalizations and deaths in 2010, 2011, and 2014 (CDC, 2018a). Additionally, stone fruits that were recalled in 2014 due to suspected presence of *L. monocytogenes* were tested and matched to isolates found in patients with listeriosis. Although stone fruits may be dehydrated to be sold as a dried fruit product, sun drying is not currently considered to be an effective and validated kill-step. Ergo, it may be necessary to treat dried fruits with the consideration that they may have been contaminated pre-processing. Although *L. monocytogenes* outbreaks are often associated with refrigerated, ready-to-eat products, such as soft cheeses, deli meats, and produce, there have been outbreaks resulting from unlikely food sources or RTE foods that were not previously identified as high-risk foods (CDC, 2018a). *Listeria* contamination of dried foods is an emerging and increasing trend for food recalls in both the US and Europe. As age expectancies rise, coupled with the rising popularity of dried food consumption, more listeriosis cases associated with a variety of foods should be anticipated (Ly, Parreira, and Farber, 2019).

3.3. Shiga Toxin-Producing Escherichia coli *(STEC)*

Shiga Toxin-producing *Escherichia coli* (STEC) are the causative agent for foodborne illnesses resulting from environmental contamination. Cattle and other ruminants serve as

asymptomatic reservoirs where STEC can colonize in the gastrointestinal system (Moxley and Acuff, 2014). Shedding through feces occurs frequently, and contaminated water and soil, as well as field workers, can introduce the pathogen to the food system.

Disease due to STEC can cause a variety of symptoms in individuals. Some illnesses result in severe abdominal cramping, vomiting, and bloody diarrhea. These infections are often mild enough to be self-limiting. Some infections, however, develop into haemorrhagic colitis (HC) or haemolytic uremic syndrome (HUS), which can lead to kidney failure, particularly in at-risk populations, such as young children or elderly individuals. The most commonly recognized STEC, in addition to O157:H7, are the "Big 6," which include O26, O45, O103, O111, O121, and O145. While many serotypes of STEC can lead to serious disease, O157:H7 is often associated with HUS (Gould et al., 2013; Thorpe, 2017). Nevertheless, recognition of other serotypes that have also been observed to result complications such as HUS is rising. Incidence of non-O157 infections rose from 0.12 to 0.95 cases per 100,000 in the United States from 2001-2010. However, O157-associated infections decreased from 2.17 to 0.95 cases per 100,000 (Crim et al., 2015).

Enterohemorrhagic *E. coli* (EHEC) strains are extremely diverse and heterogeneous, but the methods of host cell invasion are generally universal due to the locus of enterocyte effacement (LEE) pathogenicity island (Paton and Paton, 1998; Thorpe, 2017). LEE pathogenicity islands are responsible for encoding the virulence factors that are expressed to allow attachment and invasion of host cells. When the bacteria are ingested, they invade the intestinal mucosa. After the pathogen makes contact with the host cell, the Type II Secretion System passes Tir into the host cell. The pathogen uses intimin, which is produced by the *eae* gene, to interact with Tir to form attaching and effacing (A/E) lesions. A/E lesions provide an

intimate association between the host cell and pathogen. In response to the presence of H7 flagellin, lipopolysaccharide (LPS) membrane, and the release of Stx, the host cell triggers inflammatory responses from the immune system to fight against the infection. However, in addition to promoting attachment and invasion, the T3SS targets host cell pathways and proteins to disable certain immune responses in order to persist the infection and prevent cellular defense strategies. Specifically, the T3SS produces effectors that aid in degrading the host cell proteins, which typically provide cytoskeleton organization, MAPK signaling pathways, apoptosis signaling pathways, and other inflammatory responses (Zhang, Y., Pearson, and Hartland, 2017). This antagonistic action prevents host cells from addressing the infection and limiting the spread of disease.

The grave consequences that an arise from an STEC infection, such as HUS, HC, and resulting organ failure lead to high hospitalization and fatality rates. Certain STEC, namely the "Big 6" and O157:H7 were declared adulterants in the early 1990's in ground beef products following a large outbreak (Bottemiller, 2011). While ground beef and other raw meat products are commonly associated with STEC due to their natural reservoir in ruminants, the bacteria are known to contamination many other types of foods, namely produce. Any agricultural product exposed to environmental conditions is subject to STEC contamination, as seen with their association with recalls and outbreaks involving nuts (FDA, 2017; FDA, 2018).

STEC are known to survive in desiccated states or under osmotic stress in certain conditions, such as at refrigeration temperature (4°C), or in the presence of compatible solutes (Hiramatsu et al., 2005; Welsh and Herbert, 1999). They have also been associated with outbreaks involving raw flour and recalls due to the presence on nuts, further demonstrating desiccation resistance (CDC, 2019b; FDA, 2018). In some cases, STEC have developed thermal

resistance in the low-water activity environment induced by addition of solutes (Blackburn et al., 1997). Survival in low-water activity environments through desiccation or hyperosmolarity is associated with RpoS, as well as other proteins that are active in response to stress (Gunasekera, Csonka, and Paliy, 2008; Hengge-Aronis et al., 1992; Stasic, Wong, and Kaspar, 2012). Accumulation or synthesis of compatible solutes, such as trehalose, have also been associated with desiccation tolerance by using the osmoprotectants to maintain membrane stability and integrity (Zhang and Yan, 2012).

3.4. Pediococcus acidilactici

Pediococcus acidilactici, a non-pathogenic, Gram-positive, lactic acid bacterium, is a possible surrogate organism for pathogens on foods treated with specifically low-temperature thermal processes. It is well known for its use in the grain and meat industry as a starter culture and fermenter, but it is also increasingly studied for demonstrated resistance to certain food processing interventions, such as mild heat treatments (Abbasiliasi et al., 2017; Saunders, et al., 2018). *Pediococcus* has a wide growth range (30-50°C) and can tolerate temperatures below 90°C (Ceylan and Bautista, 2015; Riebel and Washington, 1990). Although the bacteria have a wide temperature range, it still requires water activity levels of a_w 0.9 or above, which suggests it would not be a spoilage threat to LWAF. Additionally, the microorganism is not commonly associated with the aforementioned foodborne pathogens and would not be considered an indicator organism, but rather could be used as a surrogate (Busta et al., 2003). Use of *Pediococcus acidilactici* as a surrogate for low-temperature, vacuum-assisted steam is further discussed in section 4.4.

While *Enterococcus faecium* NRRL 2354 has been popularly studied and approved as a surrogate for a number of different food products, including almonds, there have been foods and

intervention conditions in which it has been deemed less resistant than target pathogens (ABC, 2014; Rachon, Peñaloza, and Gibbs, 2016; Tsai et al., 2019). In one notable study directly comparing the use of *Pediococcus acidilactici* and *E. faecium* as surrogates for *Salmonella* on thermally treated pet food, it was noted that *Pediococcus* more closely resembled the *Salmonella* cocktail than *E. faecium*, designating it a more appropriate choice of surrogate when investigating thermal treatments of LWAF (Ceylan and Bautista, 2015). Using an excessively resistant surrogate, such as *E. faecium* in these particular circumstances, could result in over-processing, thus damaging the sensory characteristics of the food. For the low-temperature treatments of LWAF, *Pediococcus* may be a better choice of surrogate.

4. Relevant Research Methods

4.1. Low-Temperature, Vacuum-Assisted Steam

As minimally pocessed, organic, RTE foods gain popularity, food industries must creatively employ interventions that can enhance food safety while still maintaining the product integrity desirable by consumers. While interventions such as radiation or propylene oxide have been noted as effective in reducing pathogen populations from the surface LWAF, they cannot be used if the product is to maintain USDA organic status (CFR, 2020; Danyluk, Uesugi, and Harris, 2005; Karagöz, Moreira, and Castell-Perez, 2014). Vacuum-steam pasteurization as an intervention is more efficient in transferring heat than heated air and could be used to treat organic LWAF (Saunders et al., 2018). Examples of heightened thermal resistance have been noted in low-water activity environments, but the introduction of small amounts of moisture through the vacuum-steam treatments may make bacteria more susceptible to thermal inactivation. With the use of a vacuum, a negative-pressure environment can be created in the

chamber, thus allowing steam to form at temperatures <100°C, which may cause less damage to the food products compared to superheated steam treatments (Grasso et al., 2014).

Vacuum-steam has been successful in inactivating pathogens, such as *Salmonella* spp. on cumin and whole peppercorns (Newkirk et al., 2018; Zhou et al., 2019), cashews and macadamia nuts (Saunders, 2017), and (Shah et al., 2017); *E. coli* O157:H7 from black peppercorns (Zhou et al., 2019, Shah et al., 2017), whole flax-seed, quinoa, sunflower kernels, and milled flaxseed (Shah et al., 2017); and *L. monocytogenes* from black peppercorns (Zhou et al., 2019). Macadamia nuts were vacuum-steamed by Saunders, but only for the inactivation of *Salmonella* spp. (2017). An intermediate-water activity food, such as dried fruit, has not been investigated (2017). Each study determined vacuum-steam treatments (<90°C) to reduce pathogens by varying amounts (4-5 log CFU/g), but the treatments were determined to be successful overall. While quality was not a focal point of any study, the introduction of low amounts of moisture and mild heat could cause impacts to the shelf lives or quality of product, which should be investigated further (Duncan et al., 2017).

4.2. Inoculations and Bacterial Strains

Both wet and dry inoculation strategies have been used in studies to investigate pathogen reduction on LWAF. In the case of wet inoculation methods, the products are inoculated at high concentrations resulting in moderate changes to the water activity, and then dried back to their original water activity levels, as seen in several studies involving LWAF (Channaiah et al., 2017; Brar et al., 2015). These methods attempt to limit the amount of moisture introduced to the LWAF while still achieving even distribution of cells in the matrix, and then take steps to remove the added moisture. Dry-inoculation methods more closely mimic the physiological state of a pathogen when it exists for extended periods of in a low water activity environment. Studies

that pursue this type of inoculation first inoculate a powder, which is then transferred to the food (Hildebrandt et al., 2017). However, high target inoculation levels can be difficult to achieve with dry transfer methods, which hinder project objectives of quantifying large bacterial log reductions. A high-level inoculation, as opposed to a low-level inoculation, represents a worst-case scenario of contamination. Additionally, in the context of vacuum-steam, the minimum length of cycles for sampling is limited, in that there is a residual heating time that cannot be avoided while the vacuum is released from the chamber. Ergo, lower inoculation levels risk inabilities to approximate significant bacterial reductions. It has been hypothesized that lower contaminating populations are more realistic, and that there may be increased variability in smaller populations that should be considered (Aspridou and Koutsoumanis, 2015), but studies examining inoculation procedures of *Salmonella* on wheat flour showed no difference in D-value between low- and high-level inoculations (~3 and 8 log CFU/g) (Hildebrandt et al., 2016).

A method that compromises some advantages of both wet and dry inoculations could be accomplished by misting a liquid inoculum onto the product. Misting or spraying an inoculum has been used to inoculate many different food types, such as beef subprimal cuts, flour, walnuts, and a number of dried fruits (Beuchat and Mann, 2014; Channaiah et al., 2017; Erickson et al., 2014; Kirsch et al., 2017; Lang, Harris, and Beuchat, 2003). Beuchat and Mann observed the survival and stability of a *Salmonella* cocktail when mist-inoculated onto dried fruits and stored at different temperatures for extended periods of time (Beuchat and Mann, 2014). They noted high inoculation levels (6.6-7.0 log CFU/g) with relatively high retention rates at 4ºC, recovering 5.2 log CFU/g on raisins after 6 days of storage. They also compared their mist-inoculum results to a dry-inoculation process, using inoculated sand to coat the dried fruits. However, their retention levels were much lower (2.2-3.4 log CFU/g). In a baking validation study, flour was

mist-inoculated with a 3-strain *Salmonella* cocktail. The dried flour retained approximately 8.0 ± 0.3 log CFU/g and could be stored for 7 days (Channaiah et al., 2017).

For studies with intentional application to the food industry, a mixed inocula containing multiple species and strains, or cocktail, can be helpful in providing information that would represent multiple contamination scenarios. Additionally, a heterogenous bacterial cocktail allows for side-by-side comparison of reductions due to vacuum-steam treatments. In choosing strains to include in the cocktail, connection to the food itself is a valuable trait, as well as ability to cause disease. For vacuum-steam treatments of LWAF, strains with known heat resistance or ability to survive in a desiccated state should be considered, such as *E. coli* O121 isolated from the Gold Medal all-purpose wheat flour outbreak in 2016 (FNW19M81). Having been isolated from flour, it is likely that the pathogen survived in the a low-water activity environment for a relatively long period of time and may also have withstood some level of thermal treatment. While investigating the outbreak strain, experiments showed that the STEC survived in wheat flour for two years, without significant decreases between 6 months to 2 years (Gill et al., 2020). *E. coli* O157:H7 F4546 (alfalfa sprout outbreak isolate) has primarily been studied in relation to produce, exploring its capabilities of attachment to and internalization by fresh produce, as well as resistance to common chemical washes. It has only been briefly explored for its thermal resistance but is known for causing disease (Topalcengiz and Danyluk, 2017). *Salmonella* serovars Montevideo (1449, peppercorn-associated outbreak), Newport (allspice isolate), and Tennessee (K4643, peanut butter outbreak) have been investigated for desiccation resistance during storage on peppercorns, cashews, macadamia nuts, as well as thermal resistance (Bowman et al., 2015; Saunders et al., 2018). There are no known outbreaks of listeriosis associated with LWAF, but some studies have investigated osmotic stress resistance and

desiccation tolerance of foodborne and clinical isolates. For example, Zoz et al. noted that 1/2b serotypes displayed heightened desiccation resistance regardless of the food with which it was associated (2017).

4.3. Injury-Recovery Methods

Application of stress to bacteria can cause sublethal injury to cells, damaging cellular components without completely inactivating the cell (Wesche et al., 2009). Food processing techniques, in particular thermal treatments, can cause injury to cells that may result in phenotypic changes or inability to grow in detectable manners. However, virulence and ability to cause disease may remain. This phenomenon could result in underestimation of pathogen risk in foods that are minimally processed. To address this occurrence, laboratory methods have been developed to repair cells that may initially be unculturable due to sublethal stress. In particular, studies have shown that cells can be resuscitated on nonselective agar plates during temporary incubation, followed by a thin, selective agar overlay that selects for the pathogen (Kang and Fung, 2000; Yan, Gurtler, and Kornacki, 2006). These studies showed significantly higher recovery of bacterial populations than those from selective plating alone, highlighting the need to consider injury-recover methods when designing research methods.

4.4. Analyses and Modeling

Surrogate Determination

Surrogate organisms are nonpathogenic and useful for depicting or representing pathogens' responses to applied stresses. Surrogates can be used in place of pathogens when challenging or validating processing interventions or storage conditions. For in-plant validations, surrogates are recommended to mimic the most resistant pathogen to the applied conditions (NACMCF, 2010). For experiments intending to determine if a microorganism can be

appropriately used as a surrogate, side-by-side comparisons with the pathogen is desired. Coinoculations of pathogens and potential surrogate on the food are best for offering direct comparisons (Saunders et al., 2018). ANOVA, pairwise comparisons, and one-to-one ratio plots can be used to determine significant differences between the pathogen and surrogate.

4.5. Modeling

Mathematical modeling has been adopted by food engineers and food microbiologists to describe food processing techniques and behavior of microorganisms as they either grow, survive, or are inactivated. Using models, food processing systems can be improved in order to provide more confidence of the product's safety (Marks, 2008). Models have also been useful in describing growth of microorganisms in various environmental conditions. Furthermore, models can be employed for predictions of microbial inactivation or growth.

Primary models describe growth or inactivation under defined parameters. In the pursuit of investigating objectives proposed for the presented research, primary models will be focused on describing time of inactivation under constant temperatures. Several models will be investigated, including the first-order model, Weibull model, and Gompertz model. The first-order model describes linear inactivation, while the other two allow for nonlinear trends.

The first-order model makes generic assumptions about the microorganisms' behavior. Specifically, for this study's purposes, the first-order model assumes that the bacterial population is homogenous and behaves uniformly. For example, each bacterial cell would have the same probability of being inactivated by time at a certain temperature as any other cell in the population (van Boekel, 2002). First-order models are commonly used to determine *D*- and *z*-values, or decimal reduction times, which can be very useful in comparing resistance of

microorganisms to thermal treatments. The first-order model can be described in the following manner (Peleg, Normand, and Corradini, 2005):

$$(1) \quad logN_t - logN_0 = -kt$$

where N_t is the surviving bacterial population at time, t; N_0 is the initial bacterial population; and k is the inactivation rate (log CFU/g min^{-1}).

While first-order models have been employed to describe many bacterial inactivation scenarios, their usefulness is limited in many scenarios. Under many conditions, it should be recognized that population homogeneity is likely very rare. Rather, bacterial populations are heterogenous with slight differences in resistances between cells. This information cannot always be captured with a linear inactivation trend. Rather, models that can describe shouldering (sublethal treatment or initial resistance to the treatment) or tailing (lingering population, acquired resistance, or genetic differences) may more accurately describe inactivation kinetics (Bevilacqua et al., 2015).

The Weibull model allows for either a shouldering or tailing population, which is determined by the shape of the curve. The Weibull was developed to describe "time to failure" in mechanical and engineering systems, but the concept also applies to bacteria in terms of "time to inactivation" when the bacteria are subjected to a constant stress (van Boekel, 2002). The Weibull model can be defined as the following (Peleg, 2003):

$$(2) \quad logN_t - logN_0 = -k \cdot t^{\beta}$$

where k is a rate parameter ($k = k' / \log_e 10$) for the reduction and β indicates whether the shape is a concave upward (<1), downward (>1), or linear (=1) inactivation curve. The concave shape (β < 1) indicates a form of adaptation to the thermal inactivation or a higher level of resistance by select cells, whereas a convex shape (β > 1) represents exponential die-off as the thermal treatment persists. The Weibull model has been used to describe survival or thermal inactivation of *Salmonella* spp. in milk, tree nuts, and powders among many others (Bermúdez-Aguirre and Corradini, 2012; Chen and Hoover, 2004; Farakos, Hicks and Frank, 2013; Farakos, Pouillot, and Keller, 2017).

The Gompertz model can also describes nonlinear inactivation but can account for both shouldering and tailing of the bacterial population with a sigmoid-shaped curve. In describing inactivation, the curve initially depicts resistance to the treatment, followed by exponential inactivation and somewhat linear inactivation, lastly followed by a tailing population that is somewhat resistant to the treatment and is not fully inactivated. In its original form, the Gompertz is described in the following manner (Zwietering et al., 1990):

$$(3) \quad logN_t - logN_0 = a \cdot exp[-exp(b - ct)]$$

where a, b, and c are parameters derived to fit the shape of the sigmoidal curve with no biological meaning. A modified version of the Gompertz can be more appropriately used by microbiologists in the following format that provides biological connections to the sigmoidal curve (Zwietering et al., 1990):

$$(4) \quad logN_t - logN_0 = A \cdot exp\left\{-exp\left[\frac{\mu_M \cdot e}{A}(\lambda - t) + 1\right]\right\}$$

where μ_M (log CFU/g min^{-1}) is the maximal value of inactivation, λ is the lag time (min), and *A* is the asymptote. The Gompertz model is often used to describe growth, but similar to the presented research was used by Linton et al. to describe thermal inactivation of *L. monoctyogenes* Scott A in laboratory media (Linton et al., 1995).

To evaluate the fit of the models, a correlation coefficient (R^2) can appropriately be used for linear models. However, nonlinear models should not use the R^2, but rather the root-mean-square error (RMSE) and Akaike information criterion (AIC). These metrics can provide standardized evaluations of the models, along with attention to the confidence and prediction intervals, which describe the certainty of the models in describing and predicting future values (Marks, 2008). In addition to determining the aforementioned values, the limit of detection should also be determined and evaluated for its possible effects on the shape of the inactivation curve.

Before commissioning models to predict inactivation of microbial populations, they should be validated using other datasets to determine the robustness and flexibility (Marks, 2008). Independent data is most valuable for validation, but access to independent datasets may be limited. Comparing predicted and observed values and the resulting error can guide assessments on the model's flexibility and accuracy. Models will be challenged by comparing the fit when the foods or treatment conditions are altered.

As a growing field of food microbiology, the need for shared data in order to properly validated food processing models is vital. Combase, an online resource used to share datasets on the quantitative and predictive food microbiology, along with other databases serve as a platform for microbiologists to post and acquire external datasets. These platforms can aid in the formation of food safety plans and validation of intervention processes if employed correctly.

Experimental datasets resulting from the presented research can be added to Combase to further these endeavors.

References

1. Abbasiliasi, S., Tan, J. S., Bashokouh, F., Ibrahim, T. A. T., Mustafa, S., Vakhshiteh, F., Sivasamboo, S., and Ariff, A. B. (2017). In vitro assessment of *Pediococcus acidilactici* Kp10 for its potential use in the food industry. *BMC Microbiology, 17*(121), 1–11. http://doi.org/10.1186/s12866-017-1000-z
2. Acuff, J. C. and Ponder, M. A. (2019). "Interactions of foodborne pathogens with the food matrix." In Food Safety Engineering. Edited by Demirci A, Feng H. Springer, Boca Raton. In Press.
3. Almond Board of California (ABC). (2014). Guidelines for using *Enterococcus faecium* NRRL B-2354 as a surrogate microorganism in almond process validation. Available from: https://www.almonds.com/sites/default/files/guidelines_for_using_enterococcus_faecium_nrrl_b-2354_as_a_surrogate_microorganism_in_almond_process_validation.pdf. (Date accessed: 14 January 2020).
4. Andino, A. and Hanning, I. (2015). *Salmonella enterica*: Survival, colonization, and virulence differences among serovars. *The Scientific World Journal*, *2015*, 1–16.
5. Archer, J., Jervis, E. T., Bird, J., and Gaze, J. E. (1998). Heat resistance of *Salmonella weltevreden* in low-moisture environments. *Journal of Food Protection*, *61*(8), 969–973.
6. Archer, D. L. (2018). The evolution of FDA's policy on *Listeria monocytogenes* in ready-to-eat foods in the United States. *Current Opinion in Food Science*, *20*, 64–68. http://doi.org/10.1016/j.cofs.2018.03.007
7. Aspridou, Z. and Koutsoumanis, K. P. (2015). Individual cell heterogeneity as variability source in population dynamics of microbial inactivation. *Food Microbiology, 45*, 1–6. http://doi.org/10.1016/j.fm.2014.04.008
8. Bermúdez-Aguirre, D., and Corradini, M. G. (2012). Inactivation kinetics of *Salmonella* spp. under thermal and emerging treatments: A review. *Frin*, *45*(2), 700–712. http://doi.org/10.1016/j.foodres.2011.05.040
9. Beuchat, L. R., Komitopoulou, E., Beckers, H., Betts, R. P., Bourdichon, F., Fanning, S., Joosten, H. M., and Ter Kuile, B. H. (2013). Low–water activity foods: Increased concern as vehicles of foodborne pathogens. *Journal of Food Protection*, *76*(1), 150–172. http://doi.org/10.4315/0362-028X.JFP-12-211
10. Beuchat, L. R., and Mann, D. A. (2014). Survival of *Salmonella* on dried fruits and in aqueous dried fruit homogenates as affected by temperature. *Journal of Food Protection*, *77*, 1102–1109. http://doi.org/10.4315/0362-028X.1FP-13-549. (Date accessed: 14 June 2018).
11. Bevilacqua, A., Speranza, B., Sinigaglia, M., and Corbo, M. (2015). A focus on the death kinetics in predictive microbiology: Benefits and limits of the most important models and some tools dealing with their application in foods. *Foods*, *4*(4), 565–580. http://doi.org/10.3390/foods4040565
12. Blackburn, C. de W., Curtis, L. M., Humpheson, L., Billon, C., and McClure, P. J. (1997). Development of thermal inactivation models for *Salmonella enteritidis* and *Escherichia coli* O157:H7 with temperature, pH and NaCl as controlling factors. *International Journal of Food Microbiology*, *38*, 31–44.
13. van Boekel, M. A. J. S. (2002). On the use of the Weibull model to describe thermal inactivation of microbial vegetative cells. *International Journal of Food Microbiology*,

74, 139–159.
14. Bottemiller, H. (2011). Looking back, the story behind banning *E. coli* O157:H7. Food Safety News. Available from: https://www.foodsafetynews.com/2011/09/looking-back-in-time-the-story-behind-banning-ecoli-o157h7/. (Date accessed: 14 January 2020).
15. Bourdoux, S., Li, D., Rajkovic, A., Devlieghere, F., and Uyttendaele, M. (2016). Performance of drying technologies to ensure microbial safety of dried fruits and vegetables. *Comprehensive Reviews in Food Science and Food Safety*, *15*(6), 1056–1066. http://doi.org/10.1111/1541-4337.12224
16. Bowman, L. S., Waterman, K. M., Williams, R. C., and Ponder, M. A. (2015). Inoculation preparation affects survival of *salmonella enterica* on whole black peppercorns and cumin seeds stored at low water activity. *Journal of Food Protection*, *78,* 1259–1265. http://doi.org/10.4315/0362-028X.JFP-14-483. (Date accessed: 1 May 2018).
17. Brar, P. K., Proano, L. G., Friedrich, L. M., Harris, L. J., and Danyluk, M. D. (2015). Survival of *Salmonella*, *Escherichia coli* O157:H7, and *Listeria monocytogenes* on raw peanut and pecan kernels stored at −24, 4, and 22°C. *Journal of Food Protection, 78,* 323–332. http://doi.org/10.4315/0362-028X.JFP-14-327. (Date accessed: 1 January 2018).
18. Brežná, B., Piknová, L., and Kuchta, T. (2009). A novel real-time polymerase chain reaction method for the detection of macadamia nuts in food. *European Food Research and Technology*, *229*(3), 397–401. http://doi.org/10.1007/s00217-009-1070-8
19. Buchanan, R. L., Gorris, L. G. M., Hayman, M. M., Jackson, T. C., and Whiting, R. C. (2017). A review of *Listeria monocytogenes*: An update on outbreaks, virulence, dose-response, ecology, and risk assessments. *Food Control, 75,* 1–13. http://doi.org/10.1016/j.foodcont.2016.12.016. (Date accessed: 1 May 2018).
20. Burgess, C. M., Gianotti, A., Gruzdev, N., Holah, J., Knøchel, S., Lehner, A., Margas, E., Esser, S. S., Saldinger, S. and Tresse, O. (2016). The response of foodborne pathogens to osmotic and desiccation stresses in the food chain. *International Journal of Food Microbiology*, *221*, 37–53.
21. Busta, F. F., Suslow, T. V., Parish, M. E., Beuchat, L. R., Farber, J. N., Garrett, E. H., and Harris, L. J. (2003). The Use of indicators and surrogate microorganisms for the evaluation of pathogens in fresh and fresh-cut produce. *Comprehensive Reviews in Food Science and Food Safety*, *2*, 179–185.
22. Canadian Food Inspection Agency (CFIA). Health hazard alert: certain walnuts and walnut products may contain *E. coli* O157:H7 bacteria. (2011). http://www.inspection.gc.ca/about-the-cfia/newsroom/food-recall-warnings/complete-listing/2011-04-03/eng/1359548340192/1359548340223. (Date accessed: 24 May 2018).
23. Capozzi, V., Fiocco, D., Amodio, M. L., Gallone, A. and Spano, G. (2009). Bacterial stressors in minimally processed food. *International Journal of Molecular Sciences*, 10*(*7), 3076–3105.
24. Centers for Disease control and Prevention (CDC). (2012). Foodborne outbreak online database. http://www.cdc.gov/foodborneoutbreaks/. (Date accessed: 21 February 2019).
25. Centers for Disease Control and Prevention (CDC). (2018a). Burden of foodborne Illness: Findings. https://www.cdc.gov/foodborneburden/2011-foodborne-estimates.html (Date accessed: 30 November 2017).

26. Centers for Disease Control and Prevention (CDC). (2018b). *Salmonella*: Questions and answers. https://www.cdc.gov/salmonella/general/index.html (Date accessed: 28 January 2019).
27. Centers for Disease Control and Prevention (CDC). (2019a). Estimates of foodborne illness in the United States. https://www.cdc.gov/foodborneburden/index.html (Date accessed: 20 October 2019).
28. Centers for Disease Control and Prevention. (2019b). Reports of selected *E. coli* outbreak investigations. https://www.cdc.gov/ecoli/outbreaks.html. (Date accessed: 2 December 2019).
29. Ceylan, E., and Bautista, D. A. (2015). Evaluating *Pediococcus acidilactici* and *Enterococcus faecium* NRRL B-2354 as thermal surrogate microorganisms for *Salmonella* for in-plant validation studies of low-moisture pet food products. *Journal of Food Protection*, *78*(5), 934–939. http://doi.org/10.4315/0362-028X.JFP-14-271
30. Chang, S. S., Han, A. R., Reyes-De-Corcuera, J. I., Powers, J. R., and Kang, D. H. (2010). Evaluation of steam pasteurization in controlling *Salmonella* serotype Enteritidis on raw almond surfaces. *Letters in Applied Microbiology*, *50*(4), 393–398. http://doi.org/10.1111/j.1472-765X.2010.02809.x
31. Channaiah, L. H., Michael, M., Acuff, J. C., Phebus, R. K., Thippareddi, H., Olewnik, M., and Milliken, G. (2017). Validation of the baking process as a kill-step for controlling *Salmonella* in muffins. *International Journal of Food Microbiology, 250,* 1–6. http://doi.org/10.1016/j.ijfoodmicro.2017.03.007 (Date accessed: 29 November 2017).
32. Chen, H. and Hoover, D. G. (2004). Use of Weibull model to describe and predict pressure inactivation of *Listeria monocytogenes* Scott A in whole milk. *Innovative Food Science & Emerging Technologies*, *5*(3), 269–276. http://doi.org/10.1016/j.ifset.2004.03.002
33. Code of Federal Regulations (CFR). (2020). Title 7 – Agriculture – National Organic Program: National list of allowed and prohibited substances. Available from: https://www.ecfr.gov/cgi-bin/text-idx?c=ecfr&SID=9874504b6f1025eb0e6b67cadf9d3b40&rgn=div6&view=text&node=7:3.1.1.9.32.7&idno=7#sg7.3.205.g.sg0. (Date accessed: 20 February 2020).
34. Crim, S. M., Griffin, P. M., Tauxe, R. V., Marder, E. P., Gilliss, D., Cronquist, A. B., Cartter, M., Tobin-D'Angelo, M., Blythe, D., Smith, K., Lathrop, S., Zansky, S., Cieslak, P. R., Dunn, J., Holt, K. G., Wolpert, B., and Henao, O. L.. (2015). Preliminary incidence and trends of infection with pathogens transmitted commonly through food — Foodborne Diseases Active Surveillance Network, 10 U.S. Sites, 2006–2014. *MMWR Morbidity and Mortality Weekly Report, 64*, 495–499.
35. Cronan, J. E., Jr. (2002). Phospholipid modifications in bacteria. *Current Opinion in Microbiology*, *5*, 202–205.
36. Danyluk, M.D., Uesugi, A.R., Harris, L.J. (2005). Survival of *Salmonella* Enteritidis PT 30 on inoculated almonds after commercial fumigation with propylene oxide. *Journal of Food Protection*, *68*, 1613–1622.
37. Danyluk, M. D., Jones, T. M., Abd, S. J., Schlitt-Dittrich, F., Jacobs, M., and Harris, A. J. (2007). Prevalence and amounts of *Salmonella* found on raw California almonds. *Journal of Food Protection*, *70*(4), 820–827.
38. Davidson, G. R., Frelka, J. C., Yang, M., Jones, T. M., and Harris, L. J. (2015). Prevalence of *Escherichia coli* O157:H7 and *Salmonella* on inshell California walnuts.

Journal of Food Protection, *78*(8), 1547–1553. http://doi.org/10.4315/0362-028X.JFP-15-001

39. Deng, K., Talukdar, P. K., Sarker, M. R., Paredes-Sabja, D. and Torres, J. A. (2017). Survival of *Clostridium difficile* spores at low water activity. *Food Microbiology*, *65*, 274–278.
40. Deng, X., Li, Z., and Zhang, W. (2012). Transcriptome sequencing of *Salmonella enterica* serovar Enteritidis under desiccation and starvation stress in peanut oil. *Food Microbiology*, *30*(1), 311–315. http://doi.org/10.1016/j.fm.2011.11.001
41. Denyer, S. P., Hodges, N., and Gorman, S. P. (2011). Sterilization procedures and sterility assurance. In Denyer, S. P., Hodges, N., and Gorman, S. P. (Eds.), *Hugo & Russel's Pharmaceutical Microbiology* (pp. 346-375). Oxford: Blackwell Science Ltd. (Date accessed: 17 January 2018).
42. Duncan, S. E., Moberg, K., Amin, K. N., Wright, M., Newkirk, J. J., Ponder, M. A., Acuff, G. R., and Dickson, J. S. (2017). Processes to preserve spice and herb quality and sensory integrity during pathogen inactivation. *Journal of Food Science*, *82*(5), 1208–1215. http://doi.org/10.1111/1750-3841.13702
43. European Food Safety Authority (EFSA). (2011). Shiga toxin-producing *E. coli* (STEC) O104:H4 outbreaks in Europe: taking stock. *EFSA J* 9,2390. https://efsa.onlinelibrary.wiley.com/doi/epdf/10.2903/j.efsa.2011.2390 (Date accessed: 22 February 2019).
44. EFSA (European Food Safety Authority) (2009) The community summary report on food-borne outbreaks in the European Union in 2007. https://efsa.onlinelibrary.wiley.com/doi/epdf/10.2903/j.efsa.2009.271r (Date accessed: 22 February 2019).
45. Eglezos, S., Huang, B., and Stuttard, E. (2008). A survey of the bacteriological quality of preroasted peanut, almond, cashew, hazelnut, and brazil nut kernels received into three Australian nut-processing facilities over a period of 3 years. *Journal of Food Protection*, *71*(2), 402–404.
46. Erickson, M. C., Webb, C. C., Davey, L. E., Payton, A. S., Flitcroft, I. D., and Doyle, M. P. (2014). Internalization and fate of *Escherichia coli* O157:H7 in leafy green phyllosphere tissue using various spray conditions. *Journal of Food Protection*, *77*(5), 713–721. http://doi.org/10.4315/0362-028X.JFP-13-357. (Date accessed: 14 June 2018).
47. Faleiro, M. L. (2017). The *Listeria monocytogenes* Triad for Success: Food Matrix, Stress Response and Virulence. In J.B. Gurtler, M.P. Doyle, and J.L. Kornacki, eds., *Foodborne Pathogens: Virulence Factors and Host Susceptibility*. Cham, Switzerland: Springer International Publishing AG, pp. 93-122.
48. Farakos, S. M. S., Hicks, J. W., and Frank, J. F. (2014). Temperature resistance of *Salmonella* in low–water activity whey protein powder as influenced by salt content. *Journal of Food Protection*, *77*(4), 631–634. http://doi.org/10.4315/0362-028X.JFP-13-306
49. Farakos, S. M. S. and Frank, J. F. (2014). Challenges in the control of foodborne pathogens in low-water activity foods and spices. In Gurtler, J. B., Doyle, M. P., and Kornacki, J. L. (Eds.), *The microbiological safety of low water activity foods and spices* (pp. 15-34). New York: Springer New York.
50. Farakos, S. M. S., Pouillot, R., and Keller, S. E. (2017). *Salmonella* survival kinetics on pecans, hazelnuts, and pine nuts at various water activities and temperatures. *Journal of*

Food Protection, 80, 879–885. http://doi.org/10.4315/0362-028X.JFP-16-392 (Date accessed: 4 November 2017).
51. Farber, J. M. and Peterkin, P. I. (1991). *Listeria monocytogenes*, a food-borne pathogen. *Microbiological Reviews*, *55*(3), 476–511.
52. Finn, S., Condell, O., McClure, P., Amézquita, A., and Fanning, S. (2013). Mechanisms of survival, responses, and sources of *Salmonella* in low-moisture environments. *Frontiers in Microbiology*, 1–15. http://doi.org/10.3389/fmicb.2013.00331/abstract
53. Fudge, J., Dunn, M., Pike, O., Robison, R., and Steele, F. (2016). The isolation and identification of *Pantoea dispersa* strain JFS as a non-pathogenic surrogate for *Salmonella* Typhimurium phage type 42 in flour. *International Journal of Food Microbiology, 219,* 1–6. http://doi.org/10.1016/j.ijfoodmicro.2015.11.012 (Date accessed: 28 November 2017).
54. García, A. H. (2011). Anhydrobiosis in bacteria: From physiology to applications. *Journal of Biosciences*, 36(5), 939–950.
55. Gill, A., McMahon, T., Dussault, F., and Petronella, N. (2020). Shiga toxin-producing *Escherichia coli* survives storage in wheat flour for two years. *Food Microbiology*, *87*, 103380. http://doi.org/10.1016/j.fm.2019.103380
56. Glass, K. A., Golden, M. C., Wanless, B. J., Bedale, W., and Czuprynski, C. (2015). Growth of *Listeria monocytogenes* within a caramel-coated apple microenvironment. *mBio*, *6*(5), e01232–15–5. http://doi.org/10.1128/mBio.01232-15
57. Gould, L. H., Walsh, K. A., Vieira, A. R., Herman, K., Williams, I. T., Hall, A. J., and Cole, D. (2013). Surveillance for foodborne disease outbreaks - United States, 1998-2008. *Morbidity and Mortality Weekly Report. Surveillance Summaries (Washington, D.C. : 2002)*, *62*(2), 1–34.
58. Gradl, D. R., Sun, L., Larkin, E. L., Chirtel, S. J. and Keller, S. E. (2015). Survival of *Salmonella* during drying of fresh ginger root (*Zingiber officinale*) and storage of ground ginger. *Journal of Food Protection*, 78(11), 1954–1959.
59. Grasso, E. M., Stam, C. N., Anderson, N. M., and Krishnamurthy, K. (2014). Heat and steam treatments. In J. B. Gurtler, M. P. Doyle, and J. L. Kornacki (Eds.), *The microbiological safety of low water activity foods and spices,* pp. 403—424. LLC: Springer science + business media.
60. Greenwood, M. H. and Hooper, W. L. (1983). Chocolate bars contaminated with *Salmonella napoli*: and infectivity study. *British Medical Journal*, *286*, 1394.
61. Gruzdev, N., Pinto, R., and Saldinger, S. S. (2012). Persistence of Salmonella enterica during dehydration and subsequent cold storage. *Food Microbiology*, *32*(2), 415–422. http://doi.org/10.1016/j.fm.2012.08.003
62. Gunasekera, T. S., Csonka, L. N., and Paliy, O. (2008). Genome-wide transcriptional responses of *Escherichia coli* K-12 to continuous osmotic and heat stresses. *Journal of Bacteriology*, *190*(10), 3712–3720. http://doi.org/10.1128/JB.01990-07
63. Hansen, L. T. and Vogel, B. F. (2011). Desiccation of adhering and biofilm Listeria monocytogenes on stainless steel: Survival and transfer to salmon products. *International Journal of Food Microbiology*, *146*(1), 88–93. http://doi.org/10.1016/j.ijfoodmicro.2011.01.032
64. Hengge-Aronis, R., Lange, R., Henneberg, N., and Fischer, D. (1992). Osmotic regulation of *rpoS*-dependent oenes in *Escherichia coli*. *Journal of Bacteriology*, *175*(1), 259–265.

65. Hildebrandt, I. M., Hu, C., Grasso-Kelley, E. M., Ye, P., Anderson, N. M., and Keller, S. E. (2017). Dry transfer inoculation of low-moisture spices containing antimicrobial compounds. *Journal of Food Protection*, *80,* 338–344. http://doi.org/10.4315/0362-028X.JFP-16-279.
66. Hildebrandt, I. M., Marks, B. P., Ryser, E. T., Villa-Rojas, R., Tang, J., Garces-Vega, F. J., and Buchholz, S. E. (2016). Effects of inoculation procedures on variability and repeatability of *Salmonella* thermal resistance in wheat flour. *Journal of Food Protection*, *79*(11), 1833–1839. http://doi.org/10.4315/0362-028X.JFP-16-057
67. Hingston, P., Chen, J., Dhillon, B. K., Laing, C., Bertelli, C., Gannon, V., Tasara, T., Allen, K., Brinkman, S. L., Hansen, L. T., and Wang, S. (2017). Genotypes associated with *Listeria monocytogenes* Isolates displaying impaired or enhanced tolerances to cold, salt, acid, or desiccation stress. *Frontiers in Microbiology*, *8*, 753–20. http://doi.org/10.3389/fmicb.2017.00369
68. Hiramatsu, R., Matsumoto, M., Sakae, K., and Miyazaki, Y. (2005). Ability of shiga toxin-producing *Escherichia coli* and *Salmonella* spp. to survive in a desiccation model system and in dry foods. *Applied and Environmental Microbiology*, *71*(11), 6657–6663. http://doi.org/10.1128/AEM.71.11.6657-6663.2005
69. Hitchins, A. D. and Whiting, R. C. (2001). Food-borne *Listeria monocytogenes* risk assessment. *Food Additives and Contaminants*, *18*(12), 1108–1117. http://doi.org/10.1080/02652030110050104
70. Horn, N. and Bhunia, A. K. (2018). Food-associated stress primes foodborne pathogens for the gastrointestinal phase of infection. *Frontiers in Microbiology*, *9*, 439–16. http://doi.org/10.3389/fmicb.2018.01962
71. Ibarra, J. A. and Steele-Mortimer, O. (2009). *Salmonella*- the ultimate insider. *Salmonella* virulence factors that modulate intracellular survival. *Cellular Microbiology*, *11*(11), 1579–1586. http://doi.org/10.1111/j.1462-5822.2009.01368.x
72. Interagency Food Safety Analytics Collaboration (IFSAC). (2018). Foodborne illness source attribution estimates for 2016 for *Salmonella*, *Escherichia coli* O157, *Listeria monocytogenes*, and *Campylobacter* using multi-year outbreak surveillance data.
73. Jackson, B. R., Salter, M., Tatt, C., Conrad, A., Harvey, E., Steinbock, L., Saupe, A., Sorenson, A., Katz, L., Stroika, S., Jackson, K. A., Carleton, H., Kucerova, Z., Melka, D., Strain, E., Parish, M., and Mody, R. K. (2015). Notes from the field: listeriosis associated with stone fruit -- United States, 2014. *Morbidity and Mortality Weekly Report*, *64*(10), 282–283.
74. Juneja, V. K. and Eblen, B. S. (2000). Heat inactivation of *Salmonella typhimurium* DT104 in beef as affected by fat content. *Letters in Applied Microbiology*, *30*, 461–467.
75. Kang, D. H. and Fung, D. (2000). Application of thin agar layer method for recovery of injured *Salmonella typhimurium*. *International Journal of Food Microbiology*, *54*(1-2), 127–132.
76. Kapperud, G., Gustavsen, S., Hellesnes, I., Hansen, A. H., Lassen, J., Hirn, J., Jahkola, M., Montenegro, M. A., and Helmuth, R. (1990). Outbreak of *Salmonella typhimurium* infection traced to contaminated chocolate and caused by a strain lacking the 60-megadalton virulence plasmid. *Journal of Clinical Microbiology*, *28*(12), 2597–2601.
77. Karagöz, I., Moreira, R. G., and Castell-Perez, M. E. (2014). Radiation D10 values for *Salmonella* Typhimurium LT2 and an *Escherichia coli* cocktail in pecan nuts (Kanza cultivar) exposed to different atmospheres. *Food Control, 39(C),* 146–153.

78. Keller, S. E., Stam, C. N., Gradl, D. R., Chen, Z., Larkin, E. L., Pickens, S. R. and Chirtel, S. J. (2015). Survival of *Salmonella* on chamomile, peppermint, and green tea during storage and subsequent survival or growth following tea brewing. *Journal of Food Protection*, *78*(4), 661–667.
79. Kirsch, K. R., Tolen, T. N., Hudson, J. C., Castillo, A., Griffin, D., and Taylor, T. M. (2017). Effectiveness of a commercial lactic acid bacteria intervention applied to inhibit Shiga toxin-producing *Escherichia coli* on refrigerated vacuum-aged beef. *International Journal of Food Science*, *2017*, 1–6. http://doi.org/10.1155/2017/8070515. (Date accessed: 14 June 2018).
80. Koster, K. L. (1991). Glass formation and desiccation tolerance in seeds. *Plant Physiology*, *96*, 302–304.
81. Kothary, M. H. and Babu, U. S. (2001). Infective dose of foodborne pathogens in volunteers: A Review. *Journal of Food Safety*, *21*, 49–73.
82. Lang, M. M., Harris, L. J., and Beuchat, L. R. (2003). Evaluation of Inoculation method and inoculum drying time for their effects on survival and efficiency of recovery of *Escherichia coli* O157:H7, *Salmonella*, and *Listeria monocytogenes* inoculated on the surface of tomatoes. *Journal of Food Protection*, *67*(4), 732–441.
83. Lang, E., Iaconelli, C., Zoz, F., Guyot, S., Alvarez-Martin, P., Beney, L., Perrier-Cornet, J. M. and Gervais, P. (2017). Drying parameters greatly affect the destruction of *Cronobacter sakazakii* and *Salmonella* Typhimurium in standard buffer and milk. *Food Microbiology*, *62*, 82–91.
84. Laroche, C. and Gervais, P. (2003). Unexpected thermal destruction of dried, glass bead-immobilized microorganisms as a function of water activity. *Applied and Environmental Microbiology*, *69*(5), 3015–3019. http://doi.org/10.1128/AEM.69.5.3015-3019.2003
85. Laroche, C., Fine, F., and Gervais, P. (2005). Water activity affects heat resistance of microorganisms in food powders. *International Journal of Food Microbiology*, *97*(3), 307–315. http://doi.org/10.1016/j.ijfoodmicro.2004.04.023
86. Lee, L. E., Metz, D., Giovanni, M., and Bruhn, C. M. (2011). Consumer knowledge and handling of tree nuts: food safety implications. *Food Protection Trends*, *31*(1), 18–27.
87. Li, H., Fu, X., Bima, Y., Koontz, J., Megalis, C., Yang, F., Fleischman, G. and Tortorello, M. L. (2014). Effect of the local microenvironment on survival and thermal inactivation of *Salmonella* in low- and intermediate-moisture multi-ingredient foods. *Journal of Food Protection*, *77*(1), 67–74.
88. Linton, R. H., Carter, W. H., Pierson, M. D., and Hackney, C. R. (1995). Use of a modified Gompertz equation to model nonlinear survival curves for *Listeria monocytogenes* Scott A. *Journal of Food Protection*, *58*(9), 946–954.
89. Liu, X., Miller, P., Basu, U. and McMullen, L. M. (2014). Sodium chloride-induced filamentation and alternative gene expression of *fts*, *murZ*, and *gnd* in *Listeria monocytogenes* 08-5923 on vacuum-packaged ham. *FEMS Microbiology Letters*, *360*(2), 152–156.
90. Ly, V., Parreira, V. R., and Farber, J. M. (2019). Current understanding and perspectives on *Listeria monocytogenes* in low-moisture foods. *Current Opinion in Food Science*, *26*, 18–24. http://doi.org/10.1016/j.cofs.2019.02.012
91. Marks, B. P. (2008). Status of Microbial Modeling in Food Process Models. *Comprehensive Reviews in Food Science and Food Safety*, *7*, 137–143.

92. Maserati, A., Fink, R. C., Lourenco, A., Julius, M. L. and Diez-Gonzalez, F. (2017). General response of *Salmonella enterica* serovar Typhimurium to desiccation: A new role for the virulence factors sopD and sseD in survival. *PLoS ONE*, 12(11), 1–23. Available from: http://doi.org/10.1371/journal.pone.0187692. (Accessed 8 Nov. 2018).
93. Maurer, J. J. (2017). Factors affecting variation in *Salmonella* virulence. In J.B. Gurtler, M.P. Doyle and J.L. Kornacki, eds., *Foodborne Pathogens: Virulence Factors and Host Susceptibility*. Cham, Switzerland: Springer International Publishing AG, pp. 151-167.
94. McDonough, F. E., and Hargrove, R. E. (1968). Heat Resistance of Salmonella in Dried Milk. *Journal of Dairy Science*, *51*(10), 1587–1591. http://doi.org/10.3168/jds.S0022-0302(68)87236-4
95. McLauchlin, J., Greenwood, M. H., and Pini, P. N. (1990). The occurrence of *Listeria monocytogenes* in cheese from a manufacturer associated with a case of listeriosis. *International Journal of Food Microbiology*, *10*, 255–262.
96. Mead, P. S., Slutsker, L., Dietz, V., McCaig, L. F., Bresee, J. S., Shapiro, C., Griffin, P. and Tauxe, R. (1999). Food-Related Illness and Death in the United States. *Emerging Infectious Diseases*, *5*(5), 607–625.
97. Moxley, R. A. and Acuff, G. R. (2014). Peri- and postharvest factors in the control of Shiga toxin-producing *Escherichia coli* in beef. *Microbiology Spectrum*, *2*(6). http://doi.org/10.1128/microbiolspec.EHEC-0017-2013
98. National Advisory Committee on Microbiological Criteria for Foods. (NACMCF). (2010). Parameters for determining inoculated pack/challenge study protocols. *Journal of Food Protection, 73*(1), 140–202.
99. Newkirk, J. J., Wu, J., Acuff, J. C., Caver, C. B., Mallikarjunan, K., Wiersema, B. D., Williams, R.C., and Ponder, M.A. (2018). Inactivation of *Salmonella* enterica and surrogate *Enterococcus faecium* on whole black peppercorns and cumin seeds using vacuum steam pasteurization. *Frontiers in Sustainable Food Systems*, *2*, 252–12. http://doi.org/10.3389/fsufs.2018.00048
100. Nolan, D. A., Chamblin, D. C., and Troller, J. A. (1992). Minimal water activity levels for growth and survival of *Listeria monocytogenes* and *Listeria innocua*. *International Journal of Food Microbiology*, *16*, 323–335.
101. Ough, C. S. and Were, L. (2005). Sulfur dioxide and sulfites. In Davidson, P. M., Sofos, J. N., and Branen, A. L. (Eds.), *Antimicrobials in Food,* 3rd ed. Boca Raton, FL: CRC Press, pp.143-167.
102. Painter, J. A., Hoekstra, R. M., Ayers, T., Tauxe, R. V., Braden, C. R., Angulo, F. J., and Griffin, P. M. (2013). Attribution of foodborne illnesses, hospitalizations, and deaths to food commodities by using outbreak data, United States, 1998–2008. *Emerging Infectious Diseases*, *19,* 407–415. http://doi.org/10.3201/eid1903.111866. (Date accessed: 24 May 2018).
103. Paton, J. C. and Paton, A. W. (1998). Pathogenesis and diagnosis of Shiga toxin-producing *Escherichia coli*. *Clinical Microbiology Reviews*, *11*(3), 450–479.
104. Peleg, M. 2003. Microbial survival curves: interpretation, mathematical modeling, and utilization. *Comments on Theoretical Biology*, *8*, 357–387.
105. Peleg, M., Normand, M.D., and Corradini, M.G. (2005). Generating microbial survival curves during thermal processing in real time. *Journal of Applied Microbiology, 98*(2), 406–417.

106. Podolak, R., Enache, E., Stone, W., Black, D. G., and Elliott, P. H. (2010). Sources and Risk Factors for Contamination, Survival, Persistence, and Heat Resistance of Salmonella in Low-Moisture Foods. *Journal of Food Protection*, *73*(10), 1919–1936.
107. Poimenidou, S. V., Chatzithoma, D.-N., Nychas, G.-J., and Skandamis, P. N. (2016). Adaptive Response of *Listeria monocytogenes* to heat, salinity and low pH, after habituation on cherry tomatoes and lettuce leaves. *PLoS ONE*, *11*(10), e0165746–16. http://doi.org/10.1371/journal.pone.0165746. (Date accessed: 18 June 2018).
108. "Processing Macadamia Nuts." (2020). *The Macadamia Castle and Animal Park*. https://www.macadamiacastle.com.au/processing-macadamia-nuts/. (Date accessed: 24 June 2018).
109. Rachon, G., Peñaloza, W., Gibbs, P. A. 2016. Inactivation of *Salmonella*, *Listeria monocytogenes* and *Enterococcus faecium* NRRL B-2354 in a selection of low moisture foods. *International Journal of Food Microbiology, 231*(C), 16–25.
110. Ragon, M., Wirth, T., Hollandt, F., Lavenir, R., Lecuit, M., Le Monnier, A., & Brisse, S. (2008). A New Perspective on *Listeria monocytogenes* Evolution. *PLoS Pathogens*, *4*(9), e1000146–14. http://doi.org/10.1371/journal.ppat.1000146
111. Raponi, F., Moscetti, R., Monarca, D., Colantoni, A. and Massantini, R. (2017). Monitoring and optimization of the process of drying fruits and vegetables using computer vision: A Review. *Sustainability*, 9(2009), 1–27.
112. Ribeiro, V. B., Mujahid, S., Orsi, R. H., Bergholz, T. M., Wiedmann, M., Boor, K. J. and Destro, M. T. (2014). Contributions of σB and PrfA to *Listeria monocytogenes* salt stress under food relevant conditions. *International Journal of Food Microbiology*, *177*, 98–108.
113. Riebel, W. J. and Washington, J. A. (1990). Clinical and Microbiologic Characteristics of Pediococci. *Journal of Clinical Microbiology*, *28*(6), 1348–1355.
114. Rychlik, I. and Barrow, P. A. (2005). *Salmonella* stress management and its relevance to behaviour during intestinal colonisation and infection. *FEMS Microbiology Reviews*, *29*(5), 1021–1040. http://doi.org/10.1016/j.femsre.2005.03.005
115. Saunders, T., Wu, J., Williams, R.C., Huang, H., and Ponder, M.A. (2018). Inactivation of *Salmonella* and surrogate bacteria on cashews and macadamia nuts exposed to commercial propylene oxide processing conditions. *Journal of Food Protection*, *81*(3), 417–423.
116. Scallan, E., Hoekstra, R. M., Angulo, F. J., Tauxe, R. V., Widdowson, M., Roy, S. L., Jones, J. L., and Griffin, P. M. (2011). Foodborne illness acquired in the United States—major pathogens. *Emerging Infectious Diseases*, *17,* 7-15. https://dx.doi.org/10.3201/eid1701.P11101.
117. Scott, V. N., Chen, Y., Freier, T. A., Kuehm, J., Moorman, M., Meyer, J., Morille-Hinds, T., Post, L., Smoot, L., Hood, S., Shebuski, J., and Banks, J. (2009). Control of *Salmonella* in low-moisture foods I: Minimizing entry of *Salmonella* into a processing facility. *Food Protection Trends*, 342–353.
118. Shachar, D. and Yaron, S. (2006). Heat tolerance of *Salmonella enterica* serovars Agona, Enteritidis, and Typhimurium in peanut butter. *Journal of Food Protection*, *69*(11), 2687–2691.
119. Shah, M. K., Asa, G., Sherwood, J., Graber, K., and Bergholz, T. M. (2017). Efficacy of vacuum steam pasteurization for inactivation of *Salmonella* PT 30, *Escherichia coli* O157:H7 and *Enterococcus faecium* on low moisture foods.

International Journal of Food Microbiology, *244*(C), 111–118. http://doi.org/10.1016/j.ijfoodmicro.2017.01.003
120. Silk, B. J., Date, K. A., Jackson, K. A., Pouillot, R., Holt, K. G., Graves, L. M., Ong, K.L., Hurd, S., Meyer, R., Marcus, R., Shiferaw, B., Norton, D. M., Medus, C., Zansky, S. M., Cronquist, A. B., Henao, O. L., Jones, T. F., Vugia, D. J., Farley, M. M., and Mahon, B.E. (2012). Invasive listeriosis in the foodborne diseases active surveillance network (FoodNet), 2004–2009: Further targeted prevention needed for higher-risk groups. *Clinical Infectious Diseases*, *54*, S396–S404. http://doi.org/10.1093/cid/cis268.
121. Skandamis, P. N., Yoon, Y., Stopforth, J. D., Kendall, P. A., and Sofos, J. N. (2008). Heat and acid tolerance of *Listeria monocytogenes* after exposure to single and multiple sublethal stresses. *Food Microbiology*, *25*(2), 294–303. http://doi.org/10.1016/j.fm.2007.10.008. (Date accessed: 18 June 2018).
122. Sperber, W. H. (1983). Influence of water activity. *Journal of Food Protection*, *46*(2), 142–150.
123. Stackhouse, R. R., Faith, N. G., Kaspar, C. W., Czuprynski, C. J. and Wong, A. C. L. (2012). Survival and virulence of *Salmonella enterica* serovar Enteritidis filaments induced by reduced water activity. *Applied and Environmental Microbiology*, *78*(7), 2213–2220.
124. Stasic, A. J., Wong, A. C. L., and Kaspar, C. W. (2012). Osmotic and desiccation tolerance in *Escherichia coli* o157:h7 requires rpoS (σ38). *Current Microbiology*, *65*(6), 660–665. http://doi.org/10.1007/s00284-012-0210-8
125. Van Stelten, A., Simpson, J. M., Ward, T. J., and Nightingale, K. K. (2010). Revelation by Single-nucleotide polymorphism genotyping that mutations leading to a premature stop codon in *inlA* are common among *Listeria monocytogenes* isolates from ready-to-eat foods but not human listeriosis cases. *Applied and Environmental Microbiology*, *76*(9), 2783–2790. http://doi.org/10.1128/AEM.02651-09
126. Thorpe, C. (2017). Alterations in Shiga toxin-producing *E. coli* colonization and virulence following dietary modulation and administration of antimicrobials. In Gurtler, J. B., Doyle, M. P., and Koracki, J. L. (Eds.), *Foodborne Pathogens,* Cham, Switzerland: Springer International Publishing AG, pp.209-228.
127. Topalcengiz, Z. and Danyluk, M. D. (2017). Thermal inactivation responses of acid adapted and non-adapted stationary phase Shiga toxin-producing *Escherichia coli* (STEC), *Salmonella* spp. and *Listeria monocytogenes* in orange juice. *Food Control*, *72*(Part A), 73–82. http://doi.org/10.1016/j.foodcont.2016.07.014
128. Tsai, H.-C., Ballom, K.F., Xia, S., Tang, J., Marks, B.P., Zhu, M.-J. 2019. Evaluation of *Enterococcus faecium* NRRL B-2354 as a surrogate for *Salmonella* during cocoa powder thermal processing. Food Microbiol. 82, 135–141.
129. U.S. Department of Agriculture Economic Research Service (USDA ERS). (2015). Economic burden of major foodborne illnesses acquired in the United States. Available from: https://www.ers.usda.gov/webdocs/publications/43984/52807_eib140.pdf. (Date accessed: 20 February 2020).
130. U.S. Food and Drug Administration (FDA). (2017). FDA investigated multistate outbreak of *E. coli* O157:H7 infections linked to soynut butter. https://www.fda.gov/Food/RecallsOutbreaksEmergencies/Outbreaks/ucm544964.htm. (Date accessed: 24 May 2018).

131. U.S. Food and Drug Administration (FDA). (2018). Mauna Loa Macadamia Nut Corporation voluntarily recalls. Available from: https://www.fda.gov/safety/recalls-market-withdrawals-safety-alerts/mauna-loa-macadamia-nut-corporation-voluntarily-recalls.
132. U.S. Food and Drug Administration (FDA). (2019a). Jac. Vandenberg, Inc. recalls fresh peaches, fresh nectarines and fresh plums because they may be contaminated with *Listeria monocytogenes*. Available from: https://www.fda.gov/safety/recalls-market-withdrawals-safety-alerts/jac-vandenberg-inc-recalls-fresh-peaches-fresh-nectarines-and-fresh-plums-because-they-may-be.
133. U.S. Food and Drug Administration (FDA) (2019b). Archive for recalls, market withdrawals and safety alerts. http://www.fda.gov/Safety/Recalls/ArchiveRecalls/default.htm. (Date accessed: 21 February 2019).
134. Vázquez-Boland, J. A., Domínguez-Bernal, G., González-Zorn, B., Kreft, J., and Goebel, W. (2001). Pathogenicity islands and virulence evolution in *Listeria*. *Microbes and Infection*, *3*, 571–584.
135. Venkitanarayanan, K., Upadhyay, A., Nair, M. S., and Upadhyay, K. (2017). The Effects of Environmental Conditions and External Treatments on Virulence of Foodborne Pathogens. In J.B. Gurtler, M.P. Doyle, and J.L. Kornacki, eds., *Foodborne Pathogens: Virulence Factors and Host Susceptibility*. Cham, Switzerland: Springer International Publishing AG, pp. 305-332.
136. Ward, T. J., Gorski, L., Borucki, M. K., Mandrell, R. E., Hutchins, J., and Pupedis, K. (2004). Intraspecific Phylogeny and Lineage Group Identification Based on the prfA Virulence Gene Cluster of Listeria monocytogenes. *Journal of Bacteriology*, *186*(15), 4994–5002. http://doi.org/10.1128/JB.186.15.4994-5002.2004
137. Wemmenhove, E., Wells-Bennik, M. H. J., Stara, A., van Hooijdonk, A. C. M. and Zwietering, M. H. (2016). How NaCl and water content determine water activity during ripening of Gouda cheese, and the predicted effect on inhibition of *Listeria monocytogenes. Journal of Dairy Science*, *99*(7), 5192–5201.
138. Welsh, D. T. and Herbert, R. A. (1999). Osmotically induced intracellular trehalose, but not glycine betaine accumulation promotes desiccation tolerance in *Escherichia coli*. *FEMS Microbiology Letters*, *174*, 57–63.
139. Werber, D., Dreesman, J., Feil, F., van Treeck, U., Fell, G., Ethelberg, S., Hauri, A. M., Roggentin, P., Prager, R., Fisher, I. S. T., Behnke, S. C., Bartelt, E., Weise, E., Ellis, A., Siitonen, A., Andersson, Y., Tschäpe, H., Kramer, M. H., and Ammon, A. (2005). International outbreak of *Salmonella* Oranienburg due to German chocolate. *BMC Infectious Diseases*, *5*(1), 1–10. http://doi.org/10.1186/1471-2334-5-7
140. Wesche, A. M., Gurtler, J. B., Marks, B. P., and Ryser, E. T. (2009). Stress, sublethal injury, resuscitation, and virulence of bacterial foodborne Pathogens. *Journal of Food Protection*, *72*(5), 1121–1138.
141. Yan, Z., Gurtler, J. B., and Kornacki, J. L. (2006). A solid agar overlay method for recovery of heat-injured *Listeria monocytogenes*. *Journal of Food Protection*, *69*(2), 428–431.
142. Zhang, G., Hu, L., Melka, D., Wang, H., Laasri, A., Brown, E. W., Strain, E., Allard, M., Bunning, V. K., Musser, S. M., Johnson, R., Santillana Farakos, S. M., Scott, V. N., Pouillot, R., van Doren, J. M., and Hammack, T. S. (2017). Prevalence of

Salmonella in cashews, hazelnuts, macadamia nuts, pecans, pine nuts, and walnuts in the United States. *Journal of Food Protection*, *80*(3), 459–466. http://doi.org/10.4315/0362-028X.JFP-16-396

143. Zhang, Q. and Yan, T. (2012). Correlation of intracellular trehalose concentration with desiccation resistance of soil *Escherichia coli* populations. *Applied and Environmental Microbiology*, *78*(20), 7407–7413. http://doi.org/10.1128/AEM.01904-12
144. Zhang, Y., Pearson, J. S., and Hartland, E. L. (2017). Host innate immune factors influencing enterohemorrhagic *Escherichia coli* pathogenicity. In J.B. Gurtler, M.P. Doyle and J.L. Kornacki, eds., *Foodborne Pathogens: Food Microbiology and Food Safety*. Cham, Switzerland: Springer International Publishing AG, pp. 355-373.
145. Zhou, Z., Zuber, S., Campagnoli, M., Putallaz, T., Devlieghere, F., and Uyttendaele, M. (2019). Effect of mild steaming treatment on the inactivation of *Salmonella*, *Listeria monocytogenes, Escherichia coli* O157:H7 and their surrogates on black peppercorns. *Food Control*, *106*, 106726–8. http://doi.org/10.1016/j.foodcont.2019.106726
146. Zoz, F., Grandvalet, C., Lang, E., Iaconelli, C., Gervais, P., Firmesse, O., Guyot, S., and Beney, L. (2017). *Listeria monocytogenes* ability to survive desiccation: Influence of serotype, origin, virulence, and genotype. *International Journal of Food Microbiology*, *248*(C), 82–89. http://doi.org/10.1016/j.ijfoodmicro.2017.02.010
147. Zwietering, M. H., Jongenburger, I., Rombouts, F. M., and Riet, K. V. (1990). Modeling of the bacterial growth curve. *Applied and Environmental Microbiology*, *56,* 1875–1881. http://doi.org/0099-2240/90/061875-07$02.00/0. (Date accessed: 8 February 2018).

Chapter 3 Thermal Inactivation of *Salmonella*, Shiga Toxin-Producing *Escherichia coli*, *Listeria monocytogenes*, and a Surrogate (*Pediococcus acidilactici*) on Raisins, Apricot Halves, and Macadamia Nuts Using Vacuum-Steam Pasteurization

Jennifer C. Acuff[a], Jian Wu[a], Claire Marik[a], Kim Waterman[a], Daniel Gallagher[b], Haibo Huang[a], Robert C. Williams[a], Monica A. Ponder[a,*]

[a]Virginia Tech, Food Science and Technology,
1230 Washington St., Blacksburg, VA 24061, USA;

[b]Virginia Tech, Civil and Environmental Engineering Department,
409 Durham Hall, Blacksburg, VA 24061, USA.

Abstract:

Salmonella, Shiga toxin-producing *Escherichia coli* (STEC), and *Listeria monocytogenes* have been isolated from low water activity foods (LWAF), where they may survive for extended periods. The ready-to-eat nature of many LWAF, such as dried fruits and nuts, warrants effective post-harvest thermal treatment for the reduction of pathogens such as low-temperature, saturated steam, also known as vacuum-assisted steam pasteurization. The objective of this study was to determine reductions of *Salmonella*, STEC, *L. monocytogenes*, and a possible surrogate (*Pediococcus acidilactici*) on dried apricot halves, whole macadamia nuts, and raisins after treatment with vacuum-assisted steam at three temperatures (62°C, 72°C, or 82°C) and multiple timed intervals. Bacterial inactivation was variable between commodities, with higher temperatures and longer times necessary to achieve comparable reductions of pathogens on apricot halves and macadamia nuts compared to raisins. Reductions of the tested pathogens were comparable; therefore, one species was not more resistant than the others. Pathogens were reduced by 5-log CFU/g on apricot halves after 20 min at 72°C and after 5 min at 82°C. Longer treatment times were necessary to achieve reductions of each pathogen on macadamia nuts. Pathogens were reduced by nearly 5 log CFU/g on macadamia nuts after 38 min at 72°C (4.6-6.5 log CFU/g) and after 12 min at 82°C (4.9-5.7 log CFU/g). Reductions of pathogens on raisins were achieved at lower temperatures than necessary for the other foods. A 5-log reduction for each of the pathogens (CFU/g) on raisins occurred after 20 min at 62°C and after 5 min at 72°C. Overall, the reductions of the pathogens exceeded those of *P. acidilactici* on both the dried fruits and macadamia nuts. Statistically significant differences, indicating greater confidence as a conservative surrogate, were observed at lower treatment temperatures. Inactivation kinetics were modeled for each pathogen on each food type and temperature. Bacterial survival was best described by the Weibull model for raisins and macadamia nuts, while the Gompertz model best

described reductions on apricot halves according to Akaike information criterion (AIC) and root-mean-square error (RMSE) evaluations. Thermal inactivation kinetic models and 5-log reduction parameters can help food processors design and evaluate similar vacuum-assisted steam interventions to comply with FSMA regulations and preventive control plans. However, results or model predictions should not be extrapolated to assume the safety of other types of foods.

Key words: Vacuum-steam pasteurization, low-water activity foods, *Salmonella*, *Escherichia coli*, *Listeria monocytogenes*, inactivation kinetic model

1. Introduction

The amount of water available for biological use is a limiting factor for growth of microorganisms in foods. Low- (LWAF, a_w <0.60) and intermediate- (IWAF, a_w 0.60-0.85) water activity foods do not promote growth of human pathogens, though some organisms like *Salmonella* and Shiga toxin-producing *E. coli* (STEC) can survive for extended periods (Forghani et al., 2018; Keller et al., 2015; Suehr et al., 2019). From 2007-2012, there were 7,315 illnesses (within 41 outbreaks) reported worldwide that were associated with LWAF consumption including, but not limited to, peanut butter, spices, confectionary products, and tree nuts (CDC, 2012; CDC, 2018a; CDC, 2018b; CDC, 2019a; CDC, 2019b; CFIA, 2011; Farakos and Frank, 2014; FDA, 2016b; FDA, 2017a; Finn et al., 2013; Painter et al., 2013). In addition to the LWAF implicated in outbreaks, the detection of human pathogens including *Salmonella*, STEC, and *L. monocytogenes* have led to the recall of over 1,000 LWAF across the European Union from 2012-2019, and nearly 100 different recall events in the United States from 2017-2019 (FDA, 2019b; RASFF, 2019). Tree nuts, chiefly almonds, walnuts and hazelnuts, have been implicated numerous times in outbreaks and recalls due to contamination by *Salmonella,* STEC, and *L. monocytogenes* and many studies have sought to understand survival and identify effective treatments for pathogen reduction (FDA, 2015a; FDA, 2018a). Macadamia nuts are emerging as an under-recognized vehicle, with several recalls in 2015-2018 due to detection of *L. monocytogenes*, *Salmonella*, and STEC contamination (FDA, 2015a; FDA, 2015b; FDA, 2015c; FDA, 2016b; FDA, 2017b; FDA, 2018b). Dried fruits are also emerging as an under-recognized source of foodborne illness. A number of dried fruits including dates, dried coconut, and dried fruit mixes have been associated with outbreaks (Callejón et al., 2015; FDA, 2018a; FDA, 2019b; FSN, 2018; FSN, 2019). However they are largely unexplored in terms of pathogen prevalence, survival, or effective treatments for pathogen reduction.

Many LWAF, such as nuts and dried fruits, are considered ready-to-eat, but are also still raw or minimally processed, agricultural products. Production methods of these foods vary greatly, but contamination with human pathogens may be introduced through water, animal contact, and workers. While implementation of Good Agricultural Practices (GAPs) may reduce these events, enforcement may pose a challenge (Bourdoux et al., 2016; FAO, 2011; Munck et al., 2019). In addition, post-harvest contamination may also occur prompting researchers and the industry to identify methodologies that may reduce human pathogens on LWAF (Ban and Kang, 2016; Brar et al., 2015; Farakos et al., 2017; Jeong et al., 2011; Limcharonenchat et al., 2018). Drying methods for fruits (sun drying, microwave and infrared drying, freeze-drying, convective and heated-air drying) vary depending on the product, region, and style (FAO, 2011; Omolola et al., 2017). Effectiveness of the initial drying method for pathogen reduction has been demonstrated for only a limited number of products (Bourdoux et al., 2016). While convective, heated-air drying is effective for pathogen reductions on dried fruit, it is costly compared to sun drying and as a result, is less commonly used in some parts of the world (Nijhuis et al., 1998). Sun drying, a popular method, is not an effective technique for pathogen reductions, and exposure to the additional vehicles and permissive growth temperatures may result in additional contamination or potentially increase the microbial load (Bourdoux et al., 2016; Eze and Agbo, 2011; Karabulut et al., 2007; Wachukwu et al., 2003). *Salmonella* has been isolated from various dried fruits, but other than dates, the prevalence of pathogen contamination on other stone fruits, fresh or dried, is largely underexplored (Beuchat and Mann, 2014; Limcharoenchat et al., 2018; Whitthuhn et al., 2005). Recently, stone fruits (peaches, plums, pluots, and nectarines) have been recalled due to possible *L. monocytogenes*-contamination (FDA, 2019a; Jackson et al., 2014). If

contamination occurs pre-harvest, and the organisms survives drying, then stone fruits such as apricots, may increase consumer risk for foodborne illness.

Safety evaluations of imported RTE LWAF (dried fruits and nuts) can be challenging because they may have unknown and variable processing histories based on region and financial capabilities. Due to several large-scale outbreaks of salmonellosis on almonds, mandatory pasteurization rules were enacted in 2007 that required 4-log reductions of *Salmonella* from almond surfaces. The Preventive Control Rule of the Food Safety Modernization Act (FSMA) now requires all processors to identify possible risks and to outline and implement strategies that control or eliminate consequences of contamination events (FDA, 2016a). Pre- and post-harvest contamination risks have led researchers to investigate post-harvest pasteurization strategies such as propylene oxide, radiation, and thermal treatments to reduce presence of *Salmonella*, as well as to identify surrogate organisms for process validations (Ban and Kang, 2016; Brar et al., 2015; Danyluk et al., 2005; Farakos et al., 2017; Karagöz et al., 2014; Saunders et al., 2018). High-heat, thermal interventions such as roasting are objectional for dried fruits or some types of nuts as they may alter the color, smell, and textures in ways that consumers may find undesirable. Low temperature saturated steam pasteurization, also known vacuum-assisted steam pasteurization, is desirable because it delivers larger amounts of heat in a shorter period of time compared to roasting, dry air convection, or other thermal processes. Low-temperature, vacuum-assisted steam treatments have been shown to be effective for reduction of human pathogens on LWAF with nonuniform surfaces, such as nuts, grains, and spices due to the process's ability to transfer heat through small crevices and wrinkles while preserving qualities of the products acceptable to consumers (Chang et al., 2010; Duncan et al., 2017; Newkirk et al., 2018; Shah et al., 2017).

While low temperature saturated steam pasteurization has been verified to inactivate *Salmonella* on various commodities, it is still necessary for companies to perform process validations because temperatures, time and packaging configurations may vary. It is therefore important to identify a nonpathogenic surrogate microorganism, whose inactivation parameters are comparable to the target pathogens so that commercial facilities can validate their individual low temperature steam pasteurization processes using the surrogate microorganisms instead of pathogenic strains, reducing likelihood of facility contaminations. The almond industry relies on the use of *Enterococcus faecium* NRRL B-2354, whose heat resistance is slightly greater than *Salmonella,* to validate steam processing in a variety of packages (Stevenson et al*.,* 2014). Another lactic acid bacterium, *Pediococcus acidilactici* ATCC 8042, was demonstrated to possess greater heat resistance compared to various strains of *S. enterica* in thermally processed dry pet foods, formed jerky and nuts at a variety of temperatures, suggesting it may also serve as a conservative surrogate for processing of other low water activity food (Borowski et al*.,* 2009; Ceylan and Bautista, 2015; Saunders, 2017).

The objectives of the presented research were to 1) evaluate the efficacy of lab-scale, low-temperature, vacuum-assisted steam to reduce pathogen loads (multiple strains of *Salmonella*, STEC and *L. monocytogenes*) from the surface of LWAF and IWAF (raisins, apricot halves, and whole macadamia nuts), 2) determine if *Pediococcus acidilactici* could serve as a surrogate organism for validations of similar processes, and 3) fit models to data to provide guidance to those developing similar vacuum-assisted steam intervention strategies. For consideration as a surrogate on these dried fruits and nuts processed with low-temperature steam, *Pediococcus* needed comparable or greater survival compared to the target pathogens. Only

bacteria with consistently greater survival compared to the target pathogens should be considered conservative surrogates to use in place of pathogens within a processing facility.

2. Materials and Methods

2.1. Inoculum preparation

Bacterial strains were chosen to represent isolates from LWAF when available or various clinical isolates of relevant concern. The following strains were used: *E. coli* O121:H19 (FNW19M81, 2016 wheat flour outbreak isolate [FDA]) and O157:H7 F4546 (alfalfa sprout outbreak isolate); *L. monocytogenes* 1/2a FSL R2-499 (sliced turkey isolate), 1/2b FSL R2-502 (chocolate milk isolate), and 4b (ScottA, 1985 milk outbreak isolate); *Salmonella enterica* serotype Montevideo (1449, 2010 black pepper outbreak isolate), Newport (2010 allspice isolate), and Tennessee (K4643, 2007 peanut butter outbreak clinical isolate); and the nonpathogenic bacterium, *Pediococcus acidilactici* (ATCC 8042). Strains were stored in Tryptic Soy Broth (TSB; BD, Sparks, MD) with glycerol at -80°C prior to experiments. Each strain was streaked individually onto respective selective agars. STEC strains were streaked onto MacConkey with Sorbitol Agar (SMAC, BD, Sparks, MD), *Salmonella* onto Xylose Lysine Tergitol Agar (XLT4, Criterion, Hardy Diagnostics, Santa Maria, CA) with supplement (BD, Sparks, MD), *L. monocytogenes* onto Modified Oxford Agar (MOX, Acumedia, Neogen, Lansing, MI) with *L. monocytogenes* supplement (Dalynn Biologicals, Calgary, AB), and *Pediococcus acidilactici* onto Bile Esculine Azide Agar (BEA, Criterion, Hardy Diagnostics, Santa Maria, CA). Isolated colonies of pathogens were transferred to TSB, while *Pediococcus* was transferred to Lactobacillus MRS Broth (BD, Sparks, MD) for overnight cultures.

The bacteria were cultured on individual 100-mm petri dishes of Tryptic Soy Agar (TSA, BD, Sparks, MD) using cotton swabs (Fisherbrand, Fisher Scientific, Pittsburg, PA) saturated

with the broth overnight cultures to create lawns. Following 24-h incubation at 37°C, lawns were harvested with L-spreaders (Fisherbrand) using 1 mL of 0.1% peptone per plate. Scraped cells were collected and combined to form a cocktail containing ca. 10 log CFU/mL of each strain, which were enumerated to confirm equal amounts.

2.2. Food inoculations

Seedless Thompson Organic Sundried Raisins were purchased from a U.S. grocery store with national distribution. Turkish Organic Apricot Halves and Organic Macadamia Nuts (raw) were purchased online (Nuts.com, Cranford, NJ). Organic foods were chosen to ensure no added sulfites or salts would confound effects of the treatments. Foods were steam-sterilized at 85-88°C to eliminate the high background numbers of native microorganisms and then dried to the original water activity prior to inoculation (a_w 0.684, 0.581, and 0.563 for, apricots, macadamia nuts, and raisins, respectively). Foods were spread into a single layer in plastic tubs (1.5 gal, Rubbermaid, Atlanta, GA), placed inside a biohazard bag within a biosafety cabinet, and inoculum (1 mL/40 g) was misted using a plastic spray bottle (Up & Up™, 12 oz., 063-03-0244) so that final concentrations on foods were ~7-8 log CFU/g. Tubs were left in the biosafety cabinet with lids askew until the foods were dried to original water activity levels (measured with AQUALAB 4TE, Meter Group, Pullman, WA) and used in experiments within 24 hours.

2.3. Low-temperature, vacuum-assisted steam treatments

The general design of the vessel and its prior use was reported by Newkirk et al., 2018. Some adjustments to the system were made to connect to a medium-pressure steam line, which provided continuous steam (Figure 3.6). A steam reducing station with a regulator (Watson McDaniel, 1-2 in O-Series, Pottstown, PA) was installed to lower the gauge pressure of the

steam to <69 kPa. The steam chamber, a 25-QT canner cooker (All American®, 25 QT. #925, Manitowoc, WI) was altered to allow steam to be added through the top at a controlled rate with a valve.

Samples of inoculated foods (30 g) were placed in silicone baking cups (Wilton®, Stock 415-9424, Naperville, IL) customized with 6.35-mm holes (0.25 in), spaced approximately 20 mm apart to improve steam flow (30 holes in total). Steam could reach the product from above and through the sides. Surface temperature was monitored through the process for each of the products. Thermocouples (Type K, Omega, Norwalk, CT) were placed to reflect the surface temperature of the particles in the relative "cold spot" of the sample within three locations of the chamber. The scaffold holding the samples was lowered into the steam chamber, sealed, and placed under negative pressure with a vacuum pump (Rotary Vane Pump, Edwards Limited, A652-01-880, Burgess Hill, UK).

Once the pressure was lowered to -88 kPa (-26 in Hg), steam was injected into the chamber until the surface temperature of the product, read by the inserted thermocouples, reached the desired treatment temperature (62, 72, or 82°C; ±1.5°C), at which point the timed treatment began. Apricot halves were treated at 72°C for 0, 0.5, 1, 2, 5, 8, 14, and 20 min and 82°C for 0, 0.5, 1, 1.5, 2, 2.5, 3.5, and 5 min. Macadamia nuts were also treated at 72°C and 82°C but for longer treatments than apricot halves at 0, 1, 8, 14, 20, 28, and 38 min and 0, 1, 2, 3.5, 5, 8, and 12 min, respectively. Raisins required lower treatment temperatures for 5-log reductions. At 62°C, the treatment cycles were 0, 0.5, 1, 2, 5, 8, 14, and 20 min. For 72°C, the treatment cycles were 0, 0.5, 1, 1.5, 2, 2.5, 3.5, and 5 min. Temperature profiles representative of typical vacuum-assisted steam treatments for 62, 72, and 82°C are in Supplemental Materials (Figures 3.7-3.9). Come-up times (amount of time required to inject steam to raise products to

the target treatment temperature), treatment time, residual heating times (time spent restoring the pressure to atmosphere levels and removing the sample), and maximum and minimum temperatures can be found in Supplemental Materials (Table 3.3).

Order of the timed treatment cycles for each temperature was randomly assigned, and three or more replications were performed for each treatment. Two samples were processed in each treatment: one for bacterial enumeration and the other for water activity measurement. Apricot halves, macadamia nuts, and raisins were processed separately. Non-treated control samples that were inoculated but not vacuum-steam-treated were also enumerated to ensure consistent inoculation levels (~7-8 log CFU/g) and to show that inactivation is due to steam processing.

2.4. Enumeration

When each cycle concluded, the sample was removed from the chamber and transferred into sample bags (Whirl-Pak®, Nasco, Fort Atkinson, WI) with 90 mL of chilled, sterile 0.1% peptone that were kept refrigerated no longer than 2 hours before enumeration. Samples were hand-massaged with medium force for at least 60 sec until the foods were fully washed (nuts) or hydrated and deteriorating (dried fruits), serially diluted, and spiral plated (Dilucup® Whitley WASP Touch, Microbiology International, Don Whitley Scientific Limited, West Yorkshire, UK) on BEA agar and two plates of TSA. The two plates of TSA were overlaid after four hours of incubation with selective agars to allow for injury-recovery. Plates overlaid with MOX and XLT4 were incubated at 37°C for 24 hours. BEA plates were incubated for 48 hours at 37°C. STEC colonies on XLT4 agar appeared whitish-yellow, while *Salmonella* colonies appeared black or yellow with black centers. *L. monocytogenes* colonies on MOX agar were small and

gray with a blackened halo. *Pediococcus* colonies appeared white on BEA agar. Results were recorded with an automatic reader (ProtoCOL SR/HR, Version 1.47.0, Cambridge, UK).

Samples with no growth observed on plates were enriched with TSB, incubated overnight, and then streaked onto selective agars incubated at 37°C for 24 h to attain a positive or negative result, providing a 0.6-log CFU/g limit of detection (LOD). Samples with no initial growth, but positive enrichment results, were assigned a conservative estimate of 0.5 log CFU/g. Only samples with no growth on plates and negative enrichment results were considered to be 0.0 log CFU/g. Bacterial log reductions were calculated by subtracting the survivors of each treatment (log CFU/g) from organisms present on the 0-min treatment (log CFU/g).

2.5. Water activity and proximate analyses

Trials were conducted for proximate analyses of dried fruits and nuts of before treatment and after the longest treatments for each temperature to analyze impacts on water activity, moisture (AOAC 934.01), crude protein (AOAC 2001.11), and crude fat composition (AOAC 2003.06).

2.6. Experimental design and statistical analyses

Raisins (n=17), apricot halves (n=17), and macadamia nuts (n=15) were treated with timed cycles at low and high temperatures, and each time/temperature combination was replicated 3-5 times. ANOVA, followed by pairwise comparisons (Tukey's Honestly Significant Difference (HSD)) was used to determine significant differences between the log reductions calculated for each treatment time compared to the 0-min treatment, as well as significant differences in log reductions between the organisms within each treatment. R (Version 3.6.1) and RStudio, Inc. (Version 1.1.456, Boston, MA) with the agricolae package were used for

conducting statistical analyses. The significance level of $p < 0.05$ was chosen. Water activity and proximate measurements were analyzed using Wilcoxon ranked sum and exact tests due to smaller sample sizes, with a significance level set to $p < 0.05$ (JMP® Pro 14.0, SAS Institute Inc., v. 9.4, Cary, NC).

2.7. Model development and validation

Survivability models were fit to each dataset of food, temperature, and organism using log reductions calculated from final and initial bacterial concentrations and converted to fractional survival. Replicates of each treatment were pooled and the nlstools package in R were used for model fitting. First-order models were fit and D-values were calculated based on the inactivation rate. Weibull and Gompertz models were also fit to the data to provide more robust fits than the first-order models and D-values (Bermúdez-Aguirre and Corradini, 2012).

The log-linear or first-order model parameters were estimated from the compiled data using the equation provided by Peleg et al. (2005):

$$(1) \quad logN_t - \ logN_0 = -kt$$

where N_t is the surviving bacterial population at time, t; N_0 is the initial bacterial population; and k is the inactivation rate (log CFU/g min^{-1}).

D-values were calculated from the inverse of the first-order k, and R^2 values were used to measure the fit of the first-order linear regression models. Akaike's Information Criteria (AIC) and root mean squared error (RMSE) were calculated and used to evaluate all three models.

The Weibull model was used as an alternative to identify inactivation trends that were not first-order or linear (Peleg, 2003):

$$(2) \quad logN_t - logN_0 = -k \cdot t^{\beta}$$

where k is a rate parameter for the reduction and β indicates whether the shape is a concave upward (<1), downward (>1), or linear (=1) inactivation curve.

The Gompertz model describes inactivation trends that present as a sigmoidal curve with the following equation (Zwietering et al., 1990):

$$(3) \quad logN_t - logN_0 = a \cdot exp[-exp(b - ct)]$$

where a, b, and c are parameters derived to fit the shape of the sigmoidal curve with no biological meaning.

A modified Gompertz equation was used to model data in the presented research and was derived by Zwietering et al. (1990) with the aim of providing biological meaning to the sigmoidal curve:

$$(4) \quad logN_t - logN_0 = A \cdot exp\left\{-exp\left[\frac{\mu_M \cdot e}{A}(\lambda - t) + 1\right]\right\}$$

where μ_M (log CFU/g min^{-1}) is the maximal value of inactivation, λ is the lag time (min), and A is the asymptote.

3. Results

3.1. Bacterial reductions post vacuum steam pasteurization

Average bacterial log reductions for each treatment time and temperature combination were calculated separately for each food and microorganism. The log CFU/g of the 0-min treatment was considered the starting concentration and can be viewed in Supplemental Materials (Table 3.3).

For apricot halves processed at 72°C, it required ≥8 min of treatment to result in statistically significant bacterial reductions compared to the 0-min treatment. 5-log reductions for each pathogen on dried apricot halves occurred during 20-min treatments of 72°C (Figure 3.1a). Similarly, 82°C treatments resulted in 5-log CFU/g pathogen reductions for 5-min treatments, which were statistically different from 0-min treatments (Figure 3.1b).

Low-temperature steam processing of macadamia nuts at 72°C resulted in significant bacterial reductions for >14-min treatments. The longest 72°C treatment performed, 38 min, resulted in a 5-log reduction of *E. coli* and nearly 5-log reductions of *L. monocytogenes* and *Salmonella* (Figure 3.2a). Bacteria on products treated at 82°C experienced significant reductions compared to a 0-min treatment after 3.5 minutes of processing. After the 12-min treatment, a 5-log reduction of *E. coli* and *L. monocytogenes* occurred, while *Salmonella* was reduced just under 5 log CFU/g (Figure 3.2b).

Treatment of 82°C were excessive to achieve 5-log reductions of the pathogens on raisins, so 62°C treatments were used. Vacuum-steam treatments of raisins at 62°C resulted in significant pathogen reductions for treatments >5 min compared to the 0-min treatment, with 5-log reductions of pathogens occurring at 20 min (Figure 3.3a). Significant pathogen reductions occurred after 3.5-min treatments of 72°C compared to the 0-min treatment, with 5-log reductions of all pathogens at 5-min treatments (Figure 3.3b).

3.2. Surrogate comparison

Pathogens and *Pediococcus* were coinoculated on the foods, which allowed direct comparisons between bacterial reductions. Bacterial reductions were compared against one another using pairwise comparisons within individual treatments (Tukey's HSD). At shorter time intervals, the inactivation of the pathogens and surrogate were sometimes comparable. As the time interval increased, the reductions of the bacterial pathogens were significantly larger than those of *Pediococcus* on the dried fruits at the lower tested temperatures. For example, after the 72°C 14- and 20-min treatments of apricot halves, the pathogens' reductions were significantly larger than those of *Pediococcus* (Figure 3.1a; $p < 0.05$). The same was true for raisins treated at 62°C for 8-20 min (Figure 3.3a). However, for macadamia nuts treated at the lower temperature of 72°C, only *E. coli* reductions were significantly greater than *Pediococcus* reductions (Figure 3.2a). When higher temperatures were used the bacterial reductions of the pathogen and *Pediococcus* increased. As a result, while the average reductions of the pathogens on raisins and macadamia nuts treated with higher temperatures (72°C and 82°C, respectively) were larger, but not significantly different from those of *Pediococcus* (Figure 3.2b, 3.3b). Only apricots treated at 82°C showed reductions of pathogens that were significantly greater than those of *Pediococcus* (Figure 3.1b).

One-to-one (1:1) ratio plots were also constructed of the log reductions for each pathogen vs. the log reductions of *Pediococcus* for the different food types and treatment temperatures to provide direct comparisons (Figure 3.4a-f). There were few instances when the reduction of *Pediococcus* was greater than those of the pathogens, and these generally occurred more frequently at the higher temperatures (Figure 3.4b, d, f), although Figure 3.4a also shows a few points where the pathogen reductions were slightly less than *Pediococcus* reductions. These three points were due to an abnormally large reduction of *Pediococcus* during a particular replicate,

which is reflected in the large standard deviation reported in Table 3.4. At no point, however, was the reduction of *Pediococcus* statistically significantly greater than those of the pathogens (Figures 3.1-3.3). Lastly, as seen throughout the plots, no single pathogen stood out as consistently more heat resistant than the others.

The shaded 95% confidence and prediction intervals in each panel reflect the range of the expected mean log reductions which was impacted by the overall variation of the vacuum-assisted steam process. In general, trends showed that steam treatments of higher temperatures with short times (Figure 3.4b, d, f) yielded wider confidence intervals than lower-temperature steam treatments with a number of points lying outside the prediction intervals. Furthermore, the confidence intervals of the higher temperatures commonly showed greater and more frequent overlap with the 1:1 ratio line.

3.3. Model evaluation

Coefficients for each model parameter for each food type, organism, and temperature treatment are reported in Tables 3.1-3.2 alongside AIC, RMSE, and R_2 values of the first-order models. Table 3.1 includes D-values calculated from the inactivation rates determined from the first-order models. For pathogens on raisins, $D_{62°C}$ = 3.00-3.12 min and $D_{72°C}$ = 0.72-0.77 min. For pathogens on apricots, $D_{72°C}$ = 2.29-2.53 min and $D_{82°C}$ = 0.64-0.66 min. For pathogens on macadamia nuts, $D_{72°C}$ = 5.44-7.54 min and $D_{82°C}$ = 1.85-2.27 min. Although the R_2 shown in Table 3.1 values were generally acceptable for each first-order model (0.83-0.95), this model was not best suited for any dataset compared to the nonlinear models based on RMSE and AIC.

The Weibull and Gompertz models allowed for characterization of non-linear inactivation of microorganisms, the former describing the inactivation rate curve as having either a concave or convex shape, and the latter providing shouldering (lag) and/or tailing with exponential,

nearly linear inactivation. High inoculation levels (~7-8 log CFU/g) and low LOD (0.6 log CFU/g) for the log bacterial counts allowed confidence that the curve inflections were not impacted by the LOD or complete die-off. Overall, based on the RMSE and AIC, the bacterial inactivation on raisins and macadamia nuts datasets were best fit to the Weibull model, with the shape parameter ($\beta<1$), indicating a concave, upward curve (Table 3.2). Only 15% (6 out of 40) of the shape parameter (β) confidence intervals included 1, boosting confidence in the concave upward curve trend.

All but one of the apricot halves' datasets were best described by the Gompertz model, with a few exceptions (noted by * in Table 3.2). For this instance, the confidence intervals of the lag time parameter (λ) were quite large compared to the other datasets' lag time parameters. The lag time parameter distinguishes the Gompertz model from the Weibull by depicting a delay in the exponential population reduction.

3.4. Water activity and proximate analyses

Water activity levels were measured for each temperature and time combination of the foods. Moisture, protein, and fat were measured for non-inoculated samples. On average, a_w of apricot halves increased significantly (p=0.0119) from 0.684 ± 0.044 (SD) to 0.769 ± 0.033 and 0.783 ± 0.038 after 72°C and 82°C treatments, respectively. Percent moisture also significantly increased (p=0.002) from an initial 29.1 ± 1.6% to 31.8 ± 1.1%. Neither crude protein (2.8 ± 0.5% to 2.6 ± 1.1%; $p > 0.05$) nor crude fat (<1.0%) were significantly impacted.

On average, a_w of macadamia nuts significantly increased (p=0.0005) from an initial 0.581 ± 0.025 to 0.870 ± 0.031 and 0.885 ± 0.016 for 72°C and 82°C treatments, respectively. Percent moisture increased insignificantly ($p > 0.05$) from 13.2 ± 1.8% to approximately 14.0 ±

1.3%. Crude protein (9.6 ± 0.2% to 9.2 ± 0.4%) and crude fat (49.3 ± 1.9% to 56.3 ± 4.3%) were not significantly altered ($p > 0.05$).

On average, a_w of raisins increased significantly (p=0.0048) from an initial 0.563 ± 0.001 to an average of 0.701 ± 0.023 and 0.685 ± 0.018 for 62°C and 72°C treatments, respectively. A significant increase (p=0.0048) in moisture was noted from an initial 18.2 ± 2.5% to approximately 25.0 ± 0.4%. Neither protein (3.3 ± 0.5% to 3.5 ± 0.2%) nor fat (<1.0%) were significantly impacted ($p > 0.05$).

4. Discussion

The main objectives of this research were to evaluate the efficacy of low-temperature, vacuum-assisted steam in reducing pathogens and a potential surrogate from dried fruits and macadamia nuts. Compared to heated air, steam offers more efficient heat transfer on LWAF for pathogen reduction (Newkirk et al., 2018). Pulling a vacuum prior to injecting low-temperature steam to remove air allowed the creation of a negative pressure in the chamber so that steam could be formed and stay at lower temperatures (<90°C) to preserve quality aspects of the food. The introduction of moisture and mild heat in the presented research was observed to have some impacts on moisture content and water activity of the foods, so additional drying steps following thermal treatments would be recommended. While it would be unlikely for the pathogens used in the present studies to grow at the resulting water activity there are examples of molds that grow in this range and may compromise product quality. *Salmonella* spp. have been shown to persist in raisin homogenate at lower water activities, but only grew at a much higher water activity (a_w 0.997; Beuchat and Mann, 2014), further highlighting the need for a drying step. It was beyond the scope of the present study to examine other changes to product quality and shelf life. Future work should measure other quality indicators (color and appearance, lipid oxidation, sensory,

total microbial counts, yeast and mold counts throughout storage) to determine if the food products are affected in ways that harm resale value.

4.1 Steam treatment bacterial reductions

Currently, the U.S. Food and Drug Administration (FDA) requires processes that result in a 4-log reduction of *Salmonella* from California-grown almonds (CFR, 2007). For other products lacking risk assessments or prevalence studies, demonstration of 5-log reductions of the most resistant pathogen is suggested. The results of the present study similarly support a higher log reduction (4-5 log CFU/g) as a guideline to reduce risk to consumers, as shorter time intervals with reductions of 1-3 log CFU/g were associated with greater variability in reductions and could fail to return the expected reduction. Steam movement and heat transfer to the product were not directly measured, however the product surface temperatures were monitored using thermocouples for each replicate and used to standardize treatments. While some variability is introduced by thermocouple placement, other factors, such as how steam moves through the product, may also contribute to variability. In a similar study, Shah et al. (2017) used vacuum-assisted steam (75-85°C) to pasteurize flaxseed, quinoa, and sunflower kernels and showed similar variability in reductions of *Salmonella* and *E. coli* O157 ranging from 0.1-1.4 log CFU/g depending on temperature and treatment time. Ergo, treatments that yielded larger reductions can provide more confidence in product safety. Although a risk assessment may indicate that reasonable pathogen contamination would only require smaller bacterial reductions, it may be more difficult for processors to assert confidence in milder treatments if process variation occurs.

From the first-order models, *D*-values were calculated and provided valuable information for comparison to similar work examining inactivation trends of pathogens on dried fruits. Other studies specifically examined *D*-values of *Salmonella* serovars Typhimurium

DT104 and PT 30 on dried date fruits and found the $D_{80°C}$ to be 3.5-3.6 min (Limcharoenchat et al., 2018; Mattick et al., 2001). These were much higher $D_{82°C}$ and $D_{72°C}$ values of *Salmonella* than what was observed for the dried fruits in the presented study ($D_{82°C}$ = 0.64 min on apricots, $D_{72°C}$ = 0.77 min on raisins). One explanation for this may be the larger size of dates compared to raisins or the higher sugar content in dates (67.5%) compared to apricots (37.5%). High sugar content could contribute to the thermal resistance of the *Salmonella* by serving as a protectant for the bacteria to resist desiccation stress by forming glasses that can prevent membrane degradation (Burgess et al., 2016). The method of heat transfer may have also resulted in differences in *D*-values. In the present study, low-temperature, saturated steam was used, which efficiently transfers heat to the surface associated microorganisms compared to isothermal treatments (Limcharoenchat et al., 2018; Mattick et al., 2001).

For process validations, it is recommended that the most resistant pathogen to the selected intervention be identified and targeted for the reduction (NACMCF, 2010). *Salmonella* is frequently the target pathogen for thermal inactivation studies, process interventions, and validations on dried products due to its frequent association with outbreaks in LWAF. However, recent outbreaks associated with other pathogens on dried products, notably STEC on flour and nuts, warrant investigation of these isolates for their potential thermal resistance. In some cases, thermoresistance of *Salmonella* and other pathogens have been shown to be greater in LWAF compared to high-water activity and high-humidity conditions (Casulli et al., 2018; He et al., 2013). The designation of *Salmonella* as the most thermoresistant pathogen should be based on research, as examples of other pathogens with similar or greater thermotolerance have been described (Lang et al., 2017; Shah et al., 2017). This may be highly dependent on the food and its characteristics, such as water activity. In a study comparing thermotolerance of pathogens

(*Salmonella* and *L. monocytogenes*) on low moisture foods, the $D_{80°C}$ was greater for *Salmonella* than *L. monocytogenes* in the LWAF (chicken meat powder, confectionary mix, pet food; a_w <0.6), but the two pathogens had comparable $D_{80°C}$ in the IWAF (culinary seasoning; a_w 0.65; Rachon et al., 2016). Vacuum-steam pasteurization used in the presented research increased the water activity of all foods, including the LWAF (raisins and macadamia nuts), to intermediate levels (a_w 0.6-0.85), which could have contributed to the comparable reductions for each of the pathogens when inoculated on the same food.

In addition to water activity, inactivation trends of different pathogens may be influenced by inoculum preparation (Enache et al., 2015), food physical structure (Mogollón et al., 2009) and composition (Rachon et al., 2016). Pathogens in the presented research displayed increased heat resistance on foods with high fat content (macadamia nuts), compared to foods with little or no fat (dried fruits). Macadamia nuts with >50% fat content required longer treatment times (38 min at 72°C, 12 min at 82°C) to achieve large pathogen reductions. Foodborne pathogens were also reported to have enhanced thermal tolerance in other foods with notably high fat contents including, ground beef (19%) and meat powder (25%) compared with lower fat products (Rachon et al., 2016; Smith et al., 2001). Furthermore, models for predicting survival in non-fat food systems have been shown to be more accurate than for low fat foods (12% fat), adding to the conclusion that fat content can influence pathogen inactivation (Farakos et al., 2013; Podolak, 2010). In peanut butters with 33% fat content, increasing the carbohydrate compositions was associated with greater survival of *Salmonella* at 90°C and 126°C, indicating other food components may also be important to consider in terms of pathogen inactivation (He et al., 2013).

4.2 Surrogate comparison

In-plant validation is the most effective way to evaluate the efficacy of an intervention intended to increase product safety, but due to concerns about cross-contamination of products, human pathogen introduction to food processing plants should be avoided. In place of these pathogens, surrogates, non-pathogenic bacteria with greater resistance to the intervention than the target pathogen, may be used. When selecting a surrogate, inactivation rates, intervention method, food type and characteristics, target reduction, and correlation to the target pathogen must all be considered (Hu and Gurtler, 2017). *Enterococcus faecium* NRRL B-2354 is a popular surrogate for *Salmonella* on LWAF, but some studies have shown *E. faecium* to be less resistant to certain thermal treatments or display inactivation trends too dissimilar from the targeted pathogens (Verma et al., 2018). *E. faecium* was not deemed a suitable surrogate for *Salmonella* spp. on cocoa powder or a confectionary mix due to its greater susceptibility to the heat, but was excessively more heat resistant than both *Salmonella* and *L. monocytogenes* cocktails in a culinary seasoning mix, dried meat powder, and pet food (Rachon et al., 2016; Tsai et al., 2019) highlighting the necessity of verifying the behavior of the surrogate on the target food and using the target process. *Pediococcus acidilactici* ATCC 8042, a lactic acid bacterium, has also been used as a surrogate for pathogens, namely *Salmonella*, on LWAF. Several studies have noted it as a more suitable surrogate than *E. faecium* for *Salmonella* on thermally treated pet food (76.7-87.8°C), PPO-treated macadamia nuts, and vacuum-steam-treated macadamia nuts (Ceylan and Bautista, 2015; Saunders, 2017; Saunders et al., 2018). Differences in suitability as a surrogate may be observed due to differences in characteristics of the food or the processes used for inactivation.

Pediococcus was selected for the present study based on preliminary work on that identified *P. acidilactici* as a more comparable surrogate to *S. enterica* on vacuum steam treated

macadamia nuts compared to *E. faecium* (Saunders et al., 2018). The suitability of *Pediococcus acidilactici* as a surrogate was demonstrated by comparable reductions and inactivation trends, and in several cases, statistically lower reductions than those of the pathogens (Figures 3.1-3.3). On macadamia nuts, the *Pediococcus* reductions were consistently lower than those of the pathogens. When 5-log reductions of the pathogens occurred, the *Pediococcus* average reductions ranged from 2.4-4.9 log CFU/g. At temperatures of 62-72°C, the reductions of *Pediococcus* were often significantly smaller compared to the pathogens, providing greater confidence that a reduction of the surrogate would assure the reduction of pathogens on raisins, apricots, and macadamia nuts at these temperatures (Figures 3.1a, 3.2a, 3.3a). At the highest treatment temperatures (72°C for raisins and 82°C for apricot halves and macadamia nuts), rapid reductions of *Pediococcus* occurred that were more comparable to the target pathogens. As a result, the reductions of *Pediococcus* and the pathogens were sometimes not significantly different statistically. Even still, when the inactivation of pathogens and *Pediococcus* on individual samples were directly compared using 1:1 ratio plots, the majority of points fell below the 1:1 ratio line (Figure 3.4a-f), showing increased reduction of pathogens than surrogate. Furthermore, nearly all points fell within the 90% confidence intervals, indicating that it could be considered a suitable surrogate (Figure 3.4). In a few instances at shorter treatment times, reductions of pathogens were less than that of *Pediococcus* (points above the 1:1 ratio line, Figure 3.4), but these points generally represented < 3-log CFU/g reductions, which would not be considered adequate treatments for safety. As demonstrated in the presented research, surrogate efficacy is highly dependent on food type and process conditions, stressing the importance of specific process validations to meet FSMA regulations.

4.3 Model evaluation

Traditionally, first-order inactivation has been used to describe effects on pathogens during thermal treatments. However, treatment parameters (sublethal temperatures, dry vs. moist heat), as well as the foods' properties in terms of size, water activity, or composition could impact inactivation trends. In general, the first-order model cannot always successfully fit data when the environment or microorganisms are more complex, so nonlinear models can be helpful in describing the complex relationship between the microorganisms and the food matrix (Bevilacqua et al., 2015; Rachon et al., 2016). Non-linear Weibull and Gompertz models provided a better fit for the survival data based on the RMSE and AIC. Specifically, the upward, concave inactivation trend of the Weibull model, best fit to the raisins and macadamia nuts. These inactivation curves indicated rapid inactivation upon reaching the target treatment temperature, with a decreasing inactivation rate throughout the treatment. Other studies investigating inactivation kinetics of pathogens have also proposed the Weibull model to describe the reduction of pathogens on LWAF (Farakos et al., Keller, 2017; Mattick et al., 2001; Villa-Rojas et al., 2013). Notably, the shape and scale parameters of the Weibull models for most datasets were highly correlated (0.856-0.988). This indicates that while the model fits the data well and can appropriately be used for predictions, the high correlation prevents determining the impact of each individual parameter on the model.

In contrast to the macadamia nuts and raisins, the modified Gompertz model best described the apricot halves datasets. The sigmoidal shape depicted by the modified Gompertz model with biologically meaningful parameters accounts for a lag time, followed by rapid, exponential inactivation, ending with a slowed rate of reduction. There was little or no correlation between the three parameters of the Gompertz model. Although the Gompertz model has been primarily used to describe growth, the modified model with adjustments to describe

reduction of bacterial populations in the presented research successfully describes the bacterial inactivation on certain food commodities. Figure 3.5 provides examples to highlight the contrast in the different inactivation kinetics modeled by the Weibull and Gompertz based on food commodity.

The variation of best-fit models in the present study seemed primarily influenced by food rather than organism, leading to the hypothesis that the physical size or compositional properties of the foods (such as sugar and fat content) impacted the efficacy of the vacuum-assisted steam. A few datasets did not "fit the pattern" correlating food type and model. These exceptions (Table 3.2) can be understood by noting the confidence intervals of the parameters that influence the shape of the curves. It is highly recommended that detailed models for specific processes and foods be established. A single model likely cannot accurately predict inactivation kinetics across food categories or apply to different treatment parameters.

5.0 Conclusions

Low-temperature, vacuum-assisted steam pasteurization offers a strategy to improve the safety of dried fruits and nuts in relatively short time periods, depending on the temperature. Future studies should be performed to determine changes to quality and sensory properties. This research demonstrates that *P. acidilactici* ATCC 8042 can serve as a surrogate for certain strains of STEC, *L. monocytogenes*, and *Salmonella* on low-temperature, vacuum-assisted steam-treated whole macadamia nuts, apricot halves and raisins with comparable processing parameters as described in this study. The Gompertz and Weibull models most closely represent the survival of STEC, *L. monocytogenes*, and *Salmonella* subjected to low-temperature vacuum-assisted steam; however, the choice of model was highly influenced by food type. Processors may be able to use these models for developing low-temperature, vacuum-assisted steam

processes or predicting reductions from similar processes, but results should not be extrapolated to other types of dried fruits or nuts, or other types of thermal processing, as it may result in an unsafe product. In-plant validations with an appropriate surrogate to confirm model predictions are highly recommended.

Conflicts of interest: The authors have no known conflicts of interest or competing financial interests.

Acknowledgments: We acknowledge the efforts and expertise of Dr. Hengjian Wang in conducting all proximate analyses and the advisement of the Virginia Tech Statistical Applications and Innovations Group (SAIG) for statistical analyses of proximate results.

Funding: This work was supported by the Virginia Agricultural Experiment Station and the Hatch Program of the National Institute of Food and Agriculture, U.S. Department of Agriculture.

Figures

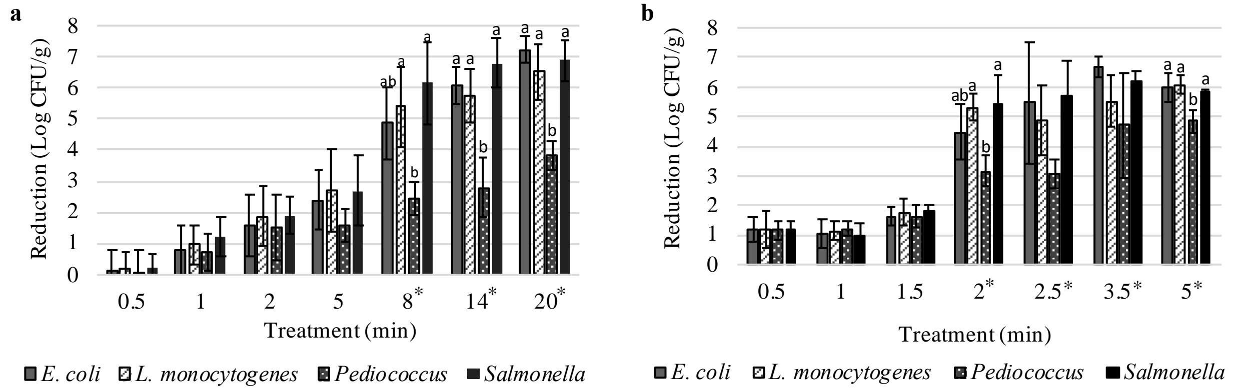

Figure 3.1. Bacterial reductions (log CFU/g ± standard deviation) on apricot halves resulting from 72°C (a) and 82°C (b) vacuum-steam treatment of various times. Asterisk (*) next to treatment times indicate significant differences of bacterial reductions from the reductions of a 0-min treatment, while lowercase letters above bars indicate significant differences between organisms within a treatment from pairwise comparisons (p<0.05). Lack of letters indicate no significant difference between organisms within the treatment.

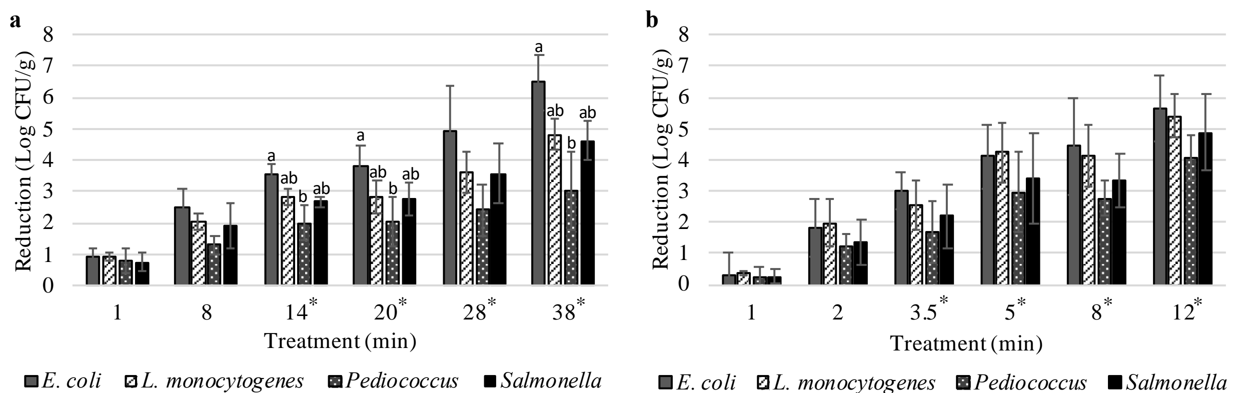

Figure 3.2. Bacterial reductions (log CFU/g ± standard deviation) on macadamia nuts resulting from 72°C (a) and 82°C (b) vacuum-steam treatment of various times. Asterisk (*) next to treatment times indicate significant differences of bacterial reductions from the reductions of a 0-min treatment, while lowercase letters above bars indicate significant differences between organisms within a treatment from pairwise comparisons (p<0.05). Lack of letters indicate no significant difference between organisms within the treatment.

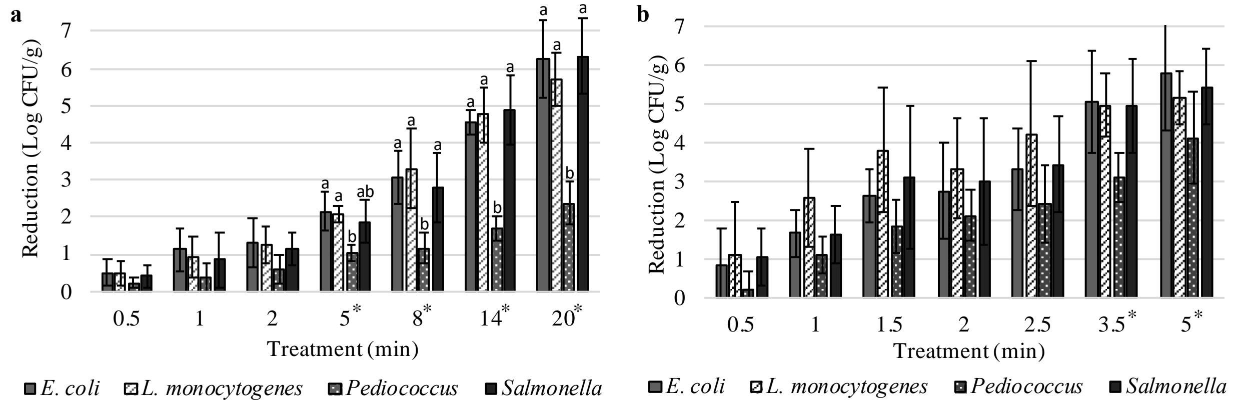

Figure 3.3. Bacterial reductions (log CFU/g ± standard deviation) on raisins resulting from 62°C (a) and 72°C (b) vacuum-steam treatment of various times. Asterisk (*) next to treatment times indicate significant differences of bacterial reductions from the reductions of a 0-min treatment, while lowercase letters above bars indicate significant differences between organisms within a treatment from pairwise comparisons ($p<0.05$). Lack of letters indicate no significant difference between organisms within the treatment.

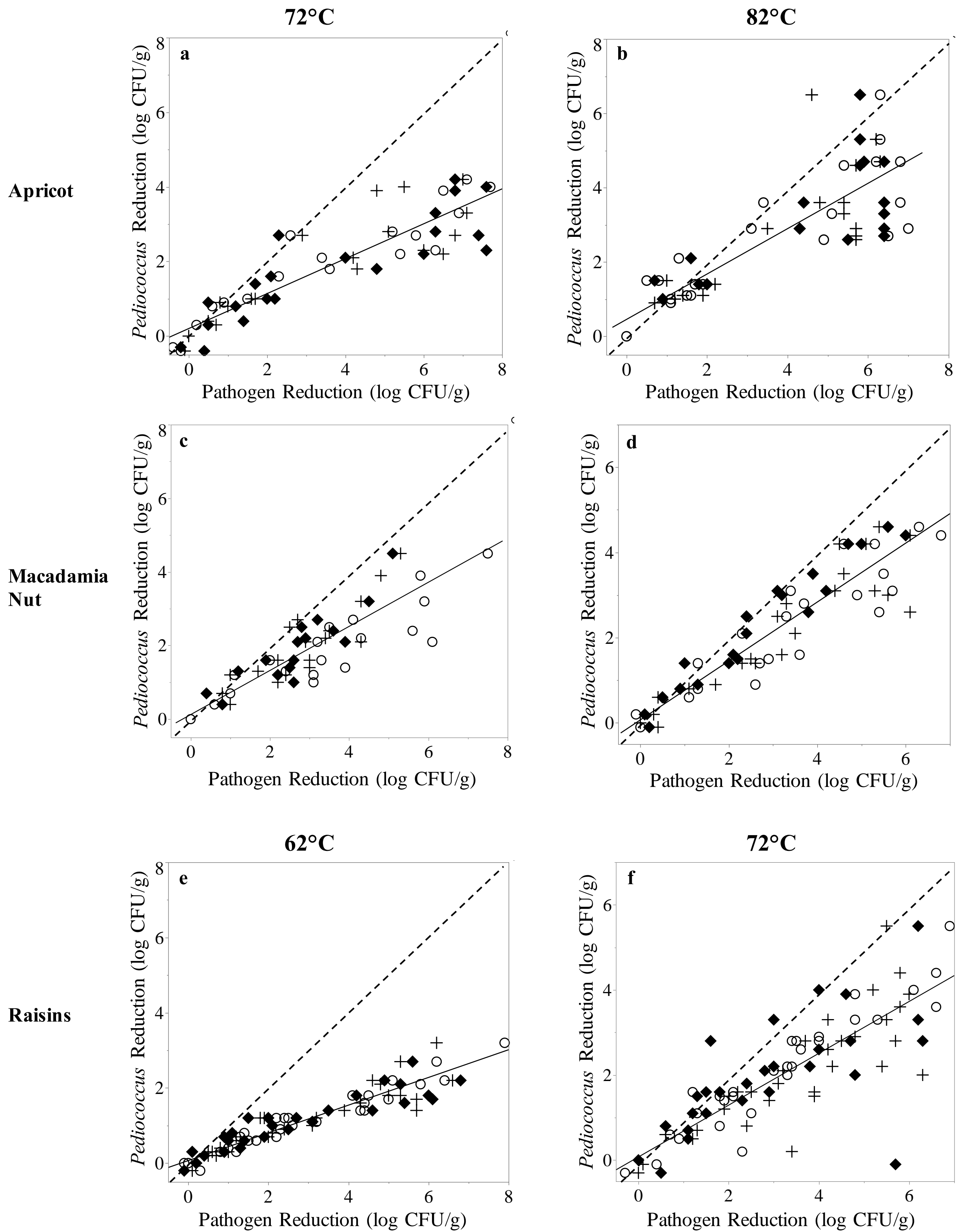

Figure 3.4. Figure 4. One-to-one ratio plots of pathogens vs. Pediococcus reductions (log CFU/g) for apricot halves (a, b), macadamia nuts (c, d), and raisins (e, f) with confidence and prediction intervals. (○*E. coli*, +*L. monocytogenes*, ◆*Salmonella*)

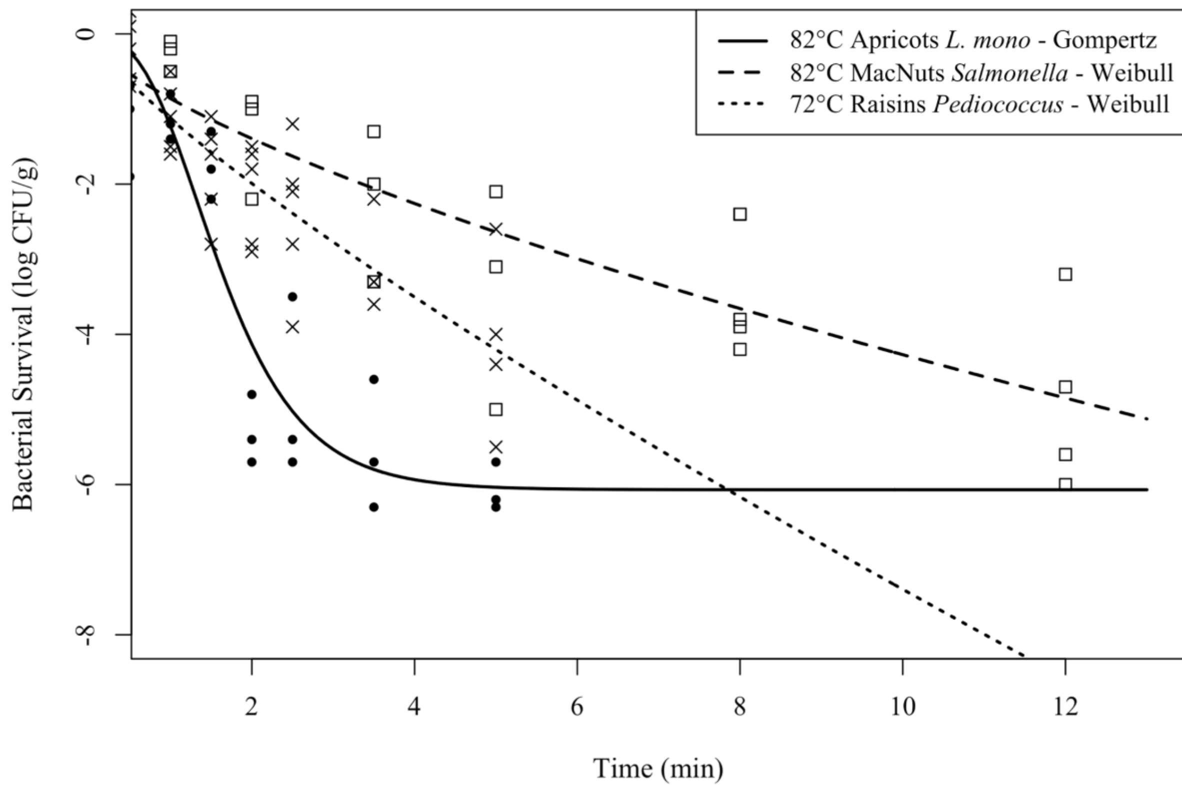

Figure 3.5. Inactivation kinetics of *L. monocytogenes*, *Salmonella*, and *Pediococcus* on apricots (●), macadamia nuts (☐), and raisins (╳), respectively, when treated with 72°C vacuum-steam treatment. Lines shown are Gompertz and Weibull models that were best fit to the datasets.

Tables

Table 3.1. First-order model inactivation rate coefficients (k [log CFU/g min^{-1}]), $D_{62°C}$, $D_{72°C}$, or $D_{82°C}$ values (min), and statistical results (R^2, RMSE [log CFU/g], AIC) for each food, organism, and treatment combination.

Food	Temp	Organism	*k*	*D*-value	R^2	RMSE	AIC
Apricot	72	*E. coli*	-0.41	2.43	0.94	1.00	70.96
		L. mono	-0.40	2.53	0.89	1.32	84.46
		Ped.	-0.21	4.70	0.87	0.80	60.21
		Sal.	-0.44	2.29	0.89	1.45	89.01
	82	*E. coli*	-1.57	0.64	0.90	1.35	85.66
		L. mono	-1.51	0.66	0.91	1.22	80.51
		Ped.	-1.16	0.86	0.93	0.82	61.68
		Sal.	-1.57	0.64	0.89	1.43	88.24
MacNut	72	*E. coli*	-0.18	5.44	0.95	0.85	60.96
		L. mono	-0.14	7.29	0.94	0.67	49.60
		Ped.	-0.10	10.32	0.89	0.69	51.30
		Sal.	-0.13	7.54	0.94	0.68	50.51
	82	*E. coli*	-0.54	1.85	0.90	1.14	83.72
		L. mono	-0.52	1.94	0.92	0.99	73.57
		Ped.	-0.37	2.73	0.91	0.76	62.49
		Sal.	-0.44	2.27	0.90	0.95	73.95
Raisins	62	*E. coli*	-0.33	3.04	0.95	0.71	85.30
		L. mono	-0.32	3.12	0.94	0.77	90.58
		Ped.	-0.13	7.96	0.91	0.39	38.36
		Sal.	-0.33	3.00	0.95	0.71	84.39
	72	*E. coli*	-1.31	0.76	0.91	0.98	109.40
		L. mono	-1.39	0.72	0.83	1.55	143.92
		Ped.	-0.90	1.11	0.91	0.69	82.58
		Sal.	-1.31	0.77	0.87	1.23	126.72

Table 3.2. Weibull (k (log CFU/g min$_{-1}$) and β) and Gompertz (A, μ_M [log CFU/g min$_{-1}$], λ [min]) model parameter coefficients and statistical results (RMSE [log CFU/g], AIC) for each food, organism, and treatment. Best values are bolded. Notable deviances from the observed trends related to food type are marked with (*).

			Weibull				Gompertz				
Food	Temp	Organism	k	β	RMSE	AIC	A	μ_M	λ	RMSE	AIC
Apricot	72	*E. coli*	-0.96	0.69	0.79	60.85	-7.22	-0.62	0.54	**0.78**	**60.78**
		L. mono	-1.24	0.58	0.98	70.89	-6.41	-0.70	0.23	**0.92**	**68.66**
		Ped.	-0.71	0.55	**0.64***	**50.62***	-3.67	-0.27	-1.00	0.71	56.64
		Sal.	-1.39	0.57	1.04	73.99	-7.13	-0.79	0.36	**0.88**	**66.91**
	82	*E. coli*	-2.15	0.75	1.28	83.75	-6.45	-4.33	0.99	**0.96**	**71.04**
		L. mono	-2.19	0.70	1.09	75.95	-6.07	-3.18	0.63	**0.89**	**67.23**
		Ped.	-1.60	0.74	0.75	58.18	-5.38	-1.50	0.15	**0.73**	**57.65**
		Sal.	-2.37	0.67	1.27	83.58	-5.94	-11.07	1.34	**0.80**	**62.03**
MacNut	72	*E. coli*	-0.69	0.60	**0.64**	**48.43**	-6.68	-0.21	-1.19	0.78	58.66
		L. mono	-0.69	0.51	**0.40**	**26.99**	-4.81	-0.15	-2.26	0.55	42.61
		Ped.	-0.46	0.53	**0.61**	**46.36**	-3.66	-0.10	-2.69	0.67	51.70
		Sal.	-0.59	0.55	**0.48**	**35.89**	-4.59	-0.16	-1.53	0.60	46.31
	82	*E. coli*	-1.23	0.63	0.98	**76.49**	-5.20	-1.00	0.49	**0.96***	76.62
		L. mono	-1.21	0.61	0.78	62.54	-5.01	-0.94	0.43	**0.76***	**62.04***
		Ped.	-0.73	0.68	**0.69**	**58.63**	-3.71	-0.57	0.34	0.72	61.81
		Sal.	-0.86	0.70	**0.88**	**71.03**	-4.48	-0.68	0.35	0.92	74.13
Raisins	62	*E. coli*	-0.74	0.70	**0.59**	**71.06**	-7.45	-0.35	-0.56	0.65	79.75
		L. mono	-0.81	0.66	**0.58**	**69.97**	-6.04	-0.40	-0.28	0.61	75.61
		Ped.	-0.33	0.64	**0.33**	**27.84**	-2.72	-0.13	-1.08	0.36	35.59
		Sal.	-0.56	0.81	**0.67**	**80.87**	-7.71	-0.38	0.37	0.69	84.53
	72	*E. coli*	-1.71	0.78	**0.94**	**106.73**	-6.54	-1.51	0.05	0.96	109.88
		L. mono	-2.53	0.49	**1.24**	**128.35**	-4.92	-2.36	0.01	**1.24***	129.19
		Ped.	-1.13	0.82	**0.67**	**81.32**	-4.24	-1.15	0.18	0.69	84.27
		Sal.	-1.94	0.67	**1.13**	**120.93**	-5.67	-1.61	-0.03	1.15	123.49

Supplemental Materials

Table 3.3. Temperature profile parameters averaged across each treatment combination.

Food	Treatment Temp (°C)	Treatment (min)	Come-up Time (sec)	Residual Heating (sec)	Maximum Temp (°C)	Minimum Temp (°C)
Apricot Halves	72	0	62.3	11.0	72.5	69.6
		0.5	47.3	3.0	73.4	69.7
		1	50.3	2.3	73.5	70.5
		2	48.7	3.3	73.4	70.5
		5	48.0	3.3	73.5	70.5
		8	50.3	2.7	73.2	70.4
		14	55.3	5.7	73.6	70.2
		20	46.7	2.7	73.8	70.4
	82	0	72.3	9.7	82.8	79.2
		0.5	68.3	1.7	83.9	79.6
		1	69.7	2.7	83.8	80.5
		1.5	65.0	2.7	83.8	80.6
		2	82.7	1.3	83.3	80.6
		2.5	65.0	4.0	83.5	79.4
		3.5	72.3	3.7	83.4	80.8
		5	70.0	3.0	83.2	80.5
Macadamia Nut	72	0	40.0	12.0	72.2	69.5
		1	38.0	1.0	73.9	70.7
		8	34.0	1.3	73.2	70.8
		14	37.3	1.0	72.9	70.6
		20	32.3	1.7	73.0	70.6
		28	33.3	4.1	73.6	70.4
		38	33.3	2.3	73.7	70.5
	82	0	45.8	14.0	83.3	78.7
		1	40.7	1.3	83.7	80.5
		2	39.7	1.3	83.0	80.7
		3.5	39.7	1.3	82.9	80.6
		5	42.3	1.7	82.9	80.8
		8	44.8	3.4	83.3	80.7
		12	41.3	2.0	83.5	80.4
Raisins	62	0	46.3	17.7	64.5	64.5
		0.5	45.3	6.0	63.4	63.4
		1	44.0	7.3	62.9	62.9
		2	67.6	7.6	65.1	62.7
		5	48.7	8.0	63.0	60.5
		8	45.8	6.8	63.6	60.4

	14	50.8	6.3	63.4	60.6
	20	52.3	14.5	63.5	60.8
72	0	50.7	17.7	72.1	69.3
	0.5	50.8	5.5	73.1	73.1
	1	39.5	5.8	73.2	73.2
	1.5	71.5	8.0	73.1	70.7
	2	45.0	6.3	73.1	70.7
	2.5	59.5	6.8	73.1	70.5
	3.5	41.8	5.8	72.9	70.8
	5	59.3	7.8	73.6	70.6

Table 3.4. Average bacterial reductions of Shiga toxin-producing *E. coli*, *L. monocytogenes*, *Salmonella*, and *Pediococcus acidilactici* on apricot halves, macadamia nuts, and raisins treated for various lengths at 62, 72, and 82°C.

			Reduction ± standard deviation (log CFU/g)			
Food	Temp (°C)	Treatment (min)	*E. coli*	*L. mono*	*Salmonella*	*Pediococcus*
Apricot Halves	72	0.5	0.1 ± 0.7	0.2 ± 0.6	0.2 ± 0.4	0.1 ± 0.7
		1	0.8 ± 0.8	1.0 ± 0.6	1.2 ± 0.6	0.7 ± 0.6
		2	1.6 ± 1.0	1.9 ± 1.0	1.9 ± 0.6	1.5 ± 1.0
		5	2.4 ± 1.0	2.7 ± 1.3	2.7 ± 1.1	1.6 ± 0.6
		8	4.9 ± 1.1	5.4 ± 1.3	6.2 ± 1.3	2.4 ± 0.6
		14	6.1 ± 0.6	5.8 ± 0.9	6.8 ± 0.8	2.8 ± 1.0
		20	7.2 ± 0.4	6.5 ± 0.9	6.9 ± 0.7	3.8 ± 0.5
	82	0.5	1.2 ± 0.4	1.2 ± 0.6	1.2 ± 0.3	1.2 ± 0.3
		1	1.0 ± 0.5	1.1 ± 0.3	1.0 ± 0.4	1.2 ± 0.3
		1.5	1.6 ± 0.3	1.8 ± 0.5	1.8 ± 0.2	1.6 ± 0.4
		2	4.5 ± 0.9	5.3 ± 0.5	5.4 ± 1.0	3.2 ± 0.5
		2.5	5.5 ± 2.1	4.9 ± 1.2	5.7 ± 1.2	3.1 ± 0.5
		3.5	6.7 ± 0.4	5.5 ± 0.9	6.2 ± 0.3	4.7 ± 1.8
		5	6.0 ± 0.5	6.1 ± 0.3	5.8 ± 0.1	4.9 ± 0.4
Macadamia Nut	72	1	0.9 ± 0.3	0.9 ± 0.1	0.7 ± 0.3	0.8 ± 0.4
		8	2.5 ± 0.6	2.0 ± 0.3	1.9 ± 0.7	1.3 ± 0.3
		14	3.5 ± 0.4	2.8 ± 0.3	2.7 ± 0.2	2.0 ± 0.6
		20	3.8 ± 0.6	2.8 ± 0.5	2.8 ± 0.5	2.0 ± 0.8
		28	4.9 ± 1.4	3.6 ± 0.7	3.6 ± 1.0	2.4 ± 0.8
		38	6.5 ± 0.9	4.8 ± 0.5	4.6 ± 0.6	3.5 ± 1.2
	82	1	0.3 ± 0.7	0.4 ± 0.1	0.3 ± 0.2	0.2 ± 0.4

		2	1.8 ± 0.9	2.0 ± 0.8	1.4 ± 0.7	1.2 ± 0.4
		3.5	3.0 ± 0.6	2.5 ± 0.8	2.2 ± 1.0	1.7 ± 1.0
		5	4.1 ± 1.0	4.2 ± 1.0	3.4 ± 1.5	3.0 ± 1.3
		8	4.4 ± 1.5	4.1 ± 1.0	3.3 ± 0.9	2.8 ± 0.5
		12	5.7 ± 1.1	5.4 ± 0.7	4.9 ± 1.2	4.1 ± 0.7
Raisins	62	0.5	0.5 ± 0.4	0.5 ± 0.3	0.4 ± 0.3	0.2 ± 0.1
		1	1.1 ± 0.6	0.9 ± 0.6	0.9 ± 0.7	0.4 ± 0.4
		2	1.3 ± 0.7	1.3 ± 0.5	1.1 ± 0.4	0.6 ± 0.4
		5	2.2 ± 0.5	2.1 ± 0.2	1.9 ± 0.6	1.0 ± 0.2
		8	3.1 ± 0.7	3.3 ± 1.1	2.8 ± 0.9	1.2 ± 0.4
		14	4.5 ± 0.3	4.8 ± 0.7	4.9 ± 0.9	1.7 ± 0.3
		20	6.3 ± 1.1	5.7 ± 0.7	6.3 ± 1.0	2.4 ± 0.6
	72	0.5	0.8 ± 1.0	1.1 ± 1.4	1.0 ± 0.7	0.2 ± 0.4
		1	1.7 ± 0.6	2.6 ± 1.3	1.6 ± 0.7	1.1 ± 0.5
		1.5	2.6 ± 0.7	3.8 ± 1.6	3.1 ± 1.8	1.8 ± 0.7
		2	2.7 ± 1.2	3.3 ± 1.3	3.0 ± 1.6	2.1 ± 0.7
		2.5	3.3 ± 1.0	4.2 ± 1.9	3.4 ± 1.2	2.4 ± 1.0
		3.5	5.0 ± 1.3	5.0 ± 0.8	5.0 ± 1.2	3.1 ± 0.6
		5	5.8 ± 1.5	5.2 ± 0.7	5.4 ± 1.0	4.1 ± 1.2

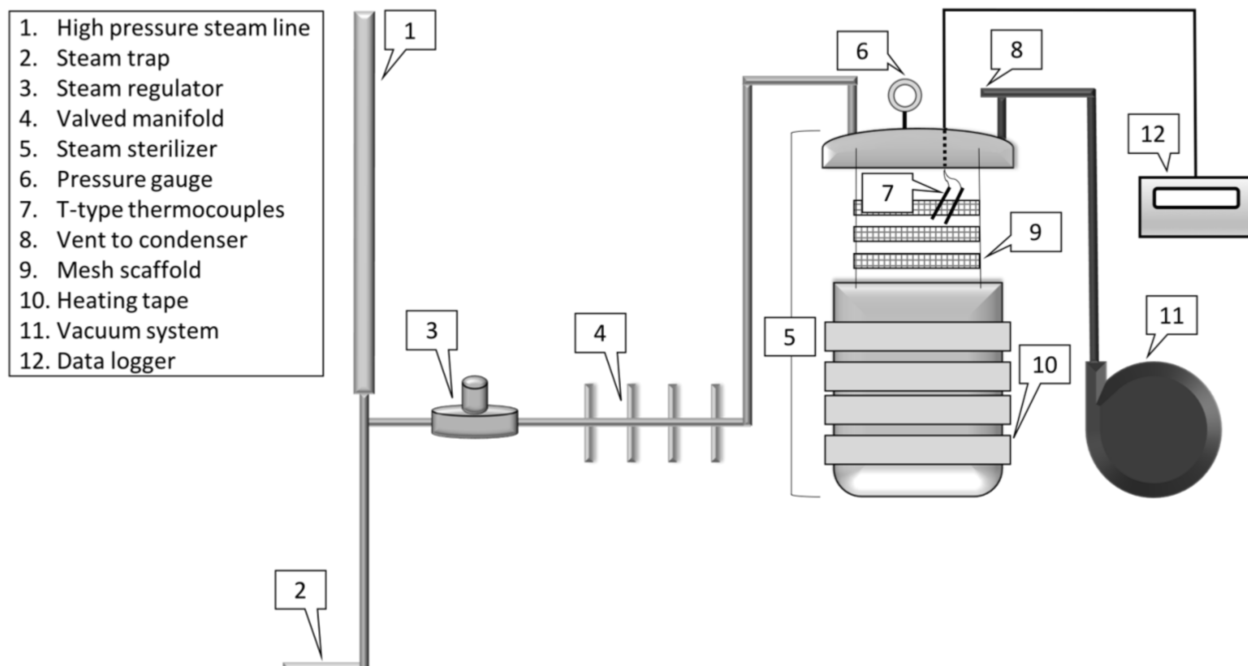

Figure 3.6. Schematic of equipment design for vacuum-assisted steam delivery.

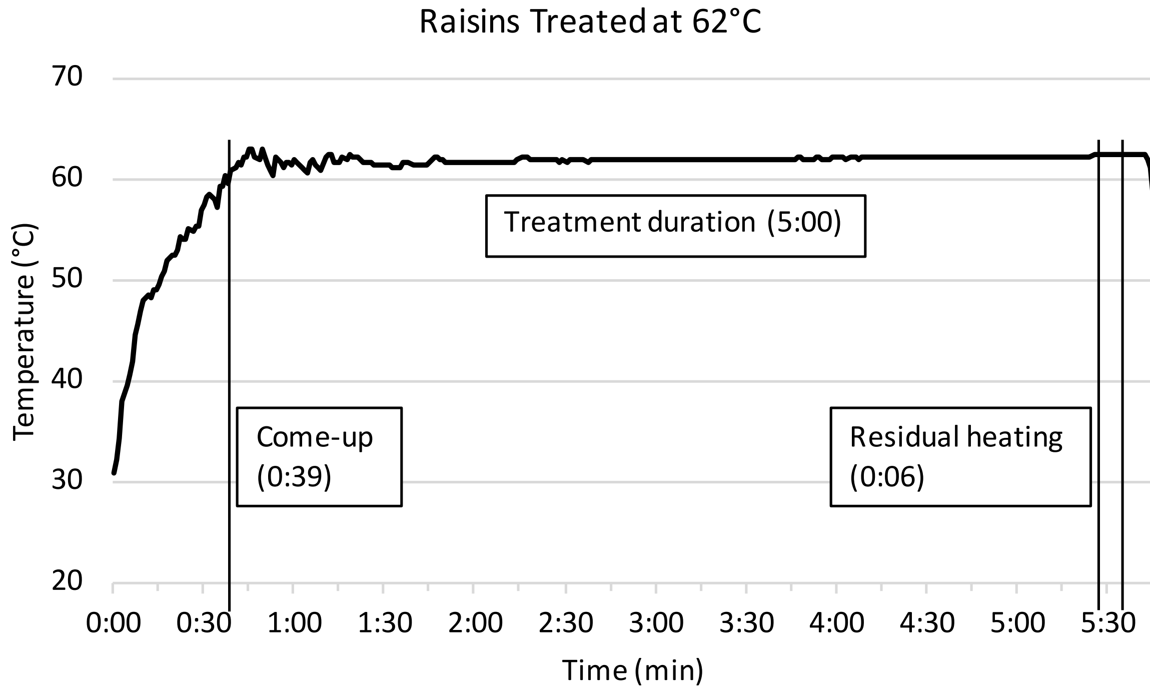

Figure 3.7. Temperature profile example of 62°C vacuum-steam cycle on raisins.

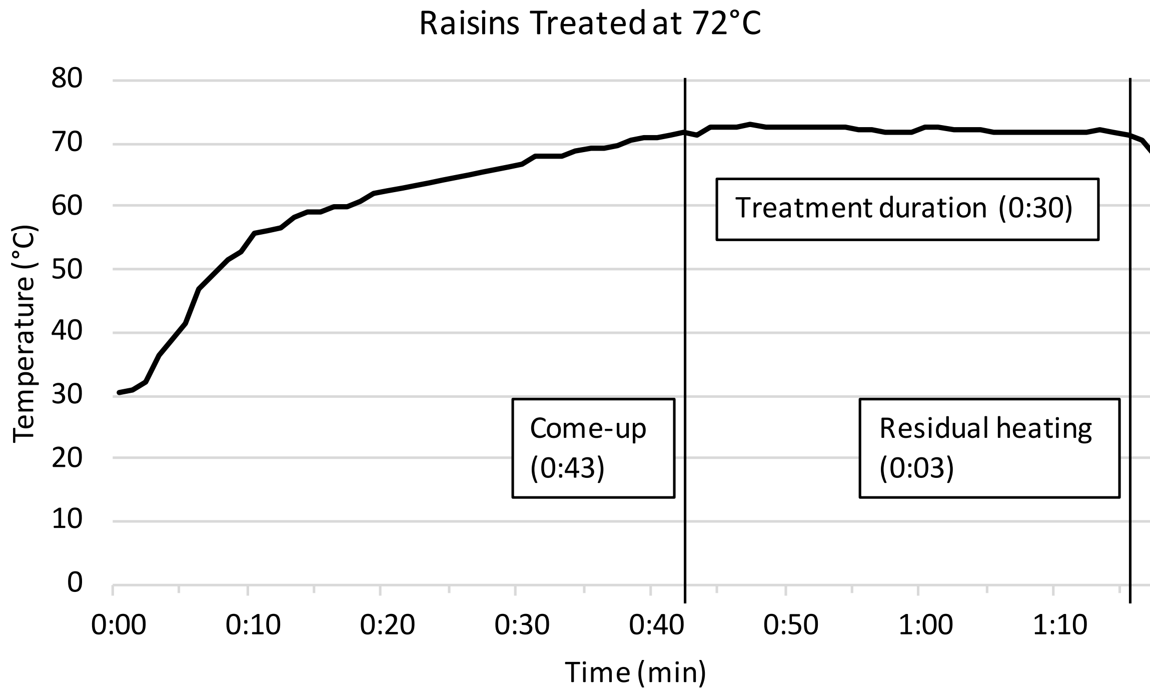

Figure 3.8. Temperature profile example of 72°C vacuum-steam cycle on raisins.

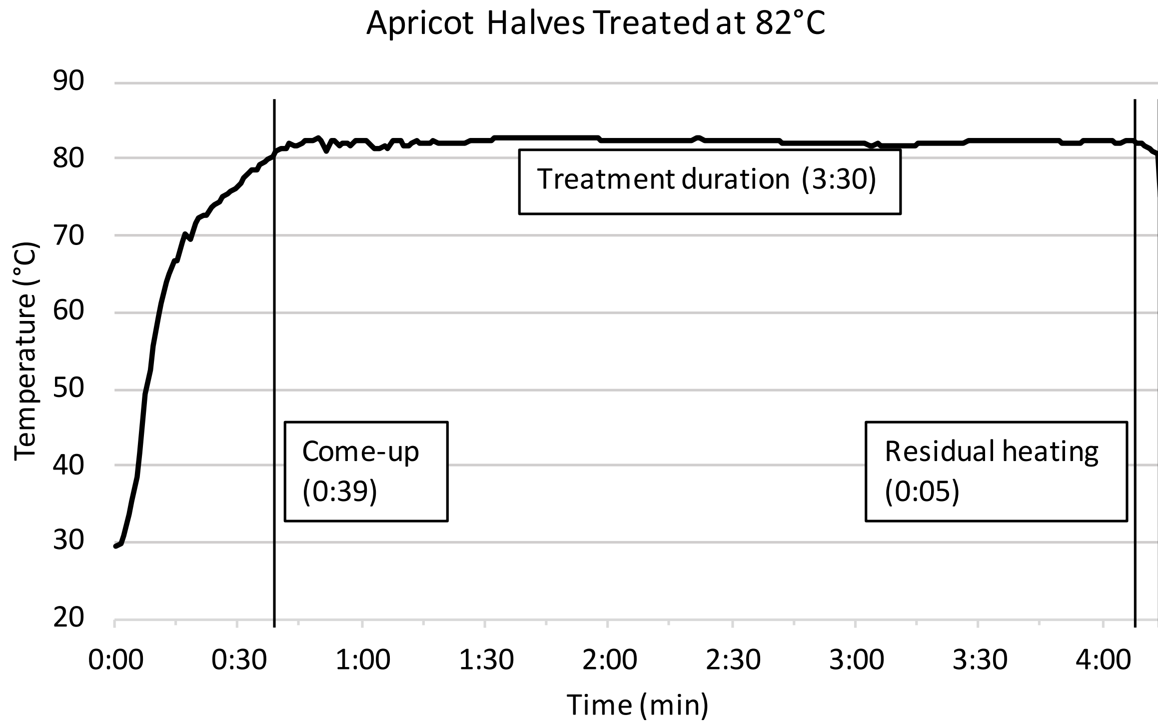

Figure 3.9. Temperature profile example of 82°C vacuum-steam cycle on apricot halves.

References

1. Andino, A. and Hanning, I. 2015. *Salmonella enterica*: Survival, colonization, and virulence differences among serovars. Sci. World J. 2015, 1–16.
2. Ban, G.-H., Kang, D.-H. 2016. Effectiveness of superheated steam for inactivation of *Escherichia coli* O157:H7, *Salmonella* Typhimurium, *Salmonella* Enteritidis phage type 30, and *Listeria monocytogenes* on almonds and pistachios. Int. J. Food Microbiol. 220(C), 19–25.
3. Bermúdez-Aguirre, D., Corradini, M.G. 2012. Inactivation kinetics of *Salmonella* spp. under thermal and emerging treatments: A review. Food Res. Int. 45(2), 700–712.
4. Beuchat, L.R., Komitopoulou, E., Beckers, H., Betts, R.P., Bourdichon, F., Fanning, S., Joosten, H.M., Ter Kuile, B.H. 2013. Low–water activity foods: increased concern as vehicles of foodborne pathogens. J. Food Prot. 76(1), 150–172.
5. Beuchat, L.R., Mann, D.A. 2014. Survival of *Salmonella* on dried fruits and in aqueous dried fruit homogenates as affected by temperature. J. Food Prot. 77(7), 1102–1109.
6. Bevilacqua, A., Speranza, B., Sinigaglia, M., Corbo, M. 2015. A focus on the death kinetics in predictive microbiology: benefits and limits of the most important models and some tools dealing with their application in foods. Foods. 4(4), 565–580.
7. Borowski, A.G., Ingham, S.C., Ingham, B.H. 2009. Validation of ground-and-formed beef jerky processes using commercial lactic acid bacteria starter cultures as pathogen surrogates. J. Food Prot. 72(6), 1234-47.
8. Bourdoux, S., Li, D., Rajkovic, A., Devlieghere, F., Uyttendaele, M. 2016. Performance of drying technologies to ensure microbial safety of dried fruits and vegetables. Compr. Rev. Food Sci. F. 15, 1056-1066.
9. Brar, P.K., Proano, L.G., Friedrich, L.M., Harris, L.J., Danyluk, M.D. 2015. Survival of *Salmonella*, *Escherichia coli* O157:H7, and *Listeria monocytogenes* on raw peanut and pecan kernels stored at −24, 4, and 22°C. J. Food Prot. 78(2), 323–332. http://doi.org/10.4315/0362-028X.JFP-14-327
10. Burgess, C.M., Gianotti, A., Gruzdev, N., Holah, J., Knøchel, S., Lehner, A., Margas, E., Esser, S.S., Sela (Saldinger), S., Tresse, O. 2016. The response of foodborne pathogens to osmotic and desiccation stresses in the food chain. Int. J. Food Microbiol. 221(C), 37–53.
11. Callejón, R.M., Rodríguez-Naranjo, M.I., Ubeda, C., Hornedo-Ortega, R., Garcia-Parrilla, M.C., Troncoso, A.M. 2015. Reported foodborne outbreaks due to fresh produce in the United States and European Union: trends and causes. Foodborne Pathog. Dis. 12(1), 32–38.
12. Canadian Food Inspection Agency (CFIA). 2011. Health hazard alert: certain walnuts and walnut products may contain *E. coli* O157:H7 bacteria. Available from: http://www.inspection.gc.ca/about-the-cfia/newsroom/food-recall-warnings/complete-listing/2011-04-03/eng/1359548340192/1359548340223.
13. Casulli, K.E., Garces-Vega, F.J., Dolan, K.D., Ryser, E.T., Harris, L.J., Marks, B.P. 2018. Impact of process temperature, humidity, and initial product moisture on thermal inactivation of *Salmonella Enteritidis* PT 30 on pistachios during hot-air heating. J. Food Prot. *81*(8), 1351–1356. http://doi.org/10.4315/0362-028X.JFP-17-439
14. Centers for Disease control and Prevention (CDC). 2012. Foodborne outbreak online database. Available from: http://www.cdc.gov/foodborneoutbreaks/.

15. Centers for Disease Control and Prevention (CDC). 2018a. Burden of foodborne illness: findings. Available from: https://www.cdc.gov/foodborneburden/2011-foodborne-estimates.html
16. Centers for Disease Control and Prevention, National Outbreak Reporting System (CDC). 2018b. NORS dashboard. Available from: https://wwwn.cdc.gov/norsdashboard/
17. Centers for Disease Control and Prevention (CDC). 2019a. Reports for selected *E. coli* outbreak investigations. Available from: https://www.cdc.gov/ecoli/outbreaks.html.
18. Centers for Disease Control and Prevention (CDC). 2019b. Reports for selected *Salmonella* outbreak investigations. Available from: https://www.cdc.gov/salmonella/outbreaks.html.
19. Chang, S.S., Han, A.R., Reyes-De-Corcuera, J.I., Powers, J.R., Kang, D.H. 2010. Evaluation of steam pasteurization in controlling *Salmonella* serotype Enteritidis on raw almond surfaces. Lett. Appl. Microbiol. 50(4), 393–398.
20. Ceylan, E., Bautista, D. 2015. Evaluating *Pediococcus acidilactici* and *Enterococcus faecium* NRRL B-2354 as a thermal surrogate microorganism for *Salmonella* for in-plant validations studies of low-moisture pet food products. J. Food Prot. 72(5), 934-939.
21. Code of Federal Regulations. 2007. Almonds grown in California; outgoing quality control requirements. Federal Register*, 72*(61), 7 CFR Part 981. Available from: http://www.almonds.com/sites/default/files/content/attachments/pasteurization_federal_register_ruling.pdf
22. Danyluk, M.D., Uesugi, A.R., Harris, L.J. 2005. Survival of *Salmonella* Enteritidis PT 30 on inoculated almonds after commercial fumigation with propylene oxide. J. Food Prot. 68, 1613–1622.
23. Duncan, S.E., Moberg, K., Amin, K.N., Wright, M., Newkirk, J.J., Ponder, M.A., Acuff, G.R., Dickson, J.S. 2017. Processes to preserve spice and herb quality and sensory integrity during pathogen inactivation. J. Food Sci. 82(5), 1208–1215.
24. Enache, E., Kataoka, A., Black, D.G., Napier, C.D., Podolak, R., Hayman, M.M. 2015. Development of a dry inoculation method for thermal challenge studies in low-moisture foods by using talc as a carrier for *Salmonella* and a surrogate (*Enterococcus faecium*). J. Food Prot. 78(6), 1106–1112. http://doi.org/10.4315/0362-028X.JFP-14-396
25. European Commission Rapid Alert System for Food & Feed (RASFF). 2019. Notifications list. Available from: https://webgate.ec.europa.eu/rasff-window/portal/?event=notificationsList&StartRow=1.
26. Eze, J., Agbo, K. 2011. Comparative studies of sun and solar drying of peeled and unpeeled ginger. Am. J. Sci. Ind. Rsch. 2(2), 136–143.
27. Farakos, S.M.S., Frank, J.F., Schaffner, D.W. 2013. Modeling the influence of temperature, water activity and water mobility on the persistence of *Salmonella* in low-moisture foods. Int. J. Food Microbiol. 166(2), 280–293.
28. Farakos, S.M.S., Frank, J.F. 2014. Challenges in the control of foodborne pathogens in low-water activity foods and spices. In Gurtler, J. B., Doyle, M. P., & Kornacki, J. L. (Eds.), *The microbiological safety of low water activity foods and spices* (pp. 15-34). New York: Springer New York.
29. Farakos, S.M.S., Pouillot, R., Keller, S.E. 2017. *Salmonella* survival kinetics on pecans, hazelnuts, and pine nuts at various water activities and temperatures. J. Food Prot. 80(5), 879–885.

30. Finn, S., Condell, O., McClure, P., Amézquita, A., Fanning, S. 2013. Mechanisms of survival, responses, and sources of *Salmonella* in low-moisture environments. Front. Micobiol. 4(331)*,* 1–15.
31. Food and Agriculture Organization of the United Nations (FAO). 2011. Information on post-harvest operations: dried fruit. Available from: http://www.fao.org/3/a-au111e.pdf.
32. Food Safety News (FSN). Snack mix recall expanded as patient count increases in *Salmonella* outbreak. 2019. Available from: https://www.foodsafetynews.com/2019/03/snack-mix-recall-expanded-as-patient-count-increases-in-salmonella-outbreak/.
33. Food Safety News (FSN). 2018. Dates from Iran linked to Hepatitis A outbreak for second time in 2 years. Available from: https://www.foodsafetynews.com/2019/05/dates-from-iran-linked-to-hepatitis-a-outbreak-in-sweden/
34. Forghani, F., den Bakker, M., Futral, A.N., Diez-Gonzalez, F. 2018. Long-term survival and thermal death kinetics of enterohemorrhagic *Escherichia coli* serogroups O26, O103, O111, and O157 in wheat flour. Appl. Environ. Microb. 84, e00283–18.
35. He, Y., Li, Y., Salazar, J.K., Yang, J., Tortorello, M.L., Zhang, W. 2013. Increased water activity reduces the thermal resistance of *Salmonella enterica* in peanut butter. Appl. Environ. Microb. 79(15), 4763–4767. http://doi.org/10.1128/AEM.01028-13
36. Hu, M., Gurtler, J.B. 2017. Selection of Surrogate Bacteria for Use in Food Safety Challenge Studies: A Review. J. Food Prot. 80(9), 1506–1536. http://doi.org/10.4315/0362-028X.JFP-16-536
37. Jackson, B.R., Salter, M., Tarr, C., Conrad, A., Harvey, E., Steinbock, L., Saupe, A., Sorenson, A., Katz, L., Stroika, S., Jackson, K.A., Carleton, H., Kucerova, Z., Melka, D., Strain, E., Parish, M., Mody, R.K. 2015. Listeriosis associated with stone fruit – United States, 2014. MMWR-Morbid. Mortal. W. 64, 282-283.
38. Jeong, S., Marks, B.P., Ryser, E.T. 2011. Quantifying the performance of Pediococcus sp. (NRRL B-2354: *Enterococcus faecium*) as a nonpathogenic surrogate for *Salmonella* Enteritidis PT30 during moist-air convection heating of almonds. J. Food Prot. 74(4), 603–609.
39. Karabulut, I., Topcu, A., Duran, A., Turan, S., Ozturk, B. 2007. Effect of hot air drying and sun drying on color values and β-carotene content of apricot (*Prunus armenica* L.). LWT-Food Sci. Technol. 40(5), 753–758.
40. Karagöz, I., Moreira, R. G., & Castell-Perez, M. E. (2014). Radiation D10 values for *Salmonella* Typhimurium LT2 and an *Escherichia coli* cocktail in pecan nuts (Kanza cultivar) exposed to different atmospheres. Food Control. 39(C), 146–153.
41. Keller, S.E., Stam, C.N., Gradl, D.R., Chen, Z., Larkin, E.L., Pickens, S.R., Chirtel, S.J. 2015. Survival of *Salmonella* on chamomile, peppermint, and green tea during storage and subsequent survival or growth following tea brewing. J. Food Prot. 78(4), 661–667.
42. Lang, E., Chemlal, L., Molin, P., Guyot, S., Alvarez-Martin, P., Perrier-Cornet, J.-M., Dantigny, P., Gervais, P. 2017. Modeling the heat inactivation of foodborne pathogens in milk powder: High relevance of the substrate water activity. Food Res. Int. 99(Part 1), 577–585. http://doi.org/10.1016/j.foodres.2017.06.028
43. Limcharoenchat, P., Buchholz, S.E., James, M.K., Hall, N.O., Ryser, E.T., Marks, B.P. 2018. Inoculation protocols influence the thermal resistance of *Salmonella* Enteritidis PT 30 in fabricated almond, wheat, and date products. J. Food Prot. 81(4), 606–613.

44. Mattick, K.L., Jorgensen, F., Wang, P., Pound, J., Vandeven, M.H., Ward, L.R., Legan, J.D., Lappin-Scott, H.M., Humphrey, T.J. 2001. Effect of challenge temperature and solute type on heat tolerance of *Salmonella* serovars at low water activity. Appl. Environ. Microb. 67(9), 4128–4136.
45. Mogollón, M., Marks, B., Booren, A., Orta, A. 2009. Effect of beef product physical structure on *Salmonella* thermal inactivation. *Interscience.Wiley.com*.
46. Munck, N., Smith, J., Bates, J., Glass, K., Hald, T., Kirk, M.D. 2019. Source attribution of *Salmonella* in macadamia nuts to animal and environmental reservoirs in Queensland, Australia. Foodborne Pathog. Dis. fpd.2019.2706–8.
47. National Advisory Committee on Microbiological Criteria for Foods. 2010. Parameters for determining inoculated pack/challenge study protocols. J. Food Prot. *73*(1), 140–202.
48. Newkirk, J.J., Wu, J., Acuff, J.C., Caver, C.B., Mallikarjunan, K., Wiersema, B.D., Williams, R.C., Ponder, M.A. 2018. Inactivation of *Salmonella enterica* and surrogate *Enterococcus faecium* on whole black peppercorns and cumin seeds using vacuum steam pasteurization. Front. in Sust. Food Sys. 2, 252–12.
49. Nijhuis, H.H., Torringa, H.M., Muresan, S., Yuksel, D., Leguijt, C., Kloek, W. 1998. Approaches to improving the quality of dried fruit and vegetables. Trends Food Sci. Tech. 9, 13–20.
50. Omolola, A.O., Jideani, A.I.O., Kapila, P.A. 2017. Quality properties of fruits as affected by drying operation. Crit. Rev. Food Sci. 57(1), 95-108.
51. Painter, J.A., Hoekstra, R.M., Ayers, T., Tauxe, R.V., Braden, C.R., Angulo, F.J., Griffin, P.M. 2013. Attribution of foodborne illnesses, hospitalizations, and deaths to food commodities by using outbreak data, United States, 1998–2008. Emerg. Infect. Dis. 19*,* 407–415.
52. Peleg, M. 2003. Microbial survival curves: interpretation, mathematical modeling, and utilization. Comment Theor. Biol. 8, 357–387.
53. Peleg, M., Normand, M.D., Corradini, M.G. 2005. Generating microbial survival curves during thermal processing in real time. J. Appl. Microbiol. 98(2), 406–417.
54. Podolak, R., Enache, E., Stone, W., Black, D.G., Elliott, P.H. 2010. Sources and risk factors for contamination, survival, persistence, and heat resistance of *Salmonella* in low-moisture foods. J. Food Prot. 73(10), 1919–1936.
55. Rachon, G., Peñaloza, W., Gibbs, P. A. 2016. Inactivation of *Salmonella*, *Listeria monocytogenes* and *Enterococcus faecium* NRRL B-2354 in a selection of low moisture foods. Int. J. Food Microbiol. 231(C), 16–25.
56. Saunders, T., Wu, J., Williams, R.C., Huang, H., Ponder, M.A. 2018. Inactivation of *Salmonella* and surrogate bacteria on cashews and macadamia nuts exposed to commercial propylene oxide processing conditions. J. Food Prot. 81(3), 417–423.
57. Saunders, T. 2017. Inactivation of *Salmonella* and surrogate bacteria on cashews and macadamia nuts exposed to saturated steam and propylene oxide treatments. (MS Thesis). Virginia Tech. Blacksburg, VA.
58. Scallan, E., Hoekstra, R.M., Angulo, F.J., Tauxe, R.V., Widdowson, M., Roy, S.L., Jones, J.L., Griffin, P.M. (2011). Foodborne illness acquired in the United States—major pathogens. Emerg. Infect. Dis. 17*,* 7-15.
59. Scott, V.N., Chen, Y., Freier, T.A., Kuehm, J., Moorman, M., Meyer, J., Morille-Hinds, T., Post, L., Smoot, L., Hood, S., Shebuski, J., Banks, J. 2009. Control of *Salmonella* in

low-moisture foods i: minimizing entry of *Salmonella* into a processing facility. Food Prot. Trend. 29, 342–353.

60. Shah, M.K., Asa, G., Sherwood, J., Graber, K., Bergholz, T.M. 2017. Efficacy of vacuum steam pasteurization for inactivation of *Salmonella* PT 30, *Escherichia coli* O157:H7 and *Enterococcus faecium* on low moisture foods. Int. J. Food Microbiol. 244(C), 111–118.
61. Smith, S.E., Maurer, J.L., Orta-Ramirez, A., Ryser, E.T., Smith, D.M. 2001. Thermal inactivation of *Salmonella* spp., *Salmonella typhimurium* DT104, and *Escherichia coli* O157:H7 in ground beef. J. Food Sci. 66(8), 1164–1168.
62. Smith, D.F., Hildebrandt, I.M., Casulli, K.E., Dolan, K.D., Marks, B.P., Marks, B.P. 2016. Modeling the effect of temperature and water activity on the thermal resistance of *Salmonella* Enteritidis PT 30 in wheat flour. J. Food Prot. 79(12), 2058–2065.
63. Stevenson, K,. Ito, K., Beuchat, L., Marks, B.P., Ashton, D. 2014. Guidelines for using *Enterococcus faecium* NRRL B-2354 as a surrogate microorganism in almond process validation. Almond Board of California. http://www.almonds.com/sites/default/files/content/attachments/guidelines_for_using_enterococcus_faecium_nrrl_b-2354_as_a_surrogate_microorganism_in_almond_process_validation.pdf. Accessed 2019, June 21.
64. Syamaladevi, R.M., Tang, J., Villa-Rojas, R., Sablani, S., Carter, B., Campbell, G. 2016. Influence of water activity on thermal resistance of microorganisms in low-moisture foods: a review. *Compr.* Rev. Food Sci. F. 15(2), 353–370.
65. Suehr, Q.J., Anderson, N.M., Keller, S.E. 2019. Desiccation and thermal resistance of *Escherichia coli* O121 in wheat flour. J. Food Prot. *82*(8), 1308–1313.
66. Tsai, H.-C., Ballom, K.F., Xia, S., Tang, J., Marks, B.P., Zhu, M.-J. 2019. Evaluation of *Enterococcus faecium* NRRL B-2354 as a surrogate for *Salmonella* during cocoa powder thermal processing. Food Microbiol. 82, 135–141.
67. U.S. Food and Drug Administration (FDA). 2015a. Natural Grocers® issues recall on 10oz. Caribbean Nut & Fruit Mix due to possible health risk. Available from: https://wayback.archive-it.org/7993/20170723005254/https://www.fda.gov/Safety/Recalls/ArchiveRecalls/2015/ucm453516.htm.
68. U.S. Food and Drug Administration (FDA). 2015b. Rocky Mountain Foods, Inc. voluntarily recalls Free Range Snack Co. Brand 16 oz. Island Fruit and Nut Trail Mix and bulk macadamia nuts because of possible health risk. Available from: https://wayback.archive-it.org/7993/20170406094935/https://www.fda.gov/Safety/Recalls/ArchiveRecalls/2015/ucm453116.htm.
69. U.S. Food and Drug Administration (FDA). 2015c. Texas Star Nut and Food Co. Inc. Natural Macadamia Nuts are being voluntarily recalled due to possible *Salmonella* contamination. Available from: https://wayback.archive-it.org/7993/20170723005958/https://www.fda.gov/Safety/Recalls/ArchiveRecalls/2015/ucm439283.htm.
70. U.S. Food and Drug Administration (FDA). 2016a. Draft guidance for industry: hazard analysis and risk-based preventive controls for human food; Chapter 4 Preventive Controls. Available from: https://www.fda.gov/media/99572/download.
71. U.S. Food and Drug Administration (FDA). 2016b. Mahina Mele Farms, LLC expands

voluntary recall of macadamia nut products due to possible health risk. Available from: https://wayback.archive-it.org/7993/20170112133521/http://www.fda.gov/Safety/Recalls/ucm484851.htm.
72. U.S. Food and Drug Administration (FDA). 2017a. FDA investigated multistate outbreak of *E. coli* O157:H7 infections linked to soynut butter. Available from: https://www.fda.gov/Food/RecallsOutbreaksEmergencies/Outbreaks/ucm544964.htm.
73. U.S. Food and Drug Administration (FDA). 2017b. Kroger recalls simple truth dry roasted macadamia nuts because of possible health risk. Available from: https://www.fda.gov/safety/recalls-market-withdrawals-safety-alerts/kroger-recalls-simple-truth-dry-roasted-macadamia-nuts-because-possible-health-risk.
74. U.S. Food and Drug Administration (FDA). 2018a. FDA investigated *Salmonella* Typhimurium outbreak linked to dried coconut. Available from: https://www.fda.gov/food/outbreaks-foodborne-illness/fda-investigated-salmonella-typhimurium-outbreak-linked-dried-coconut.
75. U.S. Food and Drug Administration (FDA). 2018b. Mauna Loa Macadamia Nut Corporation voluntarily recalls. Available from: https://www.fda.gov/safety/recalls-market-withdrawals-safety-alerts/mauna-loa-macadamia-nut-corporation-voluntarily-recalls.
76. U.S. Food and Drug Administration (FDA). 2019a. Jac. Vandenberg, Inc. recalls fresh peaches, fresh nectarines and fresh plums because they may be contaminated with *Listeria monocytogenes*. Available from: https://www.fda.gov/safety/recalls-market-withdrawals-safety-alerts/jac-vandenberg-inc-recalls-fresh-peaches-fresh-nectarines-and-fresh-plums-because-they-may-be.
77. U.S. Food and Drug Administration (FDA). 2019b. Archive for recalls, market withdrawals & safety alerts. Available from: www.fda.gov/safety/recalls-market-withdrawals-safety-alerts/archive-recalls-market-withdrawals-safety-alerts.
78. Verma, T., Wei, X., Lau, S.K., Bianchini, A., Eskridge, K.M., Subbiah, J. 2018. Evaluation of *Enterococcus faecium* NRRL B-2354 as a surrogate for *Salmonella* during extrusion of low-moisture food. J. Food Sci. 83(4), 1063–1072.
79. Villa-Rojas, R., Tang, J., Wang, S., Gao, M., Kang, D.-H., Mah, J.-H., Gray, P., Sosa-Morales, M.E., López-Maolo, A. 2013. Thermal inactivation of *Salmonella* Enteritidis PT 30 in almond kernels as influenced by water activity. J. Food Prot. 76(1), 26–32.
80. Wachukwu, C.K., Sokari, T.G., Wemedo, S.A. 2003. Effects of sun drying and smoking on *Salmonella* Typhimurium (LT 2) during cowpea-daddawa processing. Plant Food Hum. Nutr. *58*, 1–7.
81. Witthuhn, R.C., Engelbrecht, S., Joubert, E., Britz, T.J. 2005. Microbial content of commercial South African high-moisture dried fruits. J. Appl. Microbiol. 98(3), 722–726.
82. Zwietering, M.H., Jongenburger, I., Rombouts, F.M., & Riet, K.V. 1990. Modeling of the bacterial growth curve. Appl. Environ. Microb. 56(6), 1875–1881.

Chapter 4 Challenge and validation of kinetic models of bacterial thermal inactivation on whole macadamia nuts and dried apricot halves using low-temperature, vacuum-assisted steam

Jennifer C. Acuff[a], Jian Wu[a], Claire Marik[a], Kim Waterman[a], Daniel Gallagher[b], Haibo Huang[a], Robert C. Williams[a], Monica A. Ponder[a,*]

[a]Virginia Tech, Food Science and Technology,
1230 Washington St., Blacksburg, VA 24061, USA;

[b]Virginia Tech, Civil and Environmental Engineering Department,
409 Durham Hall, Blacksburg, VA 24061, USA.

Key words: Inactivation kinetics, models, thermal intervention, Shiga toxin-producing *Escherichia coli*, *Salmonella*, *Listeria monocytogenes*, surrogate

*Corresponding author: Monica A. Ponder, 540-231-5031, Mponder@vt.edu

Abstract:

Mathematical models can describe inactivation kinetics of thermally treated microorganisms. These models can be used by processors to optimize interventions and potentially predict times necessary for substantial pathogen inactivation. The presented research sought to compare bacterial inactivation models of thermally treated whole macadamia nuts and dried apricot halves to bacterial reductions on other thermally treated low-water activity foods (LWAF), with the purpose of determining model validity and application. First-order, Weibull, and Gompertz models were constructed of Shiga toxin-producing *Escherichia coli*, *Listeria monocytogenes*, *Salmonella*, and *Pediococcus acidilactici* on whole macadamia nuts, dried apricot halves, and respective smaller pieces, treated with 72 and 82°C vacuum-assisted steam. These models were compared to one another to determine each model's ability to predict times for 3-log reductions of the other microorganism, food, and temperature models. Additionally, external, independent datasets of similarly treated LWAF were acquired from literature, and times for 3-log reductions were extrapolated and compared. There was notable success of the internally constructed models, particularly first-order models, of macadamia nuts and dried apricots in predicting bacterial reductions of other steam-treated products (internal data). However, limited accuracy was observed in predicted times extrapolated from the external datasets. Trends observed in heatmaps of relative error indicated that accuracy was dictated by food category and treatment. Furthermore, non-pathogenic microorganisms, *Pediococcus acidilactici* and *Enterococcus faecium* were shown to be sufficient in predicting fail-safe times for reductions of multiple pathogens, proving to be adequate surrogates for pathogens and thermal processes in many cases. However, the two surrogates were not interchangeable. Overall, the presented work highlighted that primary inactivation models can accurately describe pathogen inactivation from thermal processes, but they can only be applied for predictions in

specific circumstances. Future work should build on these models and quantify the impacts of processing conditions and food composition on inactivation trends.

Highlights:

- Primary models constructed for LWAF were used to predict bacterial inactivation.
- Models were only accurate predictors for bacterial inactivation in certain conditions.
- Models of surrogate organisms could be used to predict inactivation of several pathogens.

1. Introduction

Low-water activity foods (LWAF) are responsible for thousands of recalls in the United States and Europe each year, as well as dozens of outbreaks of associated foodborne illnesses (CDC, 2012; CDC, 2018a; CDC, 2018b; CDC, 2019a; CDC, 2019b; CFIA, 2011; FDA, 2019; RASFF, 2019). While *Salmonella* is responsible for many of these recalls and outbreaks, *Shiga* toxin-producing *Escherichia coli* (STEC) and *Listeria monocytogenes* are also associated with numerous recalls. In terms of the overall burden of foodborne illness, LWAF are not considered high-risk foods compared to other foods more frequently associated with outbreaks, likely due to certain inherent characteristics that do not promote microbial growth. LWAF are categorized based on the lack of available water for microbial growth, but the ability of pathogens to persist for months to years on LWAF has caused scientists to investigate further the risks associated pathogen contamination on LWAF (Forghani et al., 2018; Forghani, 2019; Keller et al., 2015; Suehr et al., 2019).

The Risk-Based Preventive Controls for Human Food rule of the Food Safety Modernization Act (FSMA) recognizes the possibility of pathogen contamination of human foods and subsequent risk of consumption, and therefore requires food processors to develop and implement preventive controls (FDA, 2016). Thermal interventions are a key preventive control or strategy for food processors seeking to reduce or eliminate pathogens from foods or processing environments. In-plant validation is the best method to evaluate interventions using specific equipment, *in situ* processing conditions, and standard product volumes. These investigations provide valuable information and confirmation of effectiveness, but they can be costly and require resources or expertise that are not always accessible to food processors. It has been suggested that predictive microbiology modeling may be able to serve as a guideline to or substitute for “practical experimentation” in the food industry, such as in-plant validations, but

limitations of models must be recognized and understood for proper use of these empirical kinetic models (Marks, 2008; Membré and Lambert, 2008).

The earliest inactivation kinetic models in the food industry sought to describe thermal death of microorganisms with thermal resistance, namely *Clostridium botulinum* spores, in relation to canning sterility (Bigelow and Esty, 1920; Bigelow, 1921). These types of models, classified as primary models, represent the concentration or number of microorganisms in a population for a measured period of time (Bevilacqua et al., 2015). Primary models have been paramount in the development of predictive microbiology in their descriptions of how populations change or respond to their environment over time (McMeekin et al., 1997). Most commonly, primary models may be used to describe growth, survival, or inactivation in isothermal conditions. Linear thermal lethality curves gave rise to first-order kinetic models and *D*-values. These parameters have been used to describe and compare thermal resistances of many foodborne pathogens, such as *Salmonella*, Shiga toxin-producing *Escherichia coli*, and *Listeria monocytogenes* in a variety of conditions that aim to mimic food matrices (Enache et al., 2011; Jackson et al., 1995; Mattick et al., 2001). Over time, investigations have revealed that not all inactivation trends are linear, which gave rise to the application of nonlinear models to microbial inactivation, to describe shouldering and tailing trends (Marks, 2008; Peleg, 2003).

While primary models, particularly those developed by using an actual food matrix, can provide descriptive accounts of bacterial inactivation for food processors, they are specific and can only be applied in certain circumstances. These circumstances are dictated by the experimental parameters that may heavily influence results, such as heating mechanisms (thermal cells, water baths, steam), inoculation strategies (dry vs. wet), media or food matrices, target pathogens, and recovery techniques. Standard methods designed to account for variation in

conditions or experimental methods do not currently exist, which impact processors' abilities to apply the kinetic models in different processing environments or conditions (Marks, 2008). For these reasons, challenge and validation are vital for assessing the performance and applicability of models that are meant to be applied for predictions. Both internal and external datasets can be used for challenge and validation. Validation with internal datasets using similar treatments allow for control of processing parameters, whereas validation using external datasets allow for independent evaluation of the model's applicability.

The objective of this study was to validate and explore the application of primary kinetic models for inactivation of *Salmonella*, Shiga toxin-producing *Escherichia coli* (STEC), and *Listeria monocytogenes*, as well as a non-pathogenic microorganism (*Pediococcus acidilactici*) on vacuum-steam-treated whole macadamia nuts and dried apricot halves. Predictions of 3-log reductions were made from these models and compared against 3-log reductions of the same microorganisms on smaller particles (pieces) of vacuum-steamed macadamia nuts and dried apricots that were predicted based on models of internal data. The comparison of these internal model predictions limited the influence of food composition or process differences on the analyses. Additional consideration was given to determine if the model predictions of reduction for the non-pathogenic surrogate (*Pediococcus*) could yield similar or larger predictions compared to reductions of pathogens. The models were also compared and challenged with extrapolated and calculated 3-log bacterial reductions from data acquired from external, independent studies of other thermally treated LWAF (found in the literature) to determine if the models were valid under different conditions.

2. Materials & Methods

2.1. Vacuum-steam data collection experiments

As described in Chapter 3, whole macadamia nuts and dried apricot halves were inoculated with three pathogens and one non-pathogenic microorganism and treated with low-temperature, vacuum-assisted steam. Using thermocouples, the surface temperature of the sample was monitored, and steam was injected at various intervals to maintain a targeted temperature, making the treatment conditions isothermal. Inactivation models were fit to the datasets based on bacterial log reductions resulting from treatments at constant temperatures. The same processes were used on smaller pieces of macadamia nuts and dried apricots, which allowed for side-by-side comparison of model performances.

2.1.1. Strain preparation and food inoculation

Organic, raw whole macadamia nuts and organic Turkish dried apricot halves were purchased wholesale online (Nuts.com, Cranford, NJ). Foods were inoculated with a cocktail of the following bacterial strains: *E. coli* O121:H19 (FNW19M81, wheat flour isolate from 2016 outbreak [FDA]) and O157:H7 F4546 (alfalfa sprout isolate from outbreak); *L. monocytogenes* 1/2a FSL R2-499 (sliced turkey isolate), 1/2b FSL R2-502 (chocolate milk isolate), and 4b (ScottA, milk isolate from 1985 outbreak); *Salmonella enterica* serotype Montevideo (1449, black pepper isolate from 2010 outbreak), Newport (2010 allspice isolate), and Tennessee (K4643, peanut butter clinical isolate from 2007 outbreak); and the nonpathogenic bacterium, *Pediococcus acidilactici* (ATCC 8042). Inocula were prepared, mixed, and misted onto the whole macadamia nuts and dried apricot halves as described in Chapter 3.

Smaller pieces of macadamia nuts (5-10 mm) were also purchased (Nuts.com, Cranford, NJ), inoculated, and steamed in the same manner as whole macadamia nuts and apricot halves. In the case of apricot pieces, the halves were inoculated, dried, and then cut into pieces (8-10 mm) to avoid inoculum internalization through freshly cut surfaces.

2.1.2. Low-temperature, vacuum-assisted steam treatments

A laboratory-scale, low-temperature, vacuum-assisted steam delivery system was engineered in a Biosafety Level-2 pilot plant. Newkirk et al., 2018 reported the general design and use of the system, which included a canner cooker (25-QT canner cooker; All American®, 25 QT. #925, Manitowoc, WI) with a customized, valve-controlled steam inlet. Amendments were made to connect the system to a medium-pressure steam line and a regulating reducing station that lowered steam to <69 kPa. The steaming, sampling, enumeration, and injury-recovery methods are described in detail in Chapter 3.

Foods were steamed separately from one another. Apricot halves and pieces were treated at 72°C for 0, 0.5, 1, 2, 5, 8, 14, and 20 min and 82°C for 0, 0.5, 1, 1.5, 2, 2.5, 3.5, and 5 min. Macadamia nut pieces were also treated at 72 and 82°C for the same time intervals. Whole macadamia nuts, however, were treated for longer treatments than the dried fruit and nut pieces at 0, 1, 8, 14, 20, 28, and 38 min (72°C) and 0, 1, 2, 3.5, 5, 8, and 12 min (82°C) to achieve comparable reductions.

2.1.3. Experimental design and model development

Apricot halves (n=17), apricot pieces (n=17), whole macadamia nuts (n=15), and macadamia nut pieces (n=17) were treated with timed cycles at low and high temperatures, and each time/temperature combination was replicated 3-5 times. Order of steam cycles was randomly determined in advance.

Bacterial log reductions were calculated from initial and final bacterial concentrations (log CFU/g) of each treatment and converted on the basis of survival. Datasets of survivability for each of the pathogens treated with both temperatures on the whole macadamia nuts and apricot halves were evaluated by fit to three models that have been used to describe bacterial

inactivation on foods: first-order, Weibull, and Gompertz. Replicates of each treatment were pooled and the nlstools and nlsMicrobio packages in R (Version 3.6.1) and RStudio, Inc. (Version 1.1.456, Boston, MA) were used for model construction.

The first-order model parameters were estimated from the compiled data using the equation provided by Peleg, Normand, and Corradini (2005):

$$(1) \quad logN_t - logN_0 = -kt$$

where N_t is the surviving bacterial population at time, t; N_0 is the initial bacterial population; and k is the inactivation rate (log CFU/g min^{-1}).

The Weibull model was used as an alternative to identify inactivation trends that were not first-order or linear (Peleg, 2003):

$$(2) \quad logN_t - logN_0 = -k \cdot t^{\beta}$$

where $k = k' / \log_e 10$ and is a rate parameter for the reduction, and β indicates whether the shape is a concave upward (< 1), downward (>1), or linear (=1) inactivation curve.

The Gompertz model describes inactivation trends that present as a sigmoidal curve with the following modified equation provided by (Zwietering et al., 1990):

$$(3) \quad logN_t - logN_0 = A \cdot exp\left\{-exp\left[\frac{\mu_M \cdot e}{A}(\lambda - t) + 1\right]\right\}$$

where μ_M (log CFU/g min^{-1}) is the maximal value of inactivation, λ is the lag time (min), and A is the asymptote.

Akaike's Information Criteria (AIC) and root mean squared error (RMSE) were calculated and used to evaluate all three models for their fit to the whole macadamia nut and dried apricot datasets, as well as the pieces datasets.

2.2. Model challenge and validation

2.2.1. Models challenged using internal data

Primary models constructed according to section 2.1.3. were used to predict the times necessary to achieve 3-log reductions of bacteria on the whole macadamia nuts and dried apricot halves. The times were then compared to observed times necessary to result in 3-log reductions of the microorganisms on the smaller pieces of macadamia nuts and dried apricots. Raw error (min) was calculated as the difference between the predicted and observed times. Also calculated was relative error, as described later in section 2.2.3., which was a ratio of the raw error to the predicted times necessary for 3-log reductions on smaller pieces of the same food.

D- and *z*-values are commonly used to compare thermal resistance of microorganisms and have been used to predict inactivation of pathogens in foods. Experiments explained in Chapter 5 determined *D*- and *z*-values of STEC (O121:H19 and O157:H7) in reduced-water activity solutions (a_w 0.75 and 0.50) using the capillary tube method and isothermal treatments of 56, 59, and 62°C (Sharma et al., 2005). The reduced-water activity solutions (26% NaCl, a_w 0.75; 82% glycerol, a_w 0.50) were deemed comparable to macadamia nut and dried apricot water activity (a_w 0.68 and 0.58, respectively). Using the *z*-values, $D_{72°C}$ and $D_{82°C}$ were calculated and used to extrapolate times necessary for 3-log reductions. The times for 3-log reductions of STEC according to primary models of dried apricot and macadamia nut pieces (section 2.1.3.) were compared side-by-side to those of STEC in reduced-water activity solutions and presented as a comparison of thermal resistance of STEC in reduced-water activity environments and LWAF.

2.2.2. Models challenged using independent data

To determine if the primary models constructed according to section 2.1.3., of whole macadamia nuts and dried apricot halves could predict time necessary for 3-log bacterial reductions on other thermally treated LWAF, predicted and observed values were calculated and compared. While the primary models were not deterministic by definition, the predicted values

were point estimates. The predicted times according to the internally constructed primary models were compared against additional, independent data of thermally treated LWAF, which were considered "observed" values. Foods were compared on the basis of water activity and similarity of thermal treatments for direct comparisons and validation. Descriptions of each external dataset and respective data are outlined in Table 4.1. In one case, the external data reported were estimated times for 3-log reductions from a Weibull model of *Salmonella* Typhimurium DT104 on dried apricot paste (a_w 0.64; Mattick et al., 2001). The samples were treated within a sealed bag in a 74°C water bath, while an inoculated sugar solution of comparable water activity was heated directly. While the sugar solution was a vastly different type of inoculated sample, the water activity made the food an accurate comparison to the apricot paste and apricot halves. For another external dataset, *D*- and *z*-values were reported, which were used to extrapolate times for 3-log reductions of *Salmonella* Enteritidis PT30 on LWAF (almonds) treated with 70 and 80°C water (Harris et al., 2012). Other datasets of LWAF treated at similar temperatures, ranging from 70-80°C provided first-order and Weibull model parameters, which were used to extrapolate times for 3-log bacterial reductions (Daryaei et al., 2018, Rachon et al., 2016). Foods examined were a collection of powders of similar water activity to macadamia nuts and dried apricots. Savory seasoning (a_w 0.633), pet food (a_w 0.547), and confectionary mix (a_w 0.565) were inoculated with STEC, *L. monocytogenes*, *Salmonella*, and *Enterococcus faecium* NRRL B-2354, sealed in thermal-death-time disks, and treated within an 80°C oil bath.

Consideration was given to studies examining thermal reduction of pathogens from LWAF using vacuum-steam processes. However, in the studies available and known to the authors, these datasets were not within the scope of the presented work due to a limited amount of reported data or temperatures beyond 82°C, which made predictions of 3-log reductions at

comparable temperatures impossible. Gompertz models were not challenged with additional external data (beyond data internally collected for macadamia nut and apricot pieces), as no known datasets existed from similar thermal inactivation of pathogens on LWAF.

2.2.3. Evaluation of model performance

Raw error was calculated to quantify the difference between the predicted and observed values in minutes. Relative error was calculated to determine the fail-safe and fail-dangerous zone, as well as the acceptable zone for validation to determine if the model would be acceptable for additional usage using the following formula (Oscar, 2005):

$$RE = (observed - predicted)/predicted$$

with the targeted range for validation of accuracy between -0.30 – 0.15. If the fail-safe value was beyond 30% (-1.0 to -0.30) or the fail-dangerous value was beyond 15% (>0.15) more than the predicted value, the model was not considered validated for accuracy of predictions of the respective dataset (Oscar, 2005). However, fail-safe predictions between -1.0 to -0.30 were considered as safe, albeit excessive predicted times necessary for 3-log reductions.

To observe trends holistically and visually, heat maps of the relative error between each combination of internal and external 3-log reduction times were constructed. The maps indicated with color gradients the similarities of predicted (vertical axis) and observed (horizontal axis) values based on the fail-safe (blue) and fail-dangerous (red) interval. Heat maps compared the relative error or every possible combination of internal and external model predictions.

3. Results and Discussion

3.1. Vacuum-steam bacterial reductions and primary models

Log bacterial reductions were used to generate primary kinetic models of vacuum-steam-treated whole macadamia nuts and dried apricot halves, as well as pieces (Table 4.2). RMSE and

AIC were used to determine which models described or fit best each bacterial inactivation trend on each food type. For both dried apricot halves and pieces, the Gompertz model, in general, best described the bacterial inactivation. Bacterial inactivation on whole macadamia nuts and pieces were best fit to a Weibull model with upward concavity. The fits of the first-order models were not vastly different than those of the Weibull and Gompertz, but they were not ranked as best fit in any case.

*3.2. Comparison of reductions on LWAF and reduced-water activity solutions (*D- *and* z*-values)*

As described in Chapter 5, *D*- and *z*-values of STEC (O121:H19 and O157:H7) in reduced-water activity solutions (intermediate, a_w 0.75; low, a_w 0.50) were determined using isothermal treatments (56, 59, and 62°C) and the capillary tube method. From these values, $D_{72°C}$ and $D_{82°C}$ were calculated and used to extrapolate 3-log reductions at temperatures comparable to vacuum-steam temperatures, as described in section 2.2.1. (Table 4.3). *D*-values were also calculated from first-order models of the apricot and macadamia nut pieces and compared to those extrapolated from the STEC in reduced-water activity solutions. In this way, the inactivation kinetics of STEC under osmotic stress and isothermal treatments were compared to desiccation stress and non-isothermal treatments. Similarly, Mattick et al. constructed inactivation models that compared the death rates of *Salmonella* Typhimurium DT104 in a sugar solution (a_w 0.64) to a food of comparable water activity (2001). The sugar solution was directly heated with a submerged coil, while the inoculated foods were homogenized, spread into a single layer sealed in a bag, and placed in a heated water bath. Overall, their results showed that predictions from the sugar solutions overestimated the time needed to achieve 3-log reductions of *Salmonella* on various LWAF at temperatures between 55 and 74°C.

In contrast, the extrapolated times necessary for a 3-log reduction according to the calculated $D_{72°C}$ and $D_{82°C}$ in the presented work vastly underestimated the actual times of 3-log reductions on the LWAF (apricot halves and macadamia nuts; Table 4.3). The STEC strains displayed slower rates of inactivation on the dried foods compared to within the solutions. This may have been due to the differences in composition of the reduced-water activity solutions compared to the food matrices, cell desiccation during the drying step of the inoculated foods, or the heat transfer differences between the liquid samples in capillary tubes and steamed food samples. These results highlight that caution should be used in applying conclusions made from data acquired from laboratory media to a food matrix. Inappropriate extrapolation could result in under-processed foods with remaining pathogen populations.

3.3. Whole macadamia nuts and apricot halves models challenged with internal food pieces data

The primary models constructed according to section 2.1.3. of dried apricot and macadamia nuts (whole and pieces) were compared to determine if they could be applied in predicting times for 3-log reductions of one another. Relative error was calculated for each combination of model, food, and microorganism, which is displayed in the form of heat maps (Figures 4.4-4.9). Each heat map depicts a range of fail-safe (blue) to fail-dangerous (red) relative error values, with the inner cells (within bolded lines) showing internal data and outer cells showing results of external data that will be discussed in section 3.4. At both 72 and 82°C, first-order model predictions between apricot halves, apricot pieces, and macadamia nut pieces were relatively similar and showed that times required to reduce bacteria by 3-log were close to one another (indicated by blue and white colors). However, because whole macadamia nuts required longer treatments compared to the other products, the apricot halves, apricot pieces, and macadamia nut pieces models could predict appropriate treatment times for the whole nuts.

Similar observations were made for both the Webiull and Gompertz model predictions. Also notable were the capabilities of pathogen models to predict times for 3-log reductions of other pathogens, and the fail-safe predictions made by *Pediococcus* models on each food type for each pathogen.

Processors may design treatments and validations around reducing a particular target pathogen presumed to be most resistant to the treatment. However, our results indicated that the pathogens behaved similarly to one another, meaning a single, particularly resistant pathogen may not always be realistic. The heat maps prove that models of *Pediococcus acidilactici* could accurately predict times for reductions of multiple pathogens on the vacuum-steamed LWAF, making it an appropriate surrogate organism. For example, the Gompertz *Pediococcus* inactivation models on apricot halves predicted fail-safe times for 72 and 82°C steam treatments for 3-log reductions of every pathogen on apricot halves, apricot pieces, and macadamia nut pieces (Figures 4.8-4.9). The same was observed in other models, as well. Food composition has been observed to impact pathogen thermal resistance to varying degrees, so thermal interventions designed to target multiple pathogens based on the conservative surrogate model could be more widely applied (Juneja & Eblen, 2000).

In order to specifically determine if the whole macadamia nut and apricot halves models could be validated to predict the times for 3-log bacterial reductions on pieces of the same food, raw and relative error were calculated for specific models. The raw error was calculated to highlight the exact differences between predicted and observed times for 3-log bacterial reductions using each model (Figures 4.1-4.3). Relative error was calculated according to section 2.2.3 to compare predicted and observed values of respective whole and pieces models to indicate which predictions were within the fail-safe and fail-dangerous intervals (-0.30-0.15;

Tables 4.4-4.6). The interval was used as an indicator of accuracy and to determine if a model could be validated for use for the same food with smaller particle sizes. First-order models of dried apricot halves were accurate predictors of 3-log reductions of respective bacteria on apricot pieces 100% of the time (Figure 4.1, Table 4.4). The Weibull models accurately predicted reductions on apricot pieces 63% of the time (Figure 4.2, Table 4.5), while the Gompertz model accurately predicted times for 3-log reductions only 50% of the time (Figure 4.3, Table 4.6). These predictions, particularly from the first-order model, provided insight into the relative flexibility of the models constructed to describe bacterial inactivation on apricot halves. The dried apricot pieces were inoculated and steamed in identical manners, which was reflected in the similar inactivation curves and extrapolated times for 3-log reductions by the first-order models.

In contrast, for macadamia nuts, there was no instance in which the primary models constructed for whole macadamia nuts accurately predicted the time for 3-log reductions of bacteria on macadamia nut pieces (Figures 4.1-4.3, Tables 4.4-4.6), with the relative error consistently <-0.30, indicating extreme fail-safe predictions. However, in each of these cases, the fail-safe predicted times would result in a presumably safer product, though over-processing could be of some concern. The reason for excessively longer treatments is not fully understood, since product composition and handling were designed to be as identical as possible. The whole macadamia nuts and pieces had comparable composition, as confirmed by proximate analyses, and the size of the particles was the only known difference in physical characteristics, other than a slight difference in inoculation of macadamia nut pieces. Whereas the apricot halves were inoculated and then cut into smaller pieces, the macadamia nut pieces were purchased as smaller particles meant for baking, which were then inoculated. It is likely that the difference in size

between the large and small counterparts impacted the flow of steam throughout the sample, as well as the conductive properties of the sample, which would also impact pathogen inactivation.

3.4. Comparison of models using external, independent data of thermally treated LWAF

External, independent data was also used to challenge the models' abilities to predict times required for 3-log reductions of bacteria on other LWAF to determine the accuracy or flexibility of the constructed models, as described in section 2.2.2. Data was acquired for almonds, apricot paste, sugar solutions with reduced water activity, savory seasoning powder, pet food, and a confectionary mix that were inoculated with various pathogens and thermally treated using a variety of methods (Daryaei et al., 2018; Harris et al., 2012; Mattick et al., 2001; Rachon et al., 2016). Descriptions of reported data are in Table 4.1.

Heat maps (Figures 4.4-4.7) provided comparisons of each model's ability to predict 3-log reductions for all other food types, pathogens, and thermal processes based on relative error. Specifically, the comparisons involving external data are displayed in the outermost cells. Heat maps of the first order models (Figures 4.4-4.5) indicated that the models constructed internally could accurately and safely predict the thermal treatment times required for 3-log reductions of *Salmonella* on almonds and an STEC cocktail on pet food and seasoning powder, noted by the blue-white gradient. However, the internally constructed models could not safely predict required times for reductions of *Salmonella* on confectionary mix and seasoning powder, as indicated by the red gradient. The internally constructed Weibull models accurately predicted safe treatment times at 72°C (Figure 4.6) required for 3-log reductions of *Salmonella* in apricot paste and a sugar solution of a comparable water activity. However, times predicted for the confectionary seasoning inoculated for confectionary mix inoculated with *L. monocytogenes* were beyond the fail-dangerous interval in many of the cases. At 82°C, the many of the predicted

times from the Weibull models (Figure 4.7) fit to internal data of macadamia nuts and dried apricots were lower (fail-dangerous) than the observed or extrapolated times required for 3-log bacterial reductions on other LWAF. However, 50% of the whole macadamia nut models' predictions were considered fail-safe. Direct comparisons that determine whether or not internally constructed models could be validated for accurate predictions of other thermally treated LWAF are listed in Tables 4.4-4.5.

One of the externally acquired datasets investigated thermal inactivation of *Enterococcus faecium* NRRL B-2354 on several LWAF (Rachon et al., 2016). Their purpose was to determine if the non-pathogenic microorganism could serve as a surrogate for pathogens on thermally treated powders and culinary mixes. Similarly, the presented study included *Pediococcus acidilactici* in the inoculation of LWAF for the same objective. The extrapolated and observed times for 3-log reductions of these two surrogate organisms on LWAF were compared directly according to first-order and Weibull models (Tables 4.4-4.5). It was determined that the models of *Pediococcus* on thermally treated LWAF could not yield accurate predictions of thermal inactivation of *E. faecium* on other thermally treated LWAF. Rather, *E. faecium* required much longer treatments to achieve 3-log reductions. Both *Pediococcus* and *E. faecium* have been considered appropriate surrogates for certain pathogens, foods, and processes, but several studies have noted that *E. faecium* occasionally requires excessive treatments (Rachon et al., 2016; Tsai et al., 2019; Verma et al., 2018). In many cases, the times predicted for a 3-log reduction of *Pediococcus* were slight overestimations than what was observed for 3-log reductions of pathogens from external LWAF datasets, as seen by the heat maps (Figures 4.4-4.7). Similarly, the times predicted by *E. faecium* were overestimations for 3-log inactivations of pathogens on other LWAF. If the inactivation trend of the surrogate is not comparable to that of the target

pathogen, under- or over-processing of the food may occur. While the two non-pathogenic, surrogate organisms could not be used interchangeably to predict times for 3-log reductions, both surrogates were capable of prediction inactivation of multiple pathogens for different treatments, which was evident by the trends depicted by heat maps. Consideration of this point is key when choosing a surrogate, as well as recognition that surrogate organisms can behave differently and are not interchangeable, as observed when comparing the aforementioned research.

In summary, the internally constructed models of bacterial inactivation on dried apricots and macadamia nuts were able to predict fail-safe times required for 3-log reductions for one another. These results indicate that not only could the *Pediococcus* models on LWAF be used for predicting reductions of multiple pathogens on various foods, the internally constructed models of each of the pathogens were not vastly dissimilar. LWAF processors may find this valuable if treating a variety of foods. Furthermore, interventions could be designed to target several different pathogen genera. Internally constructed models had varied success in predicting fail-safe times for bacterial reductions on other thermally treated LWAF from external datasets. Similarly, externally developed models had varied success in predicting reductions observed according to the internal datasets of vacuum-steamed dried apricots and macadamia nuts. Experiments that yielded the external data used for comparison implemented a variety of heating methodologies, such as hot water, sealed bags within a water bath, and thermal cells within an oil bath. These resulted in differences of heat transfer, which surely impacted the efficacy of the thermal treatments and resulting parameters (and respective confidence intervals) of the kinetic models.

In addition to the variety of heating mechanisms used in the included study, the differences between food matrices likely contributed to the differences in reduction. The food

microenvironment is complex, and composition has been noted to impact inactivation, such as fat content or type of solute, which can enhance or reduce thermal stress (Goepfert et al., 1970; Juneja & Eblen, 2000, Mattick et al., 2001). Models that describe pathogen inactivation on low-water activity foods (LWAF) have the additional difficulty of capturing and describing the degree of increased thermal resistance due to the desiccation stress that has been noted for many foodborne pathogens (Archer et al., 1998; Grasso, Stam, Anderson, & Krishnamurthy, 2014; Lang, et al., 2017; Laroche & Gervais, 2003; Laroche et al., 2005). The foods used in external experiments ranged from powders, pastes, and nuts. For each these experiments, both inoculation and recovery methods were equally diverse, and it is unlikely that any single model could truly account for each of these experimental variables. As described by the trends noted in the heat maps and the model fits, pathogen inactivation trends seemed to be driven by the type of food, with influence of thermal treatment and experimental methods. These results indicate that treatment parameters and food composition require further investigation for their effects on pathogen response to thermal treatments.

Some criticisms of primary models point out the limited application, as they may sometimes be used to provide point estimates of inactivation without taking into account variability associated with the food processing environment (such as changes to pH, temperature, or water activity) or the heterogenous nature of contaminating populations (Aspridou et al., 2019, van Boekel, 2008). While the primary models constructed of macadamia nuts and dried apricots (whole and pieces) were not consistently accurate predictors for times necessary for 3-log bacterial reductions from thermally treated LWAF, the data presented indicated some success and that food type likely had great impacts on the inactivation trends and predictions. Secondary models, as opposed to primary models, use data from both primary models and environmental

factors to describe the effects of processing conditions on the model parameters (Bevilacqua et al., 2015; Marks, 2008). For example, the effects of changes in temperature on bacterial response can be illustrated through the determination of a *z*-value derived from multiple *D*-values. Other environmental factors, such as pH, pressure, or water activity, can also be incorporated into secondary models, as well as properties inherent to the foods, like size or composition. While these types of models may be more robust, they require extensive data collection followed by validation (van Doornmalen and Kopinga, 2008). The information presented in the current study can guide researchers in building secondary models by comparing pathogen inactivation under various circumstances, highlighting variables that may drive the inactivation and efficacy of the thermal treatments. Future work should continue to build on the presented models or others to characterize bacterial inactivation, measuring the effects of various manipulated variables like temperature or food composition.

4. Conclusions

The presented study showed that bacterial inactivation using vacuum-assisted steam on macadamia nuts and dried apricots could be described with first-order, Weibull, and modified Gompertz models. The models for these datasets fit well, according to the RMSE and AIC. However, the prediction capabilities were limited. First-order inactivation models of microorganisms on apricot halves predicted the respective inactivation on apricot pieces very successfully, but the Weibull and Gompertz models provided fewer accurate predictions, overestimating or underestimating the inactivation several times. The models of bacterial reductions on whole macadamia nuts overestimated the required time to achieve a 3-log reduction of the bacteria on macadamia nut pieces in all instances. While an overestimation would be considered fail-safe, vacuum-steam processing the macadamia nut pieces for the

predicted times could result in over-processing of the food, though these effects are unknown. Using externally acquired data from the literature, it was also seen that the primary models of bacterial inactivation on whole macadamia nuts and dried apricot halves were often unsuccessful at predicting times for 3-log pathogen reductions. In a few cases, the models overestimated the required processing times. These results highlight the caution of applying empirical models of pathogen inactivation on food matrices to other processes or foods.

Figures

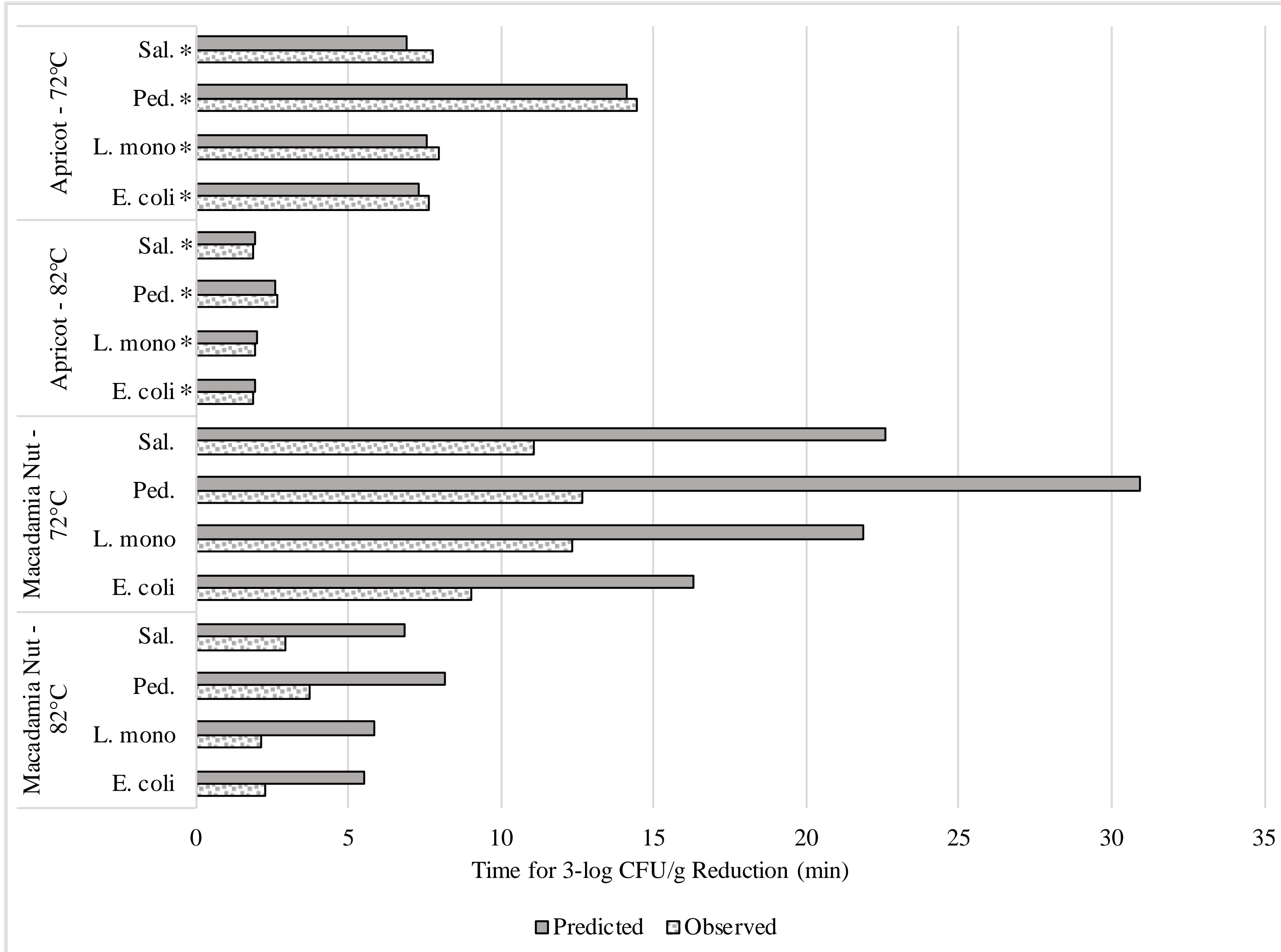

Figure 4.1. Comparisons of predicted vs. observed times for 3-log reductions made by first-order models of *Salmonella*, *Pediococcus*, *L. monocytogenes*, and *E. coli* on whole macadamia nuts (predicted) vs. macadamia nut pieces (observed) and apricot halves (predicted) vs. apricot pieces (observed) vacuum-steamed at 72 and 82°C. * indicates relative error values within the acceptable range (-0.30-0.15).

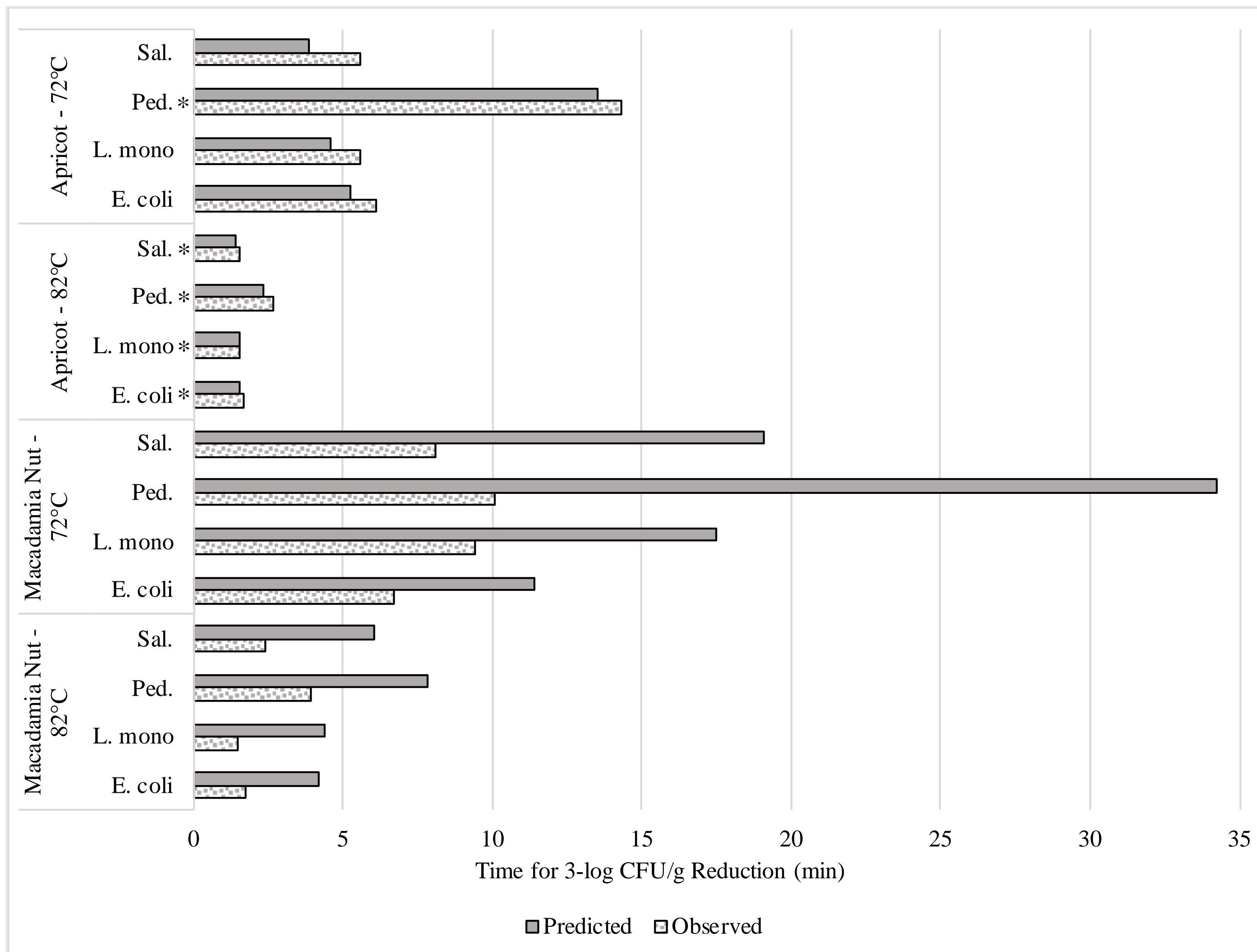

Figure 4.2. Comparisons of predicted vs. observed times for 3-log reductions made by Weibull models of *Salmonella, Pediococcus, L. monocytogenes*, and *E. coli* on whole macadamia nuts (predicted) vs. macadamia nut pieces (observed) and apricot halves (predicted) vs. apricot pieces (observed) vacuum-steamed at 72 and 82°C. * indicates relative error values within the acceptable range (-0.30-0.15).

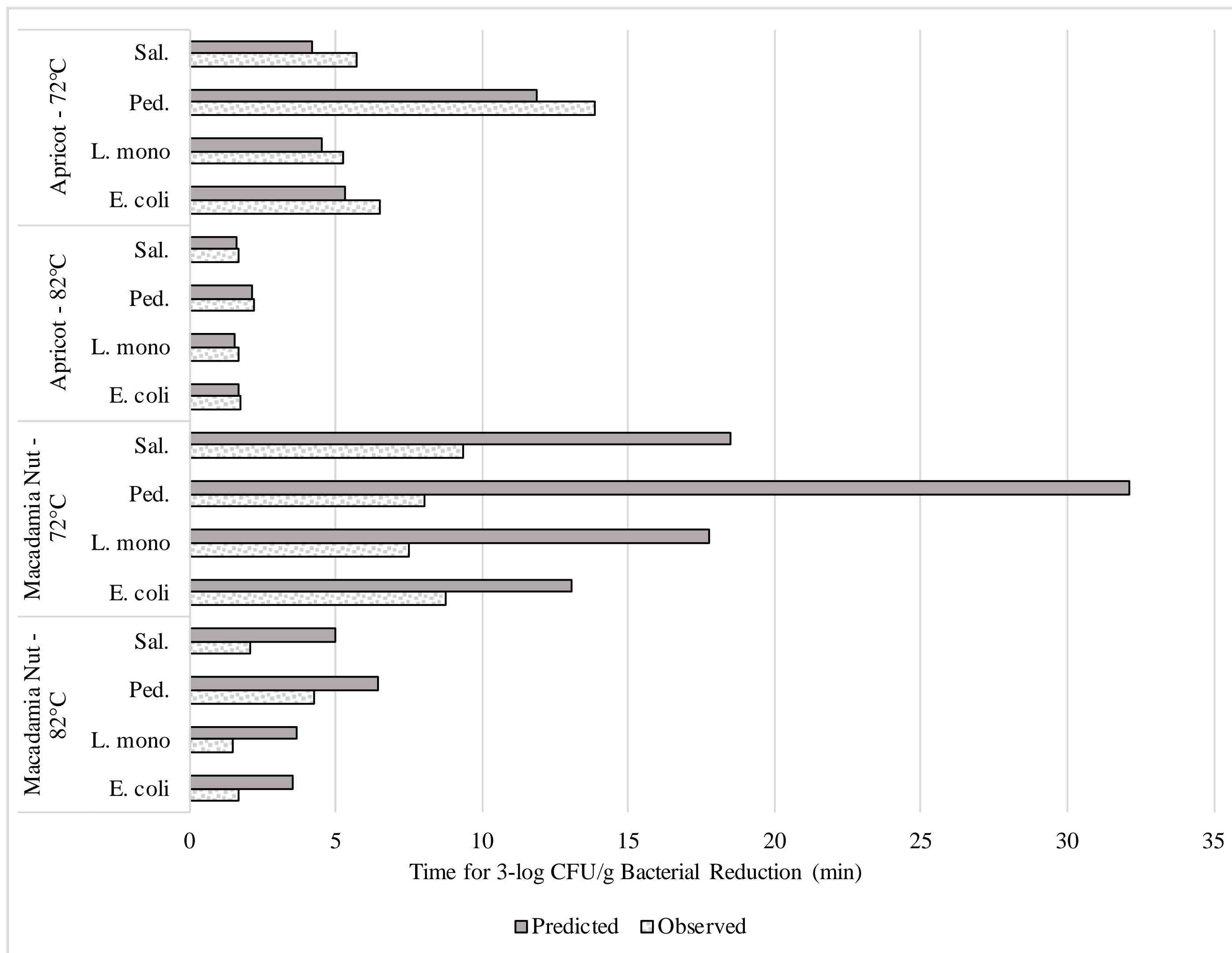

Figure 4.3. Comparisons of predicted vs. observed times for 3-log reductions made by Gompertz models of *Salmonella, Pediococcus, L. monocytogenes*, and *E. coli* on whole macadamia nuts (predicted) vs. macadamia nut pieces (observed) and apricot halves (predicted) vs. apricot pieces (observed) vacuum-steamed at 72 and 82°C. * indicates relative error values within the acceptable range (-0.30-0.15).

		Apricot Halves[a]				Whole Macadamia Nuts[a]				Apricot Pieces[a]				Macadamia Nut Pieces[a]				Almond[e]	Pet food[c]	Seasoning[b]	Confectionary[d]
		Eco	Lmo	Ped	Sal	Eco	Lmo	Ped	Sal	Eco	Lmo	Ped	Sal	Eco	Lmo	Ped	Sal	Sal	Eco	Eco	Sal
Apricot Halves[a]	Eco	0.00	0.04	0.94	-0.05	1.24	2.00	3.25	2.11	0.05	0.09	0.98	0.07	0.24	0.69	0.74	0.52	-0.51	-0.53	0.07	5.50
	Lmo	-0.04	0.00	0.86	-0.09	1.15	1.88	3.08	1.98	0.00	0.05	0.90	0.02	0.19	0.62	0.67	0.46	-0.53	-0.55	0.02	5.24
	Ped	-0.48	-0.46	0.00	-0.51	0.16	0.55	1.20	0.60	-0.46	-0.44	0.02	-0.45	-0.36	-0.13	-0.10	-0.21	-0.74	-0.76	-0.45	2.36
	Sal	0.06	0.10	1.05	0.00	1.37	2.18	3.50	2.29	0.11	0.16	1.10	0.13	0.31	0.79	0.84	0.61	-0.48	-0.50	0.13	5.88
Whole Macadamia Nuts[a]	Eco	-0.55	-0.54	-0.14	-0.58	0.00	0.34	0.90	0.38	-0.53	-0.51	-0.12	-0.52	-0.45	-0.25	-0.22	-0.32	-0.78	-0.79	-0.52	1.90
	Lmo	-0.67	-0.65	-0.36	-0.69	-0.25	0.00	0.42	0.03	-0.65	-0.64	-0.34	-0.64	-0.59	-0.44	-0.42	-0.49	-0.84	-0.84	-0.64	1.16
	Ped	-0.76	-0.75	-0.54	-0.78	-0.47	-0.29	0.00	-0.27	-0.75	-0.74	-0.53	-0.75	-0.71	-0.60	-0.59	-0.64	-0.88	-0.89	-0.75	0.53
	Sal	-0.68	-0.66	-0.38	-0.70	-0.28	-0.03	0.37	0.00	-0.66	-0.65	-0.36	-0.66	-0.60	-0.46	-0.44	-0.51	-0.84	-0.85	-0.66	1.09
Apricot Pieces[a]	Eco	-0.05	0.00	0.85	-0.10	1.14	1.87	3.06	1.97	0.00	0.04	0.89	0.02	0.18	0.62	0.66	0.45	-0.53	-0.55	0.02	5.21
	Lmo	-0.08	-0.05	0.77	-0.13	1.05	1.75	2.89	1.84	-0.04	0.00	0.81	-0.02	0.14	0.55	0.59	0.39	-0.55	-0.57	-0.02	4.95
	Ped	-0.50	-0.47	-0.02	-0.52	0.13	0.52	1.15	0.57	-0.47	-0.45	0.00	-0.46	-0.37	-0.15	-0.12	-0.23	-0.75	-0.76	-0.46	2.28
	Sal	-0.06	-0.02	0.81	-0.12	1.10	1.81	2.98	1.91	-0.02	0.02	0.86	0.00	0.16	0.58	0.63	0.43	-0.54	-0.56	0.00	5.09
Macadamia Nut Pieces[a]	Eco	-0.19	-0.16	0.56	-0.24	0.81	1.42	2.43	1.50	-0.16	-0.12	0.60	-0.14	0.00	0.36	0.40	0.23	-0.60	-0.62	-0.14	4.24
	Lmo	-0.41	-0.38	0.14	-0.44	0.33	0.78	1.51	0.84	-0.38	-0.35	0.17	-0.37	-0.27	0.00	0.03	-0.10	-0.71	-0.72	-0.37	2.84
	Ped	-0.43	-0.40	0.11	-0.46	0.29	0.73	1.44	0.78	-0.40	-0.37	0.14	-0.39	-0.29	-0.03	0.00	-0.13	-0.72	-0.73	-0.39	2.73
	Sal	-0.34	-0.32	0.27	-0.38	0.47	0.97	1.79	1.04	-0.31	-0.28	0.30	-0.30	-0.18	0.11	0.14	0.00	-0.68	-0.69	-0.30	3.27
Almond[e]	Sal	1.02	1.11	2.92	0.91	3.54	5.08	7.60	5.28	1.12	1.21	3.01	1.16	1.51	2.42	2.52	2.08	0.00	-0.04	1.16	12.15
Pet food[c]	Eco	1.11	1.20	3.09	1.00	3.74	5.34	7.98	5.56	1.21	1.31	3.18	1.26	1.62	2.57	2.68	2.21	0.04	0.00	1.26	12.74
Seasoning[b]	Eco	-0.06	-0.02	0.81	-0.12	1.10	1.81	2.98	1.91	-0.02	0.02	0.86	0.00	0.16	0.58	0.63	0.43	-0.54	-0.56	0.00	5.09
Confectionary[d]	Sal	-0.85	-0.84	-0.70	-0.85	-0.66	-0.54	-0.35	-0.52	-0.84	-0.83	-0.70	-0.84	-0.81	-0.74	-0.73	-0.77	-0.92	-0.93	-0.84	0.00

[a]Primary model predictions made for 3-log reductions of bacteria from models constructed per section 2.1.3.
[b]Data acquired from Daryaei et al., 2018 of STEC reduction on savory seasoning treated with 80°C. A 3-log reduction at 72 and 82°C was predicted using the reported *D*- and *z*-values and used as the observed value.
[c]Data acquired from Daryaei et al., 2018 of STEC reduction on pet food treated with 80°C. A 3-log reduction at 72 and 82°C was predicted using the reported *D*- and *z*-values and used as the observed value.
[d]Data acquired from Rachon et al., 2016 of *L. monocytogenes*, *Enterococcus faecium* NRRL B-2354, and *Salmonella* reduction on a confectionary mix treated with 70 and 80°C. A 3-log reduction at 72 and 82°C was predicted using the reported *D*- and *z*-values and used as the observed value.
[e]Data acquired from Harris et al., 2012 of *Salmonella* Enteritidis PT30 reduction on almond kernels treated with 70 and 80°C hot water.

Figure 4.4. Heat map of relative error between predicted and observed times required for 3-log reductions of microorganisms based on first-order model parameters of externally acquired data of thermally treated LWAF at 72°C. Area within the bold lines reflects internal generated data compared using the same vacuum-steam process. The acceptable relative error ranged from -0.30 (fail-safe; blue) to 0.15 (fail-dangerous; red). Externally acquired data is blocked and on the outside of the graph.

		Apricot Halves [a]				Whole Macadamia Nuts [a]				Apricot Pieces [a]				Macadamia Nut Pieces [a]				Almond [e]	Seasoning [b]	Pet food [c]	Confectionary [d]	Confectionary [d]
		Eco	Lmo	Ped	Sal	Eco	Lmo	Ped	Sal	Eco	Lmo	Ped	Sal	Eco	Lmo	Ped	Sal	Sal	Eco	Eco	Sal	Lmo
Apricot Halves [a]	Eco	0.00	0.04	0.36	0.00	1.90	2.05	3.28	2.57	-0.02	0.01	0.41	-0.02	0.21	0.11	0.96	0.53	0.18	-0.48	-0.61	11.59	2.53
	Lmo	-0.04	0.00	0.30	-0.04	1.78	1.92	3.11	2.42	-0.06	-0.03	0.36	-0.06	0.16	0.07	0.88	0.47	0.13	-0.50	-0.63	11.08	2.39
	Ped	-0.26	-0.23	0.00	-0.26	1.14	1.25	2.16	1.63	-0.28	-0.26	0.04	-0.28	-0.11	-0.18	0.45	0.13	-0.13	-0.62	-0.72	8.28	1.60
	Sal	0.00	0.04	0.36	0.00	1.90	2.05	3.28	2.57	-0.02	0.01	0.41	-0.02	0.21	0.11	0.96	0.53	0.18	-0.48	-0.61	11.59	2.53
Whole Macadamia Nuts [a]	Eco	-0.66	-0.64	-0.53	-0.66	0.00	0.05	0.48	0.23	-0.66	-0.65	-0.51	-0.66	-0.58	-0.62	-0.32	-0.47	-0.59	-0.82	-0.87	3.34	0.22
	Lmo	-0.67	-0.66	-0.55	-0.67	-0.05	0.00	0.41	0.17	-0.68	-0.67	-0.54	-0.68	-0.60	-0.63	-0.36	-0.50	-0.61	-0.83	-0.87	3.13	0.16
	Ped	-0.77	-0.76	-0.68	-0.77	-0.32	-0.29	0.00	-0.17	-0.77	-0.77	-0.67	-0.77	-0.72	-0.74	-0.54	-0.64	-0.72	-0.88	-0.91	1.94	-0.18
	Sal	-0.72	-0.71	-0.62	-0.72	-0.19	-0.15	0.20	0.00	-0.73	-0.72	-0.60	-0.73	-0.66	-0.69	-0.45	-0.57	-0.67	-0.85	-0.89	2.53	-0.01
Apricot Pieces [a]	Eco	0.02	0.06	0.38	0.02	1.96	2.11	3.37	2.64	0.00	0.03	0.44	0.00	0.23	0.14	1.00	0.56	0.20	-0.47	-0.61	11.84	2.60
	Lmo	-0.01	0.04	0.35	-0.01	1.88	2.03	3.26	2.54	-0.03	0.00	0.40	-0.03	0.20	0.11	0.95	0.52	0.17	-0.49	-0.62	11.51	2.51
	Ped	-0.29	-0.26	-0.04	-0.29	1.05	1.16	2.03	1.52	-0.31	-0.29	0.00	-0.31	-0.15	-0.21	0.39	0.08	-0.17	-0.63	-0.73	7.91	1.50
	Sal	0.02	0.06	0.38	0.02	1.96	2.11	3.37	2.64	0.00	0.03	0.44	0.00	0.23	0.14	1.00	0.56	0.20	-0.47	-0.61	11.84	2.60
Macadamia Nut Pieces [a]	Eco	-0.17	-0.14	0.12	-0.17	1.40	1.53	2.55	1.96	-0.19	-0.17	0.17	-0.19	0.00	-0.08	0.63	0.27	-0.02	-0.57	-0.68	9.44	1.92
	Lmo	-0.10	-0.07	0.22	-0.10	1.60	1.73	2.84	2.20	-0.12	-0.10	0.27	-0.12	0.08	0.00	0.76	0.37	0.06	-0.54	-0.65	10.29	2.16
	Ped	-0.49	-0.47	-0.31	-0.49	0.48	0.55	1.18	0.82	-0.50	-0.49	-0.28	-0.50	-0.39	-0.43	0.00	-0.22	-0.40	-0.74	-0.80	5.41	0.80
	Sal	-0.35	-0.32	-0.11	-0.35	0.90	1.00	1.80	1.33	-0.36	-0.34	-0.08	-0.36	-0.21	-0.27	0.29	0.00	-0.23	-0.66	-0.75	7.24	1.31
Almond [e]	Sal	-0.15	-0.12	0.15	-0.15	1.46	1.59	2.64	2.03	-0.17	-0.15	0.20	-0.17	0.02	-0.05	0.67	0.30	0.00	-0.56	-0.67	9.69	1.99
Seasoning [b]	Eco	0.93	1.01	1.62	0.93	4.60	4.88	7.27	5.88	0.89	0.94	1.73	0.89	1.33	1.15	2.79	1.95	1.27	0.00	-0.26	23.29	5.81
Pet food [c]	Eco	1.59	1.70	2.52	1.59	6.52	6.90	10.11	8.25	1.54	1.61	2.66	1.54	2.13	1.89	4.09	2.96	2.05	0.34	0.00	31.64	8.15
Confectionary [d]	Sal	-0.92	-0.92	-0.89	-0.92	-0.77	-0.76	-0.66	-0.72	-0.92	-0.92	-0.89	-0.92	-0.90	-0.91	-0.84	-0.88	-0.91	-0.96	-0.97	0.00	-0.72
Confectionary [d]	Lmo	-0.72	-0.70	-0.62	-0.72	-0.18	-0.14	0.21	0.01	-0.72	-0.71	-0.60	-0.72	-0.66	-0.68	-0.44	-0.57	-0.67	-0.85	-0.89	2.57	0.00
Confectionary [d]	E.fae	-0.91	-0.91	-0.88	-0.91	-0.74	-0.73	-0.62	-0.69	-0.91	-0.91	-0.88	-0.91	-0.89	-0.90	-0.83	-0.87	-0.90	-0.95	-0.97	0.11	-0.69
Seasoning [f]	Sal	-0.67	-0.65	-0.55	-0.67	-0.03	0.02	0.43	0.19	-0.67	-0.66	-0.53	-0.67	-0.60	-0.63	-0.35	-0.49	-0.61	-0.83	-0.87	3.20	0.18
Seasoning [f]	Sal	-0.76	-0.75	-0.68	-0.76	-0.31	-0.27	0.03	-0.15	-0.77	-0.76	-0.66	-0.77	-0.71	-0.73	-0.53	-0.63	-0.72	-0.88	-0.91	2.02	-0.16
Seasoning [f]	E.fae	-0.89	-0.89	-0.85	-0.89	-0.68	-0.66	-0.53	-0.61	-0.89	-0.89	-0.84	-0.89	-0.87	-0.88	-0.78	-0.83	-0.87	-0.94	-0.96	0.39	-0.61

[a]Primary model predictions made for 3-log reductions of bacteria from models constructed per section 2.1.3.
[b]Data acquired from Daryaei et al., 2018 of STEC reduction on savory seasoning treated with 80°C. A 3-log reduction at 72 and 82°C was predicted using the reported *D*- and *z*-values and used as the observed value.
[c]Data acquired from Daryaei et al., 2018 of STEC reduction on pet food treated with 80°C. A 3-log reduction at 72 and 82°C was predicted using the reported *D*- and *z*-values and used as the observed value.
[d]Data acquired from Rachon et al., 2016 of *L. monocytogenes*, *Enterococcus faecium* NRRL B-2354, and *Salmonella* reduction on a confectionary mix treated with 70 and 80°C. A 3-log reduction at 72 and 82°C was predicted using the reported *D*- and *z*-values and used as the observed value.
[e]Data acquired from Harris et al., 2012 of *Salmonella* Enteritidis PT30 reduction on almond kernels treated with 70 and 80°C hot water.
[f]Data acquired from Rachon et al., 2016 of *L. monocytogenes*, *Enterococcus faecium* B-2354, and *Salmonella* reduction on savory seasoning treated with 80°C. A 3-log reduction at 72 and 82°C was predicted using the reported *D*- and *z*-values and used as the observed value.

Figure 4.5. Heat map of relative error between predicted and observed times required for 3-log reductions of microorganisms based on first-order model parameters of externally acquired data of thermally treated LWAF at 82°C. Area within the bold lines reflects internal generated data compared using the same vacuum-steam process. The acceptable relative error ranged from -0.30 (fail-safe; blue) to 0.15 (fail-dangerous; red). Externally acquired data is blocked and on the outside of the graph.

		Confectionary [d] E.fae	Seasoning [f] Sal	Seasoning [f] Sal	Seasoning [f] E.fae
Apricot Halves [a]	Eco	10.36	2.00	3.17	8.08
	Lmo	9.91	1.88	3.01	7.71
	Ped	7.38	1.21	2.08	5.69
	Sal	10.36	2.00	3.17	8.08
Whole Macadamia Nuts [a]	Eco	2.92	0.03	0.44	2.13
	Lmo	2.73	-0.02	0.37	1.98
	Ped	1.65	-0.30	-0.03	1.12
	Sal	2.19	-0.16	0.17	1.55
Apricot Pieces [a]	Eco	10.59	2.06	3.26	8.26
	Lmo	10.29	1.98	3.15	8.02
	Ped	7.04	1.12	1.96	5.43
	Sal	10.59	2.06	3.26	8.26
Macadamia Nut Pieces [a]	Eco	8.42	1.49	2.46	6.53
	Lmo	9.19	1.69	2.74	7.14
	Ped	4.79	0.53	1.13	3.62
	Sal	6.44	0.96	1.73	4.95
Almond [e]	Sal	8.65	1.55	2.54	6.71
Seasoning [b]	Eco	20.93	4.79	7.06	16.52
Pet food [c]	Eco	28.46	6.78	9.83	22.54
Confectionary [d]	Sal	-0.10	-0.76	-0.67	-0.28
Confectionary [d]	Lmo	2.22	-0.15	0.18	1.57
Confectionary [d]	E.fae	0.00	-0.74	-0.63	-0.20
Seasoning [f]	Sal	2.79	0.00	0.39	2.03
Seasoning [f]	Sal	1.72	-0.28	0.00	1.17
Seasoning [f]	E.fae	0.25	-0.67	-0.54	0.00

[a]Primary model predictions made for 3-log reductions of bacteria from models constructed per section 2.1.3.

[b]Data acquired from Daryaei et al., 2018 of STEC reduction on savory seasoning treated with 80°C. A 3-log reduction at 72 and 82°C was predicted using the reported *D*- and *z*-values and used as the observed value.

[c]Data acquired from Daryaei et al., 2018 of STEC reduction on pet food treated with 80°C. A 3-log reduction at 72 and 82°C was predicted using the reported *D*- and *z*-values and used as the observed value.

[d]Data acquired from Rachon et al., 2016 of *L. monocytogenes*, *Enterococcus faecium* NRRL B-2354, and *Salmonella* reduction on a confectionary mix treated with 70 and 80°C. A 3-log reduction at 72 and 82°C was predicted using the reported *D*- and *z*-values and used as the observed value.

[e]Data acquired from Harris et al., 2012 of *Salmonella* Enteritidis PT30 reduction on almond kernels treated with 70 and 80°C hot water.

[f]Data acquired from Rachon et al., 2016 of *L. monocytogenes*, *Enterococcus faecium* B-2354, and *Salmonella* reduction on savory seasoning treated with 80°C. A 3-log reduction at 72 and 82°C was predicted using the reported *D*- and *z*-values and used as the observed value.

Figure 4.5 cont. Heat map of relative error between predicted and observed times required for 3-log reductions of microorganisms based on first-order model parameters of externally acquired data of thermally treated LWAF at 82°C. Area within the bold lines reflects internal generated data compared using the same vacuum-steam process. The acceptable relative error ranged from -0.30 (fail-safe; blue) to 0.15 (fail-dangerous; red). Externally acquired data is blocked and on the outside of the graph.

		Apricot Halves [a]				Whole Macadamia Nuts [a]				Apricot Pieces [a]				Macadamia Nut Pieces [a]				Apricot paste [b]	Sugar solution [c]	Confectionary [d]
		Eco	Lmo	Ped	Sal	Eco	Lmo	Ped	Sal	Eco	Lmo	Ped	Sal	Eco	Lmo	Ped	Sal	Sal	Sal	Lmo
Apricot Halves	Eco	0.00	-0.12	1.58	-0.27	1.18	2.34	5.53	2.64	0.16	0.06	1.73	0.06	0.28	0.80	0.92	0.54	-0.81	-0.52	1.77
	Lmo	0.14	0.00	1.93	-0.17	1.48	2.80	6.42	3.14	0.32	0.20	2.10	0.21	0.45	1.04	1.18	0.75	-0.78	-0.46	2.14
	Ped	-0.61	-0.66	0.00	-0.72	-0.15	0.30	1.53	0.41	-0.55	-0.59	0.06	-0.59	-0.50	-0.30	-0.26	-0.40	-0.93	-0.81	0.07
	Sal	0.36	0.20	2.52	0.00	1.98	3.56	7.91	3.97	0.59	0.45	2.72	0.45	0.74	1.45	1.62	1.10	-0.74	-0.35	2.77
Whole Macadamia Nuts	Eco	-0.54	-0.60	0.18	-0.66	0.00	0.53	1.99	0.67	-0.47	-0.51	0.25	-0.51	-0.41	-0.18	-0.12	-0.29	-0.91	-0.78	0.27
	Lmo	-0.70	-0.74	-0.23	-0.78	-0.35	0.00	0.95	0.09	-0.65	-0.68	-0.18	-0.68	-0.62	-0.46	-0.43	-0.54	-0.94	-0.86	-0.17
	Ped	-0.85	-0.87	-0.61	-0.89	-0.67	-0.49	0.00	-0.44	-0.82	-0.84	-0.58	-0.84	-0.80	-0.73	-0.71	-0.76	-0.97	-0.93	-0.58
	Sal	-0.73	-0.76	-0.29	-0.80	-0.40	-0.08	0.79	0.00	-0.68	-0.71	-0.25	-0.71	-0.65	-0.51	-0.47	-0.58	-0.95	-0.87	-0.24
Apricot Pieces	Eco	-0.14	-0.24	1.22	-0.37	0.88	1.87	4.61	2.13	0.00	-0.09	1.34	-0.09	0.10	0.54	0.65	0.32	-0.84	-0.59	1.38
	Lmo	-0.06	-0.17	1.43	-0.31	1.06	2.16	5.16	2.44	0.10	0.00	1.57	0.00	0.21	0.70	0.81	0.45	-0.82	-0.55	1.61
	Ped	-0.63	-0.68	-0.05	-0.73	-0.20	0.23	1.39	0.34	-0.57	-0.61	0.00	-0.61	-0.53	-0.34	-0.30	-0.44	-0.93	-0.83	0.01
	Sal	-0.06	-0.17	1.42	-0.31	1.05	2.14	5.14	2.43	0.09	0.00	1.56	0.00	0.20	0.69	0.81	0.45	-0.82	-0.55	1.60
Macadamia Nut Pieces	Eco	-0.22	-0.31	1.02	-0.43	0.71	1.62	4.11	1.85	-0.09	-0.17	1.14	-0.17	0.00	0.41	0.50	0.21	-0.85	-0.63	1.17
	Lmo	-0.44	-0.51	0.44	-0.59	0.22	0.86	2.64	1.03	-0.35	-0.41	0.52	-0.41	-0.29	0.00	0.07	-0.14	-0.89	-0.73	0.54
	Ped	-0.48	-0.54	0.34	-0.62	0.14	0.74	2.40	0.90	-0.39	-0.45	0.42	-0.45	-0.34	-0.07	0.00	-0.20	-0.90	-0.75	0.44
	Sal	-0.35	-0.43	0.67	-0.52	0.42	1.17	3.24	1.37	-0.24	-0.31	0.77	-0.31	-0.17	0.17	0.25	0.00	-0.88	-0.69	0.80
Apricot paste [b]	Sal	4.24	3.61	12.51	2.84	10.43	16.51	33.21	18.09	5.09	4.55	13.29	4.57	5.69	8.41	9.06	7.07	0.00	1.50	13.50
Sugar solution [c]	Eco	1.10	0.84	4.40	0.54	3.57	6.00	12.68	6.64	1.44	1.22	4.71	1.23	1.68	2.76	3.03	2.23	-0.60	0.00	4.80
Confectionary [d]	Eco	-0.64	-0.68	-0.07	-0.74	-0.21	0.21	1.36	0.32	-0.58	-0.62	-0.01	-0.62	-0.54	-0.35	-0.31	-0.44	-0.93	-0.83	0.00

[a] Primary model predictions made for 3-log reductions of bacteria from models constructed per section 2.1.3.
[b] Independent data acquired from Mattick et al., 2001 of *Salmonella* Typhimurium DT104 reduction on apricot paste treated at 74°C.
[c] Independent data acquired from Mattick et al., 2001 of *Salmonella* Typhimurium DT104 reduction in a sugar solution treated at 74°C.
[d] Independent data acquired from Rachon et al., 2016 of *L. monocytogenes*, *Enterococcus faecium* NRRL B-2354, and *Salmonella* reduction on a confectionary mix treated with 70 and 80°C. 3-log reductions were predicted using the reported shape and scale parameters.

Figure 4.6. Heat map of relative error between predicted and observed times required for 3-log reductions of microorganisms based on Weibull model parameters of externally acquired data of thermally treated LWAF at 72°C. Area within the bold lines reflects internal generated data compared using the same vacuum-steam process. The acceptable relative error ranged from -0.30 (fail-safe) to 0.15 (fail-dangerous). Externally acquired data is blocked and on the outside of the graph.

		Apricot Halves [a]				Whole Macadamia Nuts [a]				Apricot Pieces [a]				Macadamia Nut Pieces [a]				Seasoning [b]	Pet food [c]	Confectionary [d]	Confectionary [d]
		Eco	Lmo	Ped	Sal	Eco	Lmo	Ped	Sal	Eco	Lmo	Ped	Sal	Eco	Lmo	Ped	Sal	Sal	Eco	Eco	Lmo
Apricot Halves [a]	Eco	0.00	0.01	0.50	-0.09	1.67	1.83	4.01	2.86	0.09	0.01	0.71	0.00	0.11	-0.07	1.54	0.56	1.24	0.50	15.69	1.07
	Lmo	-0.01	0.00	0.49	-0.10	1.65	1.81	3.98	2.83	0.09	0.00	0.70	-0.01	0.10	-0.07	1.52	0.55	1.23	0.49	15.59	1.06
	Ped	-0.33	-0.33	0.00	-0.39	0.78	0.88	2.34	1.57	-0.27	-0.33	0.14	-0.34	-0.26	-0.38	0.69	0.04	0.49	0.00	10.13	0.38
	Sal	0.10	0.11	0.65	0.00	1.93	2.11	4.51	3.24	0.20	0.10	0.88	0.09	0.22	0.03	1.79	0.71	1.46	0.65	17.34	1.28
Whole Macadamia Nuts [a]	Eco	-0.63	-0.62	-0.44	-0.66	0.00	0.06	0.88	0.45	-0.59	-0.62	-0.36	-0.63	-0.58	-0.65	-0.05	-0.42	-0.16	-0.44	5.26	-0.22
	Lmo	-0.65	-0.64	-0.47	-0.68	-0.06	0.00	0.77	0.37	-0.61	-0.64	-0.39	-0.65	-0.61	-0.67	-0.10	-0.45	-0.21	-0.47	4.90	-0.27
	Ped	-0.80	-0.80	-0.70	-0.82	-0.47	-0.44	0.00	-0.23	-0.78	-0.80	-0.66	-0.80	-0.78	-0.81	-0.49	-0.69	-0.55	-0.70	2.33	-0.59
	Sal	-0.74	-0.74	-0.61	-0.76	-0.31	-0.27	0.30	0.00	-0.72	-0.74	-0.56	-0.74	-0.71	-0.76	-0.34	-0.60	-0.42	-0.61	3.33	-0.46
Apricot Pieces [a]	Eco	-0.08	-0.08	0.37	-0.17	1.44	1.59	3.59	2.53	0.00	-0.08	0.57	-0.09	0.02	-0.14	1.32	0.43	1.05	0.38	14.28	0.90
	Lmo	-0.01	0.00	0.49	-0.09	1.65	1.81	3.99	2.84	0.09	0.00	0.71	-0.01	0.10	-0.07	1.53	0.55	1.23	0.50	15.61	1.06
	Ped	-0.42	-0.41	-0.13	-0.47	0.56	0.65	1.92	1.25	-0.36	-0.41	0.00	-0.42	-0.35	-0.46	0.48	-0.09	0.31	-0.12	8.73	0.21
	Sal	0.00	0.01	0.51	-0.09	1.68	1.84	4.03	2.87	0.10	0.01	0.72	0.00	0.11	-0.06	1.55	0.56	1.25	0.51	15.75	1.08
Macadamia Nut Pieces [a]	Eco	-0.10	-0.09	0.35	-0.18	1.40	1.55	3.52	2.48	-0.02	-0.09	0.55	-0.10	0.00	-0.16	1.29	0.40	1.02	0.36	14.04	0.87
	Lmo	0.07	0.08	0.61	-0.03	1.85	2.03	4.37	3.13	0.17	0.08	0.84	0.07	0.19	0.00	1.72	0.67	1.40	0.61	16.87	1.22
	Ped	-0.61	-0.60	-0.41	-0.64	0.05	0.11	0.97	0.52	-0.57	-0.60	-0.32	-0.61	-0.56	-0.63	0.00	-0.39	-0.12	-0.41	5.57	-0.18
	Sal	-0.36	-0.35	-0.04	-0.42	0.71	0.82	2.22	1.48	-0.30	-0.35	0.10	-0.36	-0.29	-0.40	0.63	0.00	0.44	-0.03	9.72	0.33
Seasoning [b]	Eco	-0.55	-0.55	-0.33	-0.59	0.19	0.26	1.24	0.72	-0.51	-0.55	-0.23	-0.56	-0.50	-0.58	0.13	-0.30	0.00	-0.33	6.45	-0.08
Pet food [c]	Eco	-0.34	-0.33	0.00	-0.39	0.77	0.88	2.33	1.57	-0.27	-0.33	0.14	-0.34	-0.26	-0.38	0.69	0.04	0.49	0.00	10.10	0.38
Confectionary [d]	Sal	-0.94	-0.94	-0.91	-0.95	-0.84	-0.83	-0.70	-0.77	-0.93	-0.94	-0.90	-0.94	-0.93	-0.94	-0.85	-0.91	-0.87	-0.91	0.00	-0.88
Confectionary [d]	Lmo	-0.52	-0.51	-0.28	-0.56	0.29	0.36	1.42	0.86	-0.47	-0.51	-0.17	-0.52	-0.46	-0.55	0.23	-0.25	0.08	-0.27	7.06	0.00
Confectionary [d]	E.fae	-0.84	-0.84	-0.76	-0.85	-0.57	-0.55	-0.20	-0.38	-0.83	-0.84	-0.73	-0.84	-0.82	-0.85	-0.59	-0.75	-0.64	-0.76	1.66	-0.67
Seasoning [d]	Sal	-0.72	-0.72	-0.58	-0.75	-0.26	-0.21	0.40	0.08	-0.70	-0.72	-0.52	-0.72	-0.69	-0.74	-0.29	-0.57	-0.38	-0.58	3.65	-0.42
Seasoning [d]	Lmo	-0.72	-0.72	-0.58	-0.75	-0.26	-0.22	0.39	0.07	-0.70	-0.72	-0.53	-0.72	-0.69	-0.74	-0.30	-0.57	-0.38	-0.58	3.62	-0.43
Seasoning [d]	E.fae	-0.94	-0.94	-0.91	-0.94	-0.84	-0.83	-0.69	-0.76	-0.93	-0.94	-0.89	-0.94	-0.93	-0.94	-0.84	-0.90	-0.86	-0.91	0.02	-0.87

[a]Primary model predictions made for 3-log reductions of bacteria from models constructed per section 2.1.3.
[b]Independent data acquired from Daryaei et al., 2018 of STEC reduction on savory seasoning treated with 80°C. A 3-log reduction was predicted using the reported shape and scale parameters.
[c]Independent data acquired from Daryaei et al., 2018 of STEC reduction on pet food treated with 80°C. A 3-log reduction was predicted using the reported shape and scale parameters.
[d]Independent data acquired from Rachon et al., 2016 of *L. monocytogenes*, *Enterococcus faecium* NRRL B-2354, and *Salmonella* reduction on a savory seasoning and confectionary mix treated with 70 and 80°C. 3-log reductions were predicted using the reported shape and scale parameters.

Figure 4.7. Heat map of relative error between predicted and observed times required for 3-log reductions of microorganisms based on Weibull model parameters of externally acquired data of thermally treated LWAF at 82°C. Area within the bold lines reflects internal generated data compared using the same vacuum-steam process. The acceptable relative error ranged from -0.30 (fail-safe) to 0.15 (fail-dangerous). Externally acquired data is blocked and on the outside of the graph.

		Confectionary [d]	Seasoning [f]	Seasoning [f]	Seasoning [f]
		E.fae	Sal	Sal	E.fae
Apricot Halves [a]	Eco	5.27	2.59	2.61	15.29
	Lmo	5.23	2.56	2.59	15.18
	Ped	3.18	1.39	1.41	9.86
	Sal	5.89	2.94	2.97	16.89
Whole Macadamia Nuts [a]	Eco	1.35	0.35	0.35	5.11
	Lmo	1.22	0.27	0.28	4.76
	Ped	0.25	-0.28	-0.28	2.25
	Sal	0.62	-0.07	-0.06	3.22
Apricot Pieces [a]	Eco	4.74	2.28	2.31	13.91
	Lmo	5.24	2.57	2.59	15.21
	Ped	2.66	1.09	1.11	8.50
	Sal	5.29	2.60	2.62	15.34
Macadamia Nut Pieces [a]	Eco	4.65	2.23	2.25	13.68
	Lmo	5.71	2.84	2.87	16.43
	Ped	1.47	0.41	0.42	5.42
	Sal	3.02	1.30	1.32	9.46
Seasoning [b]	Sal	1.80	0.60	0.61	6.27
Pet food [c]	Eco	3.17	1.38	1.40	9.83
Confectionary [d]	Eco	-0.62	-0.79	-0.78	-0.02
Confectionary [d]	Sal	2.03	0.73	0.74	6.86
Confectionary [d]	Lmo	0.00	-0.43	-0.42	1.60
Seasoning [d]	E.fae	0.75	0.00	0.01	3.54
Seasoning [d]	Sal	0.74	-0.01	0.00	3.51
Seasoning [d]	E.fae	-0.62	-0.78	-0.78	0.00

[a]Primary model predictions made for 3-log reductions of bacteria from models constructed per section 2.1.3.

[b]Independent data acquired from Daryaei et al., 2018 of STEC reduction on savory seasoning treated with 80°C. A 3-log reduction was predicted using the reported shape and scale parameters.

[c]Independent data acquired from Daryaei et al., 2018 of STEC reduction on pet food treated with 80°C. A 3-log reduction was predicted using the reported shape and scale parameters.

[d]Independent data acquired from Rachon et al., 2016 of *L. monocytogenes*, *Enterococcus faecium* NRRL B-2354, and *Salmonella* reduction on a savory seasoning and confectionary mix treated with 70 and 80°C. 3-log reductions were predicted using the reported shape and scale parameters.

Figure 4.7 cont. Heat map of relative error between predicted and observed times required for 3-log reductions of microorganisms based on Weibull model parameters of externally acquired data of thermally treated LWAF at 82°C. Area within the bold lines reflects internal generated data compared using the same vacuum-steam process. The acceptable relative error ranged from -0.30 (fail-safe) to 0.15 (fail-dangerous). Externally acquired data is blocked and on the outside of the graph.

		Apricot Halves [a]				Whole Macadamia Nuts [a]				Apricot Pieces [a]				Macadamia Nut Pieces [a]			
		Eco	Lmo	Ped	Sal	Eco	Lmo	Ped	Sal	Eco	Lmo	Ped	Sal	Eco	Lmo	Ped	Sal
Apricot Halves [a]	Eco	0.00	-0.15	1.22	-0.22	1.45	2.33	5.01	2.46	0.22	-0.01	1.60	0.07	0.64	0.40	0.50	0.76
	Lmo	0.18	0.00	1.62	-0.08	1.89	2.92	6.09	3.08	0.43	0.17	2.06	0.26	0.93	0.65	0.77	1.07
	Ped	-0.55	-0.62	0.00	-0.65	0.10	0.50	1.71	0.56	-0.45	-0.55	0.17	-0.52	-0.26	-0.37	-0.32	-0.21
	Sal	0.28	0.09	1.85	0.00	2.15	3.27	6.72	3.44	0.56	0.27	2.33	0.37	1.11	0.80	0.93	1.26
Whole Macadamia Nuts [a]	Eco	-0.59	-0.65	-0.09	-0.68	0.00	0.36	1.45	0.41	-0.50	-0.60	0.06	-0.56	-0.33	-0.43	-0.39	-0.28
	Lmo	-0.70	-0.75	-0.33	-0.77	-0.26	0.00	0.81	0.04	-0.63	-0.70	-0.22	-0.68	-0.51	-0.58	-0.55	-0.47
	Ped	-0.83	-0.86	-0.63	-0.87	-0.59	-0.45	0.00	-0.42	-0.80	-0.84	-0.57	-0.82	-0.73	-0.77	-0.75	-0.71
	Sal	-0.71	-0.75	-0.36	-0.77	-0.29	-0.04	0.74	0.00	-0.65	-0.71	-0.25	-0.69	-0.53	-0.60	-0.57	-0.49
Apricot Pieces [a]	Eco	-0.18	-0.30	0.83	-0.36	1.02	1.74	3.95	1.85	0.00	-0.19	1.14	-0.12	0.35	0.15	0.24	0.45
	Lmo	0.01	-0.14	1.24	-0.21	1.48	2.36	5.08	2.50	0.23	0.00	1.63	0.08	0.66	0.42	0.52	0.78
	Ped	-0.62	-0.67	-0.15	-0.70	-0.06	0.28	1.31	0.33	-0.53	-0.62	0.00	-0.59	-0.37	-0.46	-0.42	-0.32
	Sal	-0.06	-0.21	1.08	-0.27	1.29	2.11	4.63	2.24	0.14	-0.07	1.43	0.00	0.53	0.31	0.41	0.64
Macadamia Nut Pieces [a]	Eco	-0.39	-0.48	0.35	-0.53	0.49	1.03	2.67	1.11	-0.26	-0.40	0.58	-0.35	0.00	-0.15	-0.08	0.07
	Lmo	-0.29	-0.39	0.58	-0.44	0.75	1.38	3.29	1.47	-0.13	-0.29	0.86	-0.24	0.17	0.00	0.07	0.25
	Ped	-0.33	-0.44	0.48	-0.48	0.63	1.21	3.00	1.30	-0.19	-0.34	0.73	-0.29	0.09	-0.07	0.00	0.17
	Sal	-0.43	-0.52	0.26	-0.56	0.40	0.89	2.42	0.97	-0.31	-0.44	0.48	-0.39	-0.07	-0.20	-0.14	0.00

[a]Primary model predictions made for 3-log reductions of bacteria from models constructed per section 2.1.3.

Figure 4.8. Heat map of relative error between predicted and observed times required for 3-log reductions of microorganisms based on internally constructed Gompertz model parameters of thermally treated LWAF at 72°C. The acceptable relative error ranged from -0.30 (fail-safe) to 0.15 (fail-dangerous).

		Apricot Halves [a]				Whole Macadamia Nuts [a]				Apricot Pieces [a]				Macadamia Nut Pieces [a]			
		Eco	Lmo	Ped	Sal	Eco	Lmo	Ped	Sal	Eco	Lmo	Ped	Sal	Eco	Lmo	Ped	Sal
Apricot Halves [a]	Eco	0.00	-0.07	0.29	-0.04	1.11	1.20	2.83	1.98	0.03	-0.02	0.32	0.00	-0.02	-0.12	1.53	0.22
	Lmo	0.07	0.00	0.38	0.03	1.26	1.35	3.10	2.19	0.11	0.05	0.41	0.07	0.05	-0.06	1.71	0.31
	Ped	-0.23	-0.28	0.00	-0.26	0.64	0.70	1.97	1.31	-0.20	-0.24	0.02	-0.23	-0.24	-0.32	0.96	-0.06
	Sal	0.04	-0.02	0.35	0.00	1.20	1.29	3.00	2.11	0.08	0.03	0.37	0.04	0.02	-0.08	1.64	0.27
Whole Macadamia Nuts [a]	Eco	-0.53	-0.56	-0.39	-0.55	0.00	0.04	0.81	0.41	-0.51	-0.53	-0.38	-0.53	-0.54	-0.58	0.20	-0.42
	Lmo	-0.54	-0.57	-0.41	-0.56	-0.04	0.00	0.75	0.36	-0.53	-0.55	-0.40	-0.55	-0.55	-0.60	0.15	-0.44
	Ped	-0.74	-0.76	-0.66	-0.75	-0.45	-0.43	0.00	-0.22	-0.73	-0.74	-0.66	-0.74	-0.74	-0.77	-0.34	-0.68
	Sal	-0.66	-0.69	-0.57	-0.68	-0.29	-0.26	0.29	0.00	-0.65	-0.67	-0.56	-0.67	-0.67	-0.70	-0.15	-0.59
Apricot Pieces [a]	Eco	-0.03	-0.10	0.25	-0.07	1.04	1.12	2.71	1.88	0.00	-0.05	0.27	-0.03	-0.05	-0.15	1.45	0.18
	Lmo	0.02	-0.05	0.31	-0.03	1.15	1.23	2.89	2.03	0.05	0.00	0.34	0.01	0.00	-0.11	1.57	0.24
	Ped	-0.24	-0.29	-0.02	-0.27	0.61	0.67	1.91	1.27	-0.21	-0.25	0.00	-0.24	-0.25	-0.33	0.92	-0.07
	Sal	0.00	-0.06	0.29	-0.04	1.12	1.20	2.84	1.99	0.04	-0.01	0.32	0.00	-0.02	-0.12	1.53	0.22
Macadamia Nut Pieces [a]	Eco	0.02	-0.05	0.32	-0.02	1.15	1.24	2.90	2.04	0.05	0.00	0.34	0.02	0.00	-0.10	1.58	0.24
	Lmo	0.14	0.06	0.47	0.09	1.40	1.49	3.35	2.39	0.17	0.12	0.50	0.13	0.12	0.00	1.87	0.39
	Ped	-0.60	-0.63	-0.49	-0.62	-0.16	-0.13	0.52	0.18	-0.59	-0.61	-0.48	-0.61	-0.61	-0.65	0.00	-0.52
	Sal	-0.18	-0.23	0.06	-0.21	0.73	0.80	2.14	1.44	-0.15	-0.19	0.08	-0.18	-0.20	-0.28	1.07	0.00

[a]Primary model predictions made for 3-log reductions of bacteria from models constructed per section 2.1.3.

Figure 4.9. Heat map of relative error between predicted and observed times required for 3-log reductions of microorganisms based on internally constructed Gompertz model parameters of thermally treated LWAF at 82°C. The acceptable relative error ranged from -0.30 (fail-safe) to 0.15 (fail-dangerous).

Tables

Table 4.1. External datasets acquired for independent validation and comparison against internally constructed models.

Thermal Treatment	Food	Water activity (a_w)	Organism	Reported Data	Internal Comparison Dataset	Reference
80°C	Savory seasoning	0.63	STEC	*D*- and *z*-values	*E. coli*, Apricot halves (72, 82°C)	Daryaei et al., 2018
80°C	Pet food	0.55[a]	STEC	*D*- and *z*-values	*E. coli*, Whole macadamia nuts (72, 82°C)	Daryaei et al., 2018
80°C	Savory seasoning	0.66	*L. mono*, *Salmonella*, *E. faecium*	*D*- and *z*-values	*L. mono*, *Salmonella*, *Pediococcus*; Apricot halves (72, 82°C)	Rachon et al., 2016
80°C	Confectionary mix	0.57	*L. mono*, *Salmonella*, *E. faecium*	*D*- and *z*-values	*L. mono*, *Salmonella*, *Pediococcus*; Whole macadamia nuts (72, 82°C)	Rachon et al., 2016
70, 80°C	Almond kernels	0.55	*Salmonella*	*D*-values	*Salmonella*, Whole macadamia nuts (72, 82°C)	Harris et al., 2012
74°C	Apricot paste	0.64	*Salmonella*	Time for 3-log reduction	*Salmonella*, Apricot halves (72°C)	Mattick et al., 2001
74°C	Sugar solution	0.64	*Salmonella*	Time for 3-log reduction	*Salmonella*, Apricot halves (72°C)	Mattick et al., 2001
80°C	Savory seasoning	0.63	STEC	Weibull parameters	*E. coli*, Apricot halves (82°C)	Daryaei et al., 2018
80°C	Pet food	0.55	STEC	Weibull parameters	*E. coli*, Whole macadamia nuts (82°C)	Daryaei et al., 2018
80°C	Savory seasoning	0.66	*L. mono*, *Salmonella*, *E. faecium*	Weibull parameters	*L. mono*, *Salmonella*, *Pediococcus*; Apricot halves (82°C)	Rachon et al., 2016
80°C	Confectionary mix	0.57	*L. mono*, *Salmonella*, *E. faecium*	Weibull parameters	*L. mono*, *Salmonella*, *Pediococcus*; Whole macadamia nuts (82°C)	Rachon et al., 2016

[a]Estimated (value not reported).

Table 4.2. First-order (k [log CFU/g min^{-1}]), Weibull (k (log CFU/g min^{-1}) and β) and Gompertz (A, μ_M [log CFU/g min^{-1}], λ [min]) model parameter coefficients and statistical results (RMSE [log CFU/g], AIC) for organisms (STEC, *Salmonella*, *L. monocytogenes*, and *Pediococcus acidilactici*) on dried apricot halves, whole macadamia nuts, dried apricot pieces, and macadamia nut pieces treated with 72°C and 82°C vacuum-steam.

			First-Order				Weibull				Gompertz				
Food	Temp	Bacteria	*k*	*D*-val	RMSE	AIC	*k*	*β*	RMSE	AIC	*A*	μ_M	*λ*	RMSE	AIC
Apricot Halves	72	*E. coli*	-0.41	2.43	1.00	70.96	-0.96	0.69	0.79	60.85	-7.22	-0.62	0.54	**0.78**	**60.78**
		L. mono	-0.40	2.53	1.32	84.46	-1.24	0.58	0.98	70.89	-6.41	-0.70	0.23	**0.92**	**68.66**
		Ped.	-0.21	4.70	0.80	60.21	-0.71	0.55	**0.64**	**50.62**	-3.67	-0.27	-1.00	0.71	56.64
		Sal.	-0.44	2.29	1.45	89.01	-1.39	0.57	1.04	73.99	-7.13	-0.79	0.36	**0.88**	**66.91**
	82	*E. coli*	-1.57	0.64	1.35	85.66	-2.15	0.75	1.28	83.75	-6.45	-4.33	0.99	**0.96**	**71.04**
		L. mono	-1.51	0.66	1.22	80.51	-2.19	0.70	1.09	75.95	-6.07	-3.18	0.63	**0.89**	**67.23**
		Ped.	-1.16	0.86	0.82	61.68	-1.60	0.74	0.75	58.18	-5.38	-1.50	0.15	**0.73**	**57.65**
		Sal.	-1.57	0.64	1.43	88.24	-2.37	0.67	1.27	83.58	-5.94	-11.07	1.34	**0.80**	**62.03**
Whole Macada-mia Nuts	72	*E. coli*	-0.18	5.44	0.85	60.96	-0.69	0.60	**0.64**	**48.43**	-6.68	-0.21	-1.19	0.78	58.66
		L. mono	-0.14	7.29	0.67	49.60	-0.69	0.51	**0.40**	**26.99**	-4.81	-0.15	-2.26	0.55	42.61
		Ped.	-0.10	10.32	0.69	51.30	-0.46	0.53	**0.61**	**46.36**	-3.66	-0.10	-2.69	0.67	51.70
		Sal.	-0.13	7.54	0.68	50.51	-0.59	0.55	**0.48**	**35.89**	-4.59	-0.16	-1.53	0.60	46.31
	82	*E. coli*	-0.54	1.85	1.14	83.72	-1.23	0.63	0.98	**76.49**	-5.20	-1.00	0.49	**0.96**	76.62
		L. mono	-0.52	1.94	0.99	73.57	-1.21	0.61	0.78	62.54	-5.01	-0.94	0.43	**0.76**	**62.04**
		Ped.	-0.37	2.73	0.76	62.49	-0.73	0.68	**0.69**	**58.63**	-3.71	-0.57	0.34	0.72	61.81
		Sal.	-0.44	2.27	0.95	73.95	-0.86	0.70	**0.88**	**71.03**	-4.48	-0.68	0.35	0.92	74.13
Apricot Pieces	72	*E. coli*	-0.39	2.54	0.92	88.73	-0.77	0.75	**0.83**	**82.70**	-7.88	-0.49	0.33	0.87	86.83
		L. mono	-0.38	2.65	1.03	95.42	-1.00	0.64	0.77	77.86	-6.33	-0.60	0.30	**0.75**	**77.45**
		Ped.	-0.21	4.81	0.59	59.74	-0.31	0.86	**0.58**	**60.41**	-5.26	-0.23	0.70	0.61	64.24
		Sal.	-0.39	2.59	0.94	86.98	-0.95	0.67	**0.71**	**71.09**	-6.92	-0.54	0.17	0.74	73.74
	82	*E. coli*	-1.60	0.62	1.26	105.49	-1.89	0.87	1.26	106.14	-6.78	-4.04	0.99	**0.92**	**87.50**
		L. mono	-1.56	0.64	1.45	113.86	-2.14	0.75	1.37	111.38	-6.00	-10.62	1.37	**0.62**	**63.47**
		Ped.	-1.11	0.90	0.84	77.75	-1.16	0.97	0.85	79.66	-4.75	-2.39	0.91	**0.71**	**69.78**
		Sal.	-1.60	0.62	1.38	110.75	-2.13	0.77	1.31	108.63	-6.28	-7.74	1.29	**0.70**	**70.31**
Macada-mia Nut Pieces	72	*E. coli*	-0.33	3.01	0.85	73.51	-0.89	0.64	**0.68**	**61.85**	-11.12	-0.31	-0.82	0.79	71.13
		L. mono	-0.24	4.11	0.93	78.49	-1.15	0.43	**0.50**	**44.29**	-3.87	-0.39	-1.28	0.64	58.92
		Ped.	-0.24	4.23	1.04	84.86	-1.11	0.43	**0.72**	**64.63**	-3.80	-0.37	-1.28	0.82	72.93

	Sal.	-0.27	3.69	0.89	75.78	-1.08	0.49	**0.57**	**51.55**	-6.00	-0.25	-2.87	0.70	64.66
82	*E. coli*	-1.30	0.77	0.99	79.30	-2.15	0.61	**0.77**	**66.39**	-5.44	-1.85	0.01	0.81	70.07
	L. mono	-1.41	0.71	1.20	92.86	-2.42	0.57	**0.94**	**79.65**	-5.75	-2.03	-0.01	0.96	82.12
	Ped.	-0.80	1.25	0.84	72.69	-1.50	0.50	0.64	58.32	-3.03	-1.55	0.18	**0.59**	**54.35**
	Sal.	-1.03	0.97	1.07	86.32	-1.91	0.51	**0.86**	**74.71**	-4.02	-1.55	-0.07	0.89	77.78

Best fit values are bolded.

Table 4.3. Predictions of 3-log STEC reductions based on D- and z-values acquired from thermally treated reduced-water activity solutions compared to reductions of an STEC cocktail on dried apricot halves and whole macadamia nuts.

	Sample	z-value*	3-log reduction at 72°C (min) [a]	3-log reduction at 82°C (min) [a]
a_w 0.75	O121	8.04 [b]	0.336	0.019
	O157	8.81 [b]	0.489	0.036
a_w 0.68	STEC Cocktail [d]	N/D*	7.63	1.87
a_w 0.50	O121	8.54 [c]	0.302	0.020
	O157	7.82 [c]	0.223	0.012
a_w 0.58	STEC Cocktail [e]	N/D*	9.03	2.30

* *z*-values were not determined (N/D) for dried apricots or macadamia nuts because only two temperatures were used in vacuum-steam experiments.

[a] Values extrapolated from *z*-values and *D*-values calculated for 72 and 82°C.

[b] Calculated from $D_{56°C}$, $D_{59°C}$, $D_{62°C}$, of O121:H19 (11.85, 4.36, 1.97 min) and O157:H7 (11.35, 6.01, 2.22 min) in intermediate-water activity solution.

[c] Calculated from $D_{56°C}$, $D_{59°C}$, $D_{62°C}$, of O121:H19 (7.70, 4.25, 1.49 min) and O157:H7 (8.24, 4.09, 1.41 min) in low-water activity solution.

[d] Calculated from vacuum-steam treatments of inoculated dried apricot pieces.

[e] Calculated from vacuum-steam treatments of inoculated whole macadamia nut pieces.

Supplemental Materials

Table 4.4. Comparisons of times (min) for 3-log reductions of *E. coli*, *Salmonella*, *L. monocytogenes*, and *Pediococcus acidilactici* on thermally treated low-water activity foods based on first-order models. Foods were compared based on similar water activity and comparisons were evaluated based on relative error with acceptable fail-safe, fail-dangerous range of -0.30-0.15.

		Primary Model Predictions[a]		Pieces Primary Model Observations[a]				Independent Data Observations[b]			
Temp (°C)	Organism	Food	3-log reduction (min)	Food	3-log reduction (min)	Raw Error (min)	Relative Error	Food	3-log reduction (min)	Raw Error (min)	Relative Error
72	*E. coli*	Apricot Halves	7.28	Apricot Pieces	7.63	0.35	**0.05**	Savory seasoning[c]	7.78	0.50	**0.07**
	L. mono		7.59		7.95	0.36	**0.05**	----	----	----	----
	Ped.		14.10		14.43	0.33	**0.02**	----	----	----	----
	Sal.		6.88		7.78	0.90	**0.13**	----	----	----	----
82	*E. coli*		1.91		1.87	0.04	**-0.02**	Savory seasoning[c]	0.99	0.92	-0.48
	L. mono		1.99		1.92	0.07	**-0.03**	Savory seasoning[d]	7.97	5.98	3.01
	Ped.		2.59		2.70	0.11	**0.04**	Savory seasoning[d]	17.34	14.75	5.69
	Sal.		1.91		1.87	0.04	**-0.02**	Savory seasoning[d]	5.73	3.82	2.00
72	*E. coli*	Whole Macadamia Nuts	16.33	Macadamia Nut Pieces	9.03	7.30	-0.45	Pet food[e]	3.45	12.88	-0.79
	L. mono		21.87		12.32	9.55	-0.44	Confectionary mix[f]	47.35	25.48	1.16
	Ped.		30.95		12.68	18.27	-0.59	----	----	----	----
	Sal.		22.61		11.08	11.53	-0.51	Almonds[g]	3.60	19.01	-0.84
82	*E. coli*		5.54		2.30	3.24	-0.58	Pet food[e]	0.74	4.80	-0.87
	L. mono		5.82		2.13	3.69	-0.63	Confectionary mix[f]	6.738	0.92	0.16
	Ped.		8.18		3.75	4.43	-0.54	Confectionary mix[f]	21.70	13.52	1.65
	Sal.		6.81		2.92	3.89	-0.57	Confectionary mix[f]	24.04	17.23	2.53
								Almonds[g]	2.25	4.56	-0.67

[a]Primary model predictions made for 3-log reductions of bacteria from models constructed per section 2.1.3.
[b]Independent data observations of 3-log reductions of bacteria from extrapolations from reported data or model parameters per section 2.2.2.
Bolded values represent comparison data accurately predicted by models based on relative error within the acceptable range (-0.30-0.15) per section 2.2.3.
[c]Data acquired from Daryaei et al., 2018 of STEC reduction on savory seasoning treated with 80°C. A 3-log reduction at 72 and 82°C was predicted using the reported *D*- and *z*-values and used as the observed value.
[d]Data acquired from Rachon et al., 2016 of *L. monocytogenes*, *Enterococcus faecium* NRRL B-2354, and *Salmonella* reduction on savory seasoning treated with 80°C. A 3-log reduction at 72 and 82°C was predicted using the reported *D*- and *z*-values and used as the observed value.
[e]Data acquired from Daryaei et al., 2018 of STEC reduction on pet food treated with 80°C. A 3-log reduction at 72 and 82°C was predicted using the reported *D*- and *z*-values and used as the observed value.
[f]Data acquired from Rachon et al., 2016 of *L. monocytogenes*, *Enterococcus faecium* NRRL B-2354, and *Salmonella* reduction on a confectionary mix treated with 70 and 80°C. A 3-log reduction at 72 and 82°C was predicted using the reported *D*- and *z*-values and used as the observed value.
[g]Data acquired from Harris et al., 2012 of *Salmonella* Enteritidis PT30 reduction on almond kernels treated with 70 and 80°C hot water.

Table 4.5. Comparisons of times (min) for 3-log reductions of *E. coli, Salmonella, L. monocytogenes*, and *Pediococcus acidilactici* on thermally treated low-water activity foods based on Weibull models. Foods were compared based on similar water activity and comparisons were evaluated based on relative error with acceptable fail-safe, fail-dangerous range of -0.30-0.15.

		Primary Model Predictions[a]		Pieces Primary Model Observations[a]				Independent Data Observations[b]			
Temp (°C)	Organism	Food	3-log reduction (min)	Food	3-log reduction (min)	Raw Error (min)	Relative Error	Food	3-log reduction (min)	Raw Error (min)	Relative Error
72	*E. coli*	Apricot Halves	5.24	Apricot Pieces	6.09	0.35	**0.05**	----	----	----	----
	L. mono		4.61		5.55	0.36	**0.05**	----	----	----	----
	Ped.		13.51		14.29	0.33	**0.02**	----	----	----	----
	Sal.		3.84		5.57	0.90	**0.13**	Apricot paste[c]	1.00	2.84	-0.74
						0.04	**-0.02**	Sugar solution[d]	2.50	1.34	-0.35
82	*E. coli*		1.56		1.70	0.07	**-0.03**	Savory seasoning[e]	3.49	1.93	1.24
	L. mono		1.57		1.57	0.11	**0.04**	Savory seasoning[g]	5.63	4.06	2.59
	Ped.		2.34		2.67	0.04	**-0.02**	Savory seasoning[g]	25.41	23.07	9.86
	Sal.		1.42		1.55	7.30	-0.45	Savory seasoning[g]	5.60	4.18	2.94
72	*E. coli*	Whole Macada-mia Nuts	11.43	Macada-mia Nut Pieces	6.69	9.55	-0.44	----	----	----	----
	L. mono		17.51		9.41	18.27	-0.59	Confectionary mix[g]	14.40	3.01	**-0.17**
	Ped.		34.21		10.06	11.53	-0.51	----	----	----	----
	Sal.		19.09		8.07	3.24	-0.58	----	----	----	----
82	*E. coli*		4.16		1.73	3.69	-0.63	Pet food[f]	2.35	1.81	-0.44
	L. mono		4.41		1.46	4.43	-0.54	Confectionary mix[g]	3.23	1.18	**-0.27**
	Ped.		7.82		3.96	3.89	-0.57	Confectionary mix[g]	9.78	1.96	0.25
	Sal.		6.02		2.43	0.35	**0.05**	Confectionary mix[g]	26.04	20.02	3.33

[a]Primary model predictions made for 3-log reductions of bacteria from models constructed per section 2.1.3.
[b]Independent data observations of 3-log reductions of bacteria from extrapolations from reported data or model parameters per section 2.2.2.
Bolded values represent comparison data accurately predicted by models based on relative error within the acceptable range (-0.30-0.15) per section 2.2.3.
[c]Independent data acquired from Mattick et al., 2001 of *Salmonella* Typhimurium DT104 reduction on apricot paste treated at 74°C.
[d]Independent data acquired from Mattick et al., 2001 of *Salmonella* Typhimurium DT104 reduction in a sugar solution treated at 74°C.
[e]Independent data acquired from Daryaei et al., 2018 of STEC reduction on savory seasoning treated with 80°C. A 3-log reduction was predicted using the reported shape and scale parameters.
[f]Independent data acquired from Daryaei et al., 2018 of STEC reduction on pet food treated with 80°C. A 3-log reduction was predicted using the reported shape and scale parameters.
[g]Independent data acquired from Rachon et al., 2016 of *L. monocytogenes*, *Enterococcus faecium* NRRL B-2354, and *Salmonella* reduction on a savory seasoning treated with 70 and 80°C. 3-log reductions were predicted using the reported shape and scale parameters.

Table 4.6. Comparisons of times (min) for 3-log reductions of *E. coli*, *Salmonella*, *L. monocytogenes*, and *Pediococcus acidilactici* on thermally treated low-water activity foods based on Gompertz models. Foods were compared based on similar water activity and comparisons were evaluated based on relative error with acceptable fail-safe, fail-dangerous range of -0.30-0.15.

		Primary Model Predictions[a]		Data Observations[b]			
Temp (°C)	Organism	Food	3-log reduction (min)	Food	3-log reduction (min)	Raw Error (min)	Relative Error
72	*E. coli*	Apricot Halves	5.34	Apricot Pieces	6.49	1.15	0.22
	L. mono		4.53		5.28	0.75	0.17
	Ped.		11.85		13.87	2.02	0.17
	Sal.		4.16		5.71	1.55	0.37
82	*E. coli*		1.68		1.74	0.06	**0.03**
	L. mono		1.57		1.65	0.08	**0.05**
	Ped.		2.17		2.21	0.04	**0.02**
	Sal.		1.61		1.68	0.07	**0.04**
72	*E. coli*	Whole Macadamia Nuts	13.09	Macadamia nut pieces	8.76	4.33	-0.33
	L. mono		17.77		7.48	10.29	-0.58
	Ped.		32.10		8.03	24.07	-0.75
	Sal.		18.48		9.38	9.10	-0.49
82	*E. coli*		3.55		1.65	1.90	-0.54
	L. mono		3.69		1.48	2.21	-0.60
	Ped.		6.44		4.25	2.19	-0.34
	Sal.		5.01		2.05	2.96	-0.59

[a]Primary model predictions made for 3-log reductions of bacteria from models constructed per section 2.1.3.
[b]Data observations of 3-log reductions of bacteria on apricot pieces and macadamia nut pieces from models constructed per section 2.1.3.
Bolded values represent comparison data accurately predicted by models based on relative error within the acceptable range (-0.30-0.15) per section 2.2.3.

References

1. Archer, J., Jervis, E. T., Bird, J., and Gaze, J. E. (1998). Heat resistance of *Salmonella* Weltevreden in low-moisture environments. *Journal of Food Protection*, *61*(8), 969–973.
2. Aspridou, Z., Balomenos, A., Tsakanikas, P., Manolakos, E., and Koutsoumanis, K. (2019). Heterogeneity of single cell inactivation: Assessment of the individual cell time to death and implications in population behavior. *Food Microbiology*, *80*, 85–92. http://doi.org/10.1016/j.fm.2018.12.011
3. Bevilacqua, A., Speranza, B., Sinigaglia, M., and Corbo, M. (2015). A focus on the death kinetics in predictive microbiology: benefits and limits of the most important models and some tools dealing with their application in foods. *Foods*, *4*(4), 565–580. http://doi.org/10.3390/foods4040565
4. Bigelow, W. D. (1921). The logarithmic nature of thermal death time curves. *The Journal of Infectious Diseases, 29*(5), 528-536.
5. Bigelow, W. D. and Esty, J. R. (1920). The thermal death point in relation to time of typical thermophilic organisms. *The Journal of Infectious Diseases, 27*(6), 602-617.
6. van Boekel, M. A. J. S. (2008). Kinetic modeling of food quality: a critical review. *Comprehensive Reviews in Food Science and Food Safety*, *7*, 144–158.
7. Canadian Food Inspection Agency (CFIA). (2011). Health hazard alert: certain walnuts and walnut products may contain *E. coli* O157:H7 bacteria. Available from: http://www.inspection.gc.ca/about-the-cfia/newsroom/food-recall-warnings/complete-listing/2011-04-03/eng/1359548340192/1359548340223.
8. Centers for Disease control and Prevention (CDC). (2012). Foodborne outbreak online database. Available from: http://www.cdc.gov/foodborneoutbreaks/.
9. Centers for Disease Control and Prevention (CDC). (2018a). Burden of foodborne illness: findings. Available from: https://www.cdc.gov/foodborneburden/2011-foodborne-estimates.html
10. Centers for Disease Control and Prevention, National Outbreak Reporting System (CDC). (2018b). NORS dashboard. Available from: https://wwwn.cdc.gov/norsdashboard/
11. Centers for Disease Control and Prevention (CDC). (2019a). Reports for selected *E. coli* outbreak investigations. Available from: https://www.cdc.gov/ecoli/outbreaks.html.
12. Centers for Disease Control and Prevention (CDC). (2019b). Reports for selected *Salmonella* outbreak investigations. Available from: https://www.cdc.gov/salmonella/outbreaks.html.
13. Daryaei, H., Peñaloza, W., Hildebrandt, I., Krishnamurthy, K., Thiruvengadam, P., and Wan, J. (2018). Heat inactivation of Shiga toxin-producing *Escherichia coli* in a selection of low moisture foods. *Food Control*, *85*, 48–56. http://doi.org/10.1016/j.foodcont.2017.08.029
14. van Doornmalen, J. P. C. M. and Kopinga, K. (2009). Temperature dependence of F-, *D*- and *z*-values used in steam sterilization processes. *Journal of Applied Microbiology*, *107*(3), 1054–1060. http://doi.org/10.1111/j.1365-2672.2009.04290.x
15. Enache, E., Mathusa, E. C., Elliott, P. H., Black, D. G., Chen, Y., Scott, V. N., and Schaffner, D. W. (2011). Thermal resistance parameters for Shiga toxin–producing *Escherichia coli* in apple juice. *Journal of Food Protection*, *74*(8), 1231–1237. http://doi.org/10.4315/0362-028X.JFP-10-488

16. European Commission Rapid Alert System for Food & Feed (RASFF). (2019). Notifications list. Available from: https://webgate.ec.europa.eu/rasff-window/portal/?event=notificationsList&StartRow=1.
17. Forghani, F., den Bakker, M., Futral, A.N., and Diez-Gonzalez, F. (2018). Long-term survival and thermal death kinetics of enterohemorrhagic *Escherichia coli* serogroups O26, O103, O111, and O157 in wheat flour. *Applied Environmental Microbiology, 84*, e00283–18.
18. Forghani, F. (2019). *Salmonella* and enterohemorrhagic *Escherichia coli* serogroups O45, O121, O145 in wheat flour: effects of long-term storage and thermal treatments. *Fmicb-10-00323.Tex*, 1–12. http://doi.org/10.3389/fmicb.2019.00323
19. Goepfert, J. M., Iskander, I. K., and Amundson, C. H. (1970). Relation of the heat resistance of salmonellae to the water activity of the environment. *Applied Microbiology*, *19*(3), 429–433.
20. Grasso, E. M., Stam, C. N., Anderson, N. M., and Krishnamurthy, K. (2014). Heat and steam treatments. In J. B. Gurtler, M. P. Doyle, and J. L. Kornacki (Eds.), *The microbiological safety of low water activity foods and spices* (pp. 403—424). LLC: Springer science.
21. Harris, L. J., Uesugi, A. R., Abd, S. J., and McCarthy, K. L. (2012). Survival of *Salmonella* Enteritidis PT 30 on inoculated almond kernels in hot water treatments. *Frin*, *45*(2), 1093–1098. http://doi.org/10.1016/j.foodres.2011.03.048
22. Jackson, T. C., Hardin, M. D., and Acuff, G. R. (1995). Heat resistance of *Escherichia coli* O157:H7 in a nutrient medium and in ground beef patties as influenced by storage and holding temperatures. *Journal of Food Protection*, *59*(3), 230–237.
23. Juneja, V. K., and Eblen, B. S. (2000). Heat inactivation of *Salmonella typhimurium* DT104 in beef as affected by fat content. *Letters in Applied Microbiology*, *30*, 461–467.
24. Keller, S. E., Stam, C. N., Gradl, D. R., Chen, Z., Larkin, E. L., Pickens, S. R., and Chirtel, S. J. (2015). Survival of *Salmonella* on chamomile, peppermint, and green tea during storage and subsequent survival or growth following tea brewing. *Journal of Food Protection.* 78(4), 661–667.
25. Lang, E., Iaconelli, C., Zoz, F., Guyot, S., Alvarez-Martin, P., Beney, L., Perrier-Cornet, J. M., and Gervais, P. (2017). Drying parameters greatly affect the destruction of *Cronobacter sakazakii* and *Salmonella* Typhimurium in standard buffer and milk. *Food Microbiology*, *62*(C), 82–91. http://doi.org/10.1016/j.fm.2016.10.005
26. Laroche, C., and Gervais, P. (2003). Unexpected thermal destruction of dried, glass bead-immobilized microorganisms as a function of water activity. *Applied and Environmental Microbiology*, *69*(5), 3015–3019. http://doi.org/10.1128/AEM.69.5.3015-3019.2003
27. Laroche, C., Fine, F., and Gervais, P. (2005). Water activity affects heat resistance of microorganisms in food powders. *International Journal of Food Microbiology*, *97*(3), 307–315. http://doi.org/10.1016/j.ijfoodmicro.2004.04.023
28. Marks, B. P. (2008). Status of microbial modeling in food process models. *Comprehensive Reviews in Food Science and Food Safety*, *7*, 137–143.
29. Mattick, K. L., Jorgensen, F., Wang, P., Pound, J., Vandeven, M. H., Ward, L. R., Lega, J. D., Lappin-Scott, H. M., and Humphrey, T. J. (2001). Effect of challenge temperature and solute type on heat tolerance of *Salmonella* serovars at low water activity. *Applied and Environmental Microbiology*, *67*(9), 4128–4136. http://doi.org/10.1128/AEM.67.9.4128-4136.2001

30. McMeekin, T. A., Brown, J., Krist, K., Miles, D., Neumeyer, K., Nichols, D. S., Olley, J., Presser, K., Ratkowsky. D. A., Ross, T., Salter, M., and Soontranon, S. (1997). Quantitative microbiology: a basis for food safety. *Emerging Infectious Diseases*, *3*(4), 541–549.
31. Membré, J. -M., and Lambert, R. J. W. (2008). Application of predictive modelling techniques in industry: From food design up to risk assessment. *International Journal of Food Microbiology*, *128*(1), 10–15. http://doi.org/10.1016/j.ijfoodmicro.2008.07.006
32. Newkirk, J. J., Wu, J., Acuff, J. C., Caver, C. B., Mallikarjunan, K., Wiersema, B. D., Williams, R. C., and Ponder, M. A. (2018). Inactivation of *Salmonella enterica* and surrogate *Enterococcus faecium* on whole black peppercorns and cumin seeds using vacuum steam pasteurization. *Frontiers in Sustainable Food Systems, 2*, 252–12.
33. Oscar, T. P. (2005). Development and validation of primary, secondary, and tertiary models for growth of *Salmonella* Typhimurium on sterile chicken. *Journal of Food Protection*, *68*(12), 2606–2613.
34. Peleg, M. (2003). Microbial survival curves: interpretation, mathematical modeling, and utilization. *Comments on Theoretical Biology, 8*, 357–387.
35. Peleg, M., Normand, M. D., and Corradini, M. G. (2005). Generating microbial survival curves during thermal processing in real time. *Journal of Applied Microbiology, 98*(2), 406–417.
36. Rachon, G., Peñaloza, W., & Gibbs, P. A. (2016). Inactivation of *Salmonella, Listeria monocytogenes* and *Enterococcus faecium* NRRL B-2354 in a selection of low moisture foods. *International Journal of Food Microbiology*, *231*(C), 16–25. http://doi.org/10.1016/j.ijfoodmicro.2016.04.022
37. Suehr, Q. J., Anderson, N. M., and Keller, S. E. (2019). Desiccation and thermal resistance of *Escherichia coli* O121 in wheat flour. *Journal of Food Protection, 82*(8), 1308–1313.
38. Tsai, H. -C., Ballom, K. F., Xia, S., Tang, J., Marks, B. P., Zhu, M. -J. (2019). Evaluation of *Enterococcus faecium* NRRL B-2354 as a surrogate for *Salmonella* during cocoa powder thermal processing. Food Microbiol. 82, 135–141.
39. U.S. Food and Drug Administration (FDA). (2016). Draft guidance for industry: hazard analysis and risk-based preventive controls for human food; Chapter 4 Preventive Controls. Available from: https://www.fda.gov/media/99572/download.
40. U.S. Food and Drug Administration (FDA). (2019). Archive for recalls, market withdrawals & safety alerts. Available from: www.fda.gov/safety/recalls-market-withdrawals-safety-alerts/archive-recalls-market-withdrawals-safety-alerts.
41. Verma, T., Wei, X., Lau, S.K., Bianchini, A., Eskridge, K.M., and Subbiah, J. (2018). Evaluation of *Enterococcus faecium* NRRL B-2354 as a surrogate for *Salmonella* during extrusion of low-moisture food. *Journal of Food Science, 83*(4), 1063–1072.
42. Zwietering, M. H., Jongenburger, I., Rombouts, F. M., and Riet, K. V. (1990). Modeling of the bacterial growth curve. *Applied Environmental Microbiolgy, 56*(6), 1875–1881.

Chapter 5 Inactivation Kinetics of Shiga Toxin-Producing *Escherichia coli* O121:H19 and O157:H7 as Impacted by Culture Preparation Method and Water Activity

Jennifer C. Acuff[1], Kim Waterman[1], Jahnavi Ramakrishnan[1], Monica A. Ponder[1]*

[1]Virginia Tech, Food Science and Technology,
1230 Washington St., Blacksburg, VA 24061, USA

Key words: STEC; low water activity; inactivation kinetics; isothermal treatment; osmotic stress; culture preparation

*Corresponding author: Monica A. Ponder, 540-231-5031, Mponder@vt.edu

Abstract:

Bacterial exposure to sublethal stress may provide cross-protection, increasing time necessary to achieve thermal destruction of some foodborne pathogens. On low-water activity (a_w) foods, thermal resistance of foodborne pathogens is reported to increase; however, it is unclear if enhanced thermal resistance is due to exposure to low-a_w alone, or if additional aspects of the food microenvironment impact thermal inactivation. This study investigated impacts of culture preparation method and water activity on thermal resistance of two Shiga toxin-producing *Escherichia coli* (STEC; O121:H19, O157:H7) challenged with isothermal conditions. Broth and agar (lawn cultures) were compared as culture preparation methods. To compare impacts of water activity on STEC thermal resistance, cells were suspended in high- (broth a_w 0.99), intermediate- (26% NaCl, a_w 0.75), and low- (82% glycerol, a_w 0.5) a_w solutions. $D_{56°C}$, $D_{59°C}$, and $D_{62°C}$ were determined for each isolate. $D_{56°C}$ of broth- and lawn-cultured O121:H19 (34.25 and 17.78 min) were significantly greater than those of O157:H7 (26.57 and 10.76 min), and broth cultures were significantly more resistant than lawn cultures ($p < 0.05$). $D_{59°C}$ and $D_{62°C}$ for both strains in broth cultures were also higher than lawn culture counterparts, but differences were insignificant. Across both strains, $D_{56°C}$ of the high-a_w were significantly higher than those of the intermediate- (O121 = 11.85 min; O157 = 11.35 min) and low- (O121 = 7.70; O157 = 8.24 min) a_w samples, but *D*-values of reduced-a_w cultures were not significantly different from one another. The *z*-values were not different between the STEC strains, with only some differences between high-a_w and reduced-a_w samples. These results highlight that bacterium genetics, culture method, and reduced-water activity through osmolyte addition have different implications for STEC thermal resistance, emphasizing the importance of challenge studies applied directly on foods.

Highlights

- Culture preparation method influenced thermal resistance at lower temperatures.
- STEC O121 was more heat resistant at low temperatures in broth than O157.
- Water activity alone did not increase the thermal resistance of two STEC strains.
- *D*-value experiments can be heavily influenced by multiple experimental variables.

1. Introduction

Low-water activity (a_w) food (LWAF) products, defined by having a_w <0.6, comprise a substantial amount of the food industry (*28*). Intermediate-water activity foods (IWAF; a_w 0.6-0.85) also make up a category of popularly consumed, ready-to-eat (RTE) foods, such as dried fruits. Below a_w <0.85, pathogenic bacteria cannot grow, but contaminating microorganisms may survive in the low-water activity environment (*4*). Although LWAF are not currently regarded as high-risk foods, a number of high-profile outbreaks implicating dried products have raised concerns regarding pathogen survival on dried foods (*9*, *10*, *48*). While *Salmonella* spp. have known abilities to withstand low-water activity conditions for extended periods of time and have been associated with many LWAF outbreaks, several strains of Shiga toxin-producing *Escherichia coli* (STEC) have been noted as culprits of LWAF outbreaks and recalls in products like hazelnuts, nut butter, and flour (*10*). Due to the serious nature of an STEC infection, these pathogens should be seriously considered for further investigations as possible contaminants of LWAF. Food processors must address these risks by exploring possible sources of pathogen exposure and interventions that reduce contamination.

Thermal treatments may be employed to remove pathogen contamination. For foods with higher water activity levels, a "time-temperature" combination can be more easily determined than those with low water activity due to the more consistent heat transfer through water molecules (*28*, *38*). There have been many recorded instances of increased pathogen thermal resistance (*Salmonella* spp., *Cronobacter sakazakii*, and *E. coli*) in LWAF compared to high-water activity foods or solutions, further complicating the issue of determining adequate thermal treatment parameters (*1*, *19*, *26*, *27*, *29*, *30*). Water activity of foods can be altered by addition of solutes, leading to osmotic stress, or by drying (the removal of water through evaporation),

resulting in desiccation. Both methods of reducing water activity are frequently used in food processing for preservation and safety of foods, but the bacterial responses to osmotic and desiccation stress may vary (*7*, *16*, *49*).

In addition to the water activity of the suspension or food, the physiological state of the cells at the time of a thermal treatment likely plays an important role in thermal resistance. Culture preparation methods (available nutrients, temperature, solid vs. liquid media, selective pressures) have been shown to impact stress resistance (*2*, *15*, *17*, *24*, *36*). When *E. coli* K12 was grown and maintained under glucose-starved conditions, subsequent increased thermotolerance was observed (*39*). Also, *Salmonella* spp. grown on Tryptic Soy Agar (TSA) exhibited greater survival on spices during storage than when prepared in Tryptic Soy Broth (TSB) (*6*), but no differences in thermal resistance were noted between cultures grown on the two different media (*15*). Concerning adaptation of *Salmonella*, desiccation and biofilm formation are known to prompt cross-protection against other stressors such as low-pH gastric enzymes and thermal treatments, like vacuum-assisted steam (*2*, *35*, *47*). Because *Salmonella* has been most frequently associated with outbreaks and recalls of LWAF, the majority of studies investigate *Salmonella* responses and thermal resistance in LWAF matrices. While there have been studies that examine STEC for its thermal resistance following desiccation, thermal resistance as a result of osmotic stress as it relates to LWAF has not been fully elucidated (*22*). Accordingly, assumptions that thermal resistance develops from growth or survival in a low-water activity food or solution may overlook other stressors, such as desiccation, that could contribute to results.

The presented research sought to compare thermal inactivation kinetics of two outbreak-associated isolates of STEC, O121:H19 (flour) and O157:H7 (alfalfa sprouts) grown using two different culture preparation methods (broth and lawn). Additionally, cells were suspended in

solutions of various water activity levels (a_w 0.99, 0.75, and 0.50) to investigate the impacts of osmolyte-induced reduced water activity on thermal resistance during isothermal treatments in the absence of a LWAF matrix.

2. Materials and Methods

Culture Preparation. Two strains of Shiga toxin-producing *E. coli*, O121:H19 (FNW19M81, 2016 wheat flour outbreak isolate [FDA]) and O157:H7 F4546 (1997, alfalfa sprout outbreak isolate) were chosen for the presented research. Strains were kept frozen in TSB + glycerol (BD, Sparks, MD) at -80°C until cultured onto Sorbitol MacConkey Agar (SMAC, BD, Sparks, MD) and incubated at 37°C for 24 h. Isolated colonies of each strain were transferred to 10 mL TSB in sterile, 15-mL conical tubes and incubated at 37°C for 24 h to create overnight cultures.

Sample Preparation. For each replication, broth and lawn cultures of comparable age were prepared to be used simultaneously. Lawns were prepared on TSA and harvested with 0.1% peptone (*11*). Overnight TSB cultures were grown as described above for broth samples. Capillary tubes (75 mm x 1.2 mm; Fisherbrand) were filled with either broth or lawn cultures, flame-sealed, and maintained at 4°C until use within two hours. Starting concentrations of broth and lawn cultures were 8.6-9.7 log CFU/mL and 10.4-11.1 CFU/mL, respectively.

Samples evaluating thermal resistance in reduced water activities were made from broth overnight cultures. Cultures were centrifuged (4,000 x *g*, 10 min) and the pellets were resuspended with low- (a_w 0.50 ± 0.04; 82% glycerol, %w/v) and intermediate- (a_w 0.75 ± 0.02; 26% NaCl, %w/v) water activity solutions. Capillary tubes were filled with each solution, sealed by flame, and tubes were maintained at 4°C until used within two hours. Starting concentrations for the reduced-water activity samples were 5.9-7.3 log CFU/mL. Each experiment included a

non-treated sample to check inoculation levels. An additional non-treated, post-experiment sample was enumerated for experiments using reduced-water activity solutions to ensure osmotic stress did not cause bacterial reductions during the length of the experiments.

Thermal Inactivation Experiments. Thermal inactivation studies were conducted using a water bath at three low temperatures (56, 59, and 62°C ± 0.2°C) to best visualize the decimal reductions. To ensure consistency in temperature and exposure time across samples, sets of each strain in separate capillary tubes were treated together in the same replicate (5 capillary tubes of each strain per set). Capillary tubes were fully submerged in the water, and a single tube, designated as the 0-min sample, was immediately removed and considered to be the initial bacterial concentration after exposure to the thermal treatment. Samples were removed at predetermined time intervals. Upon removal, samples were placed in an ice water bath to end the thermal treatment, sanitized, rinsed, and crushed as described by Sharma et al. (*41*). Conical tubes with the samples were vortexed extensively, serially diluted, and spiral plated (100 µL) onto TSA (Dilucup® Whitley WASP Touch, Microbiology International, Don Whitley Scientific Limited, West Yorkshire, UK). After 4-h incubation at 37°C, TSA plates were overlaid with SMAC for selective recovery of injured *E. coli* cells. Colonies counts were recorded using an automatic reader (ProtoCOL SR/HR, Version 1.47.0, Cambridge, UK).

Analyses and Experimental Design. At least three independent replications were performed for each experiment. Within each replicate, log bacterial reductions were calculated by subtracting each remaining concentration (log CFU/g) from the initial concentration at 0 min (log CFU/g). Reductions were graphed against time, and *D*-values, or decimal reductions (min), were calculated from the inverse of the linear slope. For each experiment, log *D*-values for all three temperatures within a replication were plotted against temperature and the *z*-value, or

temperature required to make a 10-fold change of the *D*-value (°C), was calculated from the inverse of the linear slope. Correlation coefficients (R^2) were used to evaluate goodness of fit.

Average *D*- and *z*- values were compared using ANOVA and Tukey's Honest Significant Difference with JMP® Pro 14.0.0 (SAS Institute Inc., Cary, NC) with a significance level of $p <$ 0.05. Comparisons of *D*- and *z*-values determined significant differences of thermal resistance between strains, culture preparation method, and water activities. Broth experiment *D*- and *z*-values were compared against those for lawn experiments, between and within STEC strains. *D*- and *z*-values of each water activity were also compared between and within STEC strains.

3. Results

Inactivation kinetics (*D*- and *z*-values) were calculated for STEC O157:H7 and O121:H19 cells immersed in solutions within capillary tubes subjected to isothermal conditions in a circulating heated water bath (Table 5.1). Log *D*-values were used to calculate *z*-values (Figures 5.1 and 5.2). Thermal inactivation experiments comparing *D*- and *z*-values for lawn- and broth-grown samples (a_w 0.99) were conducted simultaneously (Table 5.1). At 56°C with 12-min sampling intervals, average *D*-values of broth cultures for both strains were significantly greater (approximately 2x) than those of lawn cultures, and the *D*-values of O121 for both broth and lawn samples were significantly greater than those of O157 ($p < 0.05$; Table 5.1). $D_{59°C}$ and $D_{62°C}$ were determined from 5- and 1-min sampling times, respectively. Statistical analyses showed no significant differences between the $D_{59°C}$ and $D_{62°C}$ of the two strains ($p > 0.05$). Based on the log *D*-values, *z*-values were calculated and ranged from 4.33-5.75°C, with high correlation coefficients (R^2; 0.95-0.98). The calculated z-values were slightly higher for broth cultures compared to lawn cultures, but the differences were not significant ($p > 0.05$).

Experiments examining effects of reduced water activity used broth-grown cells resuspended in intermediate- (a_w 0.75) and low- (a_w 0.50) water activity suspensions and compared against the high-water activity (a_w 0.99, broth) results. These samples were treated at the same temperatures as experiments comparing broth and lawn samples but used different sampling intervals. $D_{56°C}$ of low and intermediate samples ranged from 7.70-11.85 min and were not significantly different from one another for either strain (Table 5.1; $p > 0.05$). In contrast, these *D*-values were significantly lower by approximately 3-fold ($p < 0.05$) than those of the high-water activity samples (a_w 0.99, broth). $D_{59°C}$ and $D_{62°C}$ of low and intermediate cultures were not significantly different from each other or high-water activity samples for either strain. The *z*-values of O121 at intermediate- and low-water activity were higher (29% and 33%, respectively), though not significantly ($p > 0.05$), than those for the high-water activity samples. The *z*-value of the O157 low-water activity sample was 36% higher than that of the high-water activity sample, though not significantly different ($p > 0.05$), but the intermediate sample's *z*-value was significantly higher than that of the high-water activity sample by 43% ($p < 0.05$).

4. Discussion

In the present study, we compared thermal resistances (*D*- and *z*-values) of two STEC strains (O121:H19 and O157:H7) based on culture preparation method (broth or lawn) and water activity of solutions during isothermal treatments. STEC O121:H19 strain was selected because it was isolated from a LWAF outbreak (wheat flour, 2016), which prompted the hypothesis that it may have increased resistance to low-water activity stress, with potential cross-protection against thermal treatments. STEC O157:H7 was isolated from produce (alfalfa sprouts, 1997), and was not presumed to have increased resistance against thermal or osmotic stress.

Many studies that report *D*-values of pathogens in relation to low-water activity environments or foods are highly variable, which could be due in part to cultivation methods, as well as prior cell history. Culture preparation influences the physiological state of cells. Lawn-based or colonial growth has been noted to slightly slow cell growth rate, compared to planktonic growth, because access to nutrients and substrates is limited to only a portion of the colony forming unit (*43*). Propagation methods and resulting physiology have been studied for impacts on thermal resistance and survival in low-water activity environments (*31*). TSA-grown lawns of confluent growth result in a more stable, desiccation-resistant inoculum on LWAF compared to broth-grown cells (*6*, *50*). Regarding thermal resistance, however, we observed greater thermal resistance of broth cultures ($p < 0.05$) compared to lawn cultures during isothermal treatments.

In addition to culture preparation, prior cell history, could impact conclusions made regarding thermal resistance of a microorganism in a low-water activity environment. Inherent genetic differences between microorganisms or adaptation of the microorganism to a particular stress, such as osmotic, desiccation, or thermal stress, may alter the cell's physiology and stress response. For example, Enache et al. noted that there was no difference between thermal resistance of TSA- or TSB-grown *Salmonella*, but *Enterococcus faecium* NRRL B-2354 was more thermoresistant when grown in TSB than on TSA, which was similarly observed in the presented study (*15*). The results indicate that both culture preparation and genetic differences may impact development of thermal resistance. Our experiments showed that O121 displayed greater heat resistance than O157 at 56°C, particularly in broth-grown samples. Historically, *E. coli* O157:H7 has been attributed with the majority of STEC infections, but an increasing number of non-O157-associated illnesses are occurring, prompting comparative research between serotypes (*8*, *14*, *37*, *44*). While the two strains used in this study do not currently have

published whole genome sequences, it has been reported that variation in stress response genes among STEC serotypes has been observed (*32*). In addition, Suehr et al. found O121:H19 to have increased desiccation tolerance compared to O157:H7, and even more so when acid-adapted, alluding to genomic differences between the serotypes that alter stress tolerance (*46*).

Increased thermal resistance has been observed in pathogens on LWAF (*1*, *49*). Reduced water activity can result from desiccation and the evaporation of water, or the addition of solutes to cause an osmotic shift, in which cells are surrounded by an aqueous environment rather than a gas phase void of free water molecules (*7*). Furthermore, addition of solutes to reduce the water activity can cause the accumulation of protective compatible solutes, which can increase the cells' resistance to some stressors, including heat (*3*, *47*). These conditions and stressors elicit different stress response genes, which may impact whether or not microorganisms exhibit increased thermal resistance, possibly through cross-protection (*13*, *20*, *40*, *42*, *45*). This cross-protection may be induced by overlap of gene expression in response to the two stressors (osmotic and thermal), which has been observed in *E. coli* K12 (*21*). Also, *Listeria monocytogenes* exhibited increased thermal resistance (8-fold) when shocked with 9% NaCl solutions, and even greater thermal resistance (22-fold) when grown in the hyperosmotic solution, indicating that the osmotic stress exposure impacted the pathogen's thermal resistance (*25*). In contrast, the presented research indicated decreased thermal resistance (lower *D*-values) of STEC when exposed to osmotic stress in the intermediate- and low-water activity solutions compared to the high-water activity solution.

Although thermal resistance of STEC was not induced by a reduced water activity in the presented study, it is possible that the types of solute used (NaCl and glycerol) influenced this result. The type of solute can significantly impact how the cell adjusts to the reduced water

activity (*33*). When comparing several different sugar suspensions with reduced water activity, *Salmonella* serotypes exhibited greater thermal resistance in sucrose solutions, as opposed to solutions with fructose, glycerol, and sorbitol (*18*). When using NaCl (0.5-8.5%) to decrease the water activity of TSB, strains of *E. coli* O157:H7 displayed increased *D*-values at temperatures of 54.5-64.5°C, as well as an increase in the *z*-values (*5*). In the presented work, NaCl was also used to reduce the water activity with much greater concentrations (26%, a_w 0.75). Although some studies have shown increased *D*-values when reduced-water activity solutions were used as the matrix, our results showed decreased the *D*-values for both strains in the 26% NaCl and 82% glycerol solutions. However, the *z*-values increased, indicating increased resiliency to the temperature changes and heat treatment (*5*). Other studies also observed *D*-values for both serotypes investigated in the presented work, but several of these studies used neutral buffered solution suspensions in the experiments and reported much lower $D_{56°C}$ of <2.0 min (*8*, *34*). The use of tryptic soy broth as the medium in the presented study may have contributed to the higher *D*-values determined, as the nutrients in the broth may have offered some protection from the isothermal treatments. Solutes vary in their protective capabilities for microorganisms against thermal treatments and changes in temperature (*33*). In the same way, the food matrix components also greatly impact the efficacy of a thermal treatment to inactivate microorganisms. The added complexities of the food composition, as well as the non-isothermal nature of food processing treatments make vital the careful consideration of the risks of applying laboratory-acquired thermal resistance data to food systems (*23*).

Studies investigate causes of microbial thermal resistance in LWAF using a wide variety of techniques, methods, and equipment, which certainly impact results and conclusions to unpredictable degrees (*4*). For example, when investigating differences in thermal resistance

strains of *Salmonella* as impacted by high- and low-water activity, increased resistance to the isothermal treatments in a low-water activity solution was only observed at higher temperatures (>70°C; *33*). However, there was no difference in thermal resistance of the pathogen in the different solutions at lower temperatures (<65°C), indicating temperature intensity impacts thermal resistance at a low water activity (*33*). Furthermore, while higher temperatures may induce thermal resistance associated with low water activity, observable differences between the heat resistance of pathogens may diminish. For example, *E. coli* O157:H7 were significantly more resistant to isothermal 56°C treatments in apple juice compared to non-O157 serotypes, as indicated by *D*-values, but significant differences between the serotypes were not observed at higher temperatures of >60°C (*14*). In the presented study, the only differences in *D*-values observed between the O121:H19 and O157:H7 were at the lowest tested temperature (56°C). In addition to the impacts of the intensity of thermal treatments on observed differences in thermal resistance between microorganisms, heating methods can significantly impact results. Closed systems, such as the heating method used in the presented study, prevented evaporation due to rising temperatures and reduction of water activity, which may be seen in open systems. Thermal resistance data acquired from closed, isothermal treatments should not be compared to data acquired from studies using inoculated foods and open, non-isothermal conditions (*12*).

The presented research sought to identify the impact of reduced water activities on thermal resistance without the added complexity of a food matrix or desiccation. Our results indicated that osmotic stress alone does not result in enhanced thermal resistance of STEC compared to high-water activity solutions. Evaluating thermal resistance of pathogens with *D*- and *z*-values can be valuable for LWAF processors implementing thermal treatments, as there are many recorded instances of increased thermotolerance with reduced water activities. However,

this data, along with other results, should be used with discernment, as all results from laboratory studies are likely impacted by experimental factors influencing cell stress responses and cross-protection. Specifically, isothermal resistance data gathered on a laboratory scale is not directly applicable to a non-isothermal food system with complex components.

Figures

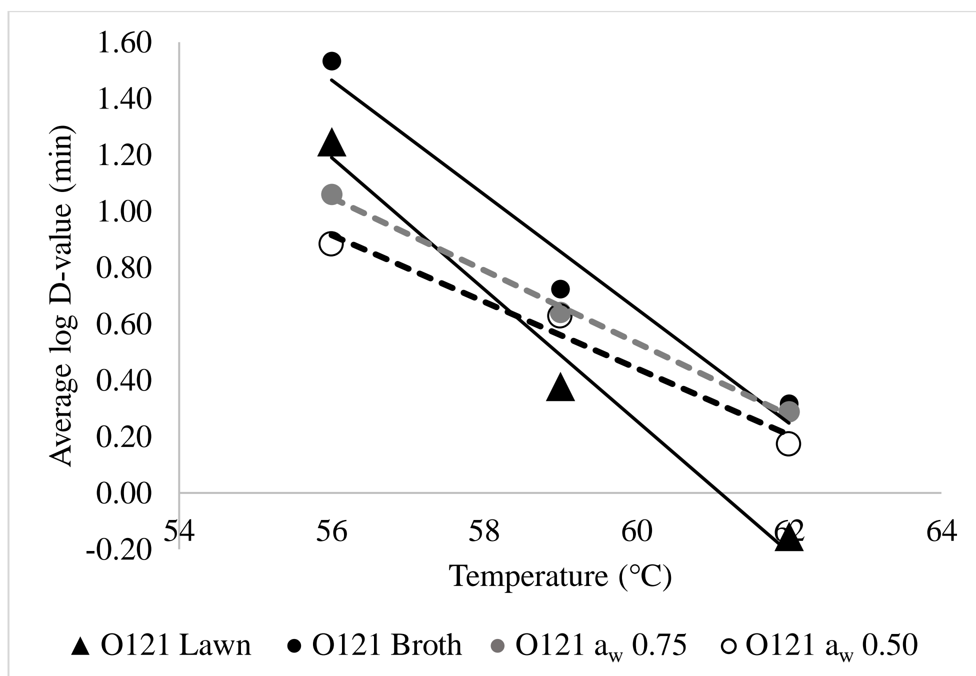

Figure 5.1. Averaged log D-values of STEC O121 lawn (▲), broth and high-water activity (a_w 0.99 ●), intermediate-water activity (a_w 0.75 ●), and low-water activity (a_w 0.50 ○).

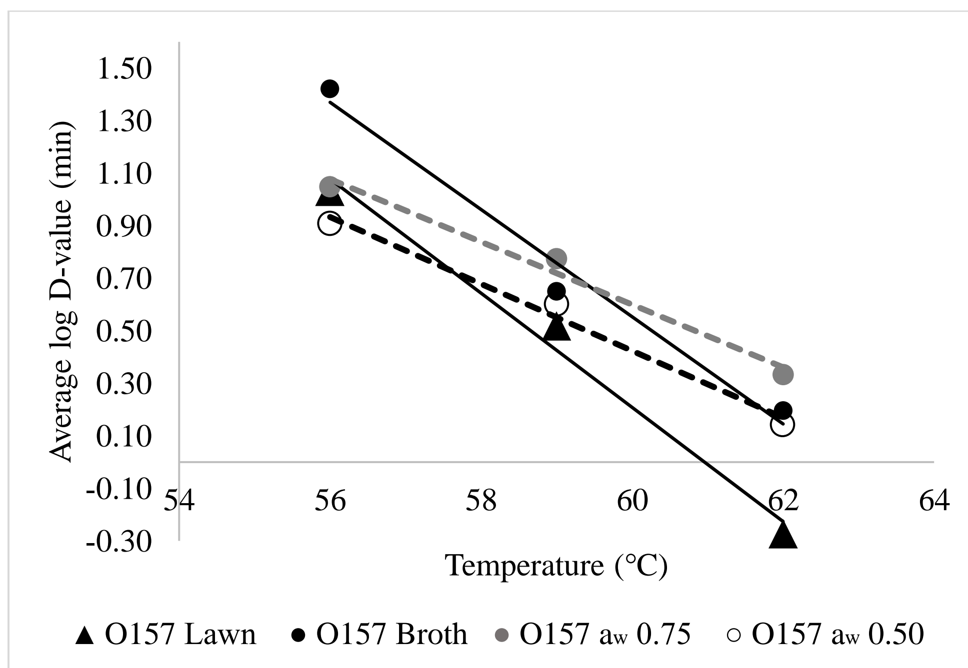

Figure 5.2. Averaged log D-values of STEC O157 lawn (▲), broth and high-water activity (a_w 0.99 ●), intermediate -water activity (a_w 0.75 ●), and low-water activity (a_w 0.50 ○).

Tables

Table 5.1. Calculated *D*-values (min ± SD) and z-values (°C ± SD) for each experiment using lawn and broth culture preparation methods and reduced-a_w solutions of *E. coli* O121:H19 and O157:H7.

	$D_{56°C}$ (min)	$D_{59°C}$ (min)	$D_{62°C}$ (min)	*z*-value (°C)	R^2 of *z*
E. coli O121					
Lawn	17.78 ± 2.70 a	2.38 ± 0.43 a	0.74 ± 0.24 ab	4.33 ± 0.36 a	0.98
Broth (a_w 0.99)	34.26 ± 4.02 b A	4.79 ± 1.41 a A	2.23 ± 1.21 a A	5.75 ± 0.47 a A B	0.95
a_w 0.75	11.85 ± 3.77 C	4.36 ± 0.28 A	1.97 ± 0.42 A	8.04 ± 1.70 A B C	0.99
a_w 0.5	7.70 ± 1.32 C	4.25 ± 0.49 A	1.49 ± 0.05 A	8.54 ± 1.02 B C	0.96
E. coli O157					
Lawn	10.76 ± 0.51 c	3.41 ± 1.08 a	0.54 ± 0.04 b	4.67 ± 0.04 a	0.97
Broth (a_w 0.99)	26.57 ± 2.86 d B	4.59 ± 1.13 a A	1.72 ± 0.74 ab A	5.04 ± 0.98 a A	0.97
a_w 0.75	11.35 ± 2.30 C	6.01 ± 1.03 A	2.22 ± 0.65 A	8.81 ± 2.30 C	0.97
a_w 0.5	8.24 ± 2.05 C	4.09 ± 1.03 A	1.41 ± 0.27 A	7.82 ± 0.21 A B C	0.99

Lowercase connecting letters within a column indicate significant differences between *D*- and *z*- values of culture preparation method ($p<0.05$). Uppercase connecting letters within a column indicate significant differences between *D*- and *z*- values of water activities ($p<0.05$).

References

1. Archer, J., E. T. Jervis, J. Bird, and E. J. Gaze. 1998. Heat resistance of *Salmonella* Weltevreden in low-moisture environments. *J. Food Prot.* 61:969–973.
2. Aviles, B., C. Klotz, J. Eifert, R. Williams, and M. Ponder. 2013. Biofilms promote survival and virulence of *Salmonella enterica* sv. Tennessee during prolonged dry storage and after passage through an in vitro digestion system. *Int. J. Food Microbiol.* 162:252–259. http://doi.org/10.1016/j.ijfoodmicro.2013.01.026.
3. Barbosa-Cánovas, G. V., A. J. Fontana Jr., and S. J. Schmidt. 2007. Water activity in foods: Fundamentals and applications (first ed.). Blackwell Publishing Professional, Ames, IA.
4. Beuchat, L. R., E. Komitopoulou, H. Beckers, R. P. Betts, F. Bourdichon, S. Fanning, H. M. Joosten, and B. H. Ter Kuile. 2013. Low–water activity foods: Increased concern as vehicles of foodborne pathogens. *J. Food Prot.* 76:150–172. http://doi.org/10.4315/0362-028X.JFP-12-211.
5. Blackburn, C. de W., L. M. Curtis, L. Humpheson, C. Billon, and P. J. McClure. 1997. Development of thermal inactivation models for *Salmonella enteritidis* and *Escherichia coli* O157:H7 with temperature, pH and NaCl as controlling factors. *Int. J. Food Microbiol.* 38:31–44.
6. Bowman, L. S., K. M. Waterman, R. C. Williams, and M. A. Ponder. 2015. Inoculation preparation affects survival of *Salmonella enterica* on whole black peppercorns and cumin seeds stored at low water activity. *J. Food Prot.* 78:1259–1265. http://doi.org/10.4315/0362-028X.JFP-14-483.
7. Burgess, C. M., A. Gianotti, N. Gruzdev, J. Holah, S. Knøchel, A. Lehner, E. Margas, S. S. Esser, S. Sela (Saldinger), and O. Tresse. 2016. The response of foodborne pathogens to osmotic and desiccation stresses in the food chain. *Int. J. Food Microbiol.* 221:37–53. http://doi.org/10.1016/j.ijfoodmicro.2015.12.014.
8. Cabrera-Diaz, E., T. Moseley, L. Lucia, J. Dickson, A. Castillo, and G. R. Acuff. 2009. Fluorescent protein-marked *Escherichia coli* Biotype I strains as surrogates for enteric pathogens in validation of beef carcass interventions. *J. Food Prot.* 72:295–303.
9. Centers for Disease Control and Prevention. 2009. Mutlistate outbreak of *Salmonella* Typhimurium infections linked to peanut butter, 2008-2009 (Final Update). Available at: https://www.cdc.gov/salmonella/2009/peanut-butter-2008-2009.html?CDC_AA_refVal=https%3A%2F%2Fwww.cdc.gov%2Fsalmonella%2Ftyphimurium%2Fupdate.html. Accessed 12 December 2019.
10. Centers for Disease Control and Prevention. 2019. Reports of selected *E. coli* outbreak investigations. Available at: https://www.cdc.gov/ecoli/outbreaks.html. Accessed 2 December 2019.
11. Channaiah, L. H., M. Michael, J. C. Acuff, R. K. Phebus, H. Thippareddi, M. Olewnik, and G. Milliken. 2017. Validation of the baking process as a kill-step for controlling *Salmonella* in muffins. *Int. J. Food Microbiol.* 250:1–6. http://doi.org/10.1016/j.ijfoodmicro.2017.03.007.
12. Chung, H. J., S. L. Birla, and J. Tang. 2008. Performance evaluation of aluminum test cell designed for determining the heat resistance of bacterial spores in foods. *Lebensm. Wiss. Technol. Food Sci. Technol.* 41:1351–1359. http://doi.org/10.1016/j.lwt.2007.08.024.

13. Deng, X., Z. Li, and W. Zhang. 2012. Transcriptome sequencing of *Salmonella enterica* serovar Enteritidis under desiccation and starvation stress in peanut oil. *Food Microbiol.* 30:311–315. http://doi.org/10.1016/j.fm.2011.11.001.
14. Enache, E., E. C. Mathusa, P. H. Elliott, D. G. Black, Y. Chen, V. N. Scott, and D. W. Schaffner. 2011. Thermal resistance parameters for Shiga toxin–producing *Escherichia coli* in apple juice. *J. Food Prot.* 74:1231–1237. http://doi.org/10.4315/0362-028X.JFP-10-488.
15. Enache, E., A. Kataoka, D. G. Black, C. D. Napier, R. Podolak, and M. M. Hayman. 2015. Development of a dry inoculation method for thermal challenge studies in low-moisture foods by using talc as a carrier for *Salmonella* and a surrogate (*Enterococcus faecium*). *J. Food Prot.* 78:1106–1112. http://doi.org/10.4315/0362-028X.JFP-14-396.
16. Esbelin, J., T. Santos, and M. Hébraud. 2018. Desiccation: An environmental and food industry stress that bacteria commonly face. *Food Microbiol.* 69:82–88. http://doi.org/10.1016/j.fm.2017.07.017.
17. Giaouris, E., G. Samoilis, N. Chorianopoulos, D. Ercolini, and G. -J. Nychas. 2013. Differential protein expression patterns between planktonic and biofilm cells of *Salmonella enterica* serovar Enteritidis PT4 on stainless steel surface. *Int. J. Food Microbiol.* 162:105–113. http://doi.org/10.1016/j.ijfoodmicro.2012.12.023.
18. Goepfert, J. M., I. K. Iskander, and C. H. Amundson. 1970. Relation of the heat resistance of salmonellae to the water activity of the environment. *Appl. Microbiol.* 19:429–433.
19. Grasso, E. M., C. N. Stam, N. M. Anderson, and K. Krishnamurthy. 2014. Heat and steam treatments, p. 403-424. *In* J. B. Gurtler, M. P. Doyle, and J. L. Kornacki (ed.), The microbiological safety of low water activity foods and spices, Springer, New York, NY.
20. Gruzdev, N., P. Pinto, and S. S. Saldinger. 2012. Persistence of *Salmonella enterica* during dehydration and subsequent cold storage. *Food Microbiol.*, 32:415–422. http://doi.org/10.1016/j.fm.2012.08.003.
21. Gunasekera, T. S., L. N. Csonka, and O. Paliy. 2008. Genome-wide transcriptional responses of *Escherichia coli* K-12 to continuous osmotic and heat stresses. *J. Bacteriol*, 190:3712–3720. http://doi.org/10.1128/JB.01990-07.
22. Hiramatsu, R., M. Matsumoto, K. Sakae, and Y. Miyazaki. 2005. Ability of Shiga Toxin-Producing *Escherichia coli* and *Salmonella* spp. to survive in a desiccation model system and in dry foods. *Appl. Environ. Microbiol.* 71:6657–6663. http://doi.org/10.1128/AEM.71.11.6657-6663.2005.
23. Huertas, J. P., A. Aznar, A. Esnoz, P. S. Fernández, A. Iguaz, P. M. Periago, and A. Palop. 2016. High heating rates affect greatly the inactivation rate of *Escherichia coli*. *Front. Microbiol.* 7:3668–8. http://doi.org/10.3389/fmicb.2016.01256
24. Jackson, T. C., M. D. Hardin, and G. R. Acuff. 1995. Heat Rresistance of *Escherichia coli* O157:H7 in a nutrient medium and in ground beef patties as influenced by storage and holding temperartures. *J. Food Prot.* 59:230–237.
25. Jørgensen, F., P. J. Stephens, and S. Knøchel. 1995. The effect of osmotic shock and subsequent adaptation on the thermotolerance and cell morphology of *Listeria monocytogenes*. *J. Appl. Bacteriol.* 79:274–281.
26. Juneja, V. K. and B. S. Eblen. 2000. Heat inactivation of *Salmonella typhimurium* DT104 in beef as affected by fat content. *Letters in Appl. Microbiol.* 30:461–467.

27. Lang, E., C. Iaconelli, F. Zoz, S. Guyot, P. Alvarez-Martin, L. Beney, J. -M. Perrier-Cornet, and P. Gervais. 2017. Drying parameters greatly affect the destruction of *Cronobacter sakazakii* and *Salmonella* Typhimurium in standard buffer and milk. *Food Microbiol.* 62:82–91. http://doi.org/10.1016/j.fm.2016.10.005.
28. Lang, E., L. Chemlal, P. Molin, S. Guyot, P. Alvarez-Martin, J. -M. Perrier-Cornet, P. Dantigny, and P. Gervais. 2017. Modeling the heat inactivation of foodborne pathogens in milk powder: High relevance of the substrate water activity. *Frin*. 99:577–585. http://doi.org/10.1016/j.foodres.2017.06.028.
29. Laroche, C. and P. Gervais. 2003. Unexpected thermal destruction of dried, glass bead-immobilized microorganisms as a function of water activity. *Appl. Environ. Microbiol.* 69:3015–3019. http://doi.org/10.1128/AEM.69.5.3015-3019.2003.
30. Laroche, C., F. Fine, and P. Gervais. 2005. Water activity affects heat resistance of microorganisms in food powders. *Int. J. Food Microbiol.* 97:307–315. http://doi.org/10.1016/j.ijfoodmicro.2004.04.023.
31. Limcharoenchat, P., S. E. Buchholz, M. K. James, N. O. Hall, E. T. Ryser, and B. P. Marks. 2018. Inoculation protocols influence the thermal resistance of *Salmonella* Enteritidis PT 30 in fabricated almond, wheat, and date products. *J. Food Prot.* 81:606–613. http://doi.org/10.4315/0362-028X.JFP-17-297.
32. Lindsey R. L., E. Trees, S. Sammons, V. Loparev, M. Frace, N. Strockbine, A. L. Sabol, E. Sowers, D. Stripling, H. Martin, K. Knipe, L. Rowe, P. Gerner-Smidt. 2014. Draft whole-genome sequences of nine non-O157 Shiga toxin-producing *Escherichia coli* strains. *Genome Announc*. 2:e00501-14. doi:10.1128/genomeA.00501-14.
33. Mattick, K. L., F. Jorgensen, P. Wang, J. Pound, M. H. Vandeven, L. R. Ward, J. D. Legan, H. M. Lappin-Scott, and T. J. Humphrey. 2001. Effect of challenge temperature and solute type on heat tolerance of *Salmonella* serovars at low water activity. *Appl. Environ. Microbiol.* 67:4128–4136. http://doi.org/10.1128/AEM.67.9.4128-4136.2001.
34. Monu, E. A., M. Valladares, D. H. D'souza, and P. M. Davidson. 2015. Determination of the thermal inactivation kinetics of *Listeria monocytogenes*, *Salmonella enterica*, and *Escherichia coli* O157:H7 and non-O157 in buffer and a spinach homogenate. *J. Food Prot.* 78:1467–1471. http://doi.org/10.4315/0362-028X.JFP-14-488.
35. Newkirk, J.J., J. Wu, J. C. Acuff, C. B. Caver, K. Mallikarjunan, B. D. Wiersema, R. C. Williams, and M. A. Ponder. 2018. Inactivation of *Salmonella enterica* and surrogate *Enterococcus faecium* on whole black peppercorns and cumin seeds using vacuum steam pasteurization. *Front. Sust. Food Sys*. 2:252–12.
36. Nielsen, M.-B., G. M. Knudsen, V. Danino-Appleton, J. E. Olsen, and L. E. Thomsen. 2013. Comparison of heat stress responses of immobilized and planktonic *Salmonella enterica* serovar Typhimurium. *Food Microbiol.* 33:221–227. http://doi.org/10.1016/j.fm.2012.09.020.
37. Perna, N. T., G. P. Ill, V. Burland, B. Mau, J. D. Glassner, D. Rose, G. F. Mayhew, P. S. Evans, J. Gregor, H. A. Kirkpatrick, G. Pósfai, J. Hackett, S. Klink, A. Boutin, Y. Shao, L. Miller, E. J. Grotbeck, N. W. Davis, A. Lim, E. T. Dimalanta, K. D. Potamousis, J. Apodaca, T. S. Anatharaman, J. Lin, G. Yen, D. C. Schwartz, R. A. Welch, and F. R. Blattner. 2001. Genome sequence of enterohaemorrhagic *Escherichia coli* O157:H7. *Nature*. 409:52953.

38. Podolak, R., E. Enache, W. Stone, D. G. Black, and P. H. Elliott. 2010. Sources and risk factors for contamination, survival, persistence, and heat resistance of *Salmonella* in low-moisture foods. *J. Food Prot.* 73:1919–1936.
39. Rockabrand, D., T. Arthur, G. Korinek, K. Livers, and P. Blum. 1995. An essential role for the *Escherichia coli* DnaK protein in starvation-induced thermotolerance, H_2O_2 resistance, and reductive division. *J. Bacteriol.* 177:3695–3703.
40. Shachar, D. and S. Yaron. 2006. Heat tolerance of *Salmonella enterica* serovars Agona, Enteritidis, and Typhimurium in peanut butter. *J. Food Prot.* 69:2687–2691.
41. Sharma, M., B. B. Adler, M. D. Harrison, and L. R. Beuchat. 2005. Thermal tolerance of acid-adapted and unadapted *Salmonella*, *Escherichia coli* O157:H7, and *Listeria monocytogenes* in cantaloupe juice and watermelon juice. *Letters in Appl. Microbiol.* 41:448–453. http://doi.org/10.1111/j.1472-765X.2005.01797.x.
42. Shen, S. and F. C. Fang. 2012. Integrated stress responses in *Salmonella*. *Int. J. Food Microbiol.* 152:75-81. http://dx.doi.org/10.1016/j.ijfoodmicro.2011.04.017.
43. Skandamis, P. N. and S. Jeanson. 2015. Colonial vs. planktonic type of growth: mathematical modeling of microbial dynamics on surfaces and in liquid, semi-liquid and solid foods. *Front. in Microbiol.* 6:382–9. http://doi.org/10.3389/fmicb.2015.01178.
44. Smith, J. L. and P. M. Fratamico. 2012. Effect of stress on non-O157 Shiga toxin–producing *Escherichia coli*. *J. Food Prot.* 75:2241–2250. http://doi.org/10.4315/0362-028X.JFP-12-255.
45. Spector, M. P. and W. J. Kenyon. 2012. Resistance and survival strategies of *Salmonella enterica* to environmental stresses. *Frin*, 45:455–481. http://doi.org/10.1016/j.foodres.2011.06.056.
46. Suehr, Q. J., F. Chen, N. M. Anderson, and S. E. Keller. 2020. Effect of pH on survival of *Escherichia coli* O157, *Escherichia coli* O121, and *Salmonella enterica* during desiccation and short-term storage. *J. Food Prot.* 83:211–220. http://doi.org/10.4315/0362-028X.JFP-19-195.
47. Syamaladevi, R. M., J. Tang, R. Villa-Rojas, S. Sablani, B. Carter, and G. Campbell. 2016. Influence of water activity on thermal resistance of microorganisms in low-moisture foods: A review. *Compr. Rev. in Food Sci. F.*15:353–370. http://doi.org/10.1111/1541-4337.12190.
48. U.S. Food and Drug Administration (FDA). 2017. FDA investigated multistate outbreak of *E. coli* O157:H7 infections linked to soynut butter. Available at: https://www.fda.gov/Food/RecallsOutbreaksEmergencies/Outbreaks/ucm544964.htm. Date accessed: 14 December 2019.
49. Villa-Rojas, R., J. Tang, S. Wang, M. Gao, D. -H. Kang, J. -H. Mah, P. Gray, M. E. Sosa-Morales, and A. López-Malo. 2013. Thermal inactivation of *Salmonella* Enteritidis PT 30 in almond kernels as influenced by water activity. *J. Food Prot.* 76:26–32. http://doi.org/10.4315/0362-028X.JFP-11-509.
50. Wiertzema, J. R., C. Borchardt, A. K. Beckstrom, K. Dev, P. Chen, C. Chen, Z. Vickers, J. Feirtag, L. Lee, R. Ruan, and D. J. Baumler. 2019. Evaluation of methods for inoculating dry powder foods with *Salmonella enterica*, *Enterococcus faecium*, or *Cronobacter sakazakii*. *J. Food Prot.* 82:1082–1088. http://doi.org/10.4315/0362-028X.JFP-18-284.

Chapter 6 Conclusions & Future Directions

The overarching goals of the presented studies were to provide information on thermal inactivation strategies of pathogens on low-water activity foods (LWAF), as well as particular factors that impact the efficacy of thermal treatments. The microbiological safety of LWAF is complex because many of these foods are raw or minimally processed and considered ready-to-eat by consumers, so typical kill steps, such as cooking are not performed. However, pathogen contamination and long-term survival are possible. *Salmonella* spp. been the focus of much of the LWAF safety research due to their high association with LWAF outbreaks and recalls, but other pathogens, such as Shiga toxin-producing *E. coli* (STEC) and *Listeria monocytogenes* are now known to survive desiccation and also exhibit potential thermotolerance in LWAF. Our research further supports the need for investigation of the risks posed by additional foodborne pathogens on LWAF, as similar thermal resistance between *Salmonella*, STEC, and *L. monocytogenes* were observed.

A common trend noted in research for the last many years is the connection between increased thermal resistance and LWAF or environments. It has been assumed that the stress placed on cells by a low water activity leads to cross-protection against thermal stress. However, it is not fully understood if water activity alone causes the observed thermal resistance, or if other factors impact this response. To further investigate this idea, *D*- and *z*-values were determined for two STEC (O121:H19 and O157:H7) in high-, intermediate-, and low-water activity solutions that were thermally treated. The high-water activity solution yielded significantly higher *D*-values than those of reduced-water activity solutions, indicating that the osmotic stress alone applied in the intermediate- and low-water activity solutions did not cause the STEC to display increased thermal resistance. Additionally, the strains were compared

because O121:H19 was hypothesized to have increased inherent thermal resistance in a low-water activity environment since it was isolated from a LWAF. This strain was significantly more resistant to some thermal treatments at the high-water activity level compared to O157:H7. Lastly, two different growth methods (broth vs. lawn) were compared for their potential impacts on thermal resistance, which revealed that the broth (planktonic growth) cultures had greater thermal resistance than the lawn (colonial growth) cultures. These thermal resistance studies highlight the many variables that can impact thermal resistance. There are examples of observed thermal resistance of pathogens on LWAF, but this is likely due to a number of factors, such as desiccation, adaptation or prior exposure to stress, or inherent resistances due to its genome.

As a practical and applied approach to address the possible thermal resistances of pathogens in LWAF, the use of low-temperature, vacuum-assisted steam was explored as an intervention on raisins, macadamia nuts, and dried apricot halves when contaminated with STEC, *L. monocytogenes*, and *Salmonella*. The vacuum-assisted steam was successful in reducing pathogen load up to 5 log CFU/g in many cases at temperatures ranging from 62-82°C. The pathogens were reduced similarly to one another, meaning a single pathogen did not stand out as having greater resistance to the treatments. The main differences observed in the efficacy of the steam were as a result of the food type: raisins required lower temperature treatments (62 and 72°C; 20 and 5 min, respectively) than apricot halves (72 and 82°C; 20 and 5 min, respectively) for 5-log reductions. Macadamia nuts required higher temperatures (72 and 82°C) and only resulted in 4-5-log reductions of pathogens at longer treatment times compared to the apricot halves (up to 38 min). The vacuum-steam was effective in reducing pathogens from the surface of LWAF, but the variation of treatment times required for significant reductions was influenced by food type. These results emphasize that caution should be used by food processors

who wish to thermally treat multiple types of foods together, for instance, in a trail mix, and that validation of effectiveness on the target commodity is needed. Particle sizes of the foods likely impacted the movement of steam and subsequent heat transfer. Food composition, such as fat and water activity, can also impact the cellular response to the thermal treatment. These factors should be further investigated to determine the degrees of impact on the treatment process by developing methods to measure relative heat transfer of the steam throughout the LWAF sample during vacuum-steam treatments and testing the vacuum-steam on other food types with varying composition.

In accordance with the Risk-Based Preventive Controls for Human Food rule of the Food Safety Modernization Act, food processors must develop and validate preventive controls and interventions to address risks associated with the food product. In-plant validations serve as the best method for proving process efficacy in reducing pathogens, which require surrogate organisms that mimic the inactivation of target pathogens. *Pediococcus acidilactici* was proposed as a non-pathogenic surrogate organism for the pathogens on these foods when treated with low-temperature, vacuum-assisted steam. At the respective lower treatment temperatures for each food, the *Pediococcus* reductions were significantly lower than pathogen reductions, indicating that it is a conservative surrogate for the pathogens under those conditions. At the respective higher temperatures, the non-pathogenic lactic acid bacterium was not significantly more resistant to the treatments compared to the pathogens, and there were a few instances where the reductions of a pathogen were slightly higher than those of *Pediococcus*. However, in no scenario was this difference significant. Overall, *Pediococcus* was determined to be a suitable surrogate for STEC, *L. monocytogenes*, and *Salmonella* on vacuum-steam-treated raisins, apricot halves, and whole macadamia nuts, particularly at lower treatment temperatures. *Enterococcus*

faecium NRRL B-2354 has also been commonly used as a surrogate organism on LWAF, but several instances have shown the microorganism to be excessively more heat resistant than target pathogens, which could lead to over-processing of foods. *Pediococcus*, however, behaved very similarly to the test pathogens. We recommend that *Pediococcus* be further investigated as a surrogate for pathogens on other LWAF and thermally challenged by other low-temperature treatments.

In addition to the use of surrogate organisms with in-plant validations, predictive models can provide information that supports and optimizes the efficacy of interventions. Thermal inactivation of pathogens can be fit to models that describe inactivation trends. If constructed and used properly, the models may provide expected predictions of pathogen reductions based on treatment conditions, such as temperature and time. Based on data gathered from the vacuum-steam treatments of the raisins, apricot halves, and whole macadamia nuts, primary models were constructed to observe if inactivation trends varied by microorganism or food type. First-order, Weibull, and Gompertz models were specifically investigated, which provided both linear and nonlinear fits. The first-order model was not best suited for any circumstance to describe inactivation trends, while the Weibull and Gompertz exhibited acceptable fits and best described the raisins/whole macadamia nuts and apricot halves, respectively. No differences in fit were observed based on microorganism, confirming that the pathogen inactivation trends were similar. The inactivation trends of *Pediococcus* were similar, but with less reduction, further confirming that the non-pathogenic microorganism could serve as an appropriate surrogate.

Similar vacuum-assisted steam experiments were also conducted on macadamia nut pieces and dried apricot pieces to explore the flexibility and limits of the primary models constructed from the larger foods, hypothesizing that the use of smaller food particles would

alter the efficacy of the treatments by impacting the flow of steam. Analyses showed that the first-order models generated for the dried apricot halves were acceptable in accurately predicting 3-log reductions of all pathogens on dried apricot pieces 100% of the time. Weibull and Gompertz models accurately predicted reductions 63% and 50% of the time for apricot pieces. In contrast, models of bacterial inactivation on whole macadamia nut models were entirely unsuccessful in accurately predicting 3-log reductions on macadamia nut pieces. However, the majority of these were fail-safe predictions that overestimated the time necessary for 3-log bacterial reductions. External datasets were also acquired of LWAF that were also thermally treated (almonds, powders, apricot paste, etc.), but the models for bacteria on whole macadamia nuts and apricot halves could not accurately predict bacterial log reductions on these foods and treatments in most cases. In many cases, the predictions recommended treatments that resulted in excessive treatments presumably safer products. While predictive models have been suggested for use in place of in-plant validations due to expense and required expertise, it should be noted that though models may exhibit good fit for specific circumstances, they must first be validated. This validation would require challenge of the models with external data and analysis of uncertainty to determine the likelihood that pathogen inactivation be overestimated. Overestimation of bacterial reductions may be costly and result in over-treated products, which could damage product quality and profit. However, a graver outcome would be an underestimation, resulting in pathogen survival that can lead to foodborne illnesses. In order to use models as a form of process validations and in place of in-plant validations, the prediction intervals should be considered, along with the development of secondary models that incorporate relevant environmental and processing factors.

The presented research demonstrated effectiveness of low-temperature, vacuum-assisted steam on a variety of food products as an intervention to reduce pathogen reduction. While this process has been utilized commercially for some food products, the process should be further explored and optimized for large batches for foods like raisins, dried apricots, and macadamia nuts, or other foods that should not be excessively heated. Commercial steam processes may treat 40-60 kg of product in sealed metal vats at a time, so potential "cold spots," times for effective heat transfer, and product heat insulation should be evaluated for various food types. While the models constructed by the presented research fit the datasets well and described the thermal inactivation of pathogens on these specific LWAF, they could only be used to accurately predict pathogen reduction for a limited set of other circumstances. In many of these cases, however, the primary models developed in the presented study overestimated the times needed for 3-log reductions on other thermally treated foods, which would presumably result in a safer product. However, the presented analyses indicated that while the two surrogate organisms examined (*Pediococcus acidilactici* and *Enterococcus faecium*) could not be used interchangeably for estimating pathogen reduction, their model parameters could accurately and safely predict reductions of multiple thermally treated pathogens. This particular discovery could be useful for processors who wish to target multiple pathogens with vacuum-assisted steam interventions.

Because steam efficacy was observed in the presented research to be impacted by food type, future directions investigating this process should expand into more food groups that would allow better understanding of how certain food characteristics, such as moisture, fat, and water activity impact the efficacy of steam treatments. Additional treatment temperatures could also allow for the construction of more robust secondary models that could expand application to other LWAF or treatments. These applications could guide the LWAF industry to design steam

treatments that are efficient in removing pathogens from the surface of certain foods, or perhaps even a mixture. Optimizing a vacuum-steam process to treat multiple food products would meet FSMA regulations and save in economic costs by combining products and reducing the likelihood of costly recalls associated with untreated LWAF. Generally, low-water activity conditions are presumed to cause an increase in bacterial thermal resistance. However, the mechanisms of this cross-protection are not fully understood. Inherent resistance of particular strains or serovars, as well as thermal resistance due to cross-protection from other stressors could be further explored to determine their impact on thermal resistance. Laboratory methods should be closely examined for their potential impacts on results, as well as how the results can be applied to large-scale food processing. LWAF are complex food matrices because of the multiple stresses (desiccation and osmotic stress) that may be placed on microorganisms and the subsequent responses and physiological states of the cells. These stressors should be further explored to elucidate risks associated with LWAF, as well as better understand the impacts the LWAF environment has on microorganisms' abilities to contaminate, persist, and resist thermal intervention.

Chapter 7 Appendix

Combined average log bacterial reductions of low-temperature, vacuum-assisted steam on raisins, apricot halves, apricot pieces, whole macadamia nuts, and macadamia nut pieces.

Table 7.1. Average bacterial reductions (Log CFU/g) of *E. coli*, *Listeria monocytogenes*, *Pediococcus*, and *Salmonella* on raisins ("Raisins"), apricot halves ("Apricot"), apricot pieces ("ApriPie"), whole macadamia nuts ("MacaNut"), and macadamia nut pieces ("MacaPie").

Food	Temperature (°C)	Organism	Treatment (min)	Reduction (log CFU/g)	St. Dev.
Apricot	72	*E. coli*	-1	-0.5	0.3
			0	0	0
			0.5	0.1	0.7
			1	0.8	0.8
			2	1.6	1.0
			5	2.4	1.0
			8	4.9	1.1
			14	6.1	0.6
			20	7.2	0.4
Apricot	72	*L. monocytogenes*	-1	-0.6	0.2
			0	0.0	0.0
			0.5	0.2	0.6
			1	1.0	0.6
			2	1.9	1.0
			5	2.7	1.3
			8	5.4	1.3
			14	5.8	0.9
			20	6.5	0.9
Apricot	72	*Pediococcus*	-1	-0.3	0.2
			0	0.0	0.0
			0.5	0.1	0.7
			1	0.7	0.6
			2	1.5	1.0
			5	1.6	0.6
			8	2.4	0.6
			14	2.8	1.0
			20	3.8	0.5
Apricot	72	*Salmonella*	-1	-0.8	0.4
			0	0.0	0.0

			0.5	0.2	0.4
			1	1.2	0.6
			2	1.9	0.6
			5	2.7	1.1
			8	6.2	1.3
			14	6.8	0.8
			20	6.9	0.7
Apricot	82	*E. coli*	-1	-1.4	0.4
			0	0.0	0.0
			0.5	1.2	0.4
			1	1.0	0.5
			1.5	1.6	0.3
			2	4.5	0.9
			2.5	5.5	2.1
			3.5	6.7	0.4
			5	6.0	0.5
Apricot	82	*L. monocytogenes*	-1	-1.6	0.3
			0	0.0	0.0
			0.5	1.2	0.6
			1	1.1	0.3
			1.5	1.8	0.5
			2	5.3	0.5
			2.5	4.9	1.2
			3.5	5.5	0.9
			5	6.1	0.3
Apricot	82	*Pediococcus*	-1	-0.8	0.4
			0	0.0	0.0
			0.5	1.2	0.3
			1	1.2	0.3
			1.5	1.6	0.4
			2	3.2	0.5
			2.5	3.1	0.5
			3.5	4.7	1.8
			5	4.9	0.4
Apricot	82	*Salmonella*	-1	-1.9	0.3
			0	0.0	0.0
			0.5	1.2	0.3
			1	1.0	0.4
			1.5	1.8	0.2
			2	5.4	1.0

			2.5	5.7	1.2
			3.5	6.2	0.3
			5	5.8	0.1
ApriPie	72	*E. coli*	-1	-0.7	0.2
			0	0.0	0.0
			0.5	0.3	0.4
			1	0.7	0.4
			2	1.4	0.3
			5	2.9	0.8
			8	3.1	0.3
			14	6.2	1.7
			20	7.1	1.3
ApriPie	72	*L. monocytogenes*	-1	-0.8	0.4
			0	0.0	0.0
			0.5	0.4	0.3
			1	0.7	0.5
			2	1.2	0.4
			5	3.2	0.9
			8	4.2	1.6
			14	5.6	1.1
			20	6.5	0.3
ApriPie	72	*Pediococcus*	-1	-0.7	0.4
			0	0.0	0.0
			0.5	0.2	0.3
			1	0.4	0.4
			2	0.7	0.3
			5	1.5	0.4
			8	1.4	0.2
			14	3.0	0.9
			20	4.1	1.3
ApriPie	72	*Salmonella*	-1	-1.3	0.3
			0	0.0	0.0
			0.5	0.4	0.4
			1	0.6	0.3
			2	1.5	0.5
			5	3.2	1.2
			8	3.7	1.0
			14	6.1	0.9
			20	6.7	0.8
ApriPie	82	*E. coli*	-1	-1.4	0.5

			0	0.0	0.0
			0.5	0.2	0.5
			1	0.9	0.8
			1.5	1.7	0.4
			2	4.4	1.2
			2.5	5.2	1.7
			3.5	6.6	0.9
			5	6.7	1.0
ApriPie	82	*L. monocytogenes*	-1	-1.5	0.3
			0	0.0	0.0
			0.5	0.3	0.4
			1	0.9	0.7
			1.5	1.4	0.5
			2	5.3	1.0
			2.5	6.0	0.7
			3.5	5.9	0.3
			5	6.0	0.7
ApriPie	82	*Pediococcus*	-1	-1.3	0.4
			0	0.0	0.0
			0.5	0.1	0.3
			1	0.4	0.2
			1.5	1.2	0.3
			2	3.0	0.8
			2.5	3.3	1.4
			3.5	4.2	1.1
			5	4.9	0.3
ApriPie	82	*Salmonella*	-1	-1.9	0.2
			0	0.0	0.0
			0.5	0.2	0.4
			1	1.0	0.7
			1.5	1.6	0.4
			2	5.0	1.3
			2.5	5.8	1.0
			3.5	6.5	0.1
			5	6.2	0.4
MacaNut	72	*E. coli*	-1	-0.4	0.3
			0	0.0	0.0
			1	0.9	0.3
			8	2.5	0.6
			14	3.5	0.4

			20	3.8	0.6
			28	4.9	1.4
			38	6.5	0.9
MacaNut	72	*L. monocytogenes*	-1	0.1	0.4
			0	0.0	0.0
			1	0.9	0.1
			8	2.0	0.3
			14	2.8	0.3
			20	2.8	0.5
			28	3.6	0.7
			38	4.8	0.5
MacaNut	72	*Pediococcus*	-1	-1.0	0.3
			0	0.0	0.0
			1	0.8	0.4
			8	1.3	0.3
			14	2.0	0.6
			20	2.0	0.8
			28	2.4	0.8
			38	3.5	1.2
MacaNut	72	*Salmonella*	-1	-0.6	0.1
			0	0.0	0.0
			1	0.7	0.3
			8	1.9	0.7
			14	2.7	0.2
			20	2.8	0.5
			28	3.6	1.0
			38	4.6	0.6
MacaNut	82	*E. coli*	-1	-1.0	0.4
			0	0.0	0.0
			1	0.3	0.7
			2	1.8	0.9
			3.5	3.0	0.6
			5	4.1	1.0
			8	4.4	1.5
			12	5.7	1.1
MacaNut	82	*L. monocytogenes*	-1	-0.7	0.6
			0	0.0	0.0
			1	0.4	0.1
			2	2.0	0.8
			3.5	2.5	0.8

			5	4.2	1.0
			8	4.1	1.0
			12	5.4	0.7
MacaNut	82	*Pediococcus*	-1	-1.4	0.2
			0	0.0	0.0
			1	0.2	0.4
			2	1.2	0.4
			3.5	1.7	1.0
			5	3.0	1.3
			8	2.8	0.5
			12	4.1	0.7
MacaNut	82	*Salmonella*	-1	-1.2	0.3
			0	0.0	0.0
			1	0.3	0.2
			2	1.4	0.7
			3.5	2.2	1.0
			5	3.4	1.5
			8	3.3	0.9
			12	4.9	1.2
MacaPie	72	*E. coli*	-1	-0.4	0.8
			0	0.0	0.0
			0.5	0.7	0.3
			1	0.9	0.4
			2	1.7	0.1
			5	2.7	0.5
			8	3.3	0.5
			14	3.5	0.3
			20	6.7	0.9
MacaPie	72	*L. monocytogenes*	-1	-0.1	0.8
			0	0.0	0.0
			0.5	0.6	0.8
			1	1.2	0.5
			2	1.8	0.4
			5	2.4	0.3
			8	2.9	0.5
			14	3.0	0.5
			20	4.4	0.3
MacaPie	72	*Pediococcus*	-1	-0.6	0.4
			0	0.0	0.0
			0.5	0.4	0.8

			1	1.3	0.8
			2	1.9	0.7
			5	2.2	0.8
			8	2.8	0.9
			14	3.1	1.1
			20	4.2	0.6
MacaPie	72	*Salmonella*	-1	-0.6	0.5
			0	0.0	0.0
			0.5	0.9	0.2
			1	1.0	0.5
			2	1.8	0.2
			5	2.5	0.6
			8	2.8	0.2
			14	3.0	0.6
			20	5.2	0.7
MacaPie	82	*E. coli*	-1	-1.4	0.8
			0	0.0	0.0
			0.5	1.1	0.7
			1	1.6	0.9
			1.5	3.3	0.5
			2	3.9	0.6
			2.5	3.5	0.8
			3.5	4.5	0.3
			5	5.7	1.4
MacaPie	82	*L. monocytogenes*	-1	-0.6	0.2
			0	0.0	0.0
			0.5	1.2	0.3
			1	2.0	1.8
			1.5	3.6	1.1
			2	3.7	0.9
			2.5	4.2	1.3
			3.5	5.0	1.1
			5	5.9	0.8
MacaPie	82	*Pediococcus*	-1	-1.7	0.4
			0	0.0	0.0
			0.5	0.5	0.7
			1	1.4	1.0
			1.5	2.1	0.2
			2	2.3	0.5
			2.5	2.7	0.2

			3.5	3.1	0.8
			5	3.0	0.9
MacaPie	82	*Salmonella*	-1	-1.4	0.3
			0	0.0	0.0
			0.5	1.2	0.6
			1	1.4	0.8
			1.5	2.8	0.3
			2	2.9	0.9
			2.5	3.1	0.8
			3.5	3.5	1.0
			5	4.3	1.7
Raisins	62	*E. coli*	-1	-0.2	0.5
			0	0.0	0.0
			0.5	0.5	0.4
			1	1.1	0.6
			2	1.3	0.7
			5	2.2	0.5
			8	3.1	0.7
			14	4.5	0.3
			20	6.3	1.1
Raisins	62	*L. monocytogenes*	-1	-0.4	0.7
			0	0.0	0.0
			0.5	0.5	0.3
			1	0.9	0.6
			2	1.3	0.5
			5	2.1	0.2
			8	3.3	1.1
			14	4.8	0.7
			20	5.7	0.7
Raisins	62	*Pediococcus*	-1	-0.2	0.5
			0	0.0	0.0
			0.5	0.2	0.1
			1	0.4	0.4
			2	0.6	0.4
			5	1.0	0.2
			8	1.2	0.4
			14	1.7	0.3
			20	2.4	0.6
Raisins	62	*Salmonella*	-1	-0.4	0.5
			0	0.0	0.0

			0.5	0.4	0.3
			1	0.9	0.7
			2	1.1	0.4
			5	1.9	0.6
			8	2.8	0.9
			14	4.9	0.9
			20	6.3	1.0
			-1	-1.1	0.4
			0	0.0	0.0
			0.5	0.8	1.0
			1	1.7	0.6
Raisins	72	*E. coli*	1.5	2.6	0.7
			2	2.7	1.2
			2.5	3.3	1.0
			3.5	5.0	1.3
			5	5.8	1.5
			-1	-1.3	0.2
			0	0.0	0.0
			0.5	1.1	1.4
			1	2.6	1.3
Raisins	72	*L. monocytogenes*	1.5	3.8	1.6
			2	3.3	1.3
			2.5	4.2	1.9
			3.5	5.0	0.8
			5	5.2	0.7
			-1	-1.1	0.3
			0	0.0	0.0
			0.5	0.2	0.4
			1	1.1	0.5
Raisins	72	*Pediococcus*	1.5	1.8	0.7
			2	2.1	0.7
			2.5	2.4	1.0
			3.5	3.1	0.6
			5	4.1	1.2
			-1	-1.4	0.3
			0	0.0	0.0
	72	*Salmonella*	0.5	1.0	0.7
			1	1.6	0.7
			1.5	3.1	1.8
Raisins			2	3.0	1.6

	2.5	3.4	1.2
	3.5	5.0	1.2
	5	5.4	1.0

Combined temperature profile parameters averaged across each treatment combination.

Table 7.2. Temperature profile parameters averaged across each treatment combination for dried apricot halves, whole macadamia nuts, raisins, dried apricot pieces, and macadamia nut pieces.

Food	Treatment Temp (°C)	Treatment Time (min)	Come-up Time (sec)	Residual Heating (sec)	Maximum Temp (°C)	Minimum Temp (°C)
Apricot Halves	72	0	62.3	11.0	72.5	69.6
		0.5	47.3	3.0	73.4	69.7
		1	50.3	2.3	73.5	70.5
		2	48.7	3.3	73.4	70.5
		5	48.0	3.3	73.5	70.5
		8	50.3	2.7	73.2	70.4
		14	55.3	5.7	73.6	70.2
		20	46.7	2.7	73.8	70.4
	82	0	72.3	9.7	82.8	79.2
		0.5	68.3	1.7	83.9	79.6
		1	69.7	2.7	83.8	80.5
		1.5	65.0	2.7	83.8	80.6
		2	82.7	1.3	83.3	80.6
		2.5	65.0	4.0	83.5	79.4
		3.5	72.3	3.7	83.4	80.8
		5	70.0	3.0	83.2	80.5
Whole Macadamia Nuts	72	0	40.0	12.0	72.2	69.5
		1	38.0	1.0	73.9	70.7
		8	34.0	1.3	73.2	70.8
		14	37.3	1.0	72.9	70.6
		20	32.3	1.7	73.0	70.6
		28	33.3	4.1	73.6	70.4
		38	33.3	2.3	73.7	70.5
	82	0	45.8	14.0	83.3	78.7
		1	40.7	1.3	83.7	80.5
		2	39.7	1.3	83.0	80.7
		3.5	39.7	1.3	82.9	80.6
		5	42.3	1.7	82.9	80.8
		8	44.8	3.4	83.3	80.7
		12	41.3	2.0	83.5	80.4
Raisins	62	0	46.3	17.7	64.5	64.5
		0.5	45.3	6.0	63.4	63.4

		1	44.0	7.3	62.9	62.9
		2	67.6	7.6	65.1	62.7
		5	48.7	8.0	63.0	60.5
		8	45.8	6.8	63.6	60.4
		14	50.8	6.3	63.4	60.6
		20	52.3	14.5	63.5	60.8
	72	0	50.7	17.7	72.1	69.3
		0.5	50.8	5.5	73.1	73.1
		1	39.5	5.8	73.2	73.2
		1.5	71.5	8.0	73.1	70.7
		2	45.0	6.3	73.1	70.7
		2.5	59.5	6.8	73.1	70.5
		3.5	41.8	5.8	72.9	70.8
		5	59.3	7.8	73.6	70.6
Apricot Pieces	72	0	105.5	9.8	72.5	70.7
		0.5	99.5	2.5	73.3	70.5
		1	74.0	2.0	73.5	70.7
		2	90.3	2.0	73.6	70.6
		5	72.8	1.8	73.7	70.8
		8	89.3	5.0	73.1	66.5
		14	73.3	2.0	73.0	69.7
		20	93.8	3.3	73.6	70.2
	82	0	79.8	11.5	82.8	79.9
		0.5	51.8	2.5	83.4	79.0
		1	73.3	2.5	83.3	80.6
		1.5	50.8	2.5	83.4	80.7
		2	47.3	3.0	83.6	80.6
		2.5	53.3	2.3	83.3	80.7
		3.5	57.0	4.0	83.4	80.3
		5	48.0	3.0	82.9	80.8
Macadamia Nut Pieces	72	0	79.8	11.8	72.7	65.7
		0.5	32.0	0.5	73.4	70.7
		1	26.7	0.3	73.3	70.7
		2	29.7	0.7	73.3	70.8
		5	28.3	1.8	73.1	70.2
		8	26.3	0.3	73.3	58.1
		14	30.7	0.3	75.1	70.7
		20	27.0	0.5	72.9	70.7
	82	0	31.0	12.3	82.6	76.6
		0.5	39.0	0.7	83.2	80.8

	1	34.3	1.0	83.4	80.5
	1.5	45.0	2.3	83.4	80.8
	2	34.5	0.5	83.3	80.9
	2.5	38.0	0.7	83.1	80.5
	3.5	37.3	5.0	83.0	78.2
	5	30.8	0.8	83.0	80.6

Bacterial reductions (log CFU/g ± standard deviation) on apricot pieces resulting from 72°C and 82°C vacuum-steam treatment of various times.

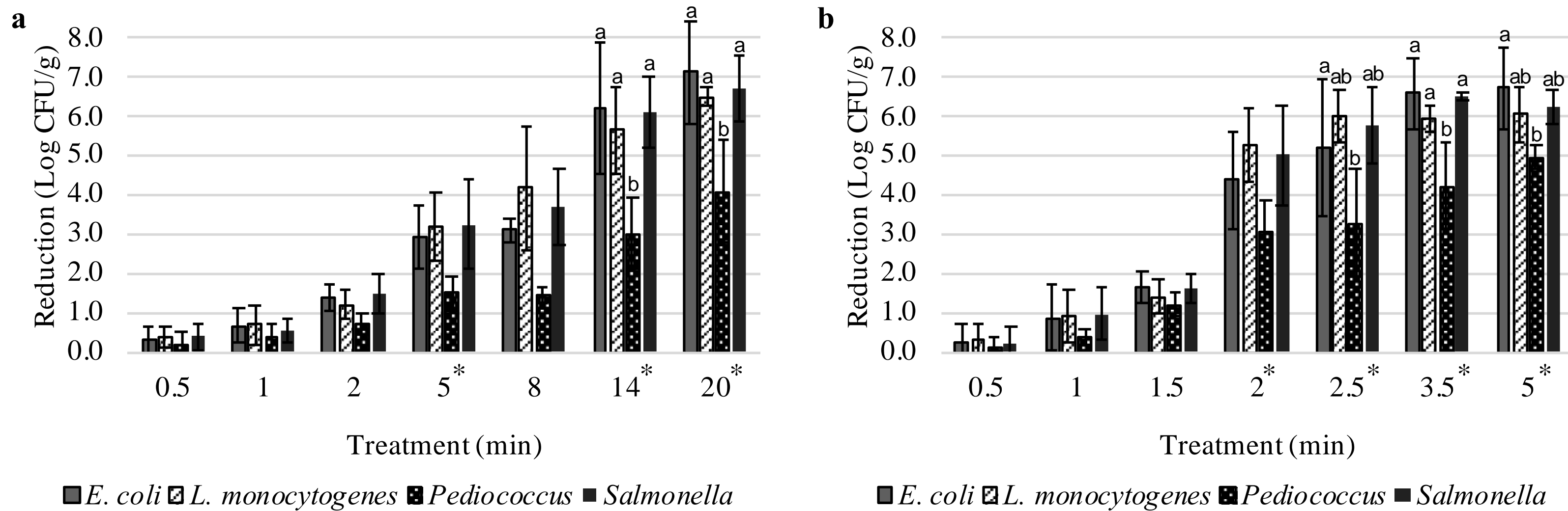

Figure 7.1. Bacterial reductions (log CFU/g ± standard deviation) on apricot pieces resulting from 72°C (a) and 82°C (b) vacuum-steam treatment of various times. Asterisk (*) next to treatment times indicate significant differences of bacterial reductions from the reductions of a 0-min treatment, while lowercase letters above bars indicate significant differences between organisms within a treatment from pairwise comparisons ($p<0.05$). Lack of letters indicate no significant difference between organisms within the treatment.

Bacterial reductions (log CFU/g ± standard deviation) on macadamia nut pieces resulting from 72°C and 82°C vacuum-steam treatment of various times.

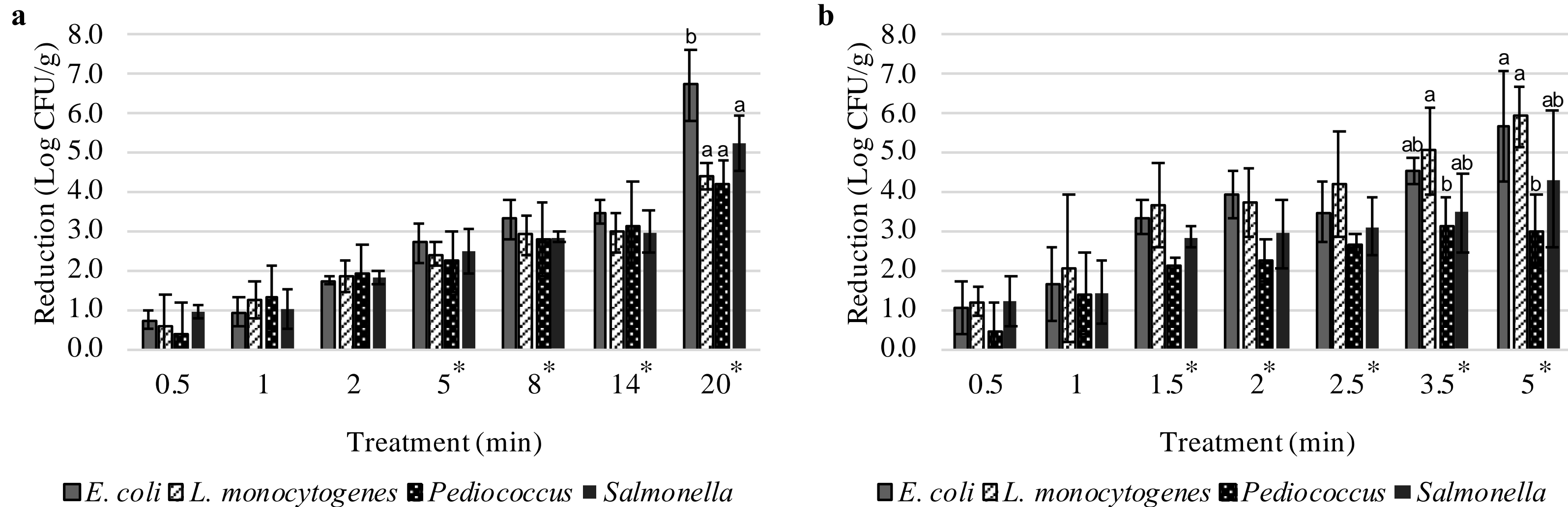

Figure 7.2. Bacterial reductions (log CFU/g ± standard deviation) on macadamia nut pieces resulting from 72°C (a) and 82°C (b) vacuum-steam treatment of various times. Asterisk (*) next to treatment times indicate significant differences of bacterial reductions from the reductions of a 0-min treatment, while lowercase letters above bars indicate significant differences between organisms within a treatment from pairwise comparisons ($p<0.05$). Lack of letters indicate no significant difference between organisms within the treatment.

Proximate analyses of raisins before and after low-temperature, vacuum-assisted treatments.

62°C Treatments - a_w										
Date	No Trt	0	0.5	1	2	5	8	14	20	
11.14.18	0.5623	0.7008	0.7416	0.6985	0.6743	0.6812	0.6741	0.6867	0.6813	
11.28.18	0.5622	0.7341	0.6822	0.6885	0.6764	0.6688	0.6672	0.6763	0.6917	Avg. of
11.30.18	0.564	0.72	0.7415	0.6647	0.7148	0.7106	0.6986	0.6832	0.7033	Treatments
Average:	0.5628	0.7183	0.7218	0.6839	0.6885	0.6869	0.6800	0.6821	0.6921	0.6942
St. Dev.	0.0010	0.0167	0.0343	0.0174	0.0228	0.0215	0.0165	0.0053	0.0110	0.0228

72°C Treatments - a_w										
Date	No Trt	0	0.5	1	1.5	2	2.5	3.5	5	
11.28.18	0.5622	0.718	0.7235	0.6841	0.6722	0.6906	0.6813	0.6694	0.6766	
11.30.18	0.564	0.6746	0.671	0.7086	0.6895	0.7026	0.6687	0.6751	0.6637	Avg. of
12.05.18	0.5677	0.6746	0.671	0.7086	0.7026	0.6687	0.6751	0.6637	0.6895	Treatments
Average:	0.5646	0.6891	0.6885	0.7004	0.6881	0.6873	0.6750	0.6694	0.6766	0.6843
St. Dev.	0.0028	0.0251	0.0303	0.0141	0.0152	0.0172	0.0063	0.0057	0.0129	0.0176

% Moisture				
Date	No Trt	62-20	72-5	
	18.5	25.6	25.4	
	18.2	24.5	23.9	
	14.9			Avg. of
7.16.19	21.1	25.8	24.8	Treatments
Average:	18.2	25.3	24.7	25.0
St. Dev.	2.5	0.7	0.8	0.6

% Protein				
Date	No Trt	62-20	72-5	
	2.86	3.29	3.42	
	2.93	3.37	3.39	
	3.26			Avg. of
07.16.19	3.98	3.44	3.86	Treatments
Average:	3.3	3.4	3.6	3.5
St. Dev.	0.5	0.1	0.3	0.2

% Fat				
Date	No Trt	62-20	72-5	
	0.219	0.128	0.141	
	0.195	0.094	0.161	
	0.149	0.239	0.911	Avg. of
07.16.19	0.16			Treatments
Average:	0.2	0.2	0.4	0.3
St. Dev.	0.0	0.1	0.4	0.3

Proximate analyses of dried apricot halves before and after low-temperature, vacuum-assisted treatments.

72°C Treatments - aw										
Date	No Trt	0	0.5	1	2	5	8	14	20	
03.13.19	0.7016	0.7426	0.7709	0.8654	0.8156	0.751	0.7686	0.8069	0.8356	
03.15.19	0.7167	0.7352	0.7638	0.7549	0.7717	0.7448	0.7612	0.758	0.7438	Avg. of Treatments
03.19.19	0.6337	0.781	0.7629	0.7693	0.7423	0.7734	0.7606	0.732	0.732	
Average:	0.6840	0.7529	0.7659	0.7965	0.7765	0.7564	0.7635	0.7656	0.7705	0.7685
St. Dev.	0.0442	0.0246	0.0044	0.0601	0.0369	0.0150	0.0045	0.0380	0.0567	0.0329

82°C Treatments - aw										
Date	No Trt	0	0.5	1	1.5	2	2.5	3.5	5	
03.13.19	0.7016	0.8302	0.8373	0.7717	0.8408	0.8201	0.8446	0.7583	0.8573	
03.15.19	0.7167	0.7439	0.7382	0.782	0.7812	0.7512	0.7587	0.7526	0.7994	Avg. of Treatments
03.19.19	0.6337	0.783	0.7115	0.773	0.7506	0.7711	0.7778	0.7874	0.7699	
Average:	0.6840	0.7857	0.7623	0.7756	0.7909	0.7808	0.7937	0.7661	0.8089	0.7830
St. Dev.	0.0442	0.0432	0.0663	0.0056	0.0459	0.0355	0.0451	0.0187	0.0445	0.0378

% Moisture				
Date	No Trt	72-20	82-5	
03.08.19	29.8	31.4	32.3	
03.08.19	30.4	30.8	32.9	
03.08.19	29.3			
7.16.19	26.7	31.5	31.5	
7.16.19		30.9	30.3	Avg. of Treatments
7.16.19		32.6	33.8	
Average:	29.1	31.4	32.2	31.8
St. Dev.	1.6	0.7	1.3	1.1

% Protein				
Date	No Trt	72-20	82-5	
03.08.19	2.98	1.61	2.38	
03.08.19	2.58	2.6	4.55	
03.08.19	3.42			
7.16.19	2.27	2.01	1.77	
7.16.19		1.75	1.75	Avg. of Treatments
07.16.19		2.16	2.58	
Average:	2.8	2.0	2.6	2.3
St. Dev.	0.5	0.4	1.1	0.9

% Fat				
Date	No Trt	72-20	82-5	
7.16.19	0.178	0.1	0.134	
7.16.19	0.046	0.116	0.485	Avg. of Treatments
7.16.19	0.041	0.281	0.119	
Average:	0.1	0.2	0.2	0.2
St. Dev.	0.1	0.1	0.2	0.2

Proximate analyses of dried apricot pieces before and after low-temperature, vacuum-assisted treatments.

72°C Treatments - a_w										
Date	No Trt	0	0.5	1	2	5	8	14	20	
04.25.19	0.6969	0.7916	0.7874	0.7829	0.8062	0.7974	0.7554	0.7819	0.7731	
04.30.19	0.7024	0.7783	0.7825	0.7764	0.796	0.8052	0.8257	0.853	0.7707	Avg. of Treatments
05.02.19	0.7286	0.7889	0.8202	0.825	0.8587	0.8291	0.8349	0.7745	0.8495	
Average:	0.7093	0.7863	0.7967	0.7948	0.8203	0.8106	0.8053	0.8031	0.7978	0.8019
St. Dev.	0.0169	0.0070	0.0205	0.0264	0.0336	0.0165	0.0435	0.0433	0.0448	0.0287

82°C Treatments - a_w										
Date	No Trt	0	0.5	1	1.5	2	2.5	3.5	5	
04.25.19	0.6969	0.7833	0.8098	0.8048	0.7999	0.8025	0.7624	0.7855	0.807	
04.30.19	0.7024	0.795	0.7853	0.7944	0.7802	0.7951	0.7919	0.7744	0.7707	Avg. of Treatments
05.02.19	0.7286	0.8279	0.8114	0.8077	0.8277	0.8189	0.828	0.8228	0.8106	
Average:	0.7093	0.8021	0.8022	0.8023	0.8026	0.8055	0.7941	0.7942	0.7961	0.7999
St. Dev.	0.0169	0.0231	0.0146	0.0070	0.0239	0.0122	0.0329	0.0254	0.0221	0.0185

% Moisture				
Date	No Trt	72-20	82-5	
07.16.19	24.7	29.7	30.1	
07.16.19	25.3	30.2	29.7	Avg. of Treatments
07.16.19	26.3	33.6	31.9	
Average:	25.4	31.2	30.6	30.9
St. Dev.	0.8	2.1	1.2	1.6

% Protein				
Date	No Trt	72-20	82-5	
07.16.19	1.69	1.88	2.11	
07.16.19	3.56	2.25	2.56	Avg. of Treatments
07.16.19	3.48	2.65	2.93	
Average:	2.9	2.3	2.5	2.4
St. Dev.	1.1	0.4	0.4	0.4

% Fat				
Date	No Trt	72-20	82-5	
07.16.19	0.087	0.1	0.059	
07.16.19	0.046	0.134	0.126	Avg. of Treatments
07.16.19	0.041	0.287	0.329	
Average:	0.1	0.2	0.2	0.2
St. Dev.	0.0	0.1	0.1	0.1

Proximate analyses of whole macadamia nuts before and after low-temperature, vacuum-assisted treatments.

72°C Treatments - a_w									
Date	No Trt	0	1	8	14	20	28	38	
05.16.19	0.5485	0.8291	0.8234	0.8652	0.8956	0.8917			
05.21.19	0.595	0.8771	0.8495	0.8981	0.8546	0.9126			
05.23.19	0.6144	0.8706	0.8869	0.8706	0.8774	0.8675	0.9433	0.8763	
05.28.19	0.5796	0.8157					0.8769	0.9042	Avg. of Treatments
05.28.19	0.5664	0.8413					0.8383	0.8466	
Average:	0.5808	0.8468	0.8533	0.8780	0.8759	0.8906	0.8862	0.8757	0.8701
St. Dev.	0.0254	0.0264	0.0319	0.0176	0.0205	0.0226	0.0531	0.0288	0.0306

82°C Treatments - a_w									
Date	No Trt	0	1	8	14	20	28	38	
05.16.19	0.5485	0.8697	0.8813	0.8993	0.8957	0.8705	0.8497		
05.21.19	0.595	0.8678	0.8858	0.8929	0.8879	0.8779	0.8922	0.9004	
05.23.19	0.6144	0.8822	0.9053	0.888	0.8929	0.8913	0.8708	0.874	
05.28.19	0.5796	0.8525					0.9075	0.9096	Avg. of Treatments
05.28.19	0.5664	0.8778					0.8883	0.8572	
Average:	0.5808	0.8700	0.8908	0.8934	0.8922	0.8799	0.8817	0.8853	0.8846
St. Dev.	0.0254	0.0114	0.0128	0.0057	0.0040	0.0105	0.0221	0.0241	0.0162

% Moisture				
Date	No Trt	72-38	82-12	
7.16.19	12.2	14.4	16	
7.16.19	12.1	13.3	14.8	Avg. of Treatments
7.16.19	15.2	12.6	12.9	
Average:	13.2	13.4	14.6	30.9
St. Dev.	1.8	0.9	1.6	1.6

% Protein				
Date	No Trt	72-38	82-12	
7.16.19	9.54	9.54	9.66	
7.16.19	9.89	8.56	9.46	Avg. of Treatments
7.16.19	9.51	9.15	8.88	
Average:	9.6	9.1	9.3	9.2
St. Dev.	0.2	0.5	0.4	0.4

% Fat				
Date	No Trt	72-38	82-12	
7.16.19	47.2	49.1	55.9	
7.16.19	49.8	56.9	53.2	Avg. of Treatments
7.16.19	50.8	62.5	60.1	
Average:	49.3	56.2	56.4	56.3
St. Dev.	1.9	6.7	3.5	4.8

Proximate analyses of macadamia nut pieces before and after low-temperature, vacuum-assisted treatments.

72°C Treatments - a_w										
Date	No Trt	0	0.5	1	2	5	8	14	20	
06.11.19	0.6187	0.8576	0.842	0.8533	0.8507	0.8714	0.8602	0.8741	0.8962	
06.13.19	0.6575	0.8788	0.8854	0.8837	0.8928	0.8882	0.8758	0.9055	0.9031	Avg. of Treatments
06.18.19	0.6225	0.8861	0.8816	0.8814	0.8675	0.8898	0.8656	0.8976	0.9398	
Average:	0.6329	0.8742	0.8697	0.8728	0.8703	0.8831	0.8672	0.8924	0.9130	0.8803
St. Dev.	0.0214	0.0148	0.0240	0.0169	0.0212	0.0102	0.0079	0.0163	0.0234	0.0210

82°C Treatments - a_w										
Date	No Trt	0	0.5	1	1.5	2	2.5	3.5	5	
06.11.19	0.6187	0.897	0.8726	0.8995	0.9071	0.88	0.8728	0.882	0.8693	
06.13.19	0.6575	0.9029	0.9066	0.9127	0.9019	0.9031	0.8969	0.88	0.8914	Avg. of Treatments
06.16.19	0.6225	0.8715	0.8877	0.867	0.895	0.8811	0.8908	0.8936	0.8692	
Average:	0.6329	0.8905	0.8890	0.8931	0.9013	0.8881	0.8868	0.8852	0.8766	0.8888
St. Dev.	0.0214	0.0167	0.0170	0.0235	0.0061	0.0130	0.0125	0.0073	0.0128	0.0139

% Moisture				
Date	No Trt	72-20	82-5	
7.16.19	15.2	14.6	16	
7.16.19	14.7	12.6	15	Avg. of Treatments
7.16.19	13	14.9	13.3	
Average:	14.3	14.0	14.8	15.8
St. Dev.	1.2	1.3	1.4	3.4

% Protein				
Date	No Trt	72-20	82-5	
7.16.19	53	56.6	55	
7.16.19	45.9	57	51.3	Avg. of Treatments
7.16.19	54.2	56.5	52.9	
Average:	51.0	56.7	53.1	56.9
St. Dev.	4.5	0.3	1.9	5.5

% Fat				
Date	No Trt	72-20	82-5	
7.16.19	9.04	9.34	8.76	
7.16.19	9.9	8.91	9.3	Avg. of Treatments
7.16.19	9.27	9.18	8.77	
Average:	9.4	9.1	8.9	8.8
St. Dev.	0.4	0.2	0.3	0.5

Code written in R for data compilation, model construction, analyses, and temperature profile compilation.

```
#Combining data; raw plate counts to usable datasets for
analyses#

#Library imports
rm(list = ls())
library(tidyverse)
library(tidyselect)
library(readxl)
library(rpart)
library(rpart.plot)
library(plotrix)
library(pracma)
library(BBmisc)

# setwd(”../../../Dropbox/vacuum decontam/R_Code”)

### user entered data###
dilutionFactor <- 4
dlCFUpermL <- 1 # detection limit, >0 to prevent -Inf logReduct
PlateCountData <- ”../Foods”
savePlotToDisk <- FALSE
today <- Sys.Date()
# combinedDataOutputFileName       <- paste(”Combined ”, today,”.csv”,
sep=””)
combinedDataOutputFileName       <- ”Combined 20190716.csv”
combinedEnrichFileName           <- ”CombinedEnrich 20190716.csv”
finalCombinedDataFileName        <- ”FinalCombinedData 20190716.csv”
#Combined data includes UniqueID assignment, addition/replacements of
enriched samples, and removal of excluded samples.
#############################


#Describing how information will be extracted from the file
names#

getOfficialOrgName <- function(filename) {
  orgname <- ””
  if (str_detect(filename, ”Eco”)) {
    orgname <- ”E. coli”
  }
  if (str_detect(filename, ”Sal”)) {
    orgname <- ”Salmonella”
  }
  if (str_detect(filename, ”Lmo”)) {
    orgname <- ”L. monocytogenes”
  }
  if (str_detect(filename, ”Ped”)) {
    orgname <- ”Pediococcus”
  }
  return(orgname)
}

getAbbreviatedOrgName <- function(filename) {
  if (str_detect(filename, ”Eco”)) {
    return (”Eco”)
  }
  if (str_detect(filename, ”Sal”)) {
    return (”Sal”)
  }
```

```
  if (str_detect(filename, ”Lmo”)) {
    return (”Lmo”)
  }
  if (str_detect(filename, ”Ped”)) {
    return (”Ped”)
  }
  return(””)
}

orgcount <- function(filename) {
  # Get the official orgname from the filename
  orgname <- getOfficialOrgName(filename)
```

#Describing how the raw data table files will be read in; filtering and massaging the data files for further use#

```
  # Read raw data
  x <- read.csv(filename, skip=28, header=FALSE, as.is=TRUE,
stringsAsFactors=FALSE)

  # Read in first 3 columns (since no changes are needed)
  x <- x[,1:3]
  x <- cbind(substr(filename, 10, 16), substr(filename, 17, 18), orgname, x)

  # Add column names
  names(x) <- c(”Food”, ”Temperature”, ”Organism”, ”Treatment”, ”PlateCount”,
”CFUpermL”)

  # Remove plate reader errors
  x <- filter(x, !str_detect(Treatment, ”Previous”))

  # Transform micro-lingo to numeric data
  x$Treatment <- ifelse(str_detect(x$Treatment, ”NoTrt”), ”-1”, x$Treatment)
  x$CFUpermL <-ifelse(x$CFUpermL<=0, dlCFUpermL, x$CFUpermL)
  # Add treat time and logCFUpermL columns
  x <- mutate(x,treatTime=Treatment, logCFUpermL =
round(log10(dilutionFactor*CFUpermL), digits = 1))
```

#Creating a unique ID that will be assigned to each data point#

```
  uniqueId = paste(substr(filename, 10, 16),
                   ”_”, substr(filename, 17, 18), ”_”,
                   substr(filename, 24, 31), ”_”,
                   getAbbreviatedOrgName(filename), ”_”,
                   x$Treatment, sep=””)
  x <- mutate(x, uniqueId = uniqueId)
  x <- mutate(x, repDate = substr(uniqueId, 12, 19))
  return(x)
}
```

#Importing files and performing tasks as described above#

```
# Program starts running here. We import all files into a data structure
# file function from https://stat.ethz.ch/R-manual/R-
devel/library/base/html/list.files.html
# take each folder and get all the file names in the folder and assign it to
this variable
filenames <- list.files(path = PlateCountData, full.name = TRUE)
for (j in 1:length(filenames)) {
  glimpse(filenames[j])
  # x1 is the data from current file we are importing
```

```
  x1 <- orgcount(filenames[j])

  # When we're on the first file, initialize a new data structure and add
file 1
  if (j == 1) {
    # comb is the data structure
    comb <- x1
  }
  # For all other files, just add it to the data structure
  else {
    comb <- rbind(comb, x1)
  }
}

# Print the data structure to the terminal
glimpse(comb)

write.csv(comb, paste("Output/",combinedDataOutputFileName,sep=""),
row.names=FALSE)
```

```
#Combining the dataset from the plate counts with the Excel data
sheet constructed manually for the enrichments#

#Read in the Enrichments file saved in Supplemental_Data
Enrichments <- read_excel("../Supplemental_Data/Enrichments.xlsx")

filter(Enrichments, Treatment != treatTime)

Combined <- read.csv(paste("Output/",combinedDataOutputFileName,sep=""),
stringsAsFactors = FALSE)
Combined <- mutate(Combined, Updated=FALSE)

CombinedEnrich <- Combined
CombinedEnrich <- full_join(CombinedEnrich, Enrichments,
by=c("uniqueId"="uniqueID"))
names(CombinedEnrich)
# Date is in the enrichment files but not the original combined file
CombinedEnrich$Food.x         <- ifelse(is.na(CombinedEnrich$Date),
CombinedEnrich$Food.x, CombinedEnrich$Food.y)
CombinedEnrich$Temperature.x  <- ifelse(is.na(CombinedEnrich$Date),
CombinedEnrich$Temperature.x, CombinedEnrich$Temperature.y)
CombinedEnrich$Organism.x     <- ifelse(is.na(CombinedEnrich$Date),
CombinedEnrich$Organism.x, CombinedEnrich$Organism.y)
CombinedEnrich$Treatment.x    <- ifelse(is.na(CombinedEnrich$Date),
CombinedEnrich$Treatment.x, CombinedEnrich$Treatment.y)
CombinedEnrich$PlateCount.x   <- ifelse(is.na(CombinedEnrich$Date),
CombinedEnrich$PlateCount.x, CombinedEnrich$PlateCount.y)
CombinedEnrich$CFUpermL       <- ifelse(is.na(CombinedEnrich$Date),
CombinedEnrich$CFUpermL, "NA")
CombinedEnrich$treatTime.x    <- ifelse(is.na(CombinedEnrich$Date),
CombinedEnrich$treatTime.x, CombinedEnrich$treatTime.y)
CombinedEnrich$logCFUpermL    <- ifelse(is.na(CombinedEnrich$Date),
CombinedEnrich$logCFUpermL, CombinedEnrich$EnrichLogCFU)
CombinedEnrich$repDate        <- ifelse(is.na(CombinedEnrich$Date),
CombinedEnrich$repDate, CombinedEnrich$Date)
CombinedEnrich$Updated        <- ifelse(is.na(CombinedEnrich$Date),
CombinedEnrich$Updated, TRUE)

write.csv(CombinedEnrich, paste("Output/", combinedEnrichFileName, sep=""),
row.names=FALSE)

#Remove columns that are no longer needed from when the Enrichment file was
joined.
```

```
finalData <- select(CombinedEnrich, -Date, -Organism.y, -Treatment.y, -
PlateCount.y,
                    -treatTime.y, -EnrichLogCFU, -Temperature.y, -Food.y, -
Temperature.y)
finalData <- rename(finalData, Organism=Organism.x,
                               Treatment=Treatment.x,
                               PlateCount=PlateCount.x,
                               treatTime=treatTime.x,
                               Temperature=Temperature.x,
                               Food=Food.x)
write.csv(finalData, paste("Output/",finalCombinedDataFileName,sep=""),
row.names=FALSE)
```

#Analyses: log reductions, model parameters#

```
# Library imports
rm(list = ls())
#
install.packages(c("plyr","tidyverse","tidyselect","readxl","rpart","rpart.pl
ot","rattle","plotrix",
# "ggplot2","minpack.lm","nlstools","nlsMicrobio","pheatmap","RColorBrewer"))
library(plyr)
library(tidyverse)
library(tidyselect)
library(readxl)
library(rpart)
library(rpart.plot)
library(rattle)
library(plotrix)
library(ggplot2)
library(minpack.lm)
library(nlstools)
library(nlsMicrobio)
library(pheatmap)
library(RColorBrewer)

# !!! NOTE: RSE are in units of the predicted variable
# !!! compare only within same model for now

### user input
inputFile <- "FinalCombinedData 20190716.csv"
outputFile <- paste("RevisedCombinedEnrich jca ", Sys.Date(), ".csv", sep="")
summaryLogReductFile <- paste("summaryLogReduction jca ", Sys.Date(), ".csv",
sep="")
savePlotToDisk <- TRUE
specify_decimal <- function(x, k) trimws(format(round(x, k), nsmall=k))

# select which models to fit
useFirstOrderAll <- TRUE
useWeibullAll <- TRUE
useGompertzAll <- TRUE
```

#Defining how the model parameters will be calculated#

```
##################################################
### first order model for 1 experiment using all points
##################################################
firstOrderAll <- function(org, food, temper, dfExper) {
# note: no intercenpt in model
  lrRes <- lm(fracSurv~treatTime-1, data=dfExper)
  aic <- AIC(lrRes)
  # organism, food, temperature, n
  # slope,
```

```
  # slope LCL, slope UCL, Rsq, RSE
  dfResultFirstOrderAll <- data.frame(org, food, temper, nrow(dfExper),
                                      coef(lrRes)[1],
                                      confint(lrRes)[1,1],
                                      confint(lrRes)[1,2],
                                      summary(lrRes)$r.squared,
                                      summary(lrRes)$sigma,
                                      aic,
                                      row.names=NULL, stringsAsFactors=FALSE)
  return(dfResultFirstOrderAll)
}
#################################################

#################################################
### Weibull model for 1 experiment using all points
#################################################
weibullAll <- function(org, food, temper, dfExper) {
  dfExper$logFracSurv <- -1 * dfExper$newLR
  nlrW <- nlsLM(newLR~-k*treatTime^a, start=list(k=1, a=1), data=dfExper)
  k <- coef(nlrW)[1]
  a <- coef(nlrW)[2]
  ci <- confint(nlrW)
  klcl <- ci[1,1]
  kucl <- ci[1,2]
  alcl <- ci[2,1]
  aucl <- ci[2,2]
  n <- nrow(dfExper)
  correlAK <- summary(nlrW, correlation=TRUE)$correlation[1,2]
  RSE <- summary(nlrW)$sigma
  aic <- AIC(nlrW)
  # organism, food, temperature, n
  # k, k LCL, k UCL,
  # a, a LCL, a UCL,
  # a-k correlation, RSE)
  dfResultWeibullAll <- data.frame(org, food, temper, n,
                                   k, klcl, kucl,
                                   a, alcl, aucl,
                                   correlAK, RSE, aic,
                                   row.names=NULL, stringsAsFactors=FALSE)

  return(dfResultWeibullAll)
}
#################################################

#################################################
### Gompertz model for 1 experiment using all points
#################################################
gompertzAll <- function(org, food, temper, dfExper) {
  # mu is the maximal value of inactivation
  # lambda is the lag time
  # a is the asymptote
  ### for test run
  ###    org =  vOrg[iOrg]
  ###    food = vFood[jFood]
  ###    temper = vTemper[kTemper]
  ###    dfExper = dfExper
  dfExper$logFracSurv <- -1 * dfExper$newLR
  e <- exp(1)
  #  dfExper$logFracSurv <- dfExper$newLR
  nlrG <- nlsLM(logFracSurv~a*exp(-exp((mu*e)/a*(lambda-treatTime)+1)),
                start=list(a=-7, mu=-0.5, lambda=0.5), data=dfExper)
  a <- coef(nlrG)[1]
  mu <- coef(nlrG)[2]
  lambda <- coef(nlrG)[3]
  ci <- confint2(nlrG)
```

```
  alcl <- ci[1,1]
  aucl <- ci[1,2]
  mulcl <- ci[2,1]
  muucl <- ci[2,2]
  lambdalcl <- ci[3,1]
  lambdaucl <- ci[3,2]
  n <- nrow(dfExper)
  RSE <- summary(nlrG)$sigma
  aic <- AIC(nlrG)
  correl_amu <- summary(nlrG, correlation=TRUE)$correlation[2,1]
  correl_alambda <- summary(nlrG, correlation=TRUE)$correlation[3,1]
  correl_mulambda <- summary(nlrG, correlation=TRUE)$correlation[3,2]

  dfResultGompertzAll <- data.frame(org, food, temper, n,
                                    a, alcl, aucl,
                                    mu, mulcl, muucl,
                                    lambda, lambdalcl, lambdaucl,
                                    correl_amu, correl_alambda,
correl_mulambda,
                                    RSE, aic,
                                    row.names=NULL, stringsAsFactors=FALSE)
  return(dfResultGompertzAll)
}
################################################
################################################
```

#Grouping all the data appropriately so log reductions can be calculated#

```
# program starts here

x <- read.csv(paste("Output/", inputFile, sep=""), stringsAsFactors=FALSE)
glimpse(x)

CombinedEnrich <- x
startData <- CombinedEnrich %>%
  group_by(Food, Organism, repDate, Temperature) %>%
  filter(treatTime==0) %>%
  ungroup()
glimpse(startData)

for (i in 1:nrow(startData)) {
  exper <- filter(CombinedEnrich,
                  Food==startData$Food[i],
                  Organism==startData$Organism[i],
                  repDate==startData$repDate[i],
                  Temperature==startData$Temperature[i])
  StartAt0 <- filter(startData,
                     Food==startData$Food[i],
                     Organism==startData$Organism[i],
                     repDate==startData$repDate[i],
                     Temperature==startData$Temperature[i])$logCFUpermL
  # glimpse(exper)
  # glimpse (StartAt0)
  exper <- arrange(exper, treatTime)
  for (j in 1:nrow(exper)) {
    exper$newLR[j] <- (StartAt0 - exper$logCFUpermL[j])
    # check. both these are logs, so log subtraction used
    exper$fracSurv[j] <- exper$logCFUpermL[j] - StartAt0
  }
  if (i==1) {
    newCombined <- exper
  } else {
    newCombined <- rbind(newCombined, exper)
```

```
  }
}

glimpse(newCombined)
write.csv(newCombined, paste("Output/fracSurv", outputFile, sep=""),
row.names=FALSE)

#################################################################
### summary statistics table of log reductions
################################################################

#Summary statistics: mean, st. dev., min, and max calculated for
all log reductions#

summaryLR <- newCombined %>%
  group_by(Organism, Food, treatTime, Temperature) %>%
  summarise(logReduct=mean(newLR, na.rm=TRUE),
            sdLR=sd(newLR, na.rm=TRUE),
            minLR=min(newLR, na.rm=TRUE),
            maxLR=max(newLR, na.rm=TRUE),
            n=n()) %>%
  ungroup()
summaryLR
write.csv(summaryLR, paste("Output/fracSurv", summaryLogReductFile, sep=""),
row.names=FALSE)
#################################################################

#################################################################
### all models with all individual points used
#################################################################

#For loop to construct models for all experiments#


vOrg <- unique(newCombined$Organism)
vFood <- unique(newCombined$Food)
vTemper <- unique(newCombined$Temperature)
# iOrg <- 1; jFood <- 1; kTemper <- 1
for (iOrg in 1:length(vOrg)) {
    for (jFood in 1:length(vFood)) {
        for (kTemper in 1:length(vTemper)) {
            dfExper <- filter(newCombined,
                              treatTime!=-1,
                              Organism == vOrg[iOrg],
                              Food == vFood[jFood],
                              Temperature == vTemper[kTemper])
            if (nrow(dfExper) > 0) {
                if (useFirstOrderAll) {
                    dfFOResult <- firstOrderAll(vOrg[iOrg],
                                                vFood[jFood],
                                                vTemper[kTemper],
                                                dfExper)
                    if (iOrg==1 & jFood==1 & kTemper==1) {
                        FirstOrderAllPoints <- dfFOResult
                    } else {
                        FirstOrderAllPoints <- rbind(FirstOrderAllPoints,
dfFOResult)
                    }
                }
                if (useWeibullAll) {
                    dfWeibullResult <- weibullAll(vOrg[iOrg],
                                                  vFood[jFood],
                                                  vTemper[kTemper],
                                                  dfExper)
                    if (iOrg==1 & jFood==1 & kTemper==1) {
```

```
                    WeibullAllPoints <- dfWeibullResult
                } else {
                  WeibullAllPoints <- rbind(WeibullAllPoints,
dfWeibullResult)
                }
            }
            if (useGompertzAll) {
                dfGompertzResult <- gompertzAll(vOrg[iOrg],
                                                vFood[jFood],
                                                vTemper[kTemper],
                                                dfExper)
                if (iOrg==1 & jFood==1 & kTemper==1) {
                    GompertzAllPoints <- dfGompertzResult
                } else {
                    GompertzAllPoints <- rbind(GompertzAllPoints,
dfGompertzResult)
                }
            }
        }
    }
  }
}

if (useFirstOrderAll) {
    names(FirstOrderAllPoints) <- c("Organism", "Food", "Temperature", "n",
                                    "FO k", "FO LCL k", "FO UCL k",
                                    "FO Rsq", "FO RSE", "FO AIC")
    write.csv(FirstOrderAllPoints,
              paste("Output/fracSurv/FirstOrderAllPoints ", Sys.Date(),
".csv", sep=""),
              row.names=FALSE)
}

if (useWeibullAll) {
    names(WeibullAllPoints) <- c("Organism", "Food", "Temperature", "n",
                                 "W k", "W k LCL", "W k UCL",
                                 "W a", "W a LCL", "W a UCL",
                                 "W a-k correlation", "W RSE", "W AIC")
    write.csv(WeibullAllPoints,
              paste("Output/fracSurv/WeibullAllPoints ", Sys.Date(), ".csv",
sep=""),
              row.names=FALSE)
}

if (useGompertzAll) {
  names(GompertzAllPoints) <- c("Organism", "Food", "Temperature", "n",
                                "G A", "G A LCL", "G A UCL",
                                "G mu", "G mu LCL", "G mu UCL",
                                "G lambda", "G lambda LCL", "G lambda UCL",
                                "G a-mu correl", "G a-lambda correl", "G mu-
lambda correl",
                                "G RSE", "G AIC")

    write.csv(GompertzAllPoints,
              paste("Output/fracSurv/GompertzAllPoints ", Sys.Date(), ".csv",
sep=""),
              row.names=FALSE)

}

#########################################################
### merge all the model parameter data

#Merging all the model parameters into one data table for
comparisons#
```

```
combinedModels <- full_join(FirstOrderAllPoints, WeibullAllPoints,
                            by=c("Organism", "Food", "Temperature", "n") )
combinedModels <- full_join(combinedModels, GompertzAllPoints,
                            by=c("Organism", "Food", "Temperature", "n") )

glimpse(combinedModels)
write.csv(combinedModels,
          file=paste("Output/fracSurv/combinedModelsAllPoints ",
                     Sys.Date(), ".csv", sep=""),
          row.names=FALSE)

### comparison
plot(combinedModels$`FO k`, combinedModels$`W k`,
     xlab="First order k", ylab="Weibull k", pch=19)
abline(a=0, b=1)
grid()

plot(combinedModels$`W k`, combinedModels$`W a`,
     xlab="Weibull k", ylab="Weibull a", pch=19)


#Graphing the models#

#################################################################
### individual experiments with model predictions
# iOrg <- 1; jFood <- 1; kTemper <- 1
tm <- seq(0,40,by=0.1)
for (iOrg in 1:length(vOrg)) {
  for (jFood in 1:length(vFood)) {
    for (kTemper in 1:length(vTemper)) {
      dfExper <- filter(newCombined,
                        treatTime!=-1,
                        Organism == vOrg[iOrg],
                        Food == vFood[jFood],
                        Temperature == vTemper[kTemper])
      if (nrow(dfExper) > 0) {
        # plot the data points on logscale
        if(savePlotToDisk) {
          fn <- paste("Output/fracSurv/graphs/",vOrg[iOrg], vFood[jFood],
                      vTemper[kTemper], ".tiff", sep="")
          tiff(filename = fn, width=8, height=6, units="in",
               comp="lzw", res=300)
        }

        plot(dfExper$treatTime, dfExper$fracSurv, pch=19, col="black",
             main=paste(vOrg[iOrg], vFood[jFood], vTemper[kTemper]),
             xlab="Time (min)", ylab="Population Reduction (log CFU/g)",
             cex.lab=1.25, cex.axis=1.15, cex=1.5)
        params <- filter(combinedModels,
                         Organism == vOrg[iOrg],
                         Food == vFood[jFood],
                         Temperature == vTemper[kTemper])

        FOPred <- params$`FO k`*tm
        lines(tm, FOPred, lwd=3, col="red")
        WPred <- params$`W k`*tm^params$`W a`
        lines(tm, WPred, lwd=3, lty=2, col="blue")
        GPred <- params$`G A` * exp(-exp((params$`G mu` * exp(1)) / params$`G
A`*(params$`G lambda`-tm)+1))
        lines(tm, GPred, lwd=4, lty=3, col="green")
        legend("bottomleft",
               legend=c(paste("First Order, RSE=",
                              specify_decimal(params$`FO RSE`,3),
                              ", AIC=",
```

```
                              specify_decimal(params$`FO AIC`,1), sep=""),
                        paste("Weibull,      RSE=",
                              specify_decimal(params$`W RSE`, 3),
                              ", AIC=",
                              specify_decimal(params$`W AIC`,1), sep=""),
                        paste("Gompertz,   RSE=",
                              specify_decimal(params$`G RSE`, 3),
                              ", AIC=",
                              specify_decimal(params$`G AIC`, 1), sep="")),
               col=c("red", "blue", "green"),
               lty=c(1,2,3), lwd=c(2,2,3))
        if(savePlotToDisk) dev.off()
      }
    }
  }
}
```

#Graphing the models for publication so that each type of model is represented on a single graph#

```
#################################################################
# Combined graphs for publication

# Utility method to filter data by food, organism, and temperature
getFoodData <- function(food, rawData) {
  foodData <- filter(rawData,
    treatTime!=-1,
    Organism == food[2],
    Food == food[1],
    Temperature == food[3])

  return (foodData)
}

apricot <- c("Apricot", "E. coli", "72")
macanut <- c("MacaNut", "Salmonella", "72")
raisins <- c("Raisins", "Pediococcus", "72")

# Get the data for each food
apricotData <- getFoodData(apricot, newCombined)
macanutData <- getFoodData(macanut, newCombined)
raisinsData <- getFoodData(raisins, newCombined)

# Save graph file
if(savePlotToDisk) {
  fn <- paste("Output/fracSurv/Graphs/CombinedGraphForPub.tiff", sep="")
  tiff(filename = fn, width=8, height=6, units="in", comp="lzw", res=300)
}

# Food and Organism (points)
par(family = "Times New Roman")
plot(macanutData$treatTime, macanutData$fracSurv, pch=0, col="black",
     main=paste("Model Fits at 72°C"),
     xlab="Time (min)", ylab="Population Reduction (log CFU/g)",
     xlim=c(1,39), ylim=c(-8,0),
     cex.lab=1.1, cex.axis=1.05, cex=1.0)
points(apricotData$treatTime, apricotData$fracSurv, "p", pch=20, col="black")
points(raisinsData$treatTime, raisinsData$fracSurv, "p", pch=4, col="black")

# Models (lines)
plotModelLine <- function(modelType, allModelData, experiment, color,
lineType) {
  temperatureSequence <- seq(0, 40, by=0.1)
```

```
  if (str_detect(modelType, "gompertz")) {
    params <- filter(allModelData, Organism == experiment[2], Food ==
experiment[1], Temperature == experiment[3])
    params <- select(params, -`FO k`,-`FO LCL k`,-`FO UCL k`,-`FO Rsq`,-`FO
RSE`,-`FO AIC`,
      -`W k`,-`W k LCL`,-`W k UCL`,-`W a`,-`W a LCL`,-`W a UCL`,-`W a-k
correlation`,-`W RSE`,-`W AIC`)
    modelData <- params$`G A` * exp(-exp((params$`G mu` * exp(1)) / params$`G
A`*(params$`G lambda`-temperatureSequence)+1))
    lines(temperatureSequence, modelData, lwd=2, lty=lineType, col=color)
  }

  else if (str_detect(modelType, "weibull")) {
    params <- filter(allModelData, Organism == experiment[2], Food ==
experiment[1], Temperature == experiment[3])
    params <- select(params, -`FO k`,-`FO LCL k`,-`FO UCL k`,-`FO Rsq`,-`FO
RSE`,-`FO AIC`,
      -`G A`,-`G A LCL`,-`G A UCL`,-`G mu`,-`G mu LCL`,-`G mu UCL`,-`G
lambda`,-`G lambda LCL`,-`G lambda UCL`,-`G RSE`,-`G AIC`)
    modelData <- params$`W k`*temperatureSequence^params$`W a`
    lines(temperatureSequence, modelData, lwd=2, lty=lineType, col=color)
  }
}

# Model lines
plotModelLine("gompertz", combinedModels, apricot, "black", 1)
plotModelLine("weibull", combinedModels, macanut, "black", 2)
plotModelLine("weibull", combinedModels, raisins, "black", 3)

# Legend
experimentLabels <- c(expression("Apricots"~paste(italic("E. coli")~"-
Gompertz")),
  expression("MacNuts"~paste(italic("Salmonella")~"- Weibull")),
  expression("Raisins"~paste(italic("Pediococcus")~"- Weibull")))
legend("topright", legend=experimentLabels, lty=c(1,2,3), lwd=c(2,2,2))

# Save graph file
if(savePlotToDisk) {
  dev.off()
}
```

Comparing the model fits#

```
##################################################################
### misc graphs
plot(FirstOrderAllPoints$`FO AIC`, WeibullAllPoints$`W AIC`,
     col="blue", pch=17,
     main="AIC comparison", xlab="First order regression AIC",
     ylab="Nonlinear regression AIC")
points(FirstOrderAllPoints$`FO AIC`, GompertzAllPoints$`G AIC`,
       col="red", pch=19)
abline(a=0,b=1)
legend("topleft", legend=c("Weibull", "Gompertz"),
       pch=c(17,19), col=c("blue","red"))

glimpse(FirstOrderAllPoints)
glimpse(WeibullAllPoints)
par(mfrow=c(3,1))
plot(FirstOrderAllPoints$`FO AIC`, WeibullAllPoints$`W AIC`,
     col="blue", pch=19,
     xlab="First order regression AIC",
     ylab="Weibull regression AIC")
abline(a=0,b=1)
plot(FirstOrderAllPoints$`FO AIC`, GompertzAllPoints$`G AIC`,
```

```
     col="red", pch=19,
     xlab="First order regression AIC",
     ylab="Gompertz regression AIC")
abline(a=0,b=1)
plot(WeibullAllPoints$`W AIC`, GompertzAllPoints$`G AIC`,
     col="green", pch=19,
     xlab="Weibull regression AIC",
     ylab="Gompertz regression AIC")
abline(a=0,b=1)
par(mfrow=c(1,1))



#Conducting ANOVA for log reductions that compares each
treatment to the 0-min treatments, and compares each
microorganism within treatments#

rm(list = ls())
library(tidyverse)
library(tidyselect)
library(readxl)
library(rpart)
library(rpart.plot)
library(plotrix)
library(ggplot2)
library(minpack.lm)
library(dplyr)
library(digest)
library(agricolae)


x <- read.csv("Output/RevisedCombinedEnrich 20190716.csv",
stringsAsFactors=FALSE)
glimpse(x)

vOrg <- unique(x$Organism)
vFood <- unique(x$Food)
vTemper <- unique(x$Temperature)
iOrg<-1; jFood<-1; kTemper<-1
cat("", file="Output/ANOVA_Treatment.txt", sep="\n")
x$Treatment<- as.factor(x$Treatment)
for (jFood in 1:length(vFood)) {
  for (kTemper in 1:length(vTemper)) {
    for (iOrg in 1:length(vOrg)) {
      dfExper <- filter(x,
                        Treatment!=-1,
                        Organism == vOrg[iOrg],
                        Food == vFood[jFood],
                        Temperature == vTemper[kTemper])
      if(nrow(dfExper) > 0) {
        xaov <- aov(newLR~Treatment, data=dfExper)
        cat(paste("\n\n", vOrg[iOrg], vFood[jFood], vTemper[kTemper], "\n"),
file="Output/ANOVA_Treatment.txt", sep="\n", append=TRUE)
        cat(capture.output(summary(xaov)), file="Output/ANOVA_Treatment.txt",
sep="\n", append=TRUE)
        cat(capture.output(HSD.test(xaov, "Treatment", group=TRUE,
console=TRUE)), file="Output/ANOVA_Treatment.txt", sep="\n", append=TRUE)
      }
    }
  }
}


x <- read.csv("Output/RevisedCombinedEnrich 20190716.csv",
stringsAsFactors=FALSE)
```

```
glimpse(x)

vTreatment <- unique(x$Treatment)
vFood <- unique(x$Food)
vTemper <- unique(x$Temperature)
iTreatment<-1; jFood<-1; kTemper<-1
cat("", file="Output/ANOVA_Organism.txt", sep="\n")
for (jFood in 1:length(vFood)) {
  for (kTemper in 1:length(vTemper)) {
    for (iTreatment in 1:length(vTreatment)) {
      dfExper <- filter(x,
                        Treatment!=-1,
                        Treatment!=0,
                        Treatment == vTreatment[iTreatment],
                        Food == vFood[jFood],
                        Temperature == vTemper[kTemper])
      if(nrow(dfExper) > 0) {
        xaov <- aov(newLR~Organism, data=dfExper)
        cat(paste("\n\n", vTreatment[iTreatment], vFood[jFood],
vTemper[kTemper], "\n"), file="Output/ANOVA_Organism.txt", sep="\n",
append=TRUE)
        cat(capture.output(summary(xaov)), file="Output/ANOVA_Organism.txt",
sep="\n", append=TRUE)
        cat(capture.output(HSD.test(xaov, "Organism", group=TRUE,
console=TRUE)), file="Output/ANOVA_Organism.txt", sep="\n", append=TRUE)
      }
    }
  }
}
```

#Reading in temperature profiles based on manually determined start and stop times#

```
# Instructions for Temperature Profiles
#
# Reading in the file:
#   "Vacuum decontam" -> "R_Code" -> "Analyses" -> "Temp Files" ->
"TempProfileTimes.xlsx"
#
# Read "File Folder" column to locate folder
# Read "File Name" column to pick a specific file (.csv) within the folder
#
# Temperatures to be read are listed in the column designated by/matching the
"Channel" column (either Channel 2 or Channel 3).
#
# Temp profile starts the amount of time listed in "TempRiseTime" column
before the time listed in "Start" column.
# Temp profile ends the amount of time listed in "ResidualTime" column after
the time listed in "End" column.
#
# Locate max temp recorded in the profile.
# Locate min temp recorded in the profile specifically between the "Start"
and "End" times.
#
# Need two output files:
#   "AllTempProfiles" - columns needed: File Folder, File Name, Food, Temp
(62, 72, or 82), Treatment, Channel, Start, End, TempRiseTime, ResidualTime,
Max Temp, Min Temp
#   "AverageTemp" - columns needed: Food, Temp (62, 72, or 82), Treatment,
Average TempRiseTime + St. Dev., Average ResidualTime + St. Dev., Average Max
+ St. Dev., Average Min + St. Dev.

rm(list = ls())
```

```
library(plyr)
library(tidyverse)
library(tidyselect)
library(readxl)
library(lubridate)
# library(chron)
x <- read_excel("./Analyses/Temp files/TempProfileTimes.xlsx")
toDisk <- TRUE
glimpse(x)
nFiles <- nrow(x)

for (i in 1:nFiles) {
    nameFolder <- x$FileFolder[i]
    nameFile <- x$FileName[i]
    # startTime <- parse_date_time(x$Start[i], "%y-%m-%d H:M:S")
    dfTemper <- read.csv(paste("./Analyses/Temp files/", nameFolder, "/",
nameFile, ".csv", sep=""),
                         skip=4, header=FALSE, stringsAsFactors=FALSE)
    dfTemper <- dfTemper[-nrow(dfTemper),]
    dfTemper <- dfTemper[,-ncol(dfTemper)]
    names(dfTemper) <-c("ToD", "SampleNumber",
                        "Channel1", "Channel2", "Channel3",
                        "Channel4", "Channel5", "Channel6",
                        "ColdJunction1",  "ColdJunction2",
                        "ColdJunction3",  "ColdJunction4")
    dfTemper <- dfTemper %>%
                mutate(vecTime=parse_date_time(dfTemper[,1], "H:M:S"))
    # glimpse(dfTemper)

    startTime <- parse_date_time(x$Start[i], "%y-%m-%d H:M:S")
    year(startTime) <- 0; month(startTime) <- 1; mday(startTime) <- 1
    # startTime
    temperProfileStart <- startTime - seconds(x$secondsRise[i])
    # temperProfileStart

    endTime <- parse_date_time(x$End[i], "%y-%m-%d H:M:S")
    year(endTime) <- 0; month(endTime) <- 1; mday(endTime) <- 1
    # endTime
    temperProfileEnd <- endTime + seconds(x$secondsResid[i])
    # temperProfileEnd

    # NOTE: the filter for the statistics runs from
    # startTime, not temperProfileStart
    dfTemperProfile <- dfTemper %>%
                       filter(vecTime >= startTime,
                              vecTime<=temperProfileEnd)
    # glimpse(dfTemperProfile)

    dfTemperProfileGraph <- dfTemper %>%
                             filter(vecTime >= temperProfileStart,
                             vecTime<=temperProfileEnd)

    useChannel <- x$Channel[i]
    vecTemper <- as.numeric(dfTemperProfile[,useChannel+2])
    minTemper <- min(vecTemper)
    maxTemper <- max(vecTemper)
    newAllTempProfileRow <- c(x$FileFolder[i],
                              x$FileName[i],
                              x$Food[i],
                              x$Tref[i],
                              x$Treatment[i],
                              x$Channel[i],
                              strftime(x$Start[i], format = "%H:%M:%S",
tz="UTC"),
```

```
                              strftime(x$End[i], format = "%H:%M:%S",
tz="UTC"),
                              x$secondsRise[i],
                              x$secondsResid[i],
                              strftime(temperProfileStart, format =
"%H:%M:%S", tz="UTC"),
                              strftime(temperProfileEnd, format = "%H:%M:%S",
tz="UTC"),
                              maxTemper,
                              minTemper,
                              use.names=FALSE)

    if (i==1) {
        allTempProfiles <- newAllTempProfileRow
    } else {
        allTempProfiles <- rbind(allTempProfiles, newAllTempProfileRow)
    }
    if (toDisk) {
       tiff(filename=paste("./Analyses/temperature output/",
                           x$FileFolder[i], " ",
                           x$FileName[i], " ",
                           x$Food[i], " ",
                           x$Tref[i], ".tiff", sep=""),
            width=6, height=6, units="in", res=300, compression="lzw")
    }
    vecTemperGraph <- as.numeric(dfTemperProfileGraph[,useChannel+2])
    plot(dfTemperProfileGraph$vecTime, vecTemperGraph,
             xlab="Time", ylab="Temperature",
             main=paste(x$FileName[i],
                        x$Food[i],
                        x$Tref[i], sep=" "))
        abline(v=startTime, lty=2)
    if (toDisk) dev.off()

}
```

#Combining all the temperature profile summaries into one Excel file, in which the averages for treatments were calculated#

```
allTempProfiles <- as.data.frame(allTempProfiles)
colnames(allTempProfiles) <- c("FileFolder", "FileName", "Food",
                            "Tref", "Treatment", "Channel",
                            "StartTime", "EndTime",
                            "SecondsRise", "SecondsResidual",
                            "ProfileStart", "ProfileEnd",
                            "MaxTemper", "MinTemper")
write.csv(allTempProfiles, "allTempProfiles.csv", row.names=FALSE)
###################################################
```

D- and *z*-values of each experiment.

Table 7.3. Decimal reduction times (min) for *E. coli* O121 broth cultures at 56, 59, and 62°C.

O121 Broth									
	R1	R2	R3	R4	Avg. D val	St. Dev.	O121 Broth		
56C	38.02	34.72	61.35	30.03	34.26	4.02	Temp	Log D-val	St. Dev.
Log 56	1.580	1.541	1.788	1.478			56	1.53	0.05
59C	3.53	6.31	7.66	4.54	4.79	1.41	59	0.72	0.15
Log 59	0.547	0.800	0.884	0.657			62	0.32	0.21
62C	1.09	3.50	2.27	2.11	2.23	1.21			
Log 62	0.036	0.544	0.356	0.325					

*Cells highlighted in orange were omitted and an additional replication was performed due to an experimental error.

Table 7.4. Decimal reduction times (min) for *E. coli* O121 lawn cultures at 56, 59, and 62°C.

O121 Lawn									
	R1	R2	R3	R4	Avg. D val	St. Dev.	O121 Lawn		
56C	19.46	19.23	33.00	14.66	17.78	2.70	Temp	Log D-val	St. Dev.
Log 56C	1.289	1.284	1.519	1.166			56	1.25	0.07
59C	2.30	2.55	2.43	2.28	2.38	0.43	59	0.38	0.02
Log 59C	0.362	0.407	0.385	0.357			62	-0.15	0.11
62C	0.61	1.02	0.66	0.59	0.74	0.24			
Log 62C	-0.216	0.008	-0.179	-0.232					

*Cells highlighted in orange were omitted and an additional replication was performed due to an experimental error.

Table 7.5. Decimal reduction times (min) for *E. coli* O156 broth cultures at 56, 59, and 62°C.

O157 Broth									
	R1	R2	R3	R4	Avg. D val	St. Dev.	O157 Broth		
56C	26.53	22.83	29.76	27.17	26.57	2.86	Temp	Log D-val	St. Dev.
Log 56C	1.424	1.359	1.474	1.434			56	1.42	0.05
59C	3.24	6.00	4.56	4.56	4.59	1.13	59	0.65	0.11
Log 59C	0.511	0.778	0.659	0.659			62	0.20	0.22
62C	0.80	2.43	1.46	2.18	1.72	0.74			
Log 62C	-0.097	0.386	0.164	0.339					

Table 7.6. Decimal reduction times (min) for *E. coli* O157 lawn cultures at 56, 59, and 62°C.

O157 Lawn									
	R1	R2	R3		Avg. D val	St. Dev.	O157 Lawn		
56C	10.24	10.80	11.25	9.07	10.76	0.51	Temp	Log D-val	St. Dev.
Log 56C	1.010	1.033	1.051	0.957			56	1.03	0.02
59C	3.02	4.94	3.25	2.44	3.41	1.08	59	0.52	0.13
Log 59C	0.480	0.694	0.512	0.387			62	-0.27	0.03
62C	0.53	0.50	0.60	0.51	0.54	0.04			
Log 62C	-0.273	-0.297	-0.225	-0.294					

Table 7.7. Decimal reduction times (min) for *E. coli* O121 in low-water activity solution (a_w 0.50) at 56, 59, and 62°C.

O121 Glycerol 0.50 a_w								
	R1	R2	R3	Avg. D val	St. Dev.	O121 Glycerol		
56C	7.82	6.33	8.96	7.70	1.32	Temp	Log D-val	St. Dev.
Log 56C	0.893	0.802	0.952			56	0.88	0.06
59C	3.69	4.61	4.44	4.25	0.49	59	0.63	0.05
Log 59C	0.567	0.664	0.647			62	0.17	0.01
62C	1.53	1.50	1.44	1.49	0.05			
Log 62C	0.186	0.176	0.159					

Table 7.8. Decimal reduction times (min) for *E. coli* O121 in intermediate-water activity solution (a_w 0.75) at 56, 59, and 62°C.

O121 NaCl 0.75 a_w								
	R1	R2	R3	Avg. D val	St. Dev.	O121 NaCl		
56C	10.98	15.97	8.59	11.85	3.77	Temp	Log D-val	St. Dev.
Log 56C	1.040	1.203	0.934			56	1.06	0.12
59C	4.53	4.52	4.04	4.36	0.28	59	0.64	0.03
Log 59C	0.656	0.655	0.606			62	0.29	0.09
62C	2.44	1.66	1.80	1.97	0.42			
Log 62C	0.388	0.219	0.255					

Table 7.9. Decimal reduction times (min) for *E. coli* O157 in low-water activity solution (a_w 0.50) at 56, 59, and 62°C.

O157 Glycerol 0.50 a_w								
	R1	R2	R3	Avg. D val	St. Dev.	O157 Glycerol		
56C	6.60	7.59	10.54	8.24	2.05	Temp	Log D-val	St. Dev.
Log 56C	0.820	0.880	1.023			56	0.91	0.10
59C	3.13	3.94	5.18	4.09	1.03	59	0.60	0.11
Log 59C	0.496	0.596	0.714			62	0.14	0.08
62C	1.16	1.37	1.70	1.41	0.27			
Log 62C	0.064	0.136	0.231					

Table 7.10. Decimal reduction times (min) for *E. coli* O157 in intermediate-water activity solution (a_w 0.75) at 56, 59, and 62°C.

O157 NaCl 0.75 aw								
	R1	R2	R3	Avg. D val	St. Dev.	O157 NaCl		
56C	10.22	14.01	9.83	11.35	2.30	Temp	Log D-val	St. Dev.
Log 56C	1.010	1.146	0.993			56	1.05	0.08
59C	6.96	4.91	6.17	6.01	1.03	59	0.77	0.08
Log 59C	0.843	0.691	0.790			62	0.33	0.13
62C	2.90	1.61	2.14	2.22	0.65			
Log 62C	0.462	0.207	0.331					

Table 7.11. Calculated *z*-values (°C) based on the log *D*-values for each culture.

z-values calculated from each replication (°C)			
	Average	St. Dev	R_2
O121 Broth	5.75	0.47	0.95
O121 Lawn	4.33	0.36	0.98
O157 Broth	5.04	0.98	0.97
O157 Lawn	4.67	0.04	0.97
O121 a_w 0.5	8.54	1.02	0.96
O157 a_w 0.5	7.82	0.21	0.99
O121 a_w 0.75	8.04	1.70	0.99
O157 a_w 0.75	8.81	2.30	0.97